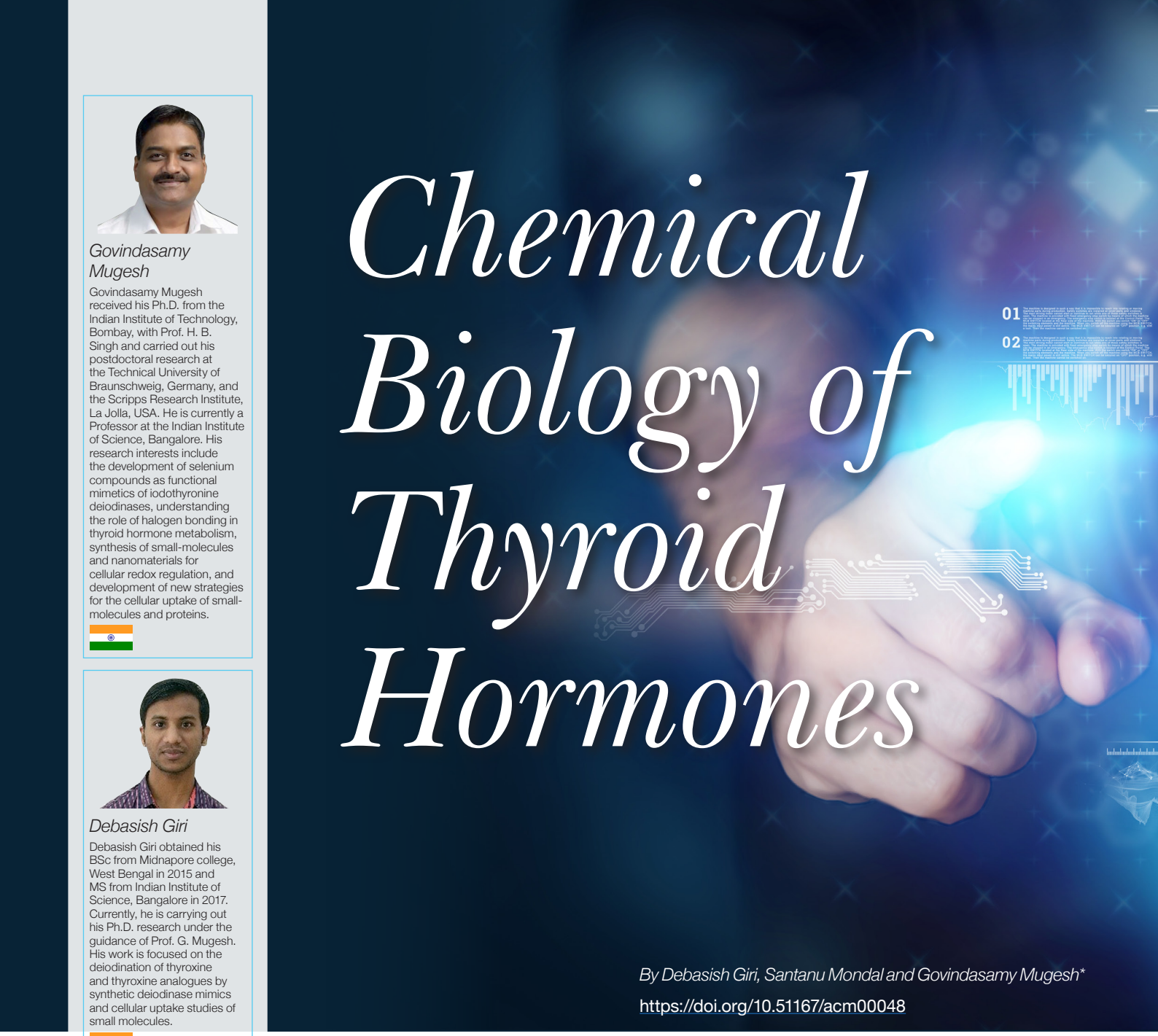

Chemical Biology of Thyroid Hormones

Govindasamy Mugesh

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Debasish Giri obtained his BSc from Midnapore college, West Bengal in 2015 and MS from Indian Institute of Science, Bangalore in 2017. Currently, he is carrying out his Ph.D. research under the guidance of Prof. G. Mugesh. His work is focused on the deiodination of thyroxine and thyroxine analogues by synthetic deiodinase mimics and cellular uptake studies of small molecules.

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https://doi.org/10.51167/acm00048

Thyroid hormones (THs) are synthesized in thyroid follicular cells and secreted by the thyroid gland. These hormones play crucial roles in regulating the body metabolism and temperature, heart rate, neuronal growth, cardiovascular, renal and brain functions. Thyroid gland mainly produces the iodine-containing prohormone L-thyroxine (T4), which undergoes monodeiodination or 5'-deiodination by the selenium-containing iodothyronine deiodinases (DIOs) to generate the biologically active hormone T3. In this general review, the chemical biology aspects of thyroid hormone actions in various organs, biochemical synthesis of T4 in thyroid gland, the role of iodine, thyroglobulin, and thyroid peroxidase in the chemical pathways of hormone synthesis, the transport of hormones by various transport proteins in the blood stream, and various metabolic pathways. The regioselective deiodination of T4 by three isoforms of DIOs to produce active and inactive metabolites and the receptor binding of thyroid hormones are also discussed.

Introduction

Thyroid gland, which is situated near the lower front part of the neck, slightly below the Adam's apple, is made up by two elongated oval-shaped lobes (right and left lobes) connected by a narrow tissue called the isthmus. It is one of the most important hormone-producing glands present in all vertebrates. The central structural unit of the thyroid gland consists of around 20-30 million follicles which produce thyroid hormones (THs), predominantly L-thyroxine or L-3,5,3',5' tetraiodothyronine (T4), an inactive form of thyroid hormones. It also produces a smaller amount of the active hormone L-3,5,3'-triiodothyronine (T3)[1,2]. After biosynthesis of thyroid hormones, they are released into the bloodstream from where they are delivered to different organs by various transporters like thyroxine-binding globulin (TBG), transthyretin (TTR), and human serum albumin (HSA). T4 then enters the target cells through membrane-associated transporters like monocarboxylate transporter 8 (MCT8),10 (MCT10) and organic anion transporter 1c1 (OATP1C1). Subsequently, T4 undergoes monodeiodination by iodothyronine deiodinase (DIOs) enzymes to produce the active form of thyroid hormone T3, which further binds with the thyroid nuclear receptors and recognizes different thyroid hormone-responsive elements (TREs) of target genes and regulates the gene translation. The secretion of THs by thyroid are controlled by hypothalamus and pituitary. Hypothalamus secretes thyrotropin-releasing hormone (TRH), a tripeptide (pyro glutamyl-histidyl-proline amide), which stimulates the pituitary to synthesize and release the thyroid-stimulating hormone (TSH) which signals the thyroid gland to make thyroid hormones (THs). Upon reaching an optimum concentration in the blood plasma, the secretion of T4 from the thyroid gland is stopped by a negative feedback mechanism.[3, 4] THs regulate different physiological functions of the human body like cardiovascular function, normal growth, and maturation of bones and overall metabolism of fat, protein, and carbohydrate. They also control the body temperature, heart rate and protein synthesis. This general article highlights the basic chemistry associated with THs synthesis, transport, and metabolism (Figure 1).

