Published in final edited form as:

Biochimie. 2010 September ; 92(9): 1227–1235. doi:10.1016/j.biochi.2010.02.013.

Efficient Use and Recycling of the Micronutrient Iodide in Mammals

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Abstract

Daily ingestion of iodide alone is not adequate to sustain production of the thyroid hormones, tri- and tetraiodothyronine. Proper maintenance of iodide *in vivo* also requires its active transport into the thyroid and its salvage from mono- and diiodotyrosine that are formed in excess during hormone biosynthesis. The enzyme iodotyrosine deiodinase responsible for this salvage is unusual in its ability to catalyze a reductive dehalogenation reaction dependent on a flavin cofactor, FMN. Initial characterization of this enzyme was limited by its membrane association, difficult purification and poor stability. The deiodinase became amenable to detailed analysis only after identification and heterologous expression of its gene. Site-directed mutagenesis recently demonstrated that cysteine residues are not necessary for enzymatic activity in contrast to precedence set by other reductive dehalogenases. Truncation of the *N*-terminal membrane anchor of the deiodinase has provided a soluble and stable source of enzyme sufficient for crystallographic studies. The structure of an enzyme•substrate co-crystal has become invaluable for understanding the origins of substrate selectivity and the mutations causing thyroid disease in humans.

Keywords

Deiodinase; Flavoprotein; Reductive Dehalogenation; Iodide Metabolism; Thyroid

Introduction

Iodide is a critical micronutrient for mammalian health, and deficiencies in either dietary iodide or iodide metabolism may lead to hypothyroidism, goiter and, in severe cases, developmental defects. The World Health Organization has identified iodide deficiency as a leading cause of brain damage worldwide [1]. More than 1.2 billion people are estimated to lack sufficient access to iodide despite our very modest daily requirement for this micronutrient (recommended daily allowance of 150 µg/day) [2]. Iodide is quite scarce in the environment, and even sea water contains a very low concentration of iodide (< 1 µM) well below that of fluoride, chloride and bromide [3]. Two thyroid proteins, the sodium/iodide symporter and iodotyrosine deiodinase, are primarily responsible for efficient utilization of dietary iodide in mammals, and mutations of either protein can cause thyroid disease [4-7].

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Our need for iodide derives solely from its requirement in the biosynthesis of 3,3',5,5'-tetraiodothyronine (T4) and 3,3',5-triiodothyronine (T3), the only mammalian hormones containing a halogen (Figure 1). These derivatives are first generated in the thyroid and then circulated throughout the body to regulate a wide variety of metabolic processes including the basal rate of metabolism, temperature regulation and expression of numerous proteins [8].

Iodide is transported and concentrated in the thyroid by the Na⁺/I⁻ symporter located on the basal membrane of thyroid follicular cells [5,9]. This promotes an accumulation of iodide within the cell to concentrations as great as 40-fold more than that found in plasma. Iodide is also excreted through anion channels into the colloid, the site of T4 biosynthesis (Figure 2). Iodide is then sequestered and stored after its oxidative coupling to tyrosyl residues of thyroglobulin in a process catalyzed by thyroid peroxidase. Thyroglobulin is the major constituent of the colloid [10], and its mono- and diiodinated tyrosyl residues serve as intermediates in the formation of T3 and T4. Despite the low molecular weight of these thyroid hormones, their biosynthesis does not involve modification of free tyrosine.

Thyroglobulin is a very large glycoprotein comprised of two identical subunits of ~330,000 daltons each. Although the homodimer contains ~ 220 tyrosyl residues, less than 25% of these are available for iodination. Even fewer of the resulting diiodotyrosyl residues are available to undergo the next step of T4 biosynthesis, the coupling of proximal diiodotyrosine residues to form an iodothyroninyl product. This second process is also catalyzed by the same thyroid peroxidase responsible for the preceding iodination reaction. Ultimately, approximately seven monoiodotyrosines (MIT), six diiodotyrosines (DIT) and one T4 are formed per thyroglobulin polypeptide [10]. Additionally, every third thyroglobulin polypeptide contains one T3. When thyroid follicular cells are activated by a signal such as thyroid-stimulating hormone, thyroglobulin is taken up from the colloid by endocytosis and hydrolyzed to release T4/T3 into the plasma.

