Thyroid Hormones

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Chemistry and Biology in the Biosynthesis and Action of Thyroid Hormones

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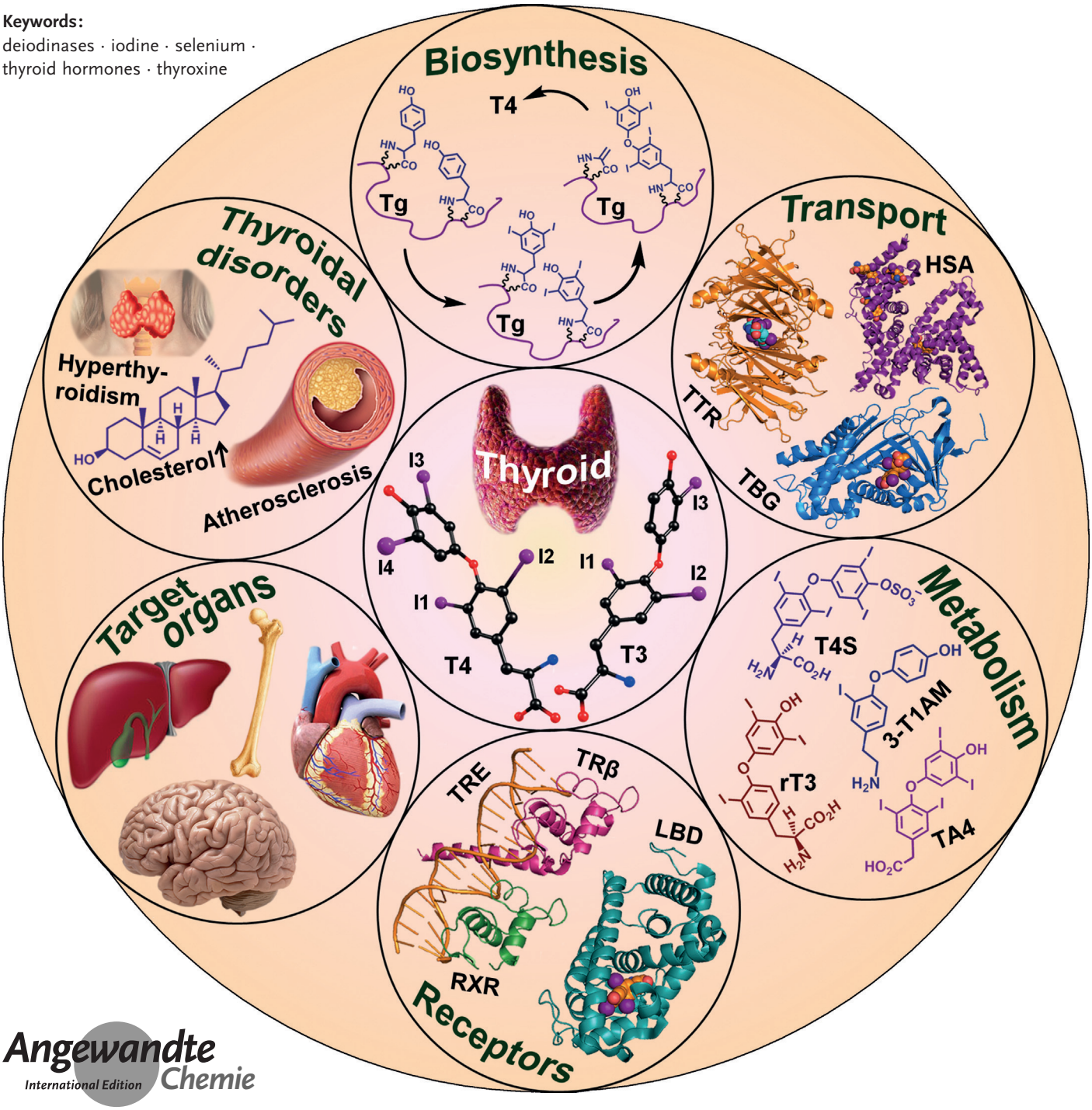

Thyroid hormones (THs) are secreted by the thyroid gland. They control lipid, carbohydrate, and protein metabolism, heart rate, neural development, as well as cardiovascular, renal, and brain functions. The thyroid gland mainly produces L-thyroxine (T4) as a prohormone, and 5′-deiodination of T4 by iodothyronine deiodinases generates the nuclear receptor binding hormone T3. In this Review, we discuss the basic aspects of the chemistry and biology as well as recent advances in the biosynthesis of THs in the thyroid gland, plasma transport, and internalization of THs in their target organs, in addition to the deiodination and various other enzyme-mediated metabolic pathways of THs. We also discuss thyroid hormone receptors and their mechanism of action to regulate gene expression, as well as various thyroid-related disorders and the available treatments.

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1. Introduction

Thyroid hormones play key roles in the human endocrine system and control the overall metabolism of the body, protein synthesis, carbohydrate and fat metabolism, neural development, normal growth and maturation of bones, as well as cardiovascular and renal functions. Thyroid hormones are secreted from the thyroid gland, which mainly produces the prohormone L-thyroxine or L-3,5,3′,5′-tetraiodothyronine (T4) and a smaller fraction of the biologically active hormone L- 3,5,3′-triodothyronine (T3; Figure 1).[1] In the endocrine system, the hypothalamus and pituitary gland control the production of THs by the thyroid gland. Neurons in the paraventricular nucleus within the hypothalamus secrete thyrotropin-releasing hormone (TRH), which stimulates the pituitary gland via a G-protein-coupled receptor to secrete thyrotropin/thyroid-stimulating hormone (TSH). The binding of TSH to its G-protein-coupled receptor in the thyroid stimulates the biosynthesis of T4 and T3, which are secreted into the plasma and exhibit specific functions at target organs, as shown in Figure 1. The set value of plasma TSH is adjusted by negative feedback of THs at the levels of the hypothalamus and pituitary (Figure 1). The combined action of the hypothalamus, pituitary gland, and thyroid gland is known as the hypothalamus-pituitary-thyroid axis and adjusts thyroid hormone levels to the physiological state of the body.[2]

The biosynthesis of T4 in the thyroid follicular cells involves the cooperation of a number of major components such as thyroglobulin (Tg), thyroid peroxidase (TPO), iodide, and hydrogen peroxide. After biosynthesis, T4 is carried to various target organs by different transfer proteins such as thyroxine-binding globulin (TBG), transthyretin (TTR), and serum albumin, and is internalized into cells by transmembrane transport proteins such as monocarboxylate transporter 8 (MCT8) and MCT10. In the target cells, T4 undergoes various types of metabolism, of which the regioselective deiodination of the phenolic and tyrosyl ring plays an important role in TH homeostasis. The deiodination reactions are catalyzed by iodothyronine deiodinases (DIOs), a set of selenoenzymes having different selectivity for deiodination. The monodeiodination at the phenolic ring converts T4 into T3, whereas deiodination at the tyrosyl ring converts T4 into a biologically inactive metabolite L-3,3′,5′-triiodothyronine (rT3). The nuclear thyroid hormone receptors (TRα and TRβ) can bind T3 with high affinity and T4 with lower affinity. Thyroid receptors (TRs) may bind as homo- and/or heterodimers, such as with the 9-*cis*-retinoic acid receptor (RXR) to form the TR/RXR complex, which can bind to thyroid hormone responsive elements (TREs) through the presence of a specific nucleotide sequence in the target genes.

The binding of the TR/RXR complex to the TREs induces the activation of co-repressor and co-activators, which ultimately regulate gene expression. As a consequence of their tissue-specific expression patterns, the actions of TRs are highly tissue-specific; for example, TRα affects the heart, whereas TRβ controls lipid and fat metabolism in the liver and mediates TH feedback in the pituitary gland. An imbalance in the thyroid hormone levels leads to various diseases such as hypothyroidism, hyperthyroidism, cardiovascular dysfunctions, atherosclerosis, and diabetes. In this Review, we discuss the basic chemistry and biology of TH action as well as recent advances in the area of TH metabolism.

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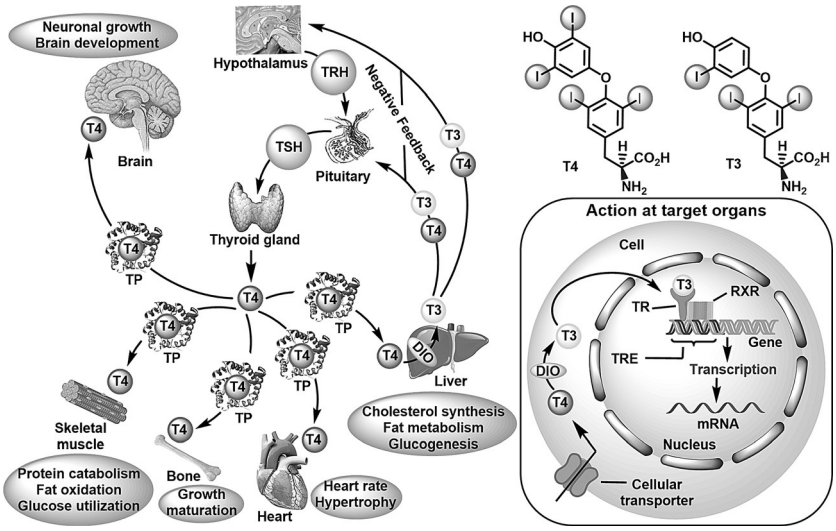

Figure 1. Schematic representation of the action of thyroid hormones in different target organs. TRH: thyrotropin-releasing hormone, TSH: thyroid-stimulating hormone, TP: transfer protein, DIO: iodothyronine deiodinase, TR: thyroid hormone receptor, RXR: retinoid X receptor, TRE: thyroid hormone responsive element.

2. Biosynthesis of Thyroid Hormones

T4 is synthesized in the thyroid follicles, which are the functional units of the mature thyroid gland (Figure 2). The spherical follicles are covered by a monolayer of polarized epithelial cells, with the basolateral and apical surfaces facing the blood stream and follicular lumen, respectively. The follicular lumen is filled with a colloid, which mainly comprises a highly cross-linked protein thyroglobulin (Tg) in a concentration range of around 100–750 mg mL^{-1}.[3] The biosynthesis of T4 takes place on Tg in five major steps: a) transfer of inorganic iodide (I^-) from the blood to thyroid follicles through the sodium iodide symporter (NIS), b) generation of hydrogen peroxide (H_2O_2) by peroxidases (DUOX1 and DUOX2), c) iodination of tyrosyl residues of Tg by thyroid peroxidase (TPO) in the presence of H_2O_2 and iodide, d) phenolic coupling of the iodotyrosyl residues on Tg by TPO to form T4, and e) proteolytic liberation of THs from Tg (Figure 2).[4] In this process, a fraction of biologically active T3 is also produced.

2.1. Iodide Uptake and Release into the Follicle

The transport of inorganic iodide into follicular cells is the first step in TH biosynthesis. Although the concentration of iodide in plasma is very low in mammals (50–300 nM), the thyroid gland has a unique transport system to acquire iodide efficiently from the blood.[5] The sodium iodide symporter (NIS) is a transmembrane protein present in the basolateral membrane of thyroid follicles that mediates the uptake of iodide from the blood. The secondary structure model of NIS indicates that the hydrophobic protein with 618 amino acids folds into 13 transmembrane segments (TMS), with the N-terminus and C-terminus projecting towards the extracellular and intracellular regions (Figure 3 A), respectively.[6] The NIS is a highly glycosylated protein, and the three N-linked glycosylation sites (one in the loop between TMS VI and VII

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Karuppusamy Raja obtained his BSc from Bharathiar University in 2006 and MSc from Anna University in 2008. He joined the Indian Institute of Science in 2010 to work in the laboratory of Prof. G. Mugesh for his PhD studies. His research is mainly focused on the deiodination of thyroxine by synthetic deiodinase mimetics.

Govindasamy Mugesh received his PhD from the Indian Institute of Technology, Bombay, in 1998 with Prof. H. B. Singh. After postdoctoral studies with Prof. W.-W. du Mont and Prof. K. C. Nicolaou, he became Assistant Professor in 2002 at the Indian Institute of Science, Bangalore, where he is currently a Professor. His research interests include the chemistry of thyroid hormone metabolism, development of novel therapeutics for endothelial dysfunction and neurodegenerative diseases, and nanomaterials for biological applications.