Biosynthesis of thyroid hormones

The biosynthesis of THs takes place in the colloidal lumen of thyroid follicular cells with the help of thyroglobulin (Tg, a highly crosslinked glycoprotein), thyroid peroxidase (TPO), iodide (I-), and hydrogen peroxide

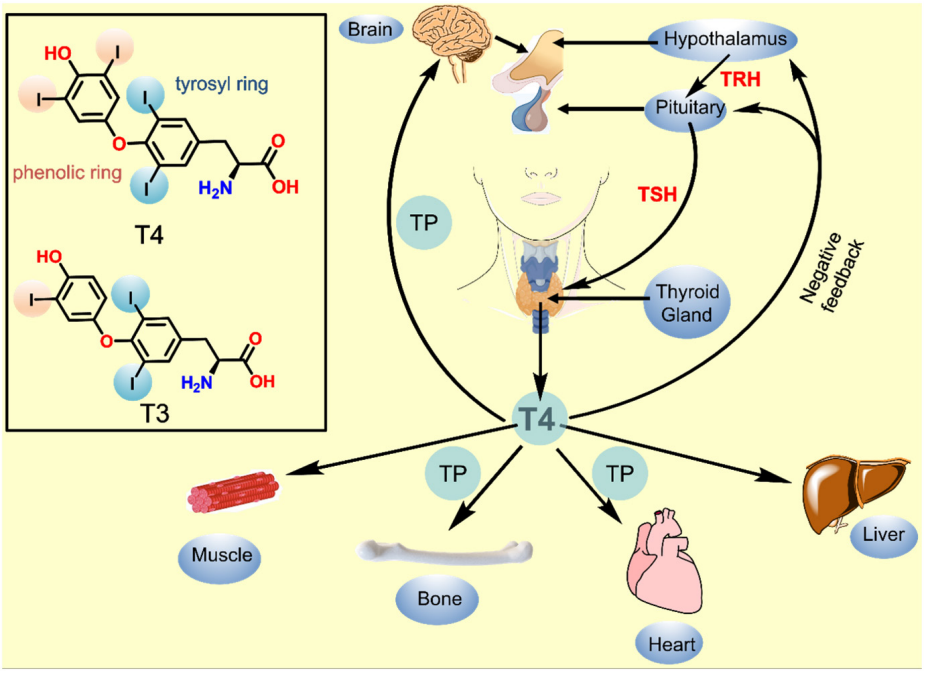

Figure 1. Schematic representation of thyroid hormones action in various organs. TRH: thyrotropin-releasing hormone, TSH: thyroid-stimulating hormone, TP: transport protein.

(H2O2) (Figure 2).[5] TH biosynthesis comprises five important steps: 1) uptake of inorganic iodide (I-) by the thyroid follicles via sodium iodide symporter (NIS);- 2) production of H2O2 by peroxidases and the oxidation of iodide; 3) iodination of tyrosyl residues present in Tg by TPO; 4) phenolic coupling of iodotyrosyl residues to afford Tg-bound THs; 5) proteolytic cleavage of Tg to release free thyroid hormones and transports them to the bloodstream. Each of these steps is briefly described in the following paragraphs

Iodide uptake

Iodine is an essential component of the THs as it compromised 65% and 58% of total weight of T4 and T3, respectively. Inorganic iodide is absorbed from the small intestine and delivered to the thyroid follicular cells by a unique membrane associated glycoprotein transporter, sodium/iodide symporter (NIS)[6] that is located near the basolateral plasma membrane of thyrocytes. Additionally, Na+/K+-ATPase plays an important role in the iodide uptake by maintaining the membrane potential. This enzyme is highly selective for the iodide ion. For each iodide, NIS transports two sodium cations (Na+) into the cell, and consequently, it raises the intracellular iodide (I-) concentration 20–50 times higher than plasma levels.[5]

Hydrogen peroxide (H2O2) production

H2O2 is a reactive oxygen species (ROS) and plays important roles in the intracellular signalling. It is crucial for the TH biosynthesis as it facilitates the iodination of tyrosyl residues and phenolic coupling between iodotyrosyl residues in Tg by TPO. H2O2 is generated in the thyroid follicular cells by two independent NADPH dual oxidases, dual oxidase 1 (DUOX1) and dual oxidase 2 (DUOX2), which are present at the apical membrane of the follicular cells.[7,1]

Formation of activated iodinated species by TPO

TPO is a member of the heme-containing superfamily of mammalian peroxidases and is essential for the synthesis of T4. TPO utilizes hydrogen peroxide and iodide to sequentially perform the iodination of tyrosyl residues in Tg and the phenolic coupling of iodotyrosyl residues.[8] During the catalytic cycle, the distal histidine residue (His95) in the active site of TPO abstracts a proton from H2O2 and facilitates its binding to the Fe+3 centre (Figure 3(A)). Then it undergoes two-electron oxidation to form an oxoferryl π-cation radical intermediate (compound I) by removing one electron from the metal centre (Fe+3 to Fe+4) and the porphyrin ring.[9] Compound I can abstract electron from aromatic amines (AH) and alcohols to afford compound II . Also, it can facilitate two-electron oxidation of I- to yield enzyme-bound active iodinating species, compound III, that can eventually iodinate the tyrosyl residues in Tg.[1]

T4 Biosynthesis in Thyroglobulin

Thyroid follicular lumen is mostly compromises with a highly crosslinked glycoprotein, thyroglobulin (Tg). Tg is homodimeric in nature and has a molecular weight of 600 kDa. It contains around 134 tyrosyl residues

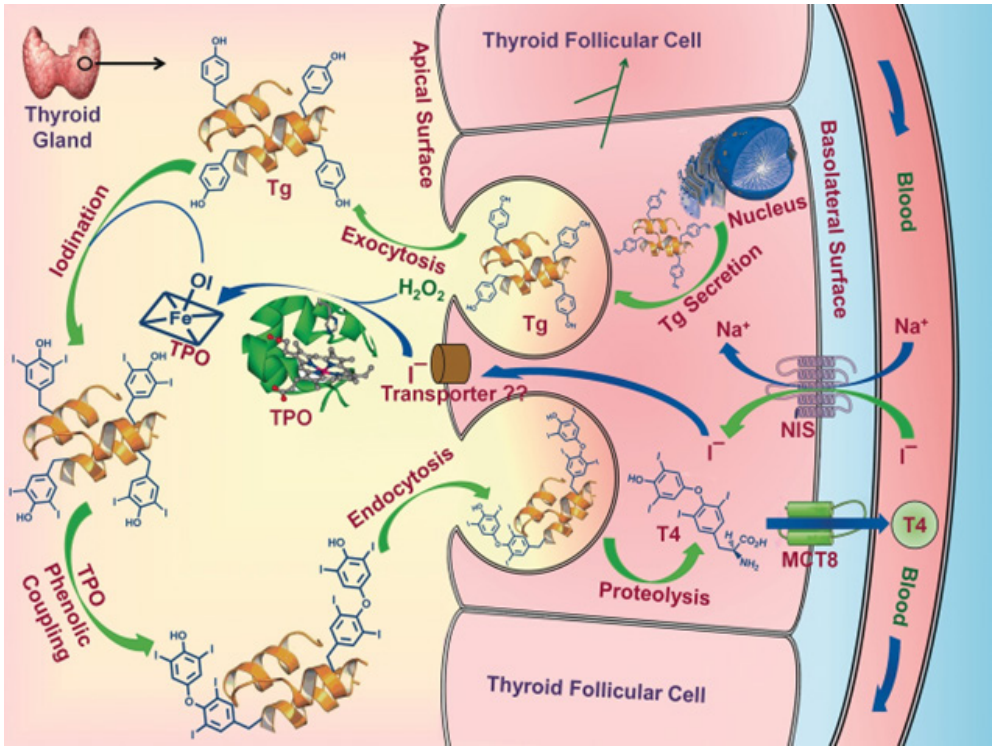

Figure 2. Biosynthesis of thyroxine (T4) and small amount of T3 in the thyroid follicular cells. Reproduced from Ref. 1.