T4 is often considered a prohormone since its T3 derivative is 10-fold more potent for regulating metabolism. Individual peripheral tissues control the ratio of these derivatives and their inactive metabolites, 3,3',5'-triiodothyronine (rT3) and the diiodothyronines, by expressing iodothyronine deiodinase, a fascinating enzyme that catalyzes an unusual reductive rather than oxidative or hydrolytic dehalogenation [11,12]. Three isoenzymes of this deiodinase are present in mammals, and each exhibits a complementary specificity for recognition and processing of the various iodothyronines. Isozyme D1 preferentially converts T4 to T3 and is primarily expressed in liver, kidney, thyroid and pituitary. Isozyme D2 almost exclusively converts T4 to T3 and is expressed in a variety of tissues including brain, heart, skeletal muscle and thyroid. Together, these enzymes support our activation of thyroid hormone. In contrast, isozyme D3 expressed in brain, skin, uterus, placenta and fetus supports our deactivation of the hormone by promoting conversion of T4 to rT3.

Although no crystal structure is yet available for D1, D2 or D3, their amino acid sequences suggest membership in the thioredoxin structural superfamily [13]. Unlike thioredoxin, however, D1-D3 contain an essential active site selenocysteine residue (Sec). The mechanism for incorporating this, the so-called 21st amino acid, in mammalian proteins was first discovered by studies of D1 [14-16]. The active site Sec is thought to be intimately involved in deiodination, and mutation of Sec to Cys reduces the catalytic activity of D1 by as much as 100-fold [11,15]. The net reaction promoted by D1 relies on the reducing power of thiols to regenerate its active site selenocysteine from a proposed selenyl iodide intermediate (Figure 3) [17,18].

The iodothyronine deiodinases are expressed in numerous organs and also contribute to iodide recycling since catalytic reduction of the iodothyronines releases iodide. Once this iodide returns to the circulatory system, it may again be transported into the thyroid by the Na^+/I^- symporter. However, the primary mechanism of iodide recycling in mammals occurs much earlier in its metabolism. Proteolysis of thyroglobulin within thyroid follicular cells releases 6-7 fold more MIT and DIT than T3 and T4 [10,19]. MIT and DIT consequently represent a major source of iodide that is recovered by a reductive dehalogenation process catalyzed by iodotyrosine deiodinase (IYD). If MIT and DIT had alternatively persisted in the thyroid or diffused away, follicular cells would be denied the iodide necessary for hormone biosynthesis [4,6,7].

IYD and D1-D3 are the only enzymes yet known to promote reductive dehalogenation in mammals, and the potential for similarities is great since at least IYD, D1 and D2 all act on an *ortho* iodophenol group. However, D1-D3 are structurally and mechanistically quite distinct from IYD. They are not members of the same structural superfamily and do not depend on equivalent residues in their active sites. Similarly, they do not share the same cofactor dependence or source of reducing equivalents. Research progress on these enzymes has been equally distinct. Advances in the study of D1-D3 have been published steadily, and a number of excellent reviews are available on this topic [11,12]. In contrast, study of IYD has been slow and variable until recent success with its heterologous expression [20,21]. This brief review focuses on historic and recent investigations on IYD and represents the first review devoted to this topic since 1984 [22].

Discovery and early characterization of iodotyrosine deiodinase (IYD)

The first report of iodotyrosine deiodination in mammals was published in 1950 [23]. Soon after this, thyroid extracts were shown to dehalogenate both [^{131}I]- iodo- and [^{82}Br]- bromotyrosine [24]. Additionally, this activity was found to be insensitive to a common drug used to treat hyperthyroidism, propylthiouracil. Iodoacetate, in contrast, inhibited dehalogenation and provided the first suggestion that a cysteinyl residue might be involved in catalysis. This early report also noted that dehalogenation was selective for halotyrosine derivatives, and no dehalogenation of iodo- or bromothyronine was detected [24]. Such selectivity is crucial to avoid a futile cycle of continual synthesis and degradation of T4 and T3. The complementary properties of D1 and IYD have since been directly compared in detail by analysis of thyroid homogenates [25].