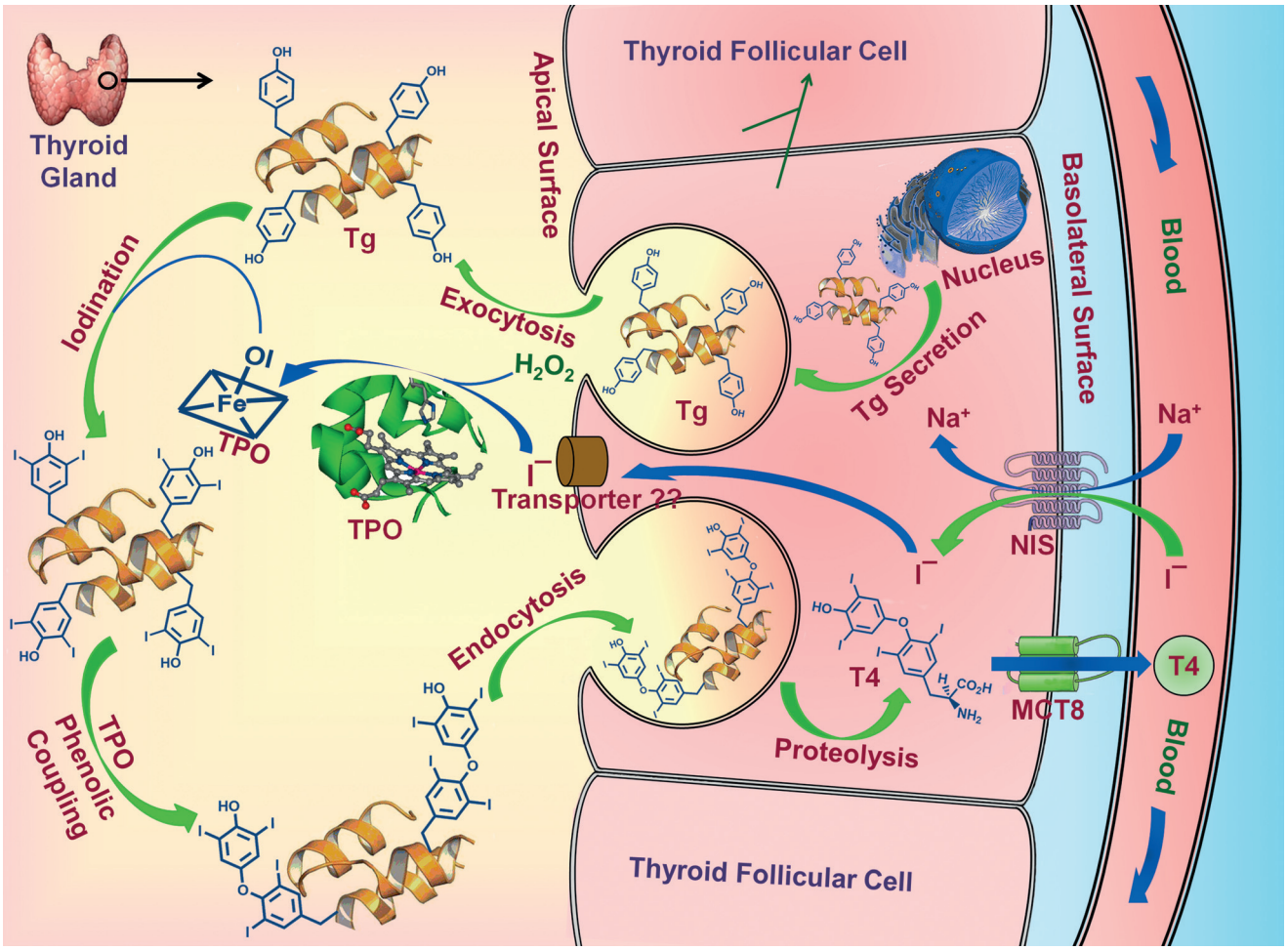


Figure 2. Biosynthesis of thyroxine (T4) in the thyroid follicular cells. A small amount of T3 is also produced in this process. Although the protein responsible for the transport of iodide through the apical membrane of the thyrocytes has been a subject of debate over the years, pendrin and anoctamin 1 are proposed to be the most probable iodide transporters.

and the other two in the loop between TMS XII and XII) are all positioned in the extracellular portion of the protein (Figure 3 A). However, iodide transport has been shown to be independent of the glycosylation of the protein.[6] The K_M values for the transport of iodide and sodium ions are 10–30 μM and 40–60 mM, respectively, thus indicating that the NIS is very selective to iodide ions.[7] However, NIS is known to mediate the translocation of other ions, such as thiocyanate (SCN^-), chlorate (ClO_3^-), nitrate (NO_3^-), and iodate (IO_4^-).[8] The NIS is inhibited by perchlorate, which is, therefore, called an endocrine-disrupting chemical. The action of the NIS depends on the Na^+/K^+-ATPase, which maintains the membrane potential of the cell and the K^+-channel KCNE2.[9] In this process, the NIS acquires a 30–60-fold higher concentration of iodide in the thyroid follicle compared to that in the blood. Iodide transport deficiency caused by mutations in the NIS (V59E, G93R, T354P, and G395R) causes congenital hypothyroidism. Biochemical studies have provided crucial information on the structure and function of NIS,[10] and helped to identify Gly93 as an important residue for the substrate specificity of the NIS.[11]

Iodide is needed in the follicular lumen for hormone biosynthesis. Pendrin (SLC26A4) was regarded as the most probable iodide transporter in the apical membrane of thyrocytes, because mutations in its gene cause Pendred's syndrome (PDS), a heritable disease associated with deafness, goiter, and an iodine organification defect (a defect in the oxidization of iodide and its incorporation into tyrosyl residues of thyroglobulin).[12] Deafness through PDS is apparently unrelated to THs, but caused by structural defects of the inner ear. More recently, anoctamin (ANO1, TMEM16A) has been suggested to be the dominant iodide transporter at the apical membrane of thyrocytes.[13] This protein was initially characterized as a Ca^{2+}-activated chloride channel composed of eight transmembrane segments, but displays specificity for iodide.

2.2. Production of Hydrogen Peroxide by Dual Oxidases

Hydrogen peroxide (H_2O_2) is an essential component for the incorporation of iodide into the tyrosyl residues of Tg (known as organification of iodide) and phenolic coupling of the iodotyrosyl residues by TPO. The generation of H_2O_2 by thyroid follicles was first reported as early as 1971.[14] Later, Björkman et al. identified that the thyroid follicles generate

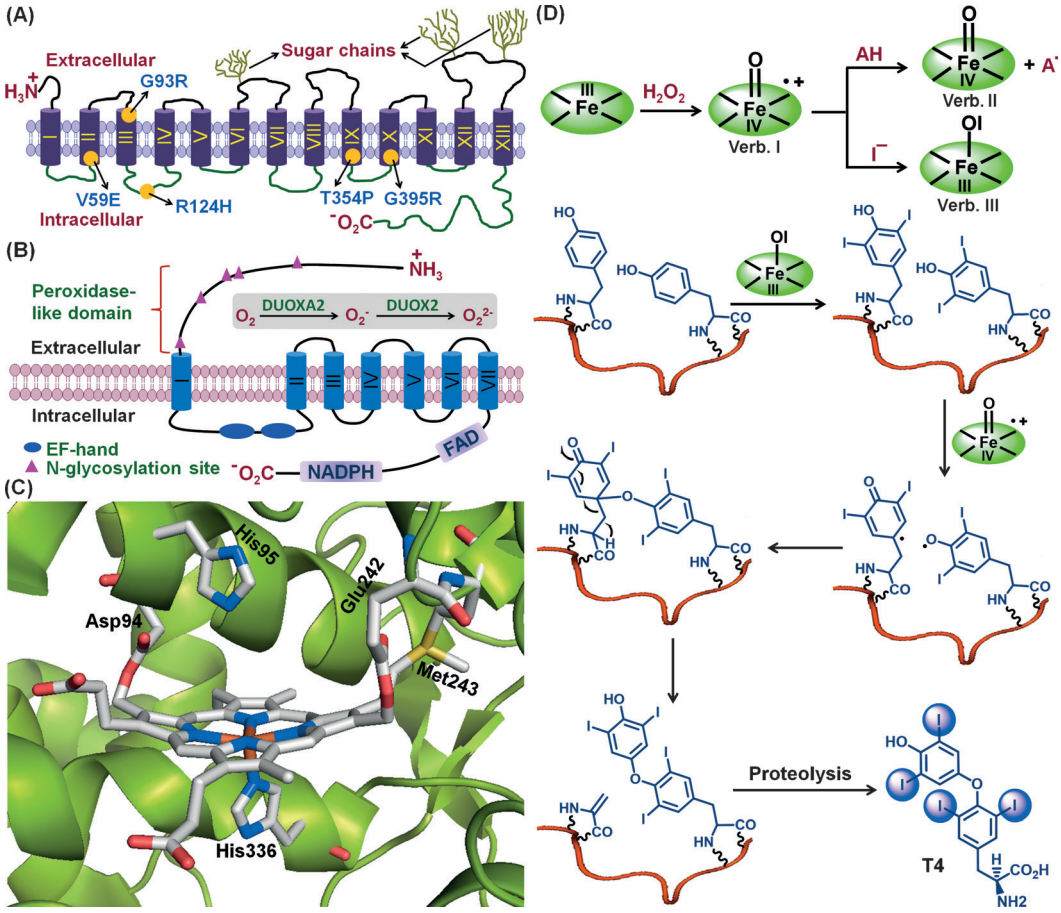


Figure 3. Schematic representation of secondary structure models of A) NIS and B) DUOX2. Some mutations in NIS that cause iodide transport deficiency (ITD) are shown by orange circles. The N-glycosylation sites in NIS have been identified to be N225, N485, and N497. C) The active site of human myeloperoxidase (MPO), indicating the covalent linkages between heme and enzyme through Asp 94, Glu 242, and Met 243 residues, distal histidine (His 95), and proximal histidine (His 336). PDB code: 1CXP.[32] D) Mechanism of biosynthesis of T4 on thyroglobulin (Tg) by thyroid peroxidase. Radical mechanism for the phenolic coupling between two iodotyrosyl residues is shown, although an ionic mechanism has also been reported.[45]

H_2O_2 with the help of NADPH oxidase (NOX) present in the apical membrane of follicular cells.[15] The genes corresponding to two independent NADPH oxidases—dual oxidase 1 (DUOX1) and dual oxidase 2 (DUOX2)—were identified from human thyroid cDNAs.[16] The human DUOX1 and DUOX2 proteins have 1551 and 1548 amino acids, respectively, and have 83% similarity in their DNA sequences. DUOXs are transmembrane glycoproteins with seven transmembrane helices, an extracellular peroxidase-like domain, a long intracellular loop between helix I and II, as well as a NADPH oxidase 2 (NOX2) like domain extending from helix II to the C-terminus (Figure 3B).[16,17]

The extracellular peroxidase-like domain has 43% similarity to TPO, although the peroxidase activity of this domain is putative and controversial.[18] The intracellular loop between transmembrane helix I and II contains two Ca^{2+}-binding EF-hand motifs, and calcium binding to DUOX2 enhances H_2O_2 production.[19] The NOX2-like domain of DUOXs contains one NADPH and one flavine adenine dinucleotide (FAD) binding site, and it retains the structural features of phagocyte NOX2 for effective electron transfer from NADPH to FAD as well as the Comp. II binding of heme and molecular oxygen.[20] Interestingly, the generation of H_2O_2 by Comp. III DUOX2 does not depend on TSH,[21] but decreases as the concentration of iodide increases.[22] However, the DUOX2-mRNA expression is 1.5—5 times higher than that of DUOX1-mRNA in the thyroid gland,[23] and mutations either in DUOX2 or in its maturation factor DUOXA2 can induce hypothyroidism as a consequence of insufficient H_2O_2 generation. As H_2O_2 is produced in the thyroid, it was hypothesised that the biosynthesis would depend on the expression of selenium-dependent peroxidases. However, no significant effect on the TH biosynthesis was observed in mice when the expression of all the selenoproteins was blocked in thyrocytes.[24] These observations suggest that the antioxidative selenoproteins may protect the thyrocytes from oxidative stress, but may not be essential for the survival of thyrocytes.

2.3. Heme-Containing Thyroid Peroxidase

Thyroid peroxidase (TPO) is a member of the heme-containing mammalian peroxidase family, which also includes lactoperoxidase (LPO), myeloperoxidase (MPO), and eosinophil peroxidase (EPO).[25] TPO plays an important role in the organification of iodide (iodination of tyrosine in Tg to form 3-iodotyrosine and 3,5-diiodotyrosine, MIT and DIT, respectively) and phenolic coupling of the iodotyrosyl residues in Tg to ultimately produce T4 and T3.[26] The TPO protein consists of 933 amino acids and forms homodimers.[27] Most parts of TPO, including the active site, remain in the extracellular region situated in the follicle lumen, whereas a small putative transmembrane region at the C-terminal end

(residues 847–871) anchors the protein to the apical thyrocyte membrane.[25b,28] In the mature enzyme, the N-terminal propeptide sequence involving amino acids 1–108 is cleaved.[29] Mutations which impair TPO production or maturation decrease TH production, thereby causing congenital hypothyroidism.[30]

TPO exhibits 47% sequence similarity with human MPO. Based on the X-ray structure of MPO, a homology model of TPO has been presented.[31] MPO also serves as a suitable model of TPO for understanding heme chemistry. The X-ray structure of MPO at 1.8 Å resolution identified the heme prosthetic group as a derivative of protoporphyrin IX, in which the methyl groups on pyrrole rings A and C are modified to allow formation of ester linkages with residues Glu242 and Asp94 (Figure 3C).[32] It has been shown that H_2O_2 mediates the modification of methyl groups on the pyrrole rings (A and C) of heme to hydroxymethyl and dihydroxymethyl groups to facilitate the formation of ester linkages between heme and LPO, which is very similar to MPO.[33] Interestingly, another covalent linkage between one of the vinyl groups in heme and the sulfur atom of Met243 of MPO that leads to the formation of a sulfonium ion has been observed in the crystal structure of MPO (Figure 3C). This covalent linkage is probably unique to MPO, as Met243 is absent from other related peroxidases. In contrast, Asp94 and Glu242 are conserved in all those peroxidases, which indicates that the ester linkages between heme and polypeptide chains may be present in all other peroxidases.[34] The distal histidine residue (His95) acts as a general acid/base catalyst during the catalytic cycle of peroxidases. It abstracts a proton from H_2O_2, thereby facilitating binding to the Fe^{III} center in the active site. Consequently, the Fe^{III} center in the active site undergoes a two-electron oxidation to form a oxoferryl π-cation radical (compound I, Comp. I, Figure 3D) intermediate.[35] In this process, one electron is removed from the metal center and the second electron is removed from the porphyrin ring. Compound I can remove one electron from a wide variety of aromatic alcohols and amines (AH) to form compound II (Comp. II, Figure 3D). In a subsequent reaction, compound II can regenerate the enzyme active site by oxidizing another molecule of AH.

Alternatively, compound I can also mediate the two-electron peroxidation of I^- to form an enzyme-bound active halogenating species, compound III (Comp. III, Figure 3D). The mechanism of the MPO-catalyzed peroxidation of I^- appears to be complex, as both H_2O_2 and I^- act as inhibitors. However, it is proposed that compound III is responsible for the iodination of tyrosyl residues on Tg.[26] The phenolic coupling of Tg-bound iodotyrosines appears to be challenging given the steric bulkiness and complexity of iodinated Tg as a substrate (see Section 2.4).