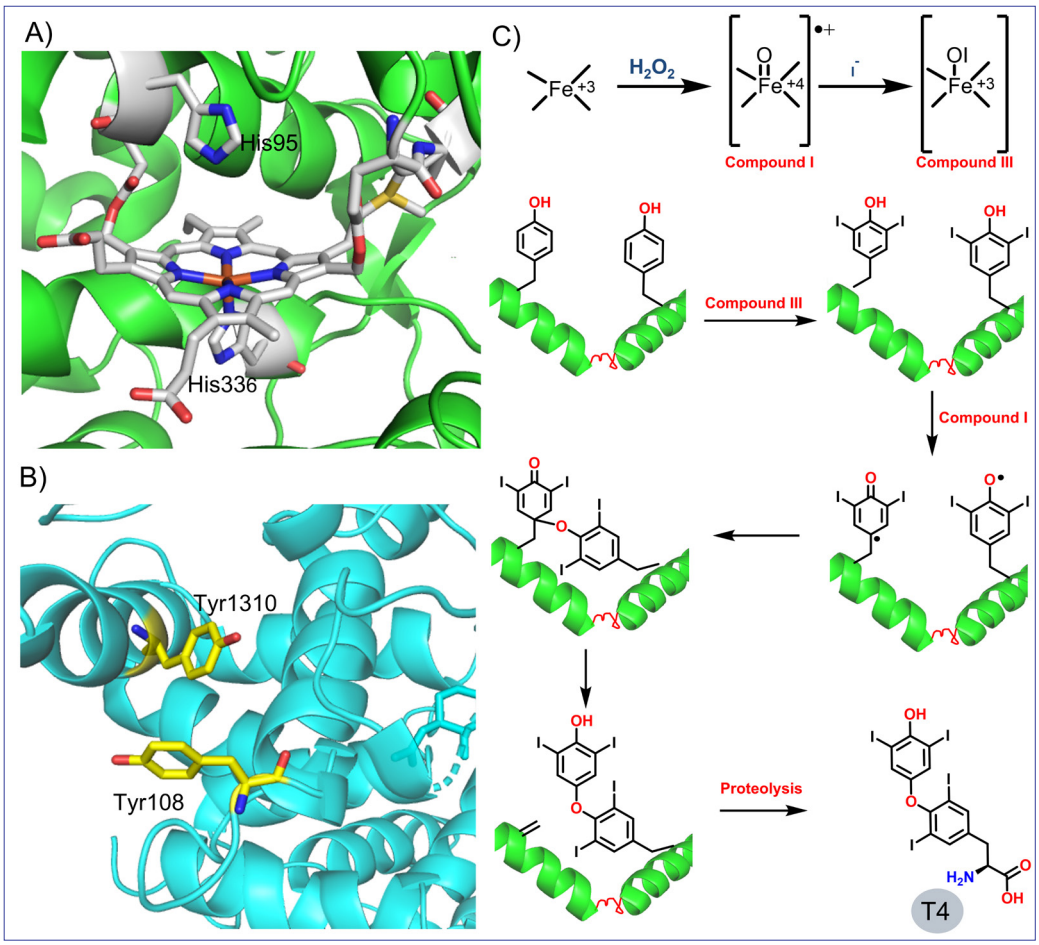

Figure 3.(A) The active site of human myeloperoxidase (MPO), indicating the presence of distal histidine (His95), and proximal histidine (His336). (PDB code: 1CXP). (B) One of the hormonogenic site in Tg. (Tyr108 acts as donor and Tyr 1310 acts as acceptor)[11] (C) Mechanism of biosynthesis of T4 on thyroglobulin.

and amongst them, approximately 30-60 residues are monoiodinated or diiodinated by compound III to afford 3-iodotyrosyl (MIT) and 3,5-iodotyrosyl (DIT), respectively . Although TPO iodinates several tyrosyl residue in Tg, only 1-4 THs are produced per molecule of Tg. These observations indicate that there are only four hormonogenic sites in T4.[1,10] These sites are characterized by the crystallographic studies and single point mutations human (Tyr24Phe, Tyr2573Phe, Tyr2766Phe and Tyr1310Phe) of Tg, four hormonogenic sites are identified where two tyrosyl residues (one act as donor and other as acceptor during synthesis of THs) are within 15Å to each other and have high flexibility in nature (Figure 3(B)). After iodination a donor DIT or MIT is transferred to a nearby acceptor DIT moiety, which yields T4 (or T3) hormone and leaves behind a dehydroalanine moiety in the backbone of the donor side.[11] Both ionic and free-radical process were proposed for the coupling between iodinated tyrosyl residue by compound I and both the mechanisms were supported by the formation of dehydroalanine (DHA) as by product.[12] In the free radical pathway compound I, which is generated by the reaction of TPO with H2O2, removes one electron from each of iodotyrosyl residue which further couples with each other to give T4 or T3 whereas in ionic mechanism it removes two electrons from one iodotyrosyl (acceptor) to form phenolate which further reacts with nearby iodotyrosyl (donor) to give T4 or T3 (Figure 3(C)).

After synthesis T4 and a small amount of T3 which is bound with Tg is retained in the follicular lumen and, via endocytosis it internalizes into follicular cells.; after proteolysis, it releases free T4 and T3 to the bloodstream. Whereas the nonhormonogenic iodotyrosyl residues undergo dehalogenation by flavoprotein iodotyrosyl deiodinase (IYD) to recycle the iodide in thyroid gland.[13]

Thyroid hormone transporter protein

As T4 contains four iodine atoms, it is highly hydrophobic, and therefore, it cannot circulate freely in the blood plasma. After T4 is produced in the thyroid gland, it reaches the bloodstream, where it immediately binds with three different types of transport proteins, namely thyroid-binding globulin (TBG), transthyretin (TTR) and human serum albumin (HSA) and is delivered to various tissues.[14] Out of these three transporters, TTR (4.6 μM) and HSA (640 μM) have comparatively higher concentration in plasma than TBG (0.27 μM), but they show moderate affinity towards T4 as identified by its fast dissociation. In comparison, TBG shows a higher affinity towards T4 and contributed to 74% of the overall T4 transport in human body. [1]

Thyroid-binding globulin

TBG, a 54 kDa protein produced in the liver and belongs to the SERPIN family, carries T4 to various tissues with a dissociation constant (Kd) of 0.1 nM. [14] It has two identical binding sites for T4 which are present between helices H-A and strands 3-5 of the β-sheet. The co-crystal of T4 bound TBG complex (PDB code: 2RIW) indicates that T4 binds with a less stable cisoid conformation where both the phenolic ring and β-alanine side chain orient towards the same side of the tyrosyl ring, and a series of hydrophobic interactions and hydrogen bonds are crucial for creating a higher binding site for T4. The carboxyl and amine group of T4 forms H-bond with nearby Arg378 and Asn273 residues, respectively, and further stabilized by stacking interaction of the phenolic group with Arg381 (Figure 4(A,B)).[15] Interestingly, a halogen bond (XB) interaction between tyrosyl ring iodine (3-I) of T4 and backbone carbonyl of Leu26 is found; such type of halogen bond is seen in numerous protein ligand complexes.[16,17] The binding mode may explain why the Ser23Thr mutation decreases binding affinity. The presence of an additional methyl group may sterically prevent T4 to bind freely with TBG. Like other SERPIN family protease inhibitors, it also has a reactive centre loop (RCL) and free movement of this loop; it creates two different binding states with a higher and lower affinity for T4. Therefore, TBG displays an allosteric type of mechanism for binding and release of T4, which is further supported by the fact that the cleavage of this loop creates an irreversible conformation of TBG.[18,19]

Transthyretin

Human Transthyretin (TTR), which is known as prealbumin with a size of 55 kDa, is one of the essential proteins responsible for the serum transport of thyroid hormones which contribute 11% of the overall T4 transport in plasma. [14] This is a crucial thyroid hormone carrier in rat or other lower vertebrates. Also, TTR is the only THs

binding protein known in cerebrospinal that is secreted from choroid plexus. It exhibits a high binding affinity for several thyroid metabolites, with values for T4 ranging from 100% to 0.7% for 3,3'-T2 and 3-T1 being less than 0.01%, indicating that the iodine atoms play key roles in binding to TTR. Furthermore, TTR transports vitamin A by combining it with the retinol-binding protein at the surface of protein.