As early as 1955, a patient exhibiting an enlarged thyroid gland at birth was found to have high levels of MIT and DIT along with low levels of T4 and T3 (Fig. 1) [26]. Defects in IYD were soon implicated in this and related cases [4,27], although the exact mutations within IYD were not identified until 2008 [6,7]. Such IYD deficiencies were confirmed in the intervening years by demonstrating a lack of iodide release from DIT and MIT after incubation with thyroid slices [28]. Modern diagnosis now includes intravenous injection of labeled MIT or DIT. Healthy patients rapidly degrade these derivatives and release iodide whereas patients with low IYD activity excrete the labeled materials unchanged [28].

Attempts to isolate IYD and characterize its catalytic reaction began in 1957 with discovery of the enzyme's association with microsomes and its requirement for NADPH but not molecular oxygen (eq. 1) [29,30]. Little more was discovered about IYD in the subsequent decade other than its sensitivity, or more often its insensitivity, to substrate analogues and potential inhibitors. Catalysis was similarly unaffected by numerous metal ions, inhibitors of electron transport, tyrosine, T4, T3 and iodide [31]. Only the keto acid derivatives of MIT and DIT weakly inhibited IYD. 3-Nitro- and 3,5-dinitrotyrosine, however, demonstrated potent inhibition of deiodination *in vitro*, and perfusion of the dinitro derivative into a rat

thyroid gland induced secretion of MIT and DIT as expected from suppression of IYD [32,33].

1

Purification and initial mechanistic investigation of IYD

Ultimately, two protocols were developed to extract IYD from microsomes [34]. One involved hydrolytic digestion of the microsomes with a crude preparation of the lipase steapsin available commercially, and the second involved extraction of IYD from microsomes using the detergent cholate. Subsequent ammonium sulfate precipitation and sequential chromatographic purification with DEAE-cellulose, hydroxylapatite and sephadex yielded homogeneous protein for the first time [34]. From this important milestone, IYD was determined to be a dimer of identical subunits containing a single essential FMN. The estimated molecular weight of IYD in retrospect suggests that proteolysis may have occurred during steapsin treatment resulting in a decrease in FMN binding affinity and consequently a sub-stoichiometric concentration of FMN in the IYD preparation. Current protocols based on heterologous expression and purification of IYD consistently yield two FMN per homodimer [35]. Removal of FMN from IYD by treatment with 1.5 M guanidine-HCl was shown to suppress all deiodinase activity, and conversely reconstitution of apoenzyme with FMN restored this activity [36]. Further analysis of this enzyme was hindered due to its arduous purification and lack of stability. Still, the discovery of FMN within IYD provided a significant foundation for later mechanistic study despite the ambiguous relationship between IYD and other flavoprotein systems such as ferredoxin/ferredoxin reductase and adrenodoxin/adrenodoxin reductase [37].

Flavoproteins serve a number of functions in nature ranging from electron transfer to metabolite reduction and oxidation [38-41]. Flavoproteins have even been associated with mercuric ion reduction [42] and natural product halogenation [43]. In contrast, very few reports have implicated flavin in reductive dehalogenation [36,44,45]. Reductive dehalogenation is rarely detected in aerobic organisms which instead typically rely on oxidation and hydrolysis for dehalogenation [46]. Only anaerobic organisms commonly depend on reductive processes to promote dehalogenation [46]. Two notable exceptions to this trend provided inspiration for an early proposal on the mechanism of IYD catalysis [47]. The precedent set by D1 was initially attractive since this enzyme also catalyzes a reductive dehalogenation and was already associated with thyroid metabolism as presented above (Fig. 3). A related mechanism described in detail for dechlorination by the bacterial enzyme tetrachlorohydroquinone dehalogenase (TCHQ) [48,49] suggested a similar precedence (eq. 2). Both D1 and TCHQ are thought to promote substrate tautomerization to form keto intermediates lacking aromatic stability (eq. 2). Subsequent nucleophilic reaction between the halogen substituent and the redox active side chain of Cys or Sec results in substrate dehalogenation and transient enzyme oxidation that is then reversed by exogenous thiols.