2.4. Thyroglobulin and T4 Biosynthesis

With the help of I^-, H_2O_2, and TPO, the biosynthesis of T4 takes place on a highly glycosylated homodimeric Tg protein with a size of 660 kDa per polypeptide.[36] Tg is the most abundant protein in the follicular lumen of the thyroid gland and almost 10% of its mass is carbohydrates. Structural analysis of Tg is challenging because of its size and complexity. Glycosylation and other heterogeneous post-translational modifications such as phosphorylation, sulfation, and finally tyrosine iodination further complicate matters. Out of a total of 134 tyrosyl residues, only 6–34 tyrosine residues can be iodinated in vivo at their 3- and 5-positions. Only 1–4 molecules of T4 are formed per molecule of Tg, thus indicating that not all the iodotyrosyl residues in Tg can undergo oxidative phenolic coupling to form TH.[37] In mature Tg, only four tyrosine residues at positions 5, 2553, 2746, and 1290 are associated with the hormone synthesis.[38] Accordingly, these four sites are designated as A, B, C, and D, with A being the major hormonogenic site. In most species, sites A and B account for 40% and 25%, respectively, of the TH production. In contrast, site C is probably associated with the production of T3 in some species, and TSH appears to favor hormonogenesis at site D in guinea pigs and rabbits.[39] A detailed study using isotopically labelled peptide fragments along with the cDNA structure of Tg showed that the iodinated tyrosine residue at position 130 couples with another one at position A to form T4.[40] This finding was further supported by mutational analysis at these two positions.[41] Model reactions on the biosynthesis of T4 suggested that only iodotyrosyl residues that are properly oriented in space can undergo coupling reactions to form T4.[42] Kinetic analysis of the iodination and oxidative coupling reactions helped to conclude that the tyrosyl residues that are iodinated during the early stages of the reactions undergo preferential phenolic coupling reaction to form T4.[43] Interestingly, a time lag between the iodination and coupling reaction has been observed; the coupling reaction always starts 3–5 min after the commencement of the iodination reaction.[44] This observation suggests that Tg may undergo a conformational change after the iodination to align the feasible iodotyrosyl residues in an orientation favorable for the coupling reaction.

Although a free-radical mechanism is shown in Figure 3D for the coupling reaction, both free-radical and ionic mechanisms are reported for the coupling reaction.[45] The major difference between these pathways is that, according to the radical mechanism, one electron is removed from each of the iodotyrosyl residues undergoing coupling, whereas according to an ionic mechanism, two electrons are removed from only one of the iodotyrosyl residues undergoing the coupling reaction. However, both mechanisms support the formation of a dehydroalanine (DHA) residue on Tg along with T4 (Figure 3D). Although the formation of DHA is widely accepted, different research groups characterized the other product formed with T4 as pyruvic acid, alanine, serine, hydroxypyruvic acid, and aminomalonic semialdehyde.[46] After iodination and phenolic coupling, Tg with bound T4 and T3 is stored in the follicular lumen as colloid. When the TSH concentration in the plasma is increased, Tg is internalized into follicular cells through endocytosis; Tg then undergoes proteolysis in the lysosomes to release T4 and T3.

During proteolysis, uncoupled DIT and MIT are released from Tg and undergo deiodination by the flavoprotein iodotyrosine deiodinase (IYD) encoded by the dehalogenase

(DEHAL1) gene.[47] IYD is expressed in the thyroid gland, liver, and kidney, and its role is the recycling of iodide for the de novo biosynthesis of THs. A deficiency of DEHAL1 leads to hypothyroidism, if the loss of iodine through MIT and DIT is not compensated by an increased iodide uptake.[48] The amount of iodide recycled in the thyroid gland is 3–5 times more than that of the iodide ions taken up from dietary sources.[49]

3. Plasma Thyroid Hormone Binding Proteins and Transmembrane Transporters

After biosynthesis in the thyroid gland, thyroid hormones enter the blood stream and associate with plasma thyroid hormone binding (transfer) proteins: thyroxine-binding globulin (TBG), transthyretin (TTR), and human serum albumin (HSA).[50] The crystal structures of complexes between these proteins and their T4 ligands have revealed different types of ligand interactions and ligand conformations. Of the three transfer proteins, TBG exhibits the highest affinity towards THs. However, the plasma concentration of TBG (0.27 μM) is much lower than that of TTR (4.6 μM) and HSA (640 μM).[50] Thus, the TH binding capacities of TTR and HSA are much higher than for TBG, but affinities are lower and TH binding to TTR and HSA is characterized by fast dissociation. To be precise, TBG, TTR, and HSA account for 74%, 11%, and 15%, respectively, of the binding of the total T4 bound by the transfer proteins.[50a] The high affinities of the transport proteins for T4 and T3 result in a slow clearance and prolonged half-life of the thyroid hormones in blood serum.

Thyroid hormones are notorious for their hydrophobicity, and without plasma binding proteins, released THs would immediately stick to blood vessels instead of being distributed in the circulation. However, THs are amino acid derivatives with carboxy and amino groups and, therefore, require membrane transporter proteins to cross cellular membranes. This concept caught on quite late among physicians (in 2004), when patients with severe psychomotor retardation and altered TH function tests were identified who carried mutations in the TH transmembrane transporter, monocarboxylate transporter 8 (MCT8).[51] THs not only enter, but also leave cells via transporters. MCT8 is the major transporter through which THs leave the thyrocyte after production.[52]

3.1. Thyroxine-Binding Globulin

TBG is a 54 kDa protein and, similar to corticosteroid-binding globulin, is a member of the serpin family of protease inhibitors and produced in the liver. Accordingly, TBG retains the structural features of a reactive center loop (RCL), with the reactive center at a position denoted as P1 and the hinge of the loop at P17. Cleavage of the RCL results in an irreversible conformational change of the protein.[53] Cleavage of the RCL in TBG by proteases during sepsis results in an almost threefold decrease in the thyroxine-binding affinity.[54] In the blood, T4 is mainly carried by TBG, with a dissociation constant (K_d) for the TBG-T4 complex of 0.1 nM.[50] A recent crystal structure of the TBG-T4 complex shows that protein carries T4 in a surface cavity between helices H and A and strands 3–5 of the B-sheet (Figure 4A).[55] The crystal structure shows two TBG molecules

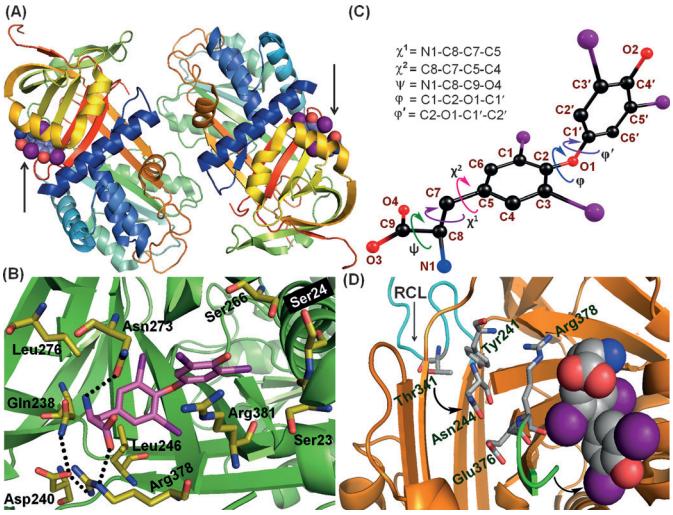


Figure 4. A) Crystal structure of thyroxine-binding globulin (TBG) complexed with T4. The arrows indicate the two T4-binding sites (PDB code: 2CEO).[55] B) Hydrogen-bonding interactions and important amino acid residues in the T4 binding site of TBG. C) Various torsional angles used to define the conformation of T4. D) Allosteric mechanism and the involved amino acid residues for the release of T4 from TBG. The RCL and the s4B-5B loop are colored cyan and green, respectively.

with two identical T4-binding sites (indicated by the arrow in Figure 4A) in the surface cavity of the asymmetric unit. T4 is bound mainly by a series of hydrophobic interactions with various residues in the binding site and a few hydrogen-bonding interactions with the help of its side-chain amine and carboxylic acid groups (Figure 4B). The amine group and carboxylic acid group of T4 form hydrogen bonds with the carbonyl group of Asn273 and the NH group of the Arg378 residue, respectively, of TBG. The side chain of the Arg381 residue forms stacking interactions with the phenolic ring of T4. This binding mode may explain the reduced binding affinity caused by the S23T mutation.[56] As Ser23 is situated close to the T4 binding site, the addition of an extra methyl group may sterically hinder T4 binding. Interestingly, T4 exhibits a less-stable cisoid conformation at both binding sites of TBG. The amino acid side chain and the phenolic ring of T4 are on the same face of the tyrosyl ring (Figure 4B). Although the T4-diethanolamine salt exhibited both the cisoid and transoid conformations,[57] only the transoid conformation was observed in the zwitterionic form of free T4.[58a] It has been shown that T4 can exhibit different conformations in both the solid state and in solution. These conformational differences can influence the physical and pharmacological properties, and most importantly, the reactivity of the C–I bonds of T4.[58] A comparison of the conformational parameters, shown in Figure 4C and Table 1, of free T4 and TBG-bound T4 clearly indicates that TBG can alter the conforma-

Table 1: Conformational parameters in the most abundant form of free T4, TBG-bound T4, human TTR bound T4, rat TTR bound T4, piscine TTR bound T4, and HAS-bound T4.

[°]	Free T4[a,b]	TBG-T4[b]	TTR-T4[b] (human)	TTR-T4[b] (rat)	TTR-T4[b] (piscine)	HSA-T4[c]
χ^1	−158.6, 64.0	73.2, 72.1	−166.3, −105.8	60.9, 178.8	−123.0, −83.1	142.6, −102.1
χ^2	−97.1, −92.1	83.2, 84.0	−91.3, −104.1	−88.3, −99.7	−72.1, −164.3	67.5, −66.3
ψ	133.6, 169.2	175.1, 174.7	−98.8, 112.4	−166.0, 118.3	−131.1, −84.7	125.5, 126.5
φ	79.7, 109.1	99.2, 100.1	13.6, 59.3	96.5, 104.4	105.4, 96.7	86.1, 102.5
φ'	37.4, −35.5	−21.6, −21.6	60.9, 5.6	−8.6, −42.7	−16.6, −2.5	32.8, −19.7

[a] Values are given for the most abundant form of T4 (form I) and are reproduced from Ref. [58a]. [b] Two values for each parameter correspond to two independent conformers of T4 in the asymmetric unit, or two molecules of T4 in each molecule of the protein. [c] Two values for each parameter correspond to two T4 molecules at the two binding sites Tr1 and Tr2.

tion of T4 to accommodate it in the binding site. It should be noted that the sign (plus or minus) and values of χ^1 and χ^2 are significantly different between the cisoid conformer of TBG-bound T4 and the transoid conformer of free T4.

Among all the transport proteins of T4, TBG exhibits an allosteric mechanism for the binding and release of T4.[55,59] TBG switches between two states that have high and low affinities for T4 by using the free movement of the RCL. In the high-affinity state, RCL (cyan-colored loop, Figure 4D) is partially inserted in the fourth strand position of the A-sheet. The initial insertion of RCL extends up to Thr341 (also named as P14 Thr) situated 14 residues before the reactive center P1. Further insertion of this loop displaces Tyr241, which consequently disturbs the hydrogen-bonding interactions in the binding pocket through a series of movements of residues Asn244, Glu376, and Arg378 as well as the s4B-5B loop (green-colored loop, Figure 4D) near the T4 binding site. The movement of the s4B-5B loop in the indicated direction in Figure 4D finally displaces T4. This mechanism was further supported by engineered deletion of four residues as well as by the addition of three extra residues in the RCL of TBG. Whereas the deletion of four residues from RCL inhibited the complete insertion of RCL into the A-sheet, thereby resulting in a stable, high-affinity form of TBG, the addition of three extra residues in RCL resulted in a stable form having completely inserted RCL in the A-sheet and with reduced affinity towards T4.[60]

3.2. Transthyretin

TTR (also known as prealbumin) is a plasma protein mainly synthesized in the liver. TTR is secreted into the cerebrospinal fluid from the choroid plexus and is the only TH-binding protein in this compartment.[61] TTR forms a stable complex with the retinol binding protein (RBP), which transports vitamin A in a pocket located in the surface of the protein.[61] The tertiary structure of TTR contains four identical 127-residue monomers (A to D), which form a β-barrel with eight strands (A to H) divided into two antiparallel β-sheets that assemble around the central hydrophobic channel of the protein.[62] Each molecule of TTR contains two sterically identical T4-binding sites in the central channel between the A and C chains (AC site or site I), and between B and D chains (BD site or site II). However, the binding of T4 at these two sites is different; the binding affinity of site I (dissociation constant $K_d = 10^{-8}$ M) is 100-times higher than that of site II ($K_d = 10^{-6}$ M). A negative cooperative effect for the binding of T4 to TTR is proposed, although the mechanism is unclear.[63] The deiodinated metabolites of T4 are also known to bind TTR, but with lower affinity—ranging from 100% for T4 to 0.7% for 3,3′-T2 to less than 0.01% for 3-T1.[64]

Single-point mutations in TTR lead to unfolding and aggregation of the protein into amyloid fibrils, thereby causing familial amyloid polyneuropathy (FAP) and senile systematic amyloidosis (SSA). The accumulation of amyloid fibrils in different tissues ultimately leads to organ failure.[65] More than 70 mutations are known in TTR, which all destabilize protein folding by conformational alterations. The binding of T4 to the TTR tetramer is known to inhibit amyloid formation by stabilizing the native structure of the protein. Small drugs that stabilize the tetramer reduce amyloid burden.[65a,66]

