Thyroid Hormones

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# Thyroid hormones—From Crystal Packing to Activity to Reactivity

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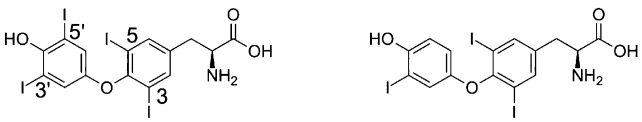
Biological molecules contain a remarkably small number of chemical elements. Almost all reactions essential to life are performed by molecules, substrates, enzymes, and their products that mostly contain the elements carbon, nitrogen, oxygen, hydrogen, phosphorus, sulfur, and small amounts of metals. It is intriguing that in humans, the element iodine seems to be exclusively needed for only two related compounds, the thyroid hormone L-thyroxine (T4, **1**, Figure 1) and its more active receptor-binding metabolite, T3 (**2**, Figure 1). Thyroid hormones not only contain iodine substituents on their aromatic rings, but these iodinated rings are also linked by a diphenylether moiety—the only one known in human biochemistry. Biosynthesis of T4 takes place exclusively in a specialized organ, the thyroid gland, or in its precursor structure in lower vertebrates and chordates. The activating conversion of T4 into T3 is accomplished via 5′-deiodination (Figure 1) catalyzed by two of the three isoforms of iodothyronine deiodinases (Dio), namely Dio1 and Dio2. 5-Deiodination (at the inner ring), which is primarily catalyzed by Dio3, converts T4 or T3 into the inactive metabolites reverse T3 and 3,3′-T2, respectively. All Dio enzymes are homologous selenoenzymes, that is, they share a basic architecture and contain selenocysteine in their active centers.[1] The molecular basis of their different regioselectivities is poorly understood. Cell-type-specific and developmentally regulated expression of different deiodinase isoforms fine tune local levels of T3, which makes these enzymes key players in thyroid hormone action.




L-thyroxine, T4
3,3', 5,5'-tetraiodothyronine

L-triiodothyronine, T3
3,3', 5-triiodothyronine

***Figure 1.*** Structures and atom numbering for the prohormone thyroxine and its more active metabolite T3. The affinity of the nuclear T3 receptor for T3 is more than one order of magnitude higher than for T4. The tyrosyl ring carrying the 3- and 5-iodines is often referred to as the inner ring and the phenolic ring as the outer ring.

Lack of the hormone leads to developmental delay or impaired development of the brain, eyes, inner ears, bones, and other organs, and it was the most common cause of mental disability until the 1970s, when diagnosis and treatment of congenital hypothyroid patients were improved.[2] Even a small deviation from a person's "normal" thyroid hormone level is perceived as impaired well-being. Thyroxine treatment is needed for tens of thousands of patients per year who have their thyroid glands removed for various reasons, and for millions of patients taking T4 to suppress thyroid-stimulating hormone (TSH) production by the pituitary gland. T4 is usually taken orally as a tablet and it is tacitly assumed that somehow the moderately water-soluble hormone is liberated from the pill and taken up during intestinal passage. Physicians know that patients are unwilling to change the brand of thyroid hormone they are adapted to. Although doctors feel uneasy with the mystery of why 100 μg of synthetic pure thyroxine from brand A should not be equivalent to 100 μg of synthetic pure thyroxine from brand B, current medical guidelines acknowledge the patients' point.[3]

The recent paper by Mondal and Mugesh on the molecular features of thyroxine provides a stunningly simple answer to this mystery.[4] The authors crystallized synthetic thyroxine from different solvents and observed two different crystal forms: a triclinic (form I) and a monoclinic (form II) form. Both polymorphs contained two molecules of T4 within the asymmetric unit. However, the crystal packing differs between the two forms, and the T4 molecules also adopt different conformations that allow different intra- and intermolecular interactions. Differences occur in particular with respect to the orientation of the amino and carboxylic acid groups relative to the diphenylether moiety and with regard to the crystal contacts, which involve a network of halogen bonds (XB). In both crystal forms, strong contacts are observed between a phenolic ring iodine and the carboxylic acid moiety from an adjacent molecule. A conformational analysis further revealed that the largest differences in geometry between the two polymorphs pertain to the

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conformation of the amino acid moiety connected to the so-called inner ring. These polymorph dissimilarities were supported by differences in in FT-Raman and solid-state $^{13}C$ NMR spectra.