2

An equivalent mechanism could be envisioned for IYD in which an active site Cys reacts with the tautomeric keto form of its substrate to generate a sulfenyl iodide that is finally reduced by the active site flavin. Reducing equivalents for the flavin reaction are expected to derive indirectly from NADPH *in vivo* and may be replaced *in vitro* by dithionite (see below). Low molecular weight thiols do not drive the reaction of IYD unlike that of D1 or TCHQ [50]. The potential participation of a Cys residue might have explained the reported sensitivity of IYD to thiol reagents [24,51]. Similarly, the possibility of a flavin-dependent reduction of sulfenyl iodide is supported by an analogous flavin-dependent reduction of a Cys-derived sulfenic acid during turnover of NADH peroxidase [52]. Despite the appeal of such precedence, IYD has now been shown to act quite uniquely.

Recent site-directed mutagenesis of the gene for IYD produced active enzyme independent of Cys residues [53]. Cys to Ala mutations did not substantially affect the ability of IYD to reduce MIT. Thus, Cys residues do not directly participate in the redox reactions promoted by IYD. Crystallographic analysis of IYD (see below) has since located the Cys residues distal to the active site [35]. The lack of Cys dependence, however, does not preclude involvement of a substrate-derived tautomer common to the mechanisms of D1 and TCHQ. The relevance of this tautomer to the turnover of IYD was examined with a series of transition-state (or intermediate) analogues based on pyridonyl derivatives of tyrosine (Fig. 4) [47]. The presence of an alkyl substituent on the heterocyclic nitrogen strongly favors its keto rather than enol form and provides steric bulk to mimic the iodo substituent of MIT. These species exhibited a very strong affinity for IYD and acted as competitive inhibitors of catalysis as expected for active site stabilization of the substrate tautomer. Preferential binding of the *N*-methyl vs. *N*-ethyl and *N*-isopropyl derivatives was somewhat surprising since the larger alkyl substituents better mimic the size of the native iodo group. Further investigations into the mechanism of IYD awaited advances in expressing soluble and active protein in large scale.

Cloning and heterologous expression of the IYD gene

Two complementary approaches were used to identify the gene (*DEHAL1*) of IYD. A classical method relied first on isolation of the enzyme from natural sources (porcine thyroids) and second on mass spectral analysis of selected peptides formed by proteolytic

digestion of the purified IYD [54]. Preparation of this protein also began with limited proteolysis to release it from microsomes. Subsequent chromatographic purification of the truncated IYD provided protein of only 70% purity. Thus, a definitive connection between IYD and the gene identified through peptide sequencing required expression of the active enzyme [21]. Such confirmation was anticipated but not guaranteed by the correlation between peptide sequences of the porcine IYD and its human homologue (geneID 389434) annotated as a flavin-dependent oxidoreductase from the NADH oxidase/flavin reductase superfamily.

A complementary genetic approach concurrently discovered the same gene through use of serial analysis of gene expression (SAGE) designed to identify thyroid specific genes [55,56]. Nucleotide sequences derived from this approach also provided the information necessary for *DEHAL1* isolation from a cDNA library (porcine) and expression in mammalian HEK293 and CHO cells [20].

The amino acid sequence of IYD consists of three domains and is highly conserved within mammals based on current sequence information available through the various genome databases. The *N*-terminal region (ca. 24 amino acids) was predicted and later confirmed to act as the sole membrane anchor of this protein [21,53]. An intermediate domain (ca. 57 amino acids) does not conform to any known structural motif. The remaining *C*-terminal region (ca. 200 amino acids) aligns with the NADH oxidase/flavin reductase (NOX/FRP) superfamily and even shares 24% sequence identity with a representative from *Thermus thermophilus* [21]. Such similarity within this superfamily allowed for the original annotation of the IYD gene during the human genome project, but similarities between the prokaryotic and mammalian proteins do not extend to their catalytic activities. Prokaryotic members of this superfamily do not associate with membranes and have not been reported to act as dehalogenases. Typically, the prokaryotic enzymes accept reducing equivalents directly from NAD(P)H in contrast to their mammalian counterpart as discussed below. The substrates reduced by these catalysts also vary widely, and for many, the natural substrates have yet to be identified. Some supply reduced flavin for bioluminescence [57] while others act as nitroreductases [58].