The crystal structure of human TTR with T4 (in the monoclinic form) reveals that T4 binds to the central channel of TTR, with the α-amino acid moiety and phenolic hydroxy group projecting towards the opening and center, respectively, of the channel.[67] In addition to the hydrogen-bonding and salt-bridge interactions of the α-amino acid moiety of T4 with the nearby Lys15 and Glu54 residues in site I, one of the phenolic ring iodine atoms of T4 forms halogen bonds with the backbone nitrogen atom of Leu110 and Ala109 residues, with N···I distances of 3.21 Å and 3.06 Å, respectively. Both of these distances are less than the sum of the van der Waals radii of nitrogen and iodine (3.53 Å; Figure 5A). However, the N···I distances in site II (Figure 5B) are higher than the sum of the van der Waals radii of nitrogen and iodine.[67] Therefore, the absence of N···I halogen bonding may also account for the poor affinity of site II for T4. In contrast to the cisoid conformation of T4 in TBG, T4 exhibits a transoid conformation in both binding sites in TTR. However, the values of different torsional angles, which define the conformation of T4, are significantly different for TTR-bound T4 and free T4 (Table 1), although both exhibit a transoid orientation of the phenolic ring and α-amino acid moiety with respect to the tyrosyl ring. Interestingly, TTR from other species, such as rat and piscine, bind T4 in different conformations compared to the human protein (Table 1).[68] These observations clearly indicate that transfer proteins can

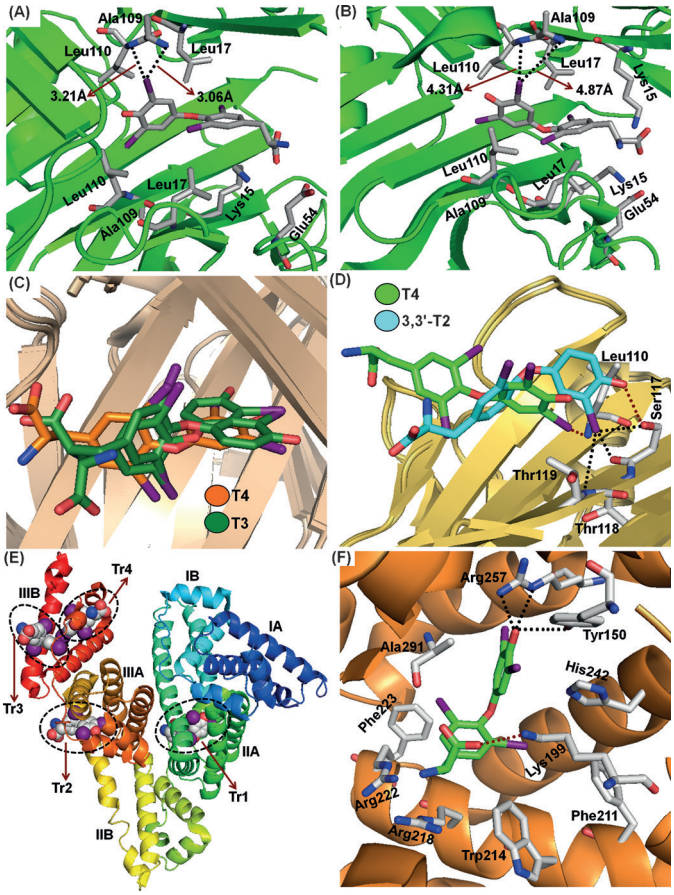


Figure 5. Noncovalent interactions between T4 and the amino acid residues in the a) binding site I and B) binding site II of the monoclinic form of human TTR (PDB code: 1ICT).[67] The formation of N···I halogen bonding was observed in site I, whereas both N···I distances are larger than the sum of the van der Waals radii of nitrogen and iodine in site II. C) Comparison of the binding modes of T4 and T3 in one of the binding sites in piscine TTR (PDB codes: 1SN0 and 1SN5).[68b] The two electron-density maps for T3 correspond to the two equivalent positions in the binding site. D) Comparison of the binding modes of T4 (PDB code: 2ROX) and 3,3′-T2 (PDB code: 1THA) in one of the binding sites of human TTR.[69] E) Different subdomains in human serum albumin (HSA), indicating the four T4-binding sites (Tr1, Tr2, Tr3, and Tr4; PDB code: 1HK1).[70] F) Hydrogen-bonding interactions between T4 and the amino acids present in the binding site located in the subdomain IIA (Tr1) in HSA.

alter the conformation of T4 to optimize the steric and noncovalent interactions in the binding site, and these altered conformations of T4 may result in different affinity of transfer proteins for T4. The cocrystal structures of TTR from *Sparus aurata* with T3 and T4 reveal different binding modes for T4 and T3 (Figure 5C). Although the tyrosyl ring iodine atoms of both T3 and T4 occupy the same halogen-binding pockets in *S. aurata* TTR, the phenolic ring iodine atom of T3 occupies a place which is generally occupied by the phenolic hydroxy group of T4.[68b] Similarly, a comparison of the cocrystal structures of human TTR with T4 (orthorhombic form) and 3,3′-T2 reveals that the binding of 3,3′-T2 to the channel is about 3.5 Å deeper than that of T4 (Figure 5D).[69] The 3-iodine substituent of 3,3′-T2 occupies the same site as the 3′-iodine atom of T4, and the 3′-iodine substituent occupies a place that is generally occupied by water molecules in the T4 complex.

3.3. Human Serum Albumin

The transport protein HSA is responsible for the transport of non-esterified fatty acids, bilirubin, bile acids, steroids, and several hydrophobic drugs. The affinity of HSA for T4 ($K_d \approx 2 \times 10^{-6}$ M) is the lowest among all three transport proteins, and a fatty meal can transiently affect the concentrations of free THs.[50] HSA is a monomeric protein with three homologous domains (I–III), each containing two subdomains (Figure 5E). The cocrystal structure of HSA with T4 reveals four T4-binding sites in domains IIA, IIIA, and IIIB, which are designated as Tr1, Tr2, Tr3, and Tr4.[70] Interestingly, T4 exhibits a cisoid conformation in the high-affinity site Tr1 (Figure 5F), whereas it exhibits transoid conformations in the other three sites. Although T4 is mainly bound in Tr1 by hydrophobic interactions, hydrogen bonding between the 4′-OH group and Arg257/Tyr150 residues as well as similar interactions between the carboxylate group of T4 and Lys199 also stabilize ligand binding (Figure 5F). The presence of Phe223, Arg222, Arg218, and Trp214 residues with large side chains forces T4 to adopt a cisoid geometry.[70] Mutation of Arg218 to His or Pro creates more space for the ligand and enhances the binding affinity of T4 by a factor of 10–15, thus increasing the total serum concentration of T4. The resulting high total plasma concentration of T4 often confuses physicians, but does not cause any known disease. This inherited syndrome is called familial dysalbuminemic hyperthyroxinemia (FDH).[71] The other T4-binding sites mainly allow hydrophobic interactions and are probably occupied by other hydrophobic ligands in vivo.

3.4. Intracellular TH-Binding Protein μ-Crystallin

μ-Crystallin (CRYM), a major protein present in the vertebrate eye lens, maintains the transparency and refractive index of the lens. CRYM is present in ear, heart, kidney, muscle, and brain in humans and mice.[72] Although CRYM does not take part in the extracellular transfer of thyroid hormones, it binds T3 inside the cell in the presence of NADPH, and overexpression of CRYM in cells can enhance the accumulation of T3.[73] The physiological function of CRYM for TH signal transduction is unclear, but mutations in CRYM which inactivate T3 binding lead to deafness.[74] Recently, the crystal structure of homodimeric CRYM in complex with NADPH and T3 has been solved (Figure 6A).[75] The binding of NADPH induces a structural rearrangement of the protein that facilitates the binding pocket to adopt an active conformation for binding T3.

Interestingly, T3 adopts a less-stable cisoid conformation in the binding site of CRYM. CRYM binds T3 mainly through hydrophobic interactions, mediated by Phe58, Phe79, and

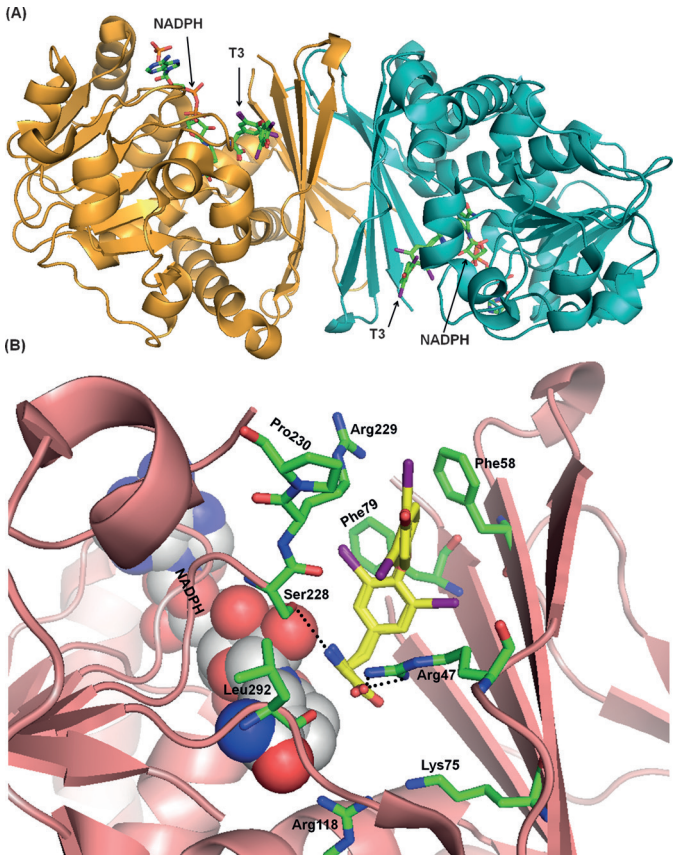


Figure 6. A) Dimer of CRYM, indicating the NADPH- and T3-binding sites on the two subunits. Two subunits are shown in different colors for clarity. PDB code: 4BVA.[75] B) Close-up view of the T3-binding sites to indicate the hydrogen-bonding interactions and amino acid residues (green C, blue N, red O) responsible for the hydrophobic interactions with T3. The bound NADPH molecule close to T3 is shown as a space-filling model (gray C, blue N, red O), whereas T3 is shown as a stick model (yellow C, purple I, blue N, red O).

Val49 as well as the aliphatic part of Arg228 (Figure 6B). Arg47 and Ser228 form direct hydrogen bonds with the carboxylic and amine groups, respectively, of T3. Lys75, Arg118, and Leu292 form water-mediated hydrogen bonds with the α-amino acid moiety of T3. The phenolic ring of T3 can adopt two different conformations in the binding site of CRYM (Figure 6B).[75] In these conformations, the 3′-iodine atom of T3 can adopt two different positions—close to (proximal position) or away from (distal position) the tyrosyl ring—similar to that observed in the crystal structure of T3.[76]

3.5. Monocarboxylate Transporter 8 and Other Transporters

THs require transmembrane transport proteins for cellular import and export.[77] Several transport proteins capable of TH transport have been identified that are related to different protein families, but work through the "rocker-switch" mechanism, passing through a series of conformations that allow alternating access to a cental binding site from the extracellular and intracellular surfaces.[78] Friesema et al. showed that rat Mct8, an orphan transporter protein located on the X chromosome, acts as an active and specific TH transporter.[79] Shortly afterwards, patients carrying inactivating mutations in the *MCT8* (*SLC16A2*) gene were identified.[51] The syndrome had been described as early as 1944 as an inherited disorder with severe mental retardation.[80] MCT8 is a neuronal TH transporter and its inactivation leads to neurodevelopmental defects in mouse models and in human patients.[81] MCT8 and the organic anion transporter OATP1C1 (SLCO1C1) are involved in T3 and T4 uptake along the blood–brain barrier.[82] MCT10 is a related transporter that is expressed in muscle and other tissues, but does not transport T4.[83]

A homology model of MCT8 has been generated, which allowed pathogenic mutations to be related to the structure and potential function.[84] The model suggests that TH enters a funnel-like cavity on the extracellular surface and interacts with His415 and His192.[85] Arg301 and Arg445 are also implicated in substrate binding, a finding reminiscent of the binding of T3 by TRs (see Section 5). After substrate binding, it is assumed that the two pseudosymmetric six-helix bundles within MCT8 tilt and twist by a few degrees, thereby closing the extracellular gate and allowing the substrate access to the intracellular side. Several pathogenic MCT8 mutant proteins respond to chemical chaperones (chemicals such as dimethylsulfoxide, genistein, or phenylbutyric acid), which help these proteins to evade the quality control mechanism of the membrane protein and reach the cell surface, despite the presence of amino acid exchanges or deletions. Interestingly, several of these mutants are functional to some extent when present in the plasma membrane.[86] The same therapeutic principle has been explored with some success in cystic fibrosis, a severe disease caused by inactivity of the transmembrane chloride channel CFTR. A side effect of the treatment of patients with tyrosine kinase inhibitors is their inhibitory action on MCT8.[87]

4. Metabolism of Thyroid Hormones

4.1. Different Metabolic Pathways

After delivery to the target tissues with the help of different transporters, thyroid hormones undergo a number of metabolic reactions by different cytosolic enzymes.[88] These metabolic pathways have different roles in the actions of thyroid hormones. The major metabolic pathway is the deiodination of T4 by iodothyronine deiodinases (DIO1, DIO2, and DIO3) to a variety of active and inactive metabolites. The regioselective removal of one iodine atom from the phenolic (outer) ring of T4 produces the biologically active hormone T3, whereas removal of an iodine atom from the tyrosyl (inner) ring leads to the formation of a biologically inactive metabolite, 3,3′,5′-triiodothyronine (rT3; Figure 7). These activation and inactivation pathways play an important role in balancing the concentration of thyroid hormones throughout the body.