The tetrameric structure of TTR is formed by four equivalent monomers (namely A, B, C, D) each having 127 residues which are assembled around the core channel of the protein. The TTR tetramer contains two sterically equivalent binding sites for thyroxine but interestingly differs in relative binding affinities.[20] Site I (dissociation constant for T4, Kd=10-8 M), which is present between monomers A and C, has a binding affinity that is 100 times greater than site II (dissociation constant for T4, Kd=10-6 M), located between monomers B and D. From the co-crystal of human TTR-T4 complex (orthorhombic form, PDB code:2ROX), T4 binds to both binding sites (I and II) in more stable transoid forms unlike in TBG where T4 is in its cisoid from. The phenolic group and β-alanine side chain of T4 are projected towards the core of the channel and the exposed surface, respectively. This type of binding is known as forward type of binding (Figure 4(C)).[21,22]

A detailed structural analysis of hormone-bound complex indicates that there are three pairs of halogen binding pockets (HBP1(HBP1'), HBP2(HBP2'), HBP3(HBP3')) which are symmetrically related to each other. These pockets are hydrophobic core surrounded by the side chain and backbone carbonyl groups of the amino acid residues.[16] In the binding site I of hTTR-T4 complex, the 3,5-iodine atoms of T4 binds at the HBP1 and HBP1' pockets and the T4 binding is stabilized by hydrophobic interactions. The phenolic ring iodine atom (3'-I) is located at the HBP3' pocket. The hydrogen bond formation between the 4'-OH group with Ser117 and salt-bridge interactions between the carboxylate group of T4 with Lys15 and Glu 54 provide further stabilization.

In the HBP3' pocket, the phenolic ring iodine forms a strong halogen bond with the oxygen backbone of Ala109 with a distance (O•••I) of 2.8 Å, which is almost 20% shorter than the sum of van der Waal's radii of oxygen and iodine (3.5 Å). The bond angles Θ1(∠CT4IO) and Θ2(∠IOCA109) are 162.0º and 94.1º, respectively.[23,24] It also has a weak interaction with the nitrogen backbone from the same residue with a distance of 3.5 Å, whereas the second iodine atom (5'-I) positioned in HBP2' pocket further stabilizes the structure through a short interaction between with amidic NH (generally less known XB acceptor as lone pair is in conjugation with carbonyl group) of Leu110' with having distance of of 3.5 Å. In contrast, in the binding site II of TBG-T4, only one such type of halogen bond is present between tyrosyl (3'-I) and Ala109 with a distance(O•••I) of 3.3 Å (Figure 4(C)). This distance is larger than that of site I (2.8 Å).[21] The weak halogen bond and the smaller number of short contacts in site II may be responsible for its lower binding affinity than the site I.

In the other type of TTR-T4 complex (monoclinic form PDB code:1ICT),[25] both the sites (I and II) show similar types of interactions (Table 1). From the crystal structures of these proteins with T4, short contacts such as halogen bond play crucial roles in molecular recognition process.[16,26] It is known that a single mutation in TTR can destabilize the native TTR structure, leading to aggregation of the protein and the formation of amyloid

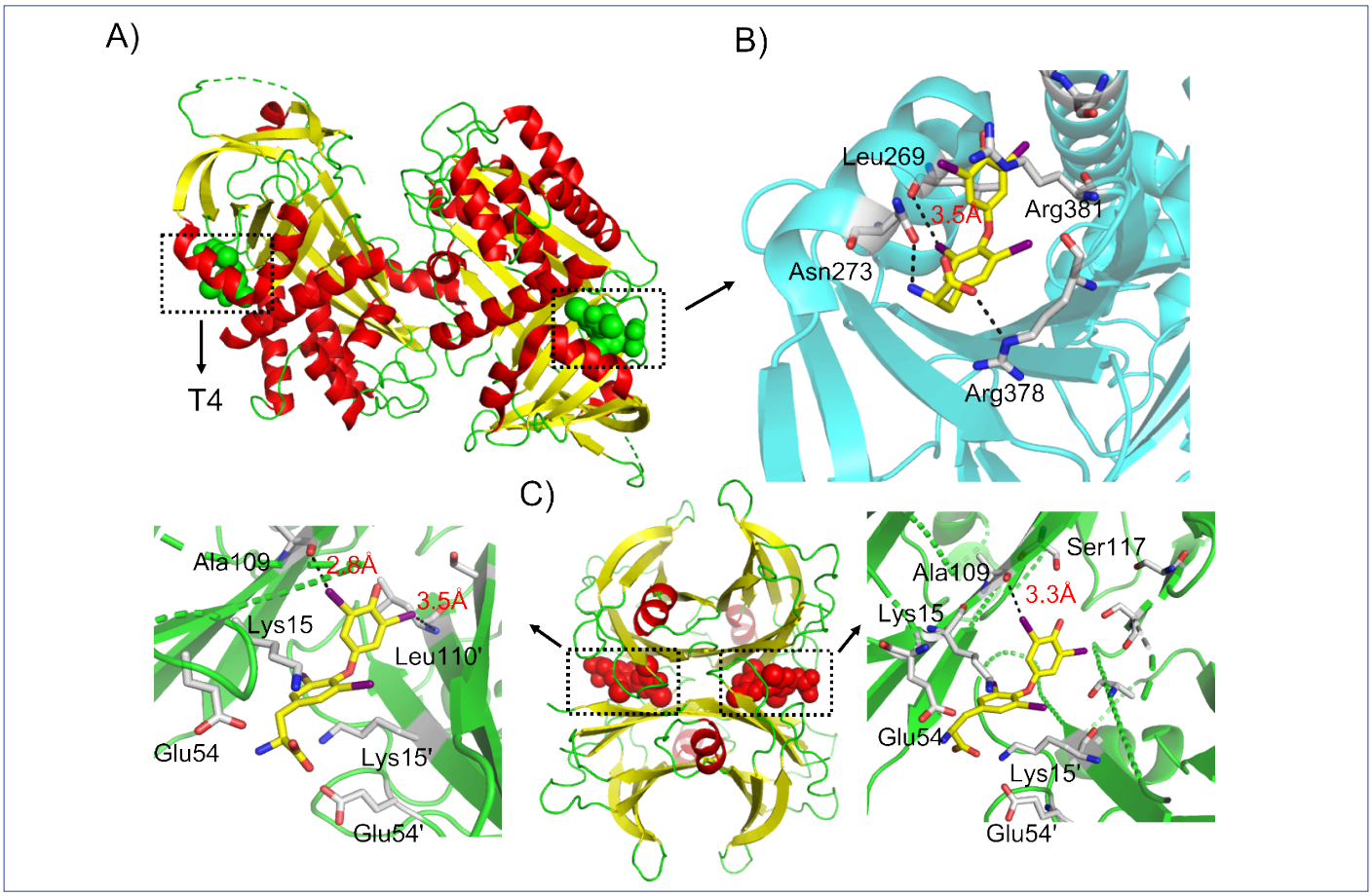

Figure 4 (A) Crystal structure of TBG with T4 showing the presence of two binding domain for T4 (PDB code:2CEO). **(B)** Different types of interactions are shown in one of the binding sites. **(C)** Crystal structure of hTTR with T4 (PDB code:2ROX) shows two binding sites for T4.