The NOX/FRP domain of IYD has yet to fold into a stable and active structure in the absence of its intermediate domain in contrast to the prokaryotic homologues that consist solely of a NOX/FRP domain [59]. Two isoforms of *DEHAL1* generated by alternative splicing of its exon 5 also produced unstable proteins that were observed to degrade rapidly when expressed in HEK293 cells [60]. However, a modest truncation of the IYD gene to remove only the *N*-terminal residues 2 - 33 allows for expression of a soluble and stable enzyme that promotes deiodination of MIT in the presence of dithionite with rate constants similar to those of the wild type, membrane-bound enzyme [53].

Use of NADPH for IYD-dependent deiodination

Initial success at solubilizing IYD from microsomes led to its loss of response to NADPH, the physiological source of reducing equivalents [50]. This has since been ascribed to the separation or inactivation of a reductase that serves to transfer the reducing equivalents of NADPH to IYD [35,36,51]. Deiodination by solubilized fractions of IYD can still be driven alternatively by reduced FMN, FAD, methyl viologen, and ferredoxin [51]. For simplicity, however, dithionite remains the most common reductant used to promote catalysis of purified IYD [22].

Expression of IYD in mammalian cell culture has recently provided additional evidence for the role of a reductase in the NADPH-dependent reaction of IYD. IYD expressed in HEK293 cells is responsive to both NADPH and dithionite, whereas IYD expressed in CHO

cells is only responsive to dithionite [20,53]. Thus, the ability of IYD to utilize NADPH does not require its expression in thyroid cells but does require an additional component such as a reductase that is not present in all mammalian cells. This other component likely associates with IYD in native membranes since the soluble form of IYD lacking its *N*-terminal membrane anchor utilizes NADPH very inefficiently in HEK293 cells when compared to the native membrane-associated enzyme [53].

Crystallographic analysis of IYD and its substrate-bound complex

The gene for the truncated IYD(Δ 2-33) (murine) with a *C*-terminal His-tag expressed to a very high level in Sf9 cells [35]. Only a single nickel-based affinity column was necessary for its purification and sufficient protein was easily obtained for structural studies. Crystallization of IYD was observed within the first 24 hrs of incubation in stark contrast to the many decades required to develop a satisfactory purification of IYD. Interpretation of the diffraction pattern was also relatively rapid by use of nine sulfur atoms in IYD to phase the data. The resulting structure (pdb 3GB5) is consistent with its preliminary assignment as part of the NOX/FRP superfamily and shares a common α - β fold, dimer of identical subunits with extensive domain swapping, and active site formed by the interface of the two subunits (Fig. 5) [35]. Although early preparation of IYD from calf thyroid recovered a single FMN per dimer [34], expression and purification of IYD from Sf9 cells yielded IYD with two FMN per dimer as evident by UV-Vis spectroscopy and X-ray crystallography [35].

The first 34 residues of IYD(Δ 2-33) along with residues 156 - 177 and 195 - 208 likely remain dynamic in the protein crystal since no electron density was detected for these regions in the absence of substrate. However, residues 156-177 and 195-208 gained structure in the presence of substrate and formed an active site lid in MIT-IYD and DIT-IYD co-crystals (pdb 3GFD and 3GH8, respectively). This lid was created by stabilizing an α -helix and loop that coordinate to the substrate directly and sequester the substrate-FMN complex from solvent (Figs 5b & 6) [35]. Substrate is tightly chelated in this complex by numerous polar interactions established by both the side chains within the lid and the pyrimidine ring of the FMN. Typically, proteins modulate the properties of their bound flavin by direct coordination to the pyrimidine ring of flavin [61,62], a region that strongly influences its reactivity [63]. IYD is very unusual for positioning the zwitterionic region of its substrate between the protein and pyrimidine ring of flavin. Consequently, the redox characteristics of the flavin are expected to be very sensitive to the presence of substrate, and future study of this cofactor in IYD should include the presence of substrate or an appropriate substrate analog.