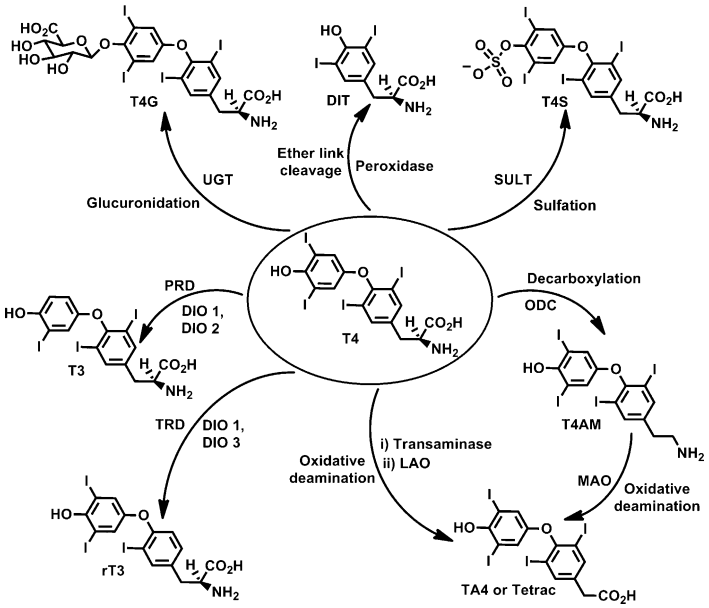


Figure 7. Metabolic pathways of thyroxine (T4). UGT: uridine 5′-diphosphate-glucuronyltransferase, SULT: sulfotransferase, DIT: 3,5-diiodotyrosine, PRD: phenolic ring deiodination, TRD: tyrosyl ring deiodination, DIO: iodothyronine deiodinase, ODC: ornithine decarboxylase, LAO: L-amino acid oxidase, MAO: monoamine oxidase.

The conjugation of the phenolic hydroxy group (4′-OH) of T4 with sulfate or glucuronic acid yields the corresponding sulfated (T4S) and glucuronidated hormone (T4G), respectively (Figure 7).[89] These metabolites show very low affinity towards the nuclear receptors, thus indicating that these pathways are inactivating pathways of TH action. In general, sulfate and glucuronic acid conjugations are known to increase the water solubility of many hydrophobic drugs and enhance their excretion through urine and/or bile.[90] However, these pathways are reversible, as the glucuronidated and sulfated metabolites undergo hydrolysis in the gastrointestinal tract and in other tissues, such as liver and brain, to regenerate the hormones. Therefore, the two metabolic pathways appear to serve as reservoirs for the biologically active thyroid hormones. Sulfation of THs is catalyzed by cytosolic sulfotransferases (SULTs), which use 3′-phosphoadenosine-5′-phosphosulfate (PAPS) as the sulfate donor.[91] SULTs are present in various tissues such as liver, kidney, and brain. Depending on the amino acid sequence and substrate specificity, SULTs are grouped into four families, SULT1, SULT2, SULT3, and SULT4. Only SULT1 accepts thyroid hormones as substrates and catalyzes the sulfation process with varied efficiency for different iodothyronines.[92] Similarly, glucuronidation is catalyzed by different isoforms of uridine 5′-diphosphate-glucuronosyltransferases (UGTs),[88,93] among which only UGT1A9, UGT2B7, and UGT1A1 are involved in the glucuronidation of T4, T3, and rT3, respectively.

The decarboxylation of the carboxy group of thyroid hormones produces iodothyronamines (TAMs; Figure 7). Recently, 3-iodothyronamine (3-T1AM) and thyronamine (T0AM) were isolated from the blood plasma of several species.[94a] TAMs with a higher iodine content have not yet been detected in the plasma. As TAMs are also accepted by DIOs as substrates,[95] it is possible that the TAMs with a higher iodine content are deiodinated by DIOs to form 3-T1AM and T0AM. The aromatic L-amino acid decarboxylase (AADC) was proposed to mediate the decarboxylation of thyroid hormones, but does not accept thyroid hormones as substrates.[96] Recently, the conversion of T4 into 3-T1AM has been observed in intestinal preparations and proceeded via 3,5-T2 and 3,5-T2AM. Ornithine decarboxylase (ODC) is present in the intestine, and recombinant ODC was able to decarboxylate iodothyronines.[97] 3-T1AM inhibits neuronal reuptake of the neurotransmitters dopamine and norepinephrine by the dopamine transporter (DAT) and norepinephrine transporter (NET), respectively.[98a] 3-T1AM may also act by modulation of α2-adrenergic receptors.[98b] 3-T1AM and T0AM exhibit some interesting pharmacological properties when injected in mice. Intraperitoneal (*i.p.*) doses of 3-T1AM induces hypothermia (decrease in body temperature), bradycardia (decrease in cardiac output), and hyperglycaemia (increase in blood sugar level) in mice.[94,99] These potentially (neuro)protective effects may be exploited for the treatment of brain ischemia or transport of organs for transplantation. Interestingly, TAMs are also accepted by SULTs as substrates and, therefore, sulfation of the 4′-OH group could be an important pathway of TAM metabolism.[100]

Recently, TAMs have been shown to undergo oxidative deamination by monoamine oxidase (MAO) to produce the iodothyroacetic acids (Figure 7).[101] Although T4 and T3 were shown to undergo oxidative deamination by transaminase and L-amino acid oxidase (LAO) to produce the corresponding iodothyroacetic acids, Tetrac (TA4) and Triac (TA3), respectively, no other iodothyronines were found to undergo such metabolism in any species.[73,102] Tetrac (3,5,3′,5′-tetraiodothyroacetic acid, TA4) and Triac (3,5,3′-triiodothyroacetic acid, TA3) show thyromimetic activity by suppressing TSH and binding to the nuclear receptors. Both in vitro and in vivo studies showed that the pituitary gland can take up TA3 rapidly and induce the suppression in TSH secretion.[103] Triac showed affinities that were almost 3.5- and 1.5-times higher than T3 towards thyroid hormone receptor β_1 ($TR\beta_1$) and $TR\alpha_1$, respectively. The higher affinity of TA3 to $TR\beta_1$ than $TR\alpha_1$ indicates that TA3 may be more efficient than T3 in treating thyroid hormone resistance syndrome (RTH), which results from mutations in the $TR\beta_1$ gene.[104] In fact, TA3 has been used effectively to treat central RTH and pituitary TSH hypersecretion.[105] Triac is also more efficient than T4 in reducing the size of goiter, with less adverse effects.[106] Triac is currently being evaluated for the treatment of MCT8-deficient patients, because TA3 enters the cells independently of MCT8, but acts on the receptors.[107] Similar to TAMs, TA4 and TA3 also undergo sulfation by SULTs and glucuronidation by UGTs, thus suggesting that the body can integrate the drugs into metabolism.[88,102] In contrast to the above-mentioned modifications, ether link cleavage (ELC) appears to be a minor pathway in the metabolism of thyroid hormones. ELC is often catalyzed by peroxidases, such as horseradish

peroxidase (HRP) and MPO, and even TPO can catalyze the conversion of T4 into DIT by ELC (Figure 7).[108]

4.2. Deiodination of Thyroid Hormones

Iodothyronine deiodinases (DIOs) are membrane-associated selenoenzymes that catalyze the deiodination of thyroid hormones.[109] Depending on the amino acid sequence and substrate specificity, three isoforms of DIOs have been identified—DIO1, DIO2, and DIO3—which are expressed in a tissue-specific pattern. For example, DIO1 is active in liver, kidney, thyroid, and pituitary, DIO2 in brain, inner ear, pituitary, thyroid, heart, and skeletal muscle, and DIO3 in placenta, brain, and in tissues during regeneration. The insertion of selenocysteine (Sec) into DIO during translation is a complicated process, since its cognate UGA codon must be recoded from the usual termination codon to an elongation codon. The required signal, which is called the selenocysteine insertion sequence (SECIS) element, resides within the mRNA.[109b,110] While the SECIS is located in the open reading frame directly adjacent to the UGA in bacteria, it is farther away in the 3′-untranslated region in eukaryotes. The UGA is then read by Sec-tRNA$^{[Ser]Sec}$, a UGA-decoding tRNA which requires its own translation elongation factor (SelB in bacteria and EF-Sec in mammals). The SECIS element is bound by SECISBP2, a protein required for the recoding of UGA in mRNA.[111] Mutations in SECISBP2 which impair SECIS binding lead to abnormal thyroid hormone function tests through reduced of DIO activites.[112]

DIO1 can remove an iodine atom from both the tyrosyl and phenolic rings of thyroid hormones, whereas DIO2 and DIO3 act selectively on the phenolic and tyrosyl ring, respectively (Figure 8).[113,114] DIO1 is important for the activation of the prohormone T4 to T3, whereas DIO3 plays an important role in protecting the tissues from excess thyroid hormones by converting T3 into biologically inactive 3,3′-T2.[113–115] The diiodo derivative 3,3′-T2 is also produced by deiodination of the phenolic ring of rT3 by DIO1 and DIO2. In fact, rT3 is the preferred substrate for the deiodination of the phenolic ring by DIO1 compared to T4 and T3.[113b,c] The other diiodo derivative 3,5-T2 exhibits selective thyromimetic activity and activates the thyroid receptors (TRs), although the biosynthesis of this derivative is not clear.[116] Although T3 has not been included as a potential substrate for the deiodination of the phenolic ring by DIO1 in many studies, homogenates of NCLP-6E monkey hepatocarcinoma cells expressing DIO1 enzymatic activity has been shown to mediate the deiodination of the phenolic ring of T3 to produce 3,5-T2.[117a,b] Interestingly, 3′-T1 undergoes deiodination of a phenolic ring by DIO1 to form T0, whereas 3-T1 does not undergo deiodination to form T0 (Figure 8).[117] These observations indicate that the three-dimensional structure and conformation of hormones may be influenced by the presence of iodine atoms in different rings and, consequently, it may alter the binding of the substrate to the active site of the enzyme.

DIOs share a significant amount of similarity in their primary structures, specifically in the region around the active

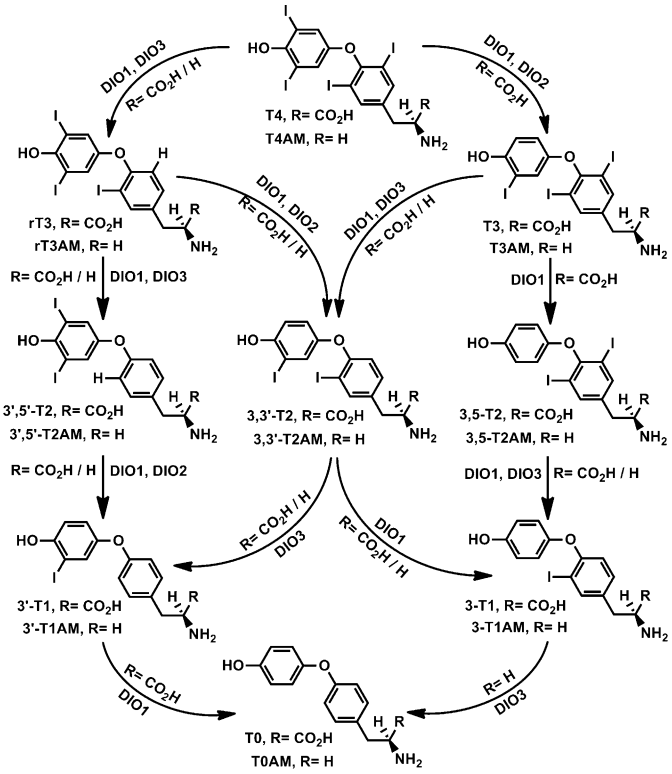


Figure 8. Deiodination of thyroid hormones and TAMs by DIOs.

site, thus suggesting that the basic mechanism of catalysis may be similar for the deiodination of both tyrosyl and phenolic rings. Selenocysteine (Sec) is conserved in all three isoforms and it plays a key role in the deiodination. The corresponding cysteine (Cys) mutants are functionally active, although the catalytic activity is found to be many fold lower than that of the wild-type enzyme.[118] A large number of mutational and kinetic studies have been carried out, but there is no X-ray structure available for any of the isoforms. Recently, the X-ray structure of the catalytic domain of mouse Dio3 (mDio3^{cat}) has been solved.[119] The construct lacks the N-terminal membrane-associated domain as well as the linker connecting it with the catalytic domain. Furthermore, Sec170 was replaced with Cys to facilitate expression in a prokaryotic host.[120] The crystal structure reveals that the enzyme adopts a previously predicted thioredoxin (Trx) fold containing a five-stranded mixed β-sheet flanked by four α-helices. A short N-terminal β-sheet followed by a 3_{10}-helix betrays an evolutionary relation to peroxiredoxins (Figure 9A,B).[119] The deiodinase-specific insertion (DIO insertion; amino acid residues 201–225) comprises a large loop D followed by a helix αD and a short strand βD aligning with the central β-sheet. The crucial Sec170 residue is located in a cleft between β1 and α1, which corresponds to the position of peroxidatic Cys in thioredoxin. The substrate-binding site on mDio3^{cat} was modeled by superimposing the His435-T3-Arg282 clamp of the T3-TRβ complex[121] on mDio3^{cat}-His202-Arg275 (Figure 9C). This binding mode places the 4′-OH group of T3 near His202, thereby favoring the formation of a hydrogen bond between them. The amino and carboxylate groups of T3

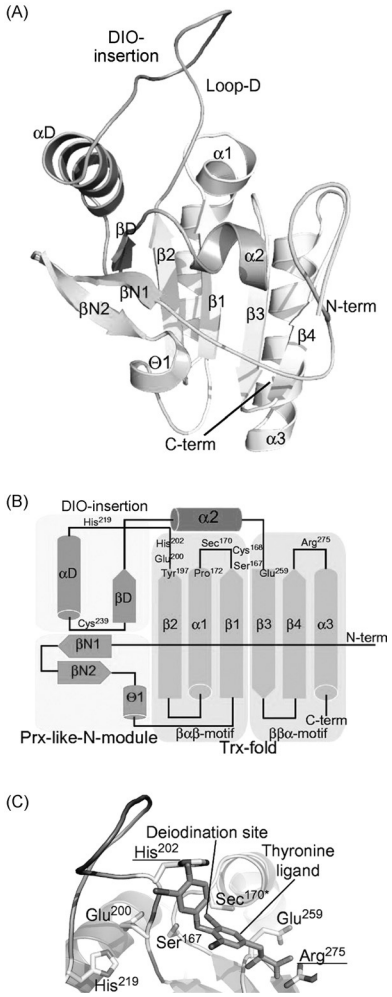


Figure 9. A) X-ray crystal structure of mDio3^{cat} (PDB code: 4TR4).[119] B) Schematic representation of the topology of mDio3^{cat}, indicating the amino acids involved in catalysis. C) Modeled substrate complex in mDio3^{cat}. Reproduced from Ref. [119] with permission.