PDB code	Ligand(I)	XB acceptor	d(Å)
2ROX	T4(3')	Ala109 (backbone O, site1)	2.8
	T4(3')	Ala109 (backbone NH, site1)	3.5
	T4(5')	Leu110' (backbone NH, site1)	3.5
	T4(3')	Ala109(backbone O, site2)	3.3
1ICT	T4(3')	Ala108 (backbone O, site1)	3.2
	T4(3')	Ala109 (backbone NH, site1)	3.1
	T4(3')	Leu110 (backbone NH, site1)	3.2
	T4(5')	Ser117'(backbone O, site1)	3.3
	T4(5')	Ser117' (sidechain O, site1)	3.4
	T4(3')	Thr119 (backbone NH, site2)	3.3
	T4(5')	Ser117' (backbone O, site2)	3.2
	T4(5')	Ser117' (sidechain O, site2)	3.2
	T4(5')	Thr119' (backbone NH, site2)	3.4
1ETA	T4(3')	Ala109 (backbone O, site1)	3.1
	T4(3')	Ala109 (backbone O, site2)	3.1
1SN5	T3(3')	Leu109 (backbone O, site1)	3.0
	T3(3')	Leu109 (backbone NH, site1)	3.4
1THA	3,3'-T2(3')	Ser117 (backbone O, site1)	3.4
	3,3'-T2(3')	Ser117 (backbone O, site2)	3.2
	3,3'-T2(3')	Ser117 (sidechain O, site2)	2.9

Table 1. XBs observed in the crystal structures of TTR in complex with various THs [16]

fibrils.[27,28] T4 acts as an inhibitor of such amyloid formation by retaining the actual structure upon binding.

Human Serum Albumin

Human Serum Albumin (HSA) is a 66 kDa monomeric protein and is responsible for transporting bilirubin, non-esterified fatty acids, steroids, and bile acids. It can also transport thyroid hormones in plasma with the lowest binding affinity towards T4 with a dissociation constant (Kd) of 2 μM. It has three homologous domains (I, II and III), each having two subdomains (A, B). The crystal structure of HSA-bound T4 (PDB code: 1HK1) is known.[29] It indicates that four T4-binding sites (Tr1, Tr2, Tr3 and Tr4) are present in HSA, out of which Tr1 and Tr2 are located in the subunit of IIA and IIIA, respectively. The IIIB subdomain contains other two binding sites, Tr3 and Tr4. Interestingly, T4 binds in a cisoid conformation at Tr1 like the binding observed with TBG. At other binding sites, T4 binds in a transoid conformation, which is similar to that of TTR. Tr1 differs from the other binding sites in forming the hydrophobic cavity from Phe223, Arg218, Arg222, and Trp214 residues, which force the hydrophilic β-alanine side chain of T4

to move to the other side of the Tyrosyl ring. In contrast, such type of interaction is absent in other binding sites. In Tr1, in addition to the hydrophobic interaction, the 4'-OH group and the carboxyl group of T4 form hydrogen bonding with Arg 257/Tyr 150 and Lys199, respectively. This is further stabilized by a halogen bond formation between the tyrosyl ring iodine atom (3-I) with an oxygen backbone of Ile290 having an (O•••I) distance of 3.2 Å (Figure 5(A)). It is found that the replacement of Arg218 to either His or Pro increases the binding affinity for T4 as Arg218 sterically inhibits the free movement of the T4 β-alanine side chain. In the binding pocket of Tr2, two hydrogen bonds are present between Tyr411 and Ser489 with the 4'-OH group. Additionally, the tyrosyl ring iodine atoms of T4 interact with the oxygen backbone of Ala406 and Leu387, having a distance of 3.5 Å and 3.4 Å, respectively (Figure 5(B)). In the third (Tr3) and fourth (Tr4) binding sites, similar halogen bond formation helps in stabilizing the T4 binding as given in Table 2. [16] Overall, the formation of a maximum number of interactions (both hydrogen bonds and XBs) in Tr1, leads to the higher affinity of Tr1 for T4.

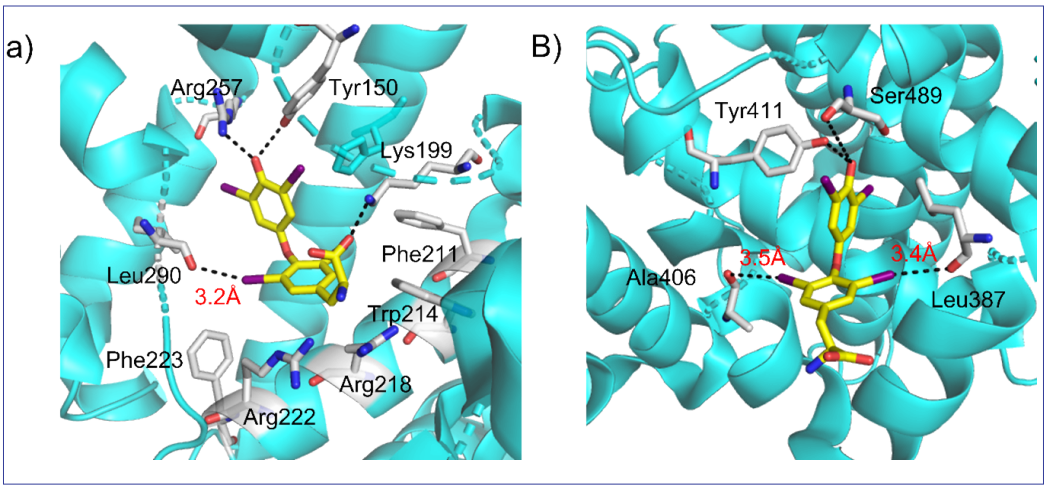

Figure 5. (A) HB and XB interactions are shown in Tr1 of HAS-T4 complex (B) HB and XB interactions are shown in Tr2 of HAS-T4 complex.

PDB code	Ligand(I)	XB acceptor	d(Å)
1hk1	T4(3)	Ile290 (backbone O, Tr1)	3.2
	T4(3)	Ala406 (backbone O, Tr2)	3.5
	T4(5)	Leu387 (backbone O, Tr2)	3.4
	T4(5)	Lys524(backbone O, Tr3)	3.5
	T4(5')	Met548(side chain S, Tr4)	3.6

Table 2. XBs observed in the crystal structures of HSA in complex with T4. [16]

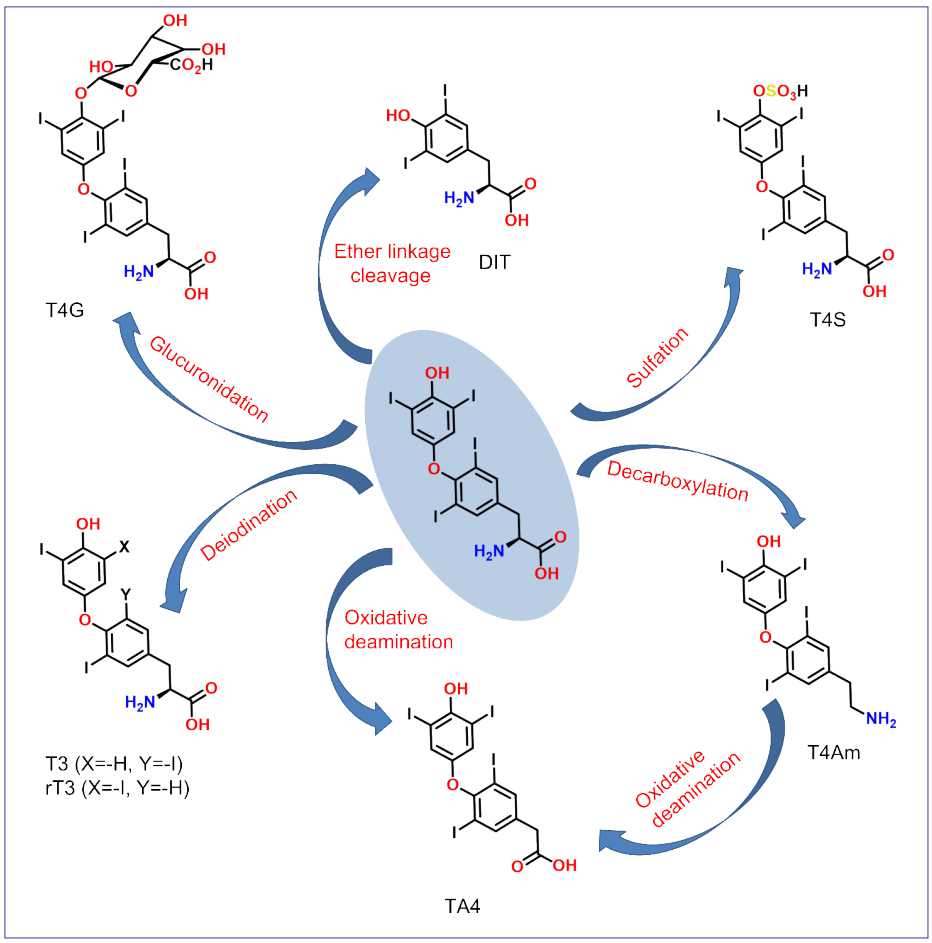

Figure 6. different metabolic pathways of thyroxine (T4).