Crystallographic data did not suggest a basis for recognition of the iodo group within the substrates. Sterics alone are not sufficient for high affinity since 3-alkyltyrosines are very weak inhibitors [32]. In contrast, 3-nitro- and 3,5-dibromotyrosine are strong inhibitors [32]. Thus, a polar substituent *ortho* to the phenolic group may enhance binding to IYD, even though active site residues of complementary polarity are not apparent in the enzyme structure. Dissociation constants for a variety of substrates and their analogues have now been measured by quenching the fluorescence of the active site FMN [64]. Consistent with the earlier trends, MIT binds to IYD with a K_D of 9 nM, and tyrosine and its 3-methyl derivative bind with K_D values of greater than 140 μ M. The substantial influence of substituents at the 3-position was investigated with a range of 3-halotyrosine derivatives. 3-Bromotyrosine binds as tightly as MIT to IYD, and even the binding of 3-fluorotyrosine is only 10-fold weaker than that of MIT despite the significant difference in size and polarity of the fluoro- and iodo-substituents. To date, binding appears to be less dependent on these properties and more influenced by the substituents' effect on the phenol pKa [64]. Full deprotonation of the phenolic group may even strengthen its association with the 2'-

hydroxyl group of FMN's ribityl chain. This interreaction first became evident from the co-crystal structure of IYD•MIT [35].

The sequence identity shared by mouse and human IYD provides confidence in use of the crystal structure to identify the structural basis for human deficiency and mutation in this enzyme. Recently, the genetic origins of a thyroid disorder diagnosed in five patients was linked to specific mutations in the IYD gene [6,7]. When tested, the concentration of serum DIT was very high compared to controls as expected for weak or non-existent deiodinase activity. The most severe consequences resulted from mutation of an Arg101 to Trp101 (Arg97 in the murine sequence corresponds to Arg101 in the human sequence) or, alternatively, deletion of Phe105 and mutation of an adjacent Ile106 to Leu106 (corresponding to residues 101 and 102 in the murine sequence) [6] (Fig 7). Both mutations caused inactivation of IYD without recovery in the presence of excess FMN (2 mM). The crystal structure now demonstrates direct contact between the Arg and the terminal phosphate of FMN [35]. Similarly, the mutant containing both a deletion and substitution is expected to prevent another key Arg (Arg 100 in the murine sequence) from coordinating the ribityl chain and pyrimidine ring of FMN. Any one of these effects likely diminishes the affinity of IYD for FMN. The two other mutants discovered to date (corresponding to murine I112T and A216T) also suppress deiodinase activity and likely act by decreasing the overall stability of the native structure [6,7].

Recent insight into the mechanism of dehalogenation promoted by IYD

Site-directed mutagenesis and crystallography confirmed that Cys residues are not involved in IYD catalysis as originally proposed from the precedence of aerobic dehalogenases [35,53]. The potential for other nucleophiles to act in place of Cys was also rejected when no appropriate amino acids were apparent in the active site. Thus, mechanisms involving polar (two electron) reactions are not supported by the existing data. Evidence for a series of one electron transfers remains indirect but still is persuasive. For example, IYD stabilizes the neutral flavin semiquinone radical after stoichiometric oxidation of IYD•FMNH₂ by substrate [64]. However, the significance of this stabilization for catalysis is not yet clear since the radical is detected for only a fraction of the enzyme (<30%), and its lifetime (days) is not consistent with enzyme turnover.