The authors went further and showed different solution behavior of the two polymorphs. Form I (which was crystallized from methanol) shows a much lower specific optical rotation in methanol than form II (crystallized from acetonitrile) in acetonitrile. This observation indicates that the conformation of thyroxine depends on the solvent. At pH 4, form I showed significantly higher solubility than form II, while form II showed higher solubility at pH 9. Together, these observations explain why thyroxine from different brands may have different solubility. They may simply be present in different polymorph forms. Moreover, these findings indicate that solubility can be an intrinsic property of the thyroxine form used, which contrasts with the former assumption that galenic formulation and additives dominate how well the tablet releases its drug.[5] Of note, liquid formulations of thyroxine are already on the market and are recommended if patients concurrently use proton-pump inhibitors, which impair gastric acid secretion.[6] Since the bioavailability of thyroxine is a persistent problem in clinical endocrinology, the paper of Mondal and Mugesh[4] constitutes a leap forward because it provides doctors and their patients with a rational grounding for their search for an optimal thyroxine therapy. This work adds thyroxine to the list of active pharmaceutical ingredients that come in different crystal forms. Polymorphs not only have an impact on the action of the medication, but they may also raise serious issues relating to patent law.[7]

The other intriguing finding in the paper by Mondal and Mugesh is that the chemical shifts of the ring carbons bonded to iodine (C3, C5, C3′, C5′) are remarkably different in the two polymorphs.[4] The authors suggest that these different chemical shifts reflect differential reactivities of the associated C–I bonds. The crystals show directional XB along the axis of a C–I bond, which leads to association of the donor with the positive electrostatic potential (σ-hole) of the iodine, and the bond strength is significantly influenced by the conformation of the molecule. This aspect is likely to be of high relevance for biochemistry, because during enzymatic deiodination, the catalytic selenolate appears to attack the σ-hole of the iodine in-line via a halogen bond, thereby weakening the C–I bond for cleavage.[8] The authors applied density functional theory (DFT) calculations to further investigate this aspect and calculated the XB energies for different T4 conformers with methylselenoate as the halogen-bond acceptor. Generally, I···Se bonds from the 3- and 5-iodines were stronger than 3′- and 5′-halogen bonds. This is in line with previous findings from Mugesh's group in which they reported the efficient 5-deiodination of T4 by a small molecule deiodinase mimetic.[9] 4′-Sulfation of iodothyronines increases the σ-hole in 5′-iodines and facilitates 5′-deiodination of T4S by the same small-molecule mimic.[10] Mondal and Mugesh then demonstrated that changing those torsional angles that define the conformation of the substituted phenyl rings of T4 along the diphenylether linkage increases the strength of the I···Se interaction such that bonds involving the 3′- and 5′-iodine approach the strength of those involving the 3- and 5-iodine.

Most intriguingly, the DFT calculations showed that the conformation of the thyronine amino acid moiety also strikingly influences XB energy. Conformations can be found in which 5′-deiodination is actually preferred over 5-deiodination. The authors recognized that the binding of iodothyronines in slightly different conformations could thus be the means by which the three otherwise highly similar deiodinase enzymes achieve their different regioselectivities.

The recently determined crystal structure of the mouse Dio3 catalytic domain ($mDio3^{cat}$) unfortunately lacked a bound substrate and we were only able to model the substrate into the active-site cleft.[11] Based on this binding mode, which is similar to the situation in the T3 thyroid hormone receptor complex[12] and in line with mutational data, we have proposed a binding of the substrate amino acid moiety to the $Arg^{275}$ and $Glu^{259}$ side chains (Figure 2).

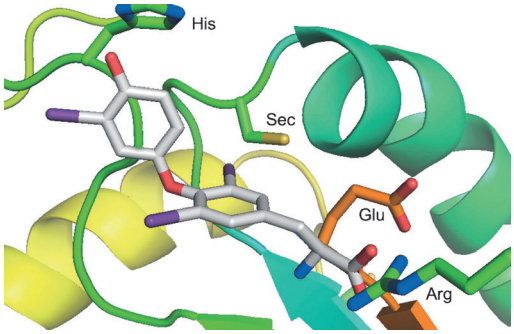


***Figure 2.*** Proposed model for the binding of T3 in $Dio3^{cat}$ based on PDB: 4TR3. The key residues His202, Sec170, Glu259, and Arg275 are labeled.

Intriguingly, among the very few amino acid differences around the active-site cleft between deiodinase isoenzymes, $Arg^{275}$ is replaced by a Lys in Dio2. In Dio1, the respective Lys residue is even shifted one position towards the N terminus, while the Glu is conserved among mammalian deiodinases. Considering the inspiring work of Mondal and Mugesh, it is conceivable that these small differences in the enzyme region that recognizes the carboxyl group of the substrate could control the regioselectivity by tweaking the binding conformation of the substrate.

In their thought-provoking work, the authors started out with crystallization of their compound from different solvents and careful and insightful conformation analysis and comparison, an almost forgotten art in our times of heavy-weight and heavily prized laboratory equipment that quickly generates large amounts of data. Only with the right questions in mind did they then employ sophisticated instrumental analytics and arrive at DFT calculations. Almost in passing, they solved the apparent mystery of the non-equivalence of thyroxine from different brands, and they provide an attractive explanation for how deiodinases might control regioselectivity through altering the substrate conformation at a distance from the reactive bond.

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