Metabolism of Thyroid Hormones

Following the internalization into target organs, THs undergo numerous metabolic processes catalyzed by various enzymes (Figure 6).[30,31] The phenolic hydroxyl group of T4 undergoes sulphation and glucuronidation to afford T4S and T4G, respectively.

These metabolites have higher solubility in water and therefore, facilitates excretion of T4 from the body.[32] Notably, these metabolites have lower affinity to the nuclear receptors and when required, they are converted to the parent hormone by sulfatases and glucuronidases. Sulfation of THs is catalyzed by a group of enzymes called sulfotransferases (SULTs) that utilizes 3'-phosphoadenosine-5'-phosphosulfate (PAPS) as a sulfate donor.[33] By contrast, glucuronidation is catalyzed by various isoforms of uridine 5'-diphosphate glucuronosyltransferases (UGTs).

Other than conjugation of 4'-OH, thyroid hormones can undergo decarboxylation of the β-alanine group by L-amino acid decarboxylase and ornithine decarboxylase to afford different iodothyronamines (TAMs), but only 3-iodothyronamine (3-T1AM) and thyronamine (T0AM) are found in organisms indicating that the TAMs with higher number of iodine atoms deiodinated by deiodinase enzymes.[34,35] Recently, it has been shown that 3-T1AM and T0AM have interesting biological properties, such as 3-T1M inhibits dopamine reuptake by dopamine transporter (DAT) and norepinephrine by norepinephrine transporter (NET); they also acts as a modulator for α2-adrenergic receptors.[36] Recently, it has been shown that monoamine oxidase (MAO) performs oxidative deamination of TAMs to afford iodothyroacetic acids (TAs), which can also be produced by the direct deamination of THs by L-amino acid oxidase (LAO). Interestingly, Tetrac (3,5,3',5'-tetraiodothyroacetic acid, TA4) and Triac (3,5,3'-triiodothyroacetic acid, TA3) can bind to the nuclear receptors and exhibit thyromimetic activity[37] and can suppress TSH level. Notably, Triac has 1.5- and 3.5-fold higher binding affinity than T3 to the thyroid nuclear receptors TRα1 and TRβ1, respectively. Since TA3 is more selective to TRβ1 than TRα1, it possesses significant potential to treat thyroid hormone resistance syndrome (RTH).[38] Except all the above-mentioned metabolic pathway, thyroid hormones also undergo ether link cleavage (ELC) by peroxidases like horseradish peroxidase (HRP) and MPO to yield DIT.[39]

Deiodination of Thyroid Hormones

The most important metabolism of T4 is the sequential deiodination by DIOs. [40] While phenolic ring deiodination of T4 produces a biologically active metabolite, T3, tyrosyl ring deiodination affords an inactive metabolite, rT3. Notably, rT3 cannot bind to the nuclear thyroid hormone receptors (Figure 7).[41] These activation and inactivation pathways are essential to control the overall thyroid hormone concentration in the blood plasma.

DIOs are selenocysteine -containing membrane-bound enzymes that activate or inactivate the THs by removing phenolic ring or tyrosyl ring iodine.[42] Three DIO isozymes, DIO1, DIO2, and DIO3 are categorized by their amino acid sequences and substrate specificity. Although the primary structures

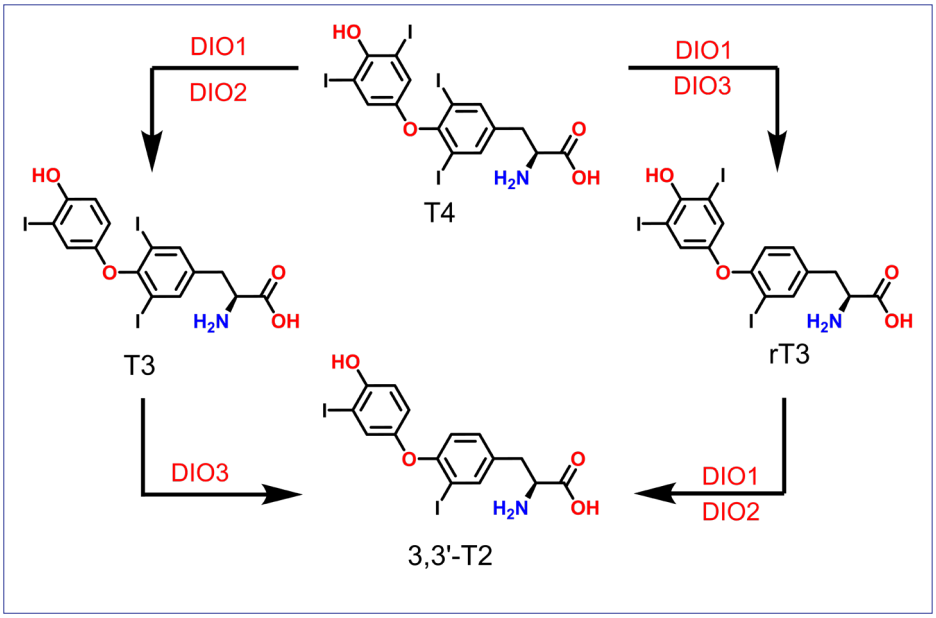

Figure 7. Deiodination of thyroxine (T4) by DIOs.