may interact with the nearby residues Arg 275 and Glu 259, which are important for catalytic activity.[119,121] The homodimerization of DIO, supported mainly by the N-terminal membrane-linked region including the linker and possibly parts of the catalytic domain, has been shown to be crucial for enzymatic activity.[122] The mDio3^{cat}structure is an inactive, monomeric conformation with Phe 258 from the α2/β3 loop sterically blocking the active site and shielding Sec from solvent. The α2/β3 loop is designated as an autoinhibitory loop, which is speculated to relax upon dimerization, thereby allowing the substrate to access the active site.[119]

The molecular mechanism by which DIOs catalyze the deiodination of thyroid hormones has been a subject of debate for several years. Small-molecule mimetics of DIOs have been developed to better understand the potential chemical mechanism of deiodination. Goto et al. showed that a sterically hindered organoselenol can remove iodine selectively from the phenolic ring of a T4 derivative at elevated temperature and in organic solvents.[123] They showed that a keto-enol tautomerism of the 4′-OH group of T4 is important for the deiodination reaction (Figure 10 A). However, this mechanism is not applicable for deiodination of the tyrosyl ring of T4. An alternative mechanism involving the formation of a halogen bond[124] between the selenium and iodine atom has been proposed by Bayse and Rafferty.[125] Recently, the first chemical model for deiodination of the tyrosyl ring of T4 and T3 has been reported.[126] The naphthyl-based diselenol **1** (Figure 10 B) mediates rapid selective deiodination of T4 and T3 at the tyrosyl ring under physiologically relevant conditions to produce rT3 and 3,3′-T2, respectively. Whereas the replacement of selenium by sulfur in **1** decreases the deiodinase activity without affecting the selectivity, such a modification with tellurium not only enhances the reactivity, but also changes the selectivity of the deiodination.[126d] The naphthyl-based selenium compounds mediate the deiodination of thyroid hormone metabolites—TAMs and sulfate conjugates—as well as the dehalogenation of halogenated nucleosides.[127] One of the selenium atoms donates electron density to the σ* (antibonding) orbital of the C–I bond, thereby, polarizing it. Furthermore, chalcogen bonding between the two selenium atoms strengthens the Se···I halogen bonding, thereby leading to cleavage of the C–I bond (Figure 10 B). As many of the amino acid residues are highly conserved in all deiodinase isoforms, the deiodinations of the phenolic and tyrosyl rings may follow a similar mechanism. As the keto-enol tautomerism proposed for deiodination of the phenolic ring is not possible for the tyrosyl ring, a halogen-bond-mediated cleavage of the C–I bond appears to be the most relevant mechanism for the deiodination of thyroid hormones by DIOs.[128] In agreement with this, modeling of the substrate complex of mDio3^{cat}places the

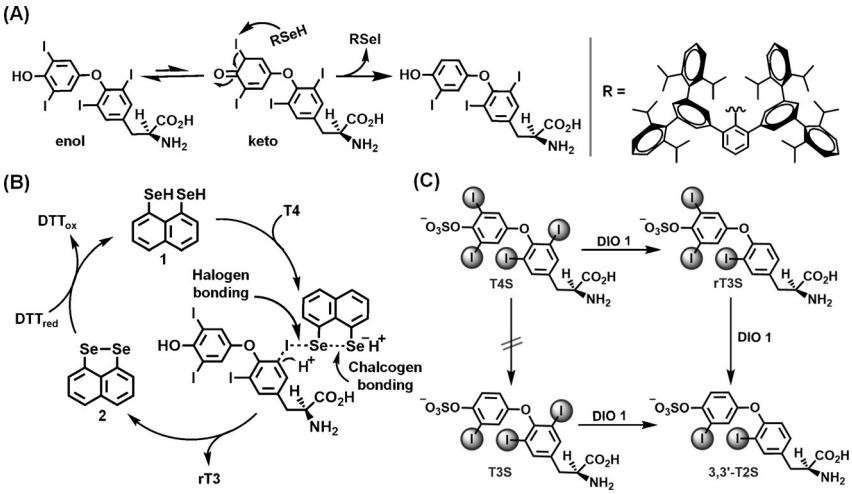


Figure 10. A) Proposed mechanism of the deiodination of the phenolic ring of T4 by a bulky selenol involving keto-enol tautomerism. B) Proposed mechanism of halogen-bond-mediated deiodination of T4 by DIO3 mimic **1**. C) Deiodination of sulfo conjugates of thyroid hormones by DIO1.

5-iodine atom of T3 only 3–4 Å away from the catalytic selenium atom, which is consistent with the observed Se···I distance in the theoretically predicted halogen-bonded geometry.[119,125–127]

The protonation of the 5-position of the substrate may occur from the back side of the tyrosyl ring with the help of a hydrogen-bond network involving His 219-Glu 200-Ser 167 along with Tyr 197 and Thr 169.[119] Cys 168 and/or Cys 239 may play a role in the regeneration of reduced Dio3, but this mechanism needs further study.

Model studies with biomimetic compounds provided valuable information regarding the mechanism of the deiodination, but the origin of the regioselectivity of DIOs still remains elusive. Mondal and Mugesh showed that the conformation of T4 at the active site of DIOs may control the reactivity of the 5- and 5′-iodine atoms.[58a] Theoretical calculations indicated that the 5-iodine atom can be more reactive than the 5′-iodine in the native conformation ($\varphi \approx 90°$, $\varphi' \approx 0°$), thus suggesting the possibility of the selective removal of 5-iodine. However, alterations in the relative orientation of the two aromatic rings of T4 make the reactivity of both the 5- and 5′-iodine atoms almost identical in certain conformations (e.g. $\varphi \approx 0°$, $\varphi' \approx 90°$). As the 5- and 5′-iodine atoms are quite distant from each other, because of the mutual perpendicularity of the two aromatic rings, the change in the relative orientation between the two rings may also bring the 5′-iodine atom close to the Sec residue of DIO1 for deiodination. Furthermore, the relative orientation of the amine and carboxylate moieties of T4 also has a significant effect on the ability of the iodine atoms to form a halogen bond with selenium.[58] In a few conformations, the 5′-iodine atom can form a stronger halogen bond than the 5-iodine atom, thus indicating that DIO2 may follow a similar strategy to selectively remove the 5′-iodine atom. However, further studies, including the binding mode of T4 at the active site of all three isoforms, are required to validate the hypothesis.

As mentioned in Section 4.2, some of the TH metabolites also undergo deiodination by DIOs. TAMs have been recognized as isozyme-specific substrates of DIOs, although T4AM and T3AM are not accepted as substrates for the deiodination of the phenolic ring by DIO1 (Figure 8).[95] A comparison of the rates of deiodination indicated that TAMs undergo much slower deiodination than THs by DIOs. Recent chemical model studies using naphthyl-based selenium compounds also indicated that TAMs undergo slower deiodination than THs.[127a] The iodine atom from Tetrac and Triac can be recycled through deiodination. In fact, Triac and Tetrac are even better substrates for DIO1 than T4 and T3.[88] For example, deiodination of the tyrosyl ring of Triac by DIO1 is almost 16 times faster than that of T3.[129] A role of DIO1 as a degrading enzyme is supported by the finding that this enzyme works well on sulfated THs, whereas DIO2 and DIO3 do not accept the conjugates as substrates,[130] thereby indicating that sulfation affects regioselectivity. In contrast to T4, T4S does not undergo deiodination of the phenolic ring by DIO1 (Figure 10C), but the deiodination of the tyrosyl ring of T4S is almost 200 times faster than that of T4.[131a] Similarly, the DIO1-mediated deiodinations of the tyrosyl ring of T3S to 3,3′-T2S and of the phenolic ring of 3,3′-T2S to 3-T1S are almost 40- and 50-times, respectively, faster than their parent thyroid hormone metabolite.[131b,c] Interestingly, the deiodination of rT3 is not affected by its sulfate conjugation. Glucuronidation also occurs with THs. Microsomes derived from rat liver and hypothyroid brain, which have DIO1 and DIO2 activity, respectively, mediate the deiodination of T4-glucuronate at almost half the rate as the deiodination of T4.[132] However, in this process, some deconjugation to T4 has been observed.

5. Thyroid Hormone Receptors

Thyroid hormone receptors (TRs) are members of the nuclear receptor (NR) superfamily, which modulate gene transcription in response to hormone binding. These receptors bind the promoter regions of target genes by recognizing specific nucleotide sequences, that is, nuclear response elements (NREs), and recruit a variety of co-activator and co-repressor proteins to the gene promoter.[133] In addition to transcriptional modulation, "nongenomic" actions of TRs are increasingly being recognized.[134] Two genes (THRA and THRB) yield four differentially spliced receptor isoforms designated TRα1, TRα2, TRβ1, and TRβ2.[133c] Expression of TR mRNA is tissue-specific: TRα1 has the highest expression in heart and skeletal muscle; TRβ1 has the highest expression in liver, brain, and kidney; TRβ2 is specific for anterior pituitary and specific regions of the hypothalamus. Both receptors cooperate in bone formation and remodeling.[135] TRα1 is associated mainly with the maintenance of cardiovascular functions, whereas TRβ1 controls the metabolism of cholesterol and lipoprotein.[133c,136] Similar to other NRs, the tertiary structure of TRs has four domains: a variable N-terminal A/B domain (NTD), a central DNA-binding domain (DBD), a C-terminal ligand-binding domain (LBD), and a linker or hinge between LBD and DBD (Figure 11A).[133c] TR-mediated regulation of gene expression involves concerted actions of all the domains. LBD can bind its ligand as well as co-regulator proteins, and it has been shown that conformational changes induced by the ligand binding help in the binding of co-regulator proteins. T3 is the natural high-affinity ligand ($K_d = 10^{-9}$–10^{-10} M) for TR isoforms, although T4 can bind to TRs with low affinity.[137] X-ray structures of LBD from both receptor isoforms complexed with T3 have been reported in the literature. The cocrystal structure of the LBD of human TRα1 and T3 show that T3 is completely buried in a hydrophobic pocket within the LBD (Figure 11B).[138] This hormone-binding pocket is formed from several helices and loops of LBD, such as residues from helix 5–6 (H5–6, Met 256-Arg 266), helix 7–8 (H7–8), and the intervening loop (Leu 287–Ile 299), helix 3 (H3, Phe 215–Arg 228), helix 11 (H11, His 381–His 387), and helix 12 (H12, Phe 401–Phe 405). T3 is bound in a hydrophobic cavity, but specific hydrogen-bonding interactions are observed between the carboxylic group of T3 and an Arg residue and between the 4′-OH group and a His residue, as described above for TRβ1. Such His-Arg clamps next to the hormone-binding site also exist in MCT8 and DIO.

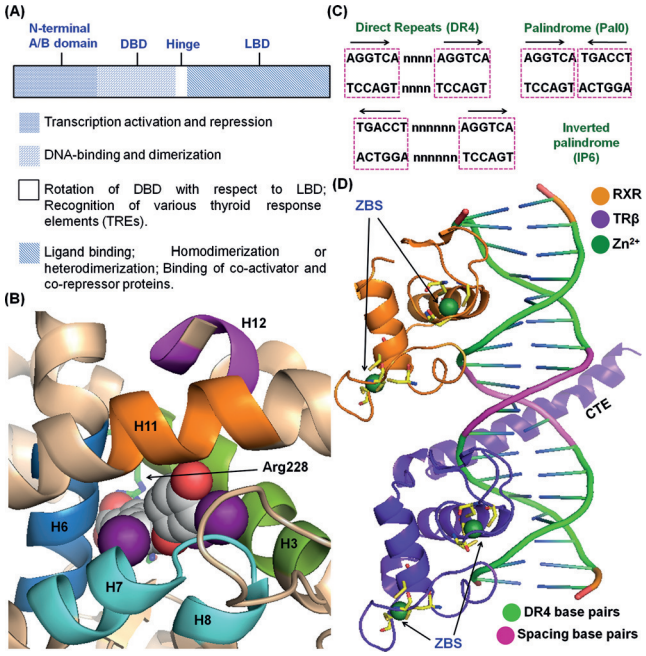


Figure 11. A) Schematic representation of different domains in thyroid hormone receptors, indicating their roles in receptor-mediated gene regulation. B) Cocrystal structure of human TRα1 and T3 showing the helices and loops responsible for ligand binding (PDB code: 2H77).[138] T3 is shown as a space-filled model and helices are shown in different colors for clarity. C) Various thyroid-response elements (TREs) with different orientations and separations of the consensus hexameric half-site 5′-AGGTCA-3′. Half-site base pairs are highlighted in the box and the spacing base pairs are indicated as "n". D) Crystal structure of the heterodimer of DBDs of TRβ and RXR bound to a direct repeat (DR4) TRE (PDB code: 2NLL).[144] Zinc fingers or the zinc-binding sites in the DBDs of RXR and TRβ are designated as ZBS. Zn^{2+} ions are shown as spheres and the surrounding cysteine residues are shown as stick models. The C-terminal extension of TRβ1 is abbreviated as CTE. DBDs of RXR and TRβ1 are colored in orange and violet, respectively. Consensus hexameric half-site base pairs in DR4 and the spacing base pairs are shown in green and magenta, respectively.

T3 adopts a transoid geometry and the iodine atom of the phenolic ring is projected outwards in a distal conformation.[138] The binding of T3 to LBD induces conformational changes in H12, thereby leading to a tight packing of the ligand at the receptor.[139] The repositioning of H12 leads to the formation of a coactivator protein binding motif LXXLL on the surface of the ligand-bound TR, which facilitates the transcriptional activity of the activation function-2 (AF-2) domain of TR. In the absence of ligand, the co-repressor is recruited to the TRs. Therefore, the ligand-induced changes in H12 govern the effect of the ligand on gene expression.[140] Note that in contrast to steroid receptors, TRs do not shuttle in a ligand-dependent manner between the cytosol and nucleus, but nuclear TRs exchange their co-regulators in response to T3 binding.