In contrast to the proposal that IYD promotes a series of one electron transfers, reactions promoted by the NOX/FRP superfamily appear to involve two electron processes. In particular, description of the nitroreductases in this superfamily as O₂ insensitive implies an inability to promote single electron transfer and reduce O₂ [65]. One exception to this general trend is the catalysis of BluB, one of the newest members of the superfamily. This enzyme promotes an O₂-dependent degradation of its bound FMN to produce one of the ligands for vitamin B₁₂ [66]. Reaction with O₂ certainly implies an initial one electron transfer step, and certain structural similarities between BluB and IYD suggest that IYD also promotes one electron processes. IYD most closely resembles BluB within the superfamily despite a sequence identity of only 19% [35]. This similarity in structure is in part based on the location of the amino acid sequences used to create their active site lids. Equivalent sequences in the two previously defined sub-categories of this superfamily are located in different regions of their genes [35]. Future investigations will hopefully supply more definitive information on the possibility of catalyzing dehalogenation by single electron transfer from reduced flavin to bound substrate. A likely mechanism for this may still involve substrate tautomerization (eq. 3), but at least one alternative should also be considered that is independent of tautomerization (eq. 4). Processes related to each mechanism have precedence in chemistry, and their relevance in biochemistry awaits further experiments.

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The ultimate mechanism describing IYD catalysis will also have to accommodate the ability of IYD to dehalogenate 3-bromo- and 3-chlorotyrosine as well. Catalytic debromination by IYD was first suggested over 50 years ago using radiolabeled bromotyrosine [24]. This reaction, in addition to dechlorination, has also now been detected by the ability of the halotyrosines to oxidize IYD-FMN_H and generate stoichiometric quantities of tyrosine [64]. The physiological significance of these reactions is not yet clear, but IYD may be involved in the metabolism of endogenous 3-chloro- and 3-bromotyrosine since reports have suggested the presence of IYD in liver and kidney cells [30,67]. The presence of these halotyrosine derivatives in serum likely results from oxidation of proteins by hypochlorite and hypobromite generated from myeloperoxidase after neutrophil activation [68-70]. Both halotyrosines have been used as indicators of lung disease and asthma [69,71], and their proposed degradation *in vivo* includes dehalogenation by a processes not yet identified [72].

Conclusions

Until recently, investigations of IYD have been limited by the cumbersome and discontinuous assay of [¹²⁵I] -iodide release from substrate and the lack of efficient preparation of homogeneous enzyme [22]. Success with heterologous expression of a truncated and soluble form of IYD and the ability to monitor the oxidation and reduction of flavin bound to IYD have recently combined to accelerate investigations on its structure and catalysis. This enzyme promotes a highly unusual activity, and its mechanism is expected to present a new precedent in flavin chemistry and aerobic dehalogenation. Additionally, human diseases caused by IYD mutation can now be analyzed from a structural perspective based on crystallographic data of IYD and its substrate complexes. The physiological importance of IYD in the thyroid has been known for many generations, and its significance in other organs may soon be discovered as well.

Acknowledgments

This review is dedicated to all of our group members and collaborators who devoted themselves to investigations of iodotyrosine deiodinase. This work was supported in part by the National Institutes of Health (DK 084186 to S.R.) and a Herman Kraybill Biochemistry Fellowship (P.M.).

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Figure 1.
Thyroid hormones and the side products diiodotyrosine (DIT) and monoiodotyrosine (MIT) formed during their biosynthesis.

Figure 2.
Iodide uptake, transport, metabolism, and salvage in thyroid follicular cells.

Figure 3.
The proposed involvement of selenocysteine (Sec) in the mechanism of iodothyronine deiodinase.

Figure 4.

N-Alkylpyridones mimic the non-aromatic tautomer of a proposed intermediate formed during catalytic dehalogenation by iodotyrosine deiodinase.

Figure 5.

The crystal structure of iodotyrosine deiodinase. (A) Identical subunits of the dimeric enzyme are differentiated by color to illustrate their general fold and assembly. Disordered regions connect the internal sequences marked by the five- and six-pointed stars. (B) The electrostatic surface of the enzyme is illustrated with an overlay of the structure induced within the co-crystal of IYD-MIT.

Figure 6. Numerous polar contacts are established between the substrate MIT, FMN and the active site residues of IYD.

Figure 7.
The locations of mutations in Human IYD are mapped onto the corresponding residues within the structure of murine IYD.