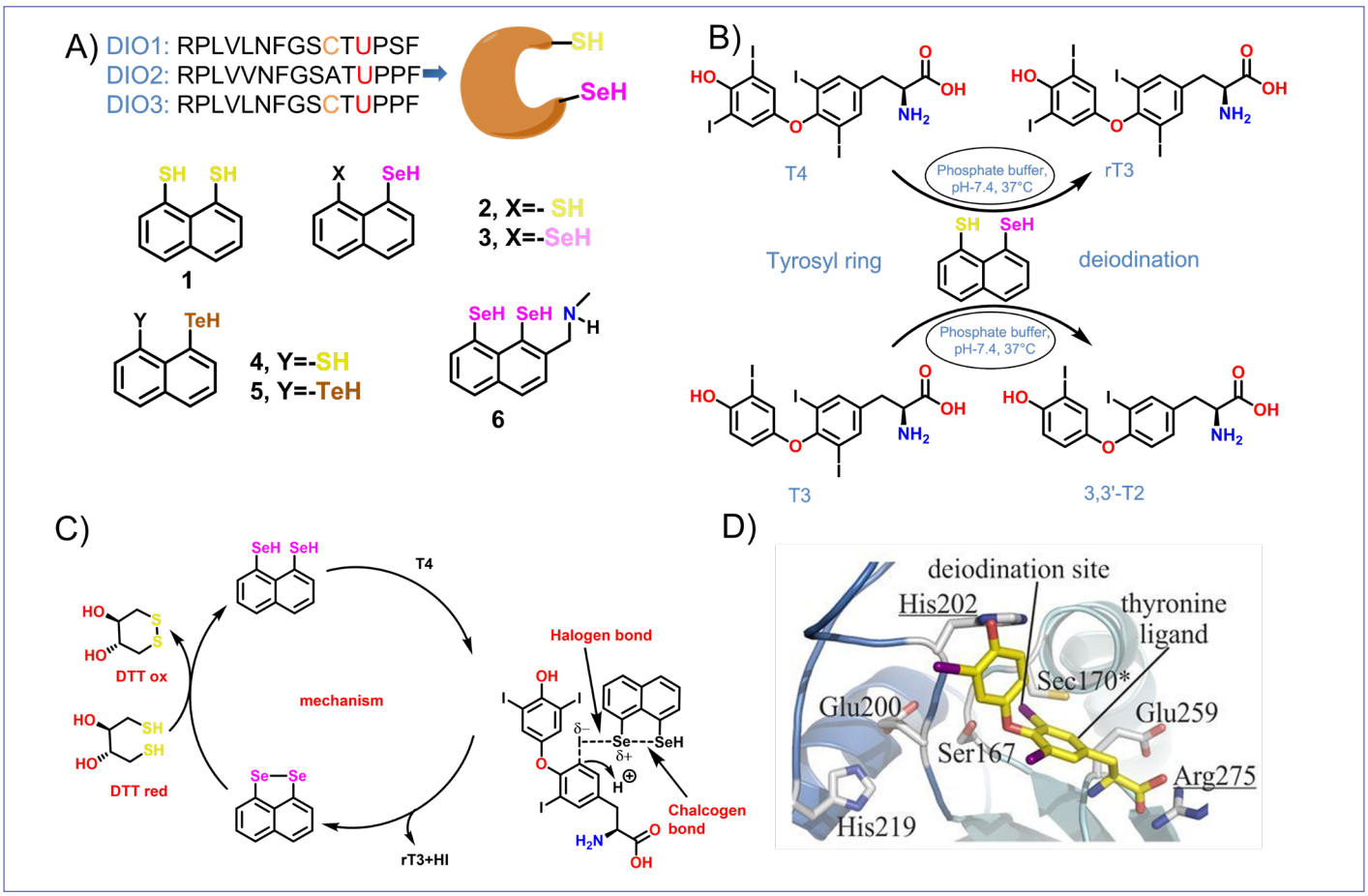

Figure 8. (A) Chemical structures of DIO3 mimics. (B) Deiodination of T4 and T3 by compound 2. (C) Proposed mechanism of halogen-bond-mediated deiodination of T4. (D) T4-binding site on mDIO3cat. Reproduced, with permission, Copyright: 2014, National Academy of Sciences.

of these enzymes are similar, particularly in the active site region, they exhibit different regioselectivities of deiodination. DIO1 can remove iodine from both phenolic and tyrosyl rings of T4, whereas DIO2 and DIO3 are selective to the phenolic and tyrosyl ring, respectively. DIOs also accept other iodothyronines as substrates, although they exhibit similar regioselectivity as observed for T4.

While the deiodination of T4 and other metabolites by DIOs was well-established, the mechanism of deiodination remained elusive for several decades. DIO1 and 3 contain a conserved selenocysteine and cysteine in the active site. These residues are crucial for TH deiodination as mutation of either of them results in a significant drop in the rate of deiodination. Inspired by this precedence, we developed the first small molecule DIO3 mimic that contains a selenol and thiol at the peri-positions (2) of a naphthyl ring.[45,47] Owing to the rigidity of the aromatic ring, the selenol and thiol moieties are hold close to each other, mimicking the primary structure of the DIO1 and 3. Interestingly, this compound regioselectivity deiodinates T4 and T3 at their tyrosyl rings to afford rT3 and 3, 3'-T2, respectively (Figure 8(B)). While replacement of the selenol with a thiol (1) leads to slower deiodination, substitution of the thiol with another selenol (3) dramatically increases the rate of deiodination. [46] These results are in agreement with the previous studies that show that a Sec170Cys DIO3 mutant exhibit significantly lower activity than the wild-type enzyme. [44] Notably, none of the DIO3 mimics exhibit altered regioselectivity of deiodination even at higher concentrations. [47] Although a keto-enol tautomerism of the phenolic hydroxyl group was proposed for the phenolic ring deioddination of T4 [48], such mechanism is not feasible for the tyrosyl ring deiodination. Electron density around the halogen atom is anisotropically distributed in the organic halides, leading to a significant positive charge (σ-hole) on the halogen atom along the C-X (X = halogen) axis. σ-hole facilitates the non-covalent interaction of halogens with an electron donor through the formation of highly directional XBs. Theoretical investigations indicate that the iodine atoms in T4 contain significant positive charge on the σ-hole that enable them to form XBs with chalcogens. Interestingly, the tyrosyl ring iodine of T4 contains a larger σ-hole than the phenolic ring iodine that lead to regioselective tyrosyl deiodination THs by the small molecule mimics[49] Owing to the formation of a Se···I XB, selenium in the naphthyl-based mimic becomes electron deficient. Therefore, the thiol group in the close proximity subsequently interacts with the selenium, leading to the formation of a Se···S chalcogen bond (ChB). Both Se···I and Se···S interactions are equally important for the polarisation and reductive cleavage of the C-I bond (Figure 8(C)).[50] These observations are further supported by the substitution of the selenol with a tellurol (4,5) that can form even stronger XB. The tellurium-containing mimics are extremely reactive (>1000-fold) towards deiodination, and they can mediate both phenolic and tyrosyl ring deiodination of T4 to produce all possible deiodinated products, including rT3, T3, 3,3'-T2, 3',5'-T2, 3-T1 and T0.).[51]

The introduction of a basic amino group in the close proximity enhances the rate of the reaction, although such substitution does not alter the selectivity of deiodination reactions.

As mentioned earlier, sulphated and decarboxylated TH metabolites also undergo enzymatic deiodinations by DIOs. Therefore, we also investigated the biomimetic deiodination of these metabolites. In contrast to

THs, sulphated metabolites undergo both phenolic and tyrosyl ring deiodination in the presence of naphthyl-based mimics, while decarboxylated metabolites undergo regioselective tyrosyl ring deiodination. Furthermore, deiodination of sulphated and decarboxylated metabolites are found to be faster and slower, respectively, than THs. These observations agree with the observed rates of enzymatic deiodinations of sulphated and decarboxylated metabolites by DIOs. The observed rates of deiodination are explained based on the formation of a stronger and weaker XB by sulphated and decarboxylated metabolites, respectively, with selenium. Furthermore, the phenolic and tyrosyl ring iodine of sulphated metabolites form equally strong XB with selenium, while the decarboxylated metabolites, similar to THs, form stronger XB with their tyrosyl ring iodine. These results also explain the observed regioselectivity of biomimetic deiodination of the sulphated and decarboxylated TH metabolites.[54,55,56]

Following our studies on the biomimetic deiodination of THs, crystal structure of DIO3 was reported by Schweizer et al.[43] (Figure 8(D)). Interestingly, modelling of the active site with T3 indicates the tyrosyl ring iodine of T3 is in fact located at a strikingly close distance (3-4 Å) to the active site selenol, leading to the formation of a XB and polarisation of the C-I bond. This binding mode is further supported the H-bonding interactions involving the β-alanine side chain and 4'-hydroxyl group of T3. Following the iodine abstraction, the active site selenol is presumably converted to a selenenyl iodide that may react with Cys239 to afford a selenenyl sulphide intermediate. This intermediate may get further reduced by nearby Cys268 to regenerate the enzyme active site. The conserved selenocysteine residue in all three DIOs indicates that a similar mechanism may be responsible for the deiodination of THs. However, the origin of regioselectivity of deiodination by DIOs still remains a mystery. Detailed theoretical investigations suggested that DIOs may bind THs in different conformations that may allow selective activation of either phenolic or tyrosyl ring iodine. As such, T4 is known to exist in at least two different stable forms with different three-dimensional structures and XB-formation capabilities. However, the binding of THs to DIOs in different conformations is yet to be experimentally verified. [52,53]