The DBD of TRs is a central domain located in the amino half of the receptor, and it is the most highly conserved domain among the members of NRs superfamily.[133c] It contains two "zinc-finger" DNA-binding motifs, each of which contains four highly conserved Zn^{2+}-coordinating Cys residues.[141] The DBD binds to specific nucleotide sequences, called thyroid hormone response elements (TREs) located in the promoter regions of target genes. A consensus nucleotide sequence 5′-AGGTCA-3′ is known to form one half-site, which is recognized by one TR. Functional TREs contain two half-sites and bind an NR dimer. TREs can be arranged in various ways, such as a head to tail arrangement in direct repeats (DRs), as in DR4, a head to head arrangement in palindrome (Pal), as in Pal0, and a tail to tail arrangement in inverted palindrome (IP), as in IP6 (Figure 11 C). Furthermore, these repeats can be separated by a number of base pairs in between, often indicated by a number at the end of the TRE classification.[142] Of the three classes of TREs, DRs are the most abundant. Besides homodimerization, the heterodimerization of TR with the NR 9-*cis*-retinoic acid receptor (RXR), is also frequently observed.[143] The crystal structure of a complex of DBDs from RXR and TRβ1 bound to a direct repeat TRE, DR4, is shown in Figure 11 D.[144] This structure reveals that the TRβ1 C-terminal extension (CTE), which adopts an α-helical conformation and is absent in RXR, plays an important role in DNA binding and the positioning of TRβ1 DBD in the complex. The CTE forms extensive contacts with the phosphate backbone of the spacer nucleotide and, thereby, specifies the spacing between the half-sites recognized by DBDs.[144] The specific interaction between the LBD and DBD of NRs is believed to define the preferred oligomerization pattern of the NREs, and communication between LBD and DBD is facilitated by the intervening hinge, which can adopt multiple conformations to facilitate rotation between the DBD and LBD and permit NRs to adapt to various NREs.[133c,138] The mechanism by which the co-activators or co-repressors regulate the transcription is not well-established. However, the recruitment of other proteins has been shown to alter the chromatin structure by changing the acetylation states of lysine residues in the histone proteins.[145]

Mutations in the THRB gene cause the syndrome of resistance to thyroid hormone, which was discovered 40 years ago. These patients show increased TSH secretion and increased plasma TH levels as a result of impaired feedback at the level of the pituitary. However, while some organs (e.g. liver and heart) show signs of hyperthyroidism, other organs or tissues (e.g. pituitary and inner ear) appear to be "resistant" to T3. The underlying mutations often affect the co-regulator or ligand-binding sites.[146] Only recently have patients been identified with mutations in the THRA gene. These patients do not have altered TH levels (because pituitary feedback is mediated via TRβ2), but show a growth defect of long bones, persistent constipation, and usually a reduced IQ. The symptoms correlate well with the expression pattern of TRα.[147]

6. Thyroid-Related Diseases

Thyroid hormones control many aspects of growth and development, regeneration, and metabolism. Hypo- and hyperthyroidism, therefore, affect many organ systems and are linked to, among others, cardiovascular diseases, obesity, and type II diabetes.[1d,133c,148] Deviations in both directions

from normal TH levels are quite frequent, very unpleasant for the patients, and are a major problem in autoimmune thyroid disease and thyroid cancer. Drugs that can control the TH function or replace the TH are, therefore, the most frequently prescribed drugs. These all have their limitations and have been on the market for decades, and thus there is current interest in new lead structures or therapeutic principles.

6.1. Hypothyroidism and Hyperthyroidism

Whereas TH replacement therapy is straightforward in hypothyroidism, hyperthyroidism is more difficult to treat. For example, in Graves disease, agonistic auto-antibodies form against the TSH receptor and lead to uncontrolled stimulation of the thyroid gland with increased T4 and T3 levels in the plasma. Even if the pituitary decreases its release of TSH, overproduction of TH persists under control of the antibodies.[149] Excess TH leads to increased heart rate and growth, sweating, muscle wasting, osteoporosis, and fatigue. Goiter may develop and the TSH-like stimulation of retro-orbital fibroblasts leads to their proliferation ultimately pushing the eyes from behind—Graves orbitopathy, which can lead to damage of the optic nerve or the eyes. Another frequent destructive thyroid auto-immune disease is Hashimoto's thyroiditis. Here, T cells attack the thyroid, and phases of hyperthyroidism caused by the onslaught of the immune system will later be followed by inactivity of the gland.

Hyperthyroidism is treated with thyroid-blocking thiourea-based drugs such as carbimazole (CBZ), methimazole (MMI), and 6-*n*-propylthiouracil (PTU; Figure 12A).[150,151] As CBZ is converted rapidly into MMI in the body, the dosage and effects of CBZ and MMI are quite similar.[150] The side effects of commonly used antithyroid drugs include rashes, arthralgia, gastrointestinal disorders, and changes in taste and smell.[151] Several molecular mechanisms for the action of antithyroid drugs have been proposed. For example, the thiourea-based compounds may divert the active iodinating species away from Tg, thereby leading to the preferred iodination of the sulfur moiety of the drug instead of the tyrosyl residues of Tg. The iodination of MMI results in the formation of an unstable sulfenyl iodide, which may undergo a disproportion reaction to form the corresponding disulfide. The MMI disulfide may undergo spontaneous degradation to form the desulfurated molecule, *N*-methylimidazole.[152] An alternative proposed mechanism for the antithyroid action involves the competitive inhibition of TPO by coordination of the thione moiety to the Fe^{III} center.[153]

A recent cocrystal structure of lactoperoxidase (LPO) with PTU shows that PTU binds to LPO in the substrate-binding site on the distal heme side (Figure 12B), thus supporting the metal coordination pathway. Whereas the distance between the PTU molecule and heme is 2.7 Å, the distance between PTU and the distal histidine residue (His109) is 2.8 Å. The orientation of the PTU molecule allows van der Waals interactions with Gln105, His109, Phe113, Ala114, Arg255, Glu258, and Arg248. The amino acid sequences near the substrate-binding site of TPO and LPO are almost identical, except for Ala114 of LPO (Thr205 in TPO). Thr205 is expected to form stronger van der Waals interactions with PTU, which is in agreement with the observed IC_{50} values of PTU (37 μM for TPO and 47 μM for LPO).[154]

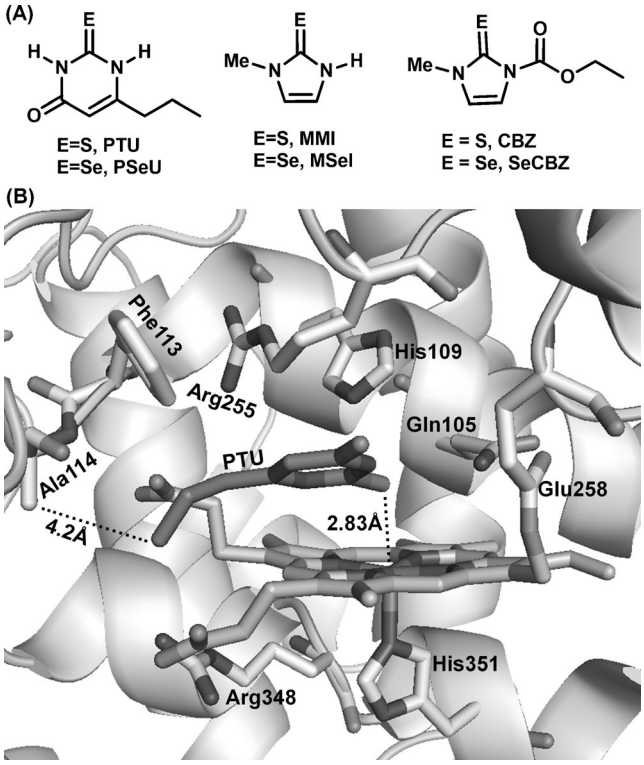


Figure 12. A) Chemical structure of antithyroid drugs. B) Crystal structure of LPO bound to PTU, showing that TPO binds in the substrate binding domain to heme iron (PDB code: 4qyq).[154]

The selenium analogues of antithyroid drugs have attracted significant attention because of the higher nucleophilicity of the selenium atom compared to sulfur. The selenium analogue of MMI, MSeI (Figure 12A), exists in a zwitterionic form with the the selenium atom carrying negative charge.[155] However, in vivo studies showed that the inhibition of TPO-catalyzed oxidation and iodination reactions by MSeI is 50 times weaker than that of MMI, thus suggesting that MMI and MSeI may inhibit TPO by different mechanisms.[156] It has been reported that the inhibition of the LPO-catalyzed iodination of L-tyrosine by MSeI is reversible and the enzyme activity can be completely recovered by increasing the concentration of H_2O_2. These results indicate that the selenium analogue of MMI acts as a H_2O_2 scavenger rather than a true enzyme inhibitor. In contrast, an increase in the concentration of H_2O_2 does not restore the enzyme activity in the case of MMI, which suggests that MMI inhibits LPO by interacting with the heme center.[155,157] Although the H_2O_2-scavenging ability of the selenium analogues may be beneficial under oxidative stress conditions, the poor accumulation of the selenium compounds in the thyroid gland may restrict their applicability.[157,158]

Although thyroidectomy may ultimately be required to control Graves hyperthyroidism if drugs are not effective, radioiodine (RAI) treatment is an alternative.[159] RAI is used

more frequently in the USA for treating Graves hyperthyroidism than in Europe and Asia.[160] This therapy exploits the NIS-mediated accumulation of $^{131}I^-$, which exposes the thyroid to radiation damage, while sparing other tissues.[161] RAI treatment is associated with the development of ophthalmopathy in 20% of the cases, but observed only in 5% of the patients undergoing treatment with antithyroid drugs.[162] Furthermore, an increased risk of cancer of the gastrointestinal tract for patients with multinodular goiter has been observed in the case of RAI treatment.[163]

6.2. Thyroid Hormones in Cardiovascular Diseases

Cardiovascular diseases are one of the most common causes of death worldwide and are often treated with statins. Statins are hydroxymethyl-glutaryl-coenzyme A (HMGCo-A) reductase inhibitors. This enzyme catalyzes the de novo biosynthesis of cholesterol. Cholesterol-deficient cells induce expression of the low-density lipoprotein (LDL) receptor, ultimately reducing circulating cholesterol levels.[164] Thyroid hormones affect lipid metabolism in the liver, and several thyroid hormone analogues or thyromimetics have been developed in the last few decades to modulate lipid metabolism. Several thyromimetics that can control plasma cholesterol levels have entered clinical trials.[165] As discussed earlier, THs exert their biological effects by binding to the ligand-binding domain of TRα and TRβ, of which TRβ is mainly associated with lipid metabolism.[148a,166] THs facilitate the elimination of LDL by upregulating LDL receptors (LDLRs) in the liver.[167] Increased plasma LDL is responsible for the deposition of cholesterol in arterial walls in the form of atherosclerotic plaques. THs further reduce plasma cholesterol and triglyceride levels by inhibiting sterol regulatory element binding protein-1 (SREBP1) transcription, a transcription factor that itself regulates fatty acid biosynthesis and the assembly of very low density lipoproteins (VLDL).[168] Cholesterol is removed from the circulation by reverse cholesterol transport (RCT) through integration of high-density lipoprotein (HDL). Increasing the level of HDL can be a potential strategy to reduce the risk of cardiovascular diseases.[169]

THs induce the expression of HDL receptors (scavenger receptor class B type 1, SRB1) in the liver, thus enhancing the rate of HDL reuptake, and increasing the biosynthesis of HDL by increasing the production of apolipoprotein A1 (APOA1), a component of the HDL particle.[168,170] THs also induce the expression of cholesterol 7-α-hydroxylase (CYP7A1), the enzyme which initiates the convertion of cholesterol into bile acids, the only form in which cholesterol can be excreted (Figure 13A).[168]

The development of thyromimetics for therapeutic applications, such as dyslipidaemia, has been a challenge because of their unwanted side effects, such as increased heart rate, bone loss, and muscle wasting.[165] The D enantiomer of T4 (D-thyroxine) has been tested for improving the survival of patients who had suffered a heart attack. However, the drug was discontinued due to an increase in mortality, although D-T4 improved plasma cholesterol.[171] The endogenous thyroid hormone metabolite Triac has been suggested for the treatment of dyslipidaemia, but further studies were not performed because of its unwanted effects on heart rate, oxygen consumption, and bone turnover.[172] A summary of synthetic thyromimetic compounds that underwent clinical trials is summarized in Table 2.