Thyroid Hormone Receptors

Thyroid hormone receptors (TRs), which are members of the nuclear receptor family, control gene expression when they bind with the active form TH, T3. Additionally, TRs bind the target gene's promoter region by recognizing nucleotide sequences known as thyroid hormone response elements (TREs).[57] Two different types of thyroid receptors (TRα and TRβ) are present which further spliced into four different isoforms, TRα1, TRα2, TRβ1, and TRβ2. The expression of isoforms is highly tissue specific and modulates different activities. For example, TRα1, which is highly expressed in the heart and muscle is involved in maintaining the cardiovascular function, whereas TRβ1 which is expressed in the liver, kidney and brain regulates the body's overall metabolism.[58] Both the receptors (TRα and TRβ) are similar in structure and amino

acid sequence. The only major difference is found in the ligand binding domain, involving one change in amino acid (Ser277 in TRα and Asn331 in TRβ). From the co-crystal structure of human TRα1 bound T3 (monoclinic form, PDB code: 2H77), it can be seen that a hydrophobic pocket forms around LBD with nearby amino acid residues from the different helix like H5-6 (Met 256-Arg266) H7-8, H-3 (Phe215-Arg 228), H-11(His381-His387) and H-12 (Figure 9(A)).[59] T3 binds in more stable transoid form at the hydrophobic core through hydrogen bonding interactions between the β-alanine group and the 4'-OH of T3 with Arg228 and His381, respectively. Interestingly, this binding is further stabilized by a halogen bond (XB) interaction between the backbone carbonyl of Phe218 and the 3-iodine atom of T3 (I•••O) with a dI•••O of 3.1 Å.[16] Similarly, from the co-crystal of TRβ1 bound T3 (PDB code: 1XZX), similar type of binding is found in the LBD with T3 where the β-alanine group of T3 forms hydrogen bond with the Arg282 and Asn331. In contrast, in the case of TRα1, it is only with Arg228. The presence of XB is found with the 3-iodine of T3 and backbone carbonyl of Phe272 with dI•••O of 3.2 Å (Figure 9(B)) .[16,60] The thyroid receptor has a higher binding affinity towards T3 (Kd= 0.06 nM) than T4 (Kd=2 nM). The 30-fold increase in the affinity for T3 can be explained by comparing the crystal structure of T4-bound TRβ1 (PDB code: 1Y0X) with that of T3, which reveals that a deformation of ligand binding domain (LBD) is created in order to accommodate two bulky iodine atoms of T4 in the phenolic ring, creating more steric hindrance (Figure 9(C)).

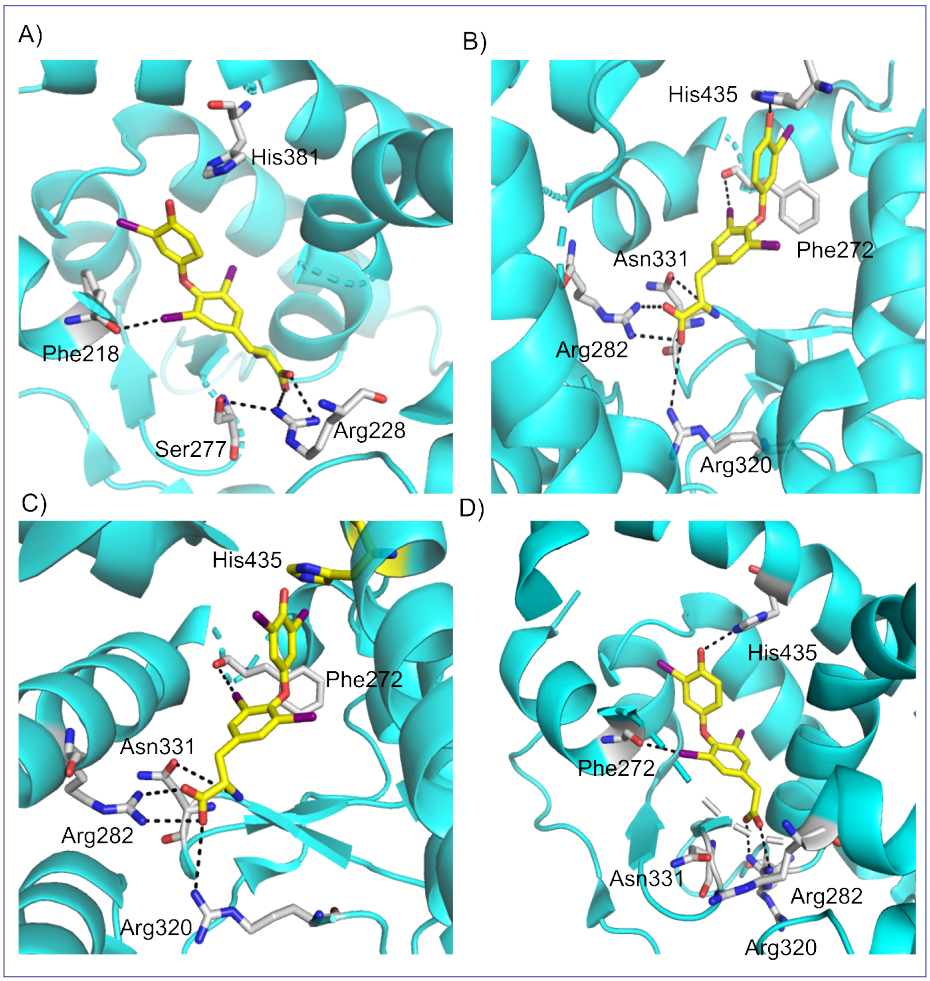

Figure 9. (A) Hydrogen bonding and halogen bonds observed in the T3-binding sites of Trα1 (PDB codes: 2H77). (B,C) Crystal structures of TRβ in complex with T3, (PDB code: 1XZX) and T4, (PDB code: 1Y0X).) (D) TRβ (PDB code: 3JZC) in complex with triiodothyroacetic acid.

3,5,3'-triiodothyoacetic acid (TA3), which shows thyromimetic activity, has almost three-fold higher binding affinity towards TRβ than that of TRα. A comparison of the co-crystals of TRα-TA3 (PDB code: 3JZB) and TRβ-TA3 (PDB code: 3JZC), indicates that T3A forms a hydrogen bond with Arg266 in TRα, whereas in TRβ, it forms two hydrogen bonds with Arg320 and Asn331. These interactions resemble similar interactions found in the T3-TRs complexes (Figure 9(D)). The presence of Asn331 in TRβ makes the ligand binding domain more flexible and it creates higher ligand binding volume (500 Å3 in TRβ) as compared to that of TRα (461 Å3). These bonding motifs may explain the better selectivity of TA3 towards TRβ.[61]

Summary and Future Perspectives

Thyroid hormones (THs) play key roles in almost every cell in the body. They regulate our body metabolism, increase the basal metabolic rate, help in protein synthesis, regulate bone and neuronal growth and cardiovascular functions. They are essential for the proper development and differentiation of all cells. The thyroid hormones, particularly the prohormone T4, are synthesized in thyroid follicular cells of the thyroid gland and transported to various organs to carry out tissue-specific and cell-specific functions. It is interesting how these hormones use the trace element iodine not only for their synthesis, but also for the transport and metabolism. The formation of halogen bond appears to be a key factor in controlling the transport as well as the metabolism such as regioselective deiodination of the thyroid hormones.

The future work in this area may involve development of more relevant mimics of deiodinases that can regioselectively remove iodine from T4 to produce T3 or its inactive metabolites. These studies may help in understanding the active sites of iodothyronine deiodinases (DIOs), which exert their catalytic activity through selenocysteine residues. The modulation of the activities of DIOs appears to be a promising strategy for treating various thyroid disorders. In particular, the type 3 enzyme, DIO3, can be an interesting drug target for diseases including cancer and future research may focus on the development of isoform-specific inhibitors for these enzymes. Additional studies on the structure and function of deiodinases may help understanding the selectivity and biochemical mechanism of regioselective deiodination.

Acknowledgements

This study was supported by the Science and Engineering Research Board (SERB, JBR-2020-000003), Department of Science and Technology (DST), New Delhi. D.G. and S.M. acknowledge the Indian Institute of Science, Bangalore, for a research fellowship.

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