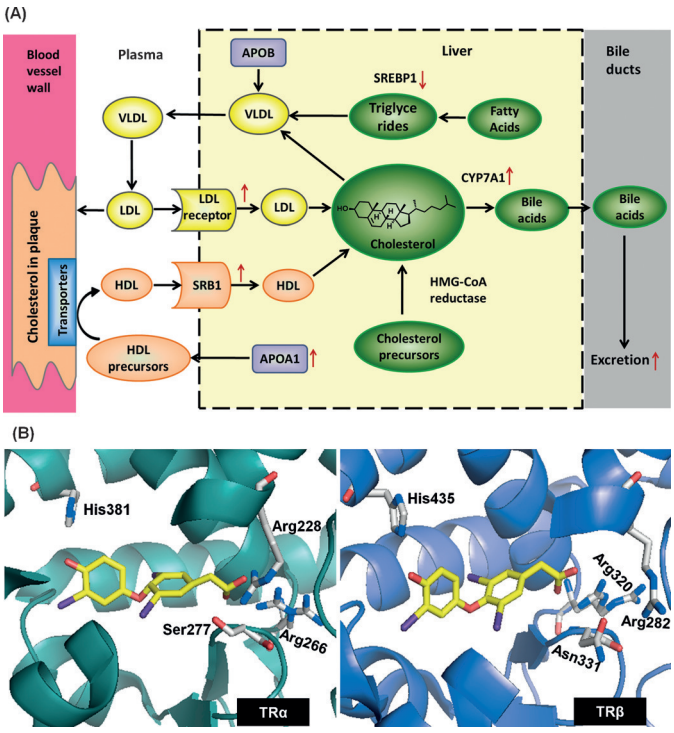
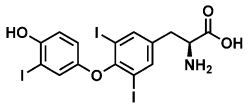
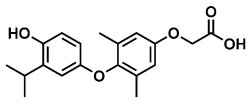
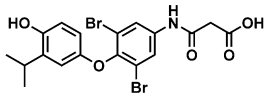
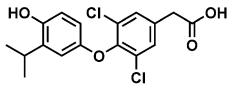
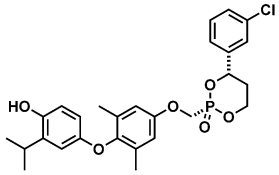
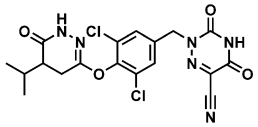
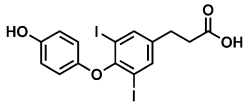


Figure 13. A) Cholesterol metabolism and the effect of thyromimetics. The red arrows show the pathways that are influenced by thyromimetics and the direction of arrows indicate the upregulation and downregulation steps (modified from Ref. [165]). B) Ligand-binding domain (LBD) of TRα (PDB code: 3jzb) and TRβ (PDB code: 3jzc) showing that only one amino acid residue (Ser277 of TRα versus Asn331of TRβ) is different among the residues interacting with the ligand.[137c]

From the crystal structures of TRs (TRα and TRβ), it has been observed that the highly homologous ligand-binding sites of TRα and TRβ differ only in one amino acid (Ser277 in TRα and Asn331 in TRβ), which forms direct contact with T3 (Figure 13B). As TRβ mainly regulates lipid metabolism, it is quite challenging to develop TRβ-selective thyromimetics that do not exhibit the TRα-associated deleterious effects.[173] Similar to THs, thyromimetics with TRβ specificity and liver selectivity can facilitate the absorption of plasma LDL by expressing LDL receptors, and reduce plasma cholesterol levels by enhancing the rate of the RCT pathway by inducing the expression of SRB1 and the synthesis of APOA1 (Figure 13B).[165,166] TRβ-selective thyromimetics can also improve the cholesterol and triglyceride levels without inducing harmful effects such as tachycardia and arrhythmias, which are generally observed with T3 and is mediated by TRα.[165,166] TRβ- and liver-selective thyromimetics include GC-1 (5–20 times more selective to TRβ), GC-24 (40–100 times more selective to TRβ), KB141 (14 times more selective to TRβ), KB2115 (TRβ selectivity not disclosed),

Table 2: Thyromimetic compounds, indications, and findings from human trials.[a] [165,166]

Compound	Indications	Clinical trials
 L-Triiodothyronine	endogenous hormone	not applicable
 GC-1 (Sorbetirome)	dyslipidaemias, high cholesterol level, obesity, NAFLD	phase Ia, LDL-cholesterol↓
 KB2115 (Eprotirome)	dyslipidaemias, high cholesterol level, FH	phase II, LDL cholesterol↓, LPA and TG↓, statin synergy
 KB141	none, discontinued	not applicable
 MB07811	dyslipidaemias, high cholesterol level,	phase Ib, LDL cholesterol↓, TG↓
 MGL3196	dyslipidaemias, high cholesterol, NAFLD, FH	phase Ib, LDL cholesterol↓, TG↓
 DITPA	dyslipidaemias, high cholesterol level, heart failure	phase Ib, LDL cholesterol↓, TG↓, statin synergy

[a] NAFLD: Non-alcoholic fatty liver disease, FH: familial hypercholesterolemia, LPA: apolipoprotein(a), LDL: low-density lipoprotein, TG: triglycerides.

MB07811 (10 times more selective to TRβ), MGL-3196 (28 times more selective to TRβ; Table 2) and many of them have entered clinical trials.[166]

The TRβ selectivity of thyromimetics appears to be associated with side-chain interactions. For example, Arg282 of TRβ interacts strongly with compounds GC-1 and KB141 to form a stable hydrogen-bonding network.[165,166] Another reason for TRβ selectivity may be associated with helical displacement. Compounds with large outer-ring extensions (e.g. GC-24) can directly interact with residues near the ligand-binding site, thereby leading to large displacements of the helices. The structural arrangements in the region (H3 and H11) to accommodate a bulky phenyl group is more flexible in the case of TRβ than TRα.[165,166] The third reason for TRβ selectivity may arise from the entropy contribution. In the case of Triac, TRβ selectivity may be due to the presence of a carboxy group, which may allow more water molecules to enter the ligand-binding site. The resulting ligand–water interactions may favor increased mobility of the ligands.[165,174]

The selectivity to the liver has been attributed to first-pass metabolism. For example, the prodrug MB07811 undergoes first-pass extraction and cleavage in the liver by cytochrome P450 to generate methylphosphonic acid and MB07344, which is an active thyromimetic compound.[147,175] Alternatively, the liver selectivity may also be achieved through selective uptake by specific transporters. It has been suggested that SLC10A1, a hepatic luminal bile acid transporter (sodium taurocholate transporting polypeptide, NTCP) selectively expressed in the liver, transports KB2115 and thereby shows a unique liver-specific mechanism for a thyromimetic compound. In contrast, if KB2115 cannot enter the heart (i.e. is not an MCT8 substrate), no cardiac side effects would occur.[166]

6.3. Thyroid Hormones and Obesity

Thyroid hormones induce weight loss by increasing the basal metabolic rate, oxygen consumption, body temperature, and enhancing β-oxidation of fatty acids.[176] These effects are mediated by TRα and, therefore, a decrease in body weight is coupled with increased heart rate as well as the breakdown of muscle and bone. The role of TRβ is smaller in regulating the body temperature and metabolic rate.[177] TRs activate thermogenesis on brown, beige, and white adipose tissues by inducing the transcription of uncoupling proteins (UCPs).[178] *DIO2* is a target of the cAMP-dependent transcription in brown fat and is activated through adrenergic stimulation of the autonomous nervous system. DIO2 then locally converts T4 into T3, which directly induces the *UCP2* gene. UCP decouples mitochondrial electron transport from ATP synthesis, thus inducing heat production.[178b] THs also increase mitochondrial biogenesis and oxidative phosphorylation.[176] The generation of heat is further facilitated through the induction of fultile cycles in the metabolism of glucose and fat by THs.[179] TRβ-selective compounds are known to increase oxygen consumption in cholesterol-fed mice (GC-1) and rats (GC-1 and KB141). The required doses are higher than those required for the reduction of cholesterol, but lower than those required to produce harmful effects.[177,180] Non-alcoholic fatty liver disease (NAFLD) or hepatic steatosis is caused by fat deposition in the liver, and thyromimetics (such as GC-1) and T3 were found to prevent and reverse NAFLD in rats fed a choline/methionine-deficient diet. Similarly, MB07811 also decreased liver fat in normal and cholesterol-fed mice.[181]

6.4. Thyroid Hormones and Type 2 Diabetes

As of 2010, over 285 million people worldwide were suffering from type 2 diabetes,[182] which is a metabolic disorder characterized by high blood glucose as a result of insulin resistance.[183] THs increase glucose uptake in non-

hepatic tissues through increased expression of glucose transporters such as glucose transporter type 4 (GLUT4).[184] Glucose uptake can be mediated by the interaction of TRβ with PI3 kinase.[185] Pancreatic β-cell mass is controlled by the balance between cell proliferation and death, and diabetes occurs when the number of β-cells decrease below what is needed. The number of pancreatic β-cells is increased by THs through proliferation and can restore insulin secretion and increase glucose tolerance in diabetic mice. This effect is mediated by TRα-dependent activation of the cyclin D1/CDK/Rb pathway.[186] TRβ is associated with T3-induced proliferation of hepatocytes and pancreatic acinar cells.[187] Glucose dynamics were improved in horses when treated with THs, and was associated with the reduction of fat.[188] Polymorphism in the *DIO2* gene (Thr92Ala) reduces the enzymatic activity of DIO2, and is associated with the development of insulin resistance. Therefore, T3 may play a role in insulin resistance.[189] The thyromimetics KB141 and MB07811 are known to reduce the blood glucose level, and the effect is similar to the standard therapies with metformin or rosiglitazone.[190]

6.5. Deiodinases as Potential Therapeutic Targets

Hyperthyroidism can be controlled by blocking thyroidal TH production or peripheral T4 into T3 conversion by DIO1 or DIO2. Under normal conditions, the thyroid produces only 6% T3, but this fraction can increase up to 57% in severe hyperthyroidism.[191] DIO1 and DIO2 are highly expressed in the thyroid gland. The *DIO1* gene responds positively to THs, thus opening the possibility that DIO1 increases T3 through a feed-forward mechanism.[192] Inhibition of DIO1 could thus represent a possibility to treat hyperthyroidism. The antithyroid drug PTU also inhibits DIO1, likely by reacting with the oxidized enzyme intermediate (Figure 14A). The selenium compound PSeU also inhibits DIO1 activity (IC_{50}: 1.7 μM in the presence of 20 mM DTT) with an inhibitory effect similar to that of PTU. The inhibitory effects of PTU and PSeU are thought to result from the formation of a selenenylsulfide or diselenide, respectively, with the enzyme and the stabilization of the enzyme–inhibitor complexes through hydrogen bonding of the amide and carbonyl functional groups of the inhibitors at the DIO1 active site.[193] The introduction of an aromatic ring onto PTU increased the inhibition of DIO1 at least tenfold.[128,194] When the concentration of DTT is low (0.2 mM), PTU can weakly inhibit DIO2.[194] In contrast to PTU, the imidazole-based compounds MMI and MSeI were not effective inhibitors of DIO1. An emerging concept is the role of DIO3 in protecting proliferating cells (stem cells, during wound healing, or in tumors) from the differentiation-inducing action of THs. Stem cells and tumors often overexpress DIO3, and the inhibition of DIO3 expression attenuates malignancy.[195] Thus, DIO3 represents a useful novel drug target.

Gold complexes, such as gold thioglucose (GTG; Figure 14B), inhibit DIO1 and DIO2 activity by reacting with the Sec to form stable gold–selenolate complexes.[113d] Amiodarone, an antiarrthymic drug, is a weak noncompetitive inhibitor of DIO1 and DIO2, and its metabolite desethyamiodarone is a strong inhibitor of these enzymes. Iopanoic acid, a cholescystographic agent, also inhibits all three isoforms by acting as a substrate.[196] Many other compounds, such as halogenated dyes,[197] plant metabolites (e.g. phloretin),[198] flavonoids (e.g. genistein),[199] and endocrine-disrupting chemicals such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs; for example, 5′-OH-BDE-99)[200] (Figure 14B), have reported DIO inhibitory activity. Structural similarities seem to exist among these compounds, as some of these also bind TH transfer proteins, transmembrane transporter proteins, and THRs.

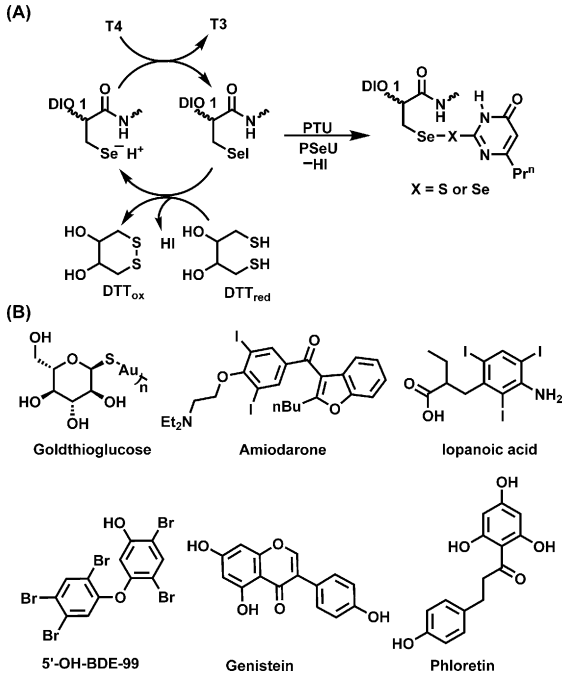


Figure 14. A) Reactions of antithyroid drugs GTG with the enzyme active site selenol and of PTU/PSeU with selenenyl iodide B) DIO Inhibitors reported in the literature.

7. Summary and Outlook

The thyroid hormones (THs)—triiodothyronine (T3) and its prohormone thyroxine (T4)—regulate fundamental biological processes such as development and metabolism. Proper development of brain, cochlea, eye, bones, and intestine; regulation of heart rate, body temperature, and basal metabolic rate; and activation of lipid, carbohydrate, and protein metabolism all depend on THs. In this Review, we have discussed various aspects related to the chemistry and biology of TH biosynthesis and action. We have illustrated both aspects with genetic diseases which interfere in specific ways with the functions of individual components of TH biosynthesis and function. We have shown that both hypo- and hyperthyroidism are serious disorders, but TH replacement therapy is much less complicated than blocking the thyroid function under conditions of hyperthyroidism. We have discussed pharmacological rationales of antithyroid and

thyromimetic drugs. Recent evidence suggests that modulating the activities of TH-metabolizing enzymes, for example, DIO, is emerging as a new strategy for treating thyroid disorders. DIO3, in particular, represents a new drug target for a variety of diseases, including cancer, and future research may focus on the development of isoform-specific inhibitors for DIO enzymes. Further studies, such as determination of the X-ray crystal structure of DIOs, to understand the mode of binding of THs and inhibitors at the enzyme active sites may shed light on the selectivity and biochemical mechanism of regioselective deiodination. Furthermore, the design and chemical synthesis of physiologically relevant sulfur- and selenium-based mimetics of these enzymes may help in understanding the chemistry of the activation and inactivation pathways of TH.

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