

A Mammalian Reductive Deiodinase has Broad Power to Dehalogenate Chlorinated and Brominated Substrates

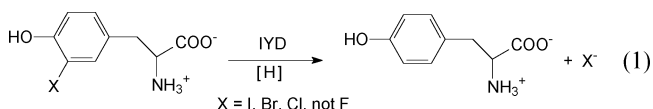
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Halogenated compounds and solvents have great economic value but pose long-term threats to the environment. Chlorinated compounds in particular resist microbial degradation and accumulate in soil and water. Only a limited number of organisms have demonstrated an ability to metabolize such compounds and hence have become central to developing biological methods of remediation.¹ Anaerobes contribute significantly to this process by catalyzing reductive dehalogenation.^{2,3} A number of catalytic strategies utilizing a range of cofactors are associated with these processes. For example, both cobalamin and Fe/S-centers are involved in the reductive dechlorination of tetrachloroethene and chlorophenol.⁴ Porphyrins, factor F430, ferredoxins, and even flavin may also participate in various other reductive dechlorinations although no direct role for flavin has yet to be established.^{3,5,6}

In contrast to anaerobes, aerobes rarely support reductive dehalogenation despite a few notable exceptions.⁷ Perhaps the best characterized exception is a bacterial dehalogenase that couples reductive dechlorination of tetrachlorohydroquinone to the oxidation of glutathione.⁸ Human health depends on another exception, iodothyronine deiodinase, that promotes reductive deiodination of the thyroid hormone thyroxine.⁹ Neither of these two exceptions require a cofactor. Instead, active site Cys and Sec (selenocysteine) residues respectively are directly responsible for substrate reduction. Yet another reductive dehalogenase, iodotyrosine deiodinase (IYD), is also critical for human health. Proper thyroid function depends on this enzyme's ability to salvage iodide from 3-iodo- and 3,5-diiodotyrosine that are generated as byproducts of thyroxine biosynthesis (eq 1). A deficiency in this enzyme can result in a lack of adequate iodide retention and ultimately hypothyroidism.¹⁰



IYD is the only dehalogenase known to contain flavin mononucleotide (FMN).^{11,12} Reaction is thought to depend on reducing equivalents from NADPH *in vivo* and can be reconstituted with dithionite *in vitro*.¹³ Thiols alone do not promote this reaction, and cysteine residues within the enzyme are not required for activity.^{14,15} Flavins are known to promote a wide range of one and two electron processes including activation of molecular oxygen, reduction of disulfides and sulfenic acid, transfer of hydride and electrons, and even halogenation of natural products,¹⁶ but no mechanistic precedent exists to date for involvement of flavin in reductive dehalogenation. The standard assay used for detecting iodide release from iodotyrosine is complex and contains free flavin, dithionite, methimazole, and 2-mercaptoethanol.¹⁴ As described for the first time below, the active site flavin is solely responsible for reduction and deiodination of 3-iodotyrosine (I-Tyr). This enzyme has also been discovered to act as a general dehalogenase by promoting reductive dehalogenation of 3-bromo- and 3-chlorotyrosine (Br-

Tyr and Cl-Tyr, respectively). Only 3-fluorotyrosine (3-F-Tyr) remains inert to the reduced form of IYD.

Recent success in heterologous expression of IYD provided sufficient quantities of the enzyme for detailed studies. The structure of an I-Tyr/IYD cocrystal determined at 2.45 Å resolution revealed an intimate association between the substrate and oxidized form of the active site-bound flavin.¹⁷ Despite this new wealth of structural information, the chemical requirements and scope of dehalogenation remained unknown. Accordingly, the reduced form of IYD was generated in the absence of the typical array of additives used in the standard activity assay by titration with a stoichiometric quantity of dithionite under anaerobic conditions. Reduction was monitored by the characteristic loss of absorbance by flavin in the visible spectrum (Figures 1 and S1). Subsequent addition of I-Tyr regenerated the spectrum of the oxidized flavin suggesting a direct discharge of electrons to the halogenated substrate (Figure S2). IYD was also competent to undergo additional cycles of reduction and oxidation as expected for a catalytic process. Chromatographic analysis of the titration mixture confirmed consumption of ca. 1 equiv of I-Tyr and a concomitant production of 1 equiv of tyrosine (Tyr) per redox cycle of IYD (Figure S3).

A broad absorbance band from 550–650 nm was additionally evident during substrate-dependent oxidation of IYD. This is attributed to a neutral flavin radical and represents ca. 33% of the flavin species based on an estimated ϵ_{585} of 4900 M⁻¹ cm⁻¹.¹² EPR analysis confirmed the presence of this radical by its *g*-factor of 2.0027 and line width of 20.3 G (Figure 2).¹⁸ The radical is surprisingly stable and persists at 4 °C under aerobic conditions for many days as monitored by A₅₈₅. However, quantitation of the EPR signal suggested that only 8% of the flavin species remained as a radical after 16 h.

Br-Tyr and Cl-Tyr are also capable of oxidizing reduced IYD under the same conditions described above for I-Tyr, and similarly, these substrates are dehalogenated to form tyrosine as well (Table 1 and Figures S3, S4, and S5). Thus, the reduced flavin bound to

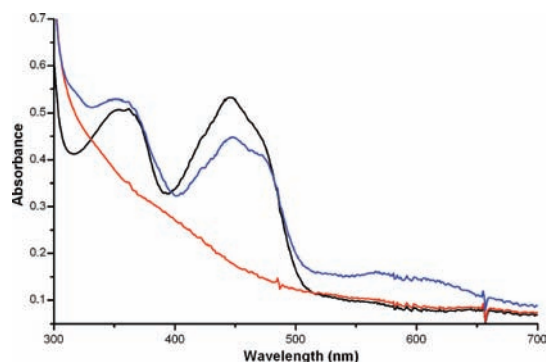


Figure 1. Absorbance spectra of reduced and oxidized IYD under anaerobic conditions. The oxidized flavin of IYD (black) was fully reduced by addition of 1 equiv of dithionite (red). Electrons were discharged from this reduced flavin after addition of excess I-Tyr (2 equiv) (blue).

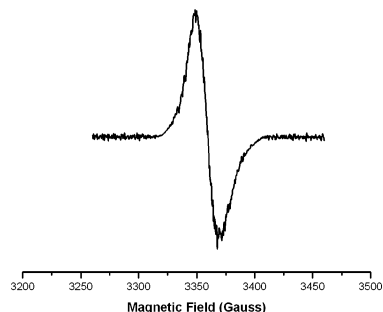


Figure 2. EPR spectrum of IYD following anaerobic reduction and subsequent discharge of electrons by substrate. X-band EPR measurements were made with an IYD sample (250 μM) stored 16 h at 4 $^{\circ}\text{C}$ under aerobic conditions following addition of I-Tyr.

the active site of IYD sustains a catalytic power sufficient to promote cleavage of the carbon–bromine and carbon–chlorine bond in addition to the much weaker carbon–iodine bond. Only F-Tyr was unable to discharge electrons from the reduced flavin of IYD or undergo defluorination. This lack of reaction is not surprising due to the resilience of the carbon–fluorine bond and cannot be attributed to its affinity for IYD. Substrate and ligand binding to IYD was measured independently from enzyme turnover by monitoring fluorescence quenching of the flavin (Table 1, Figure S6). The dissociation constants for I-Tyr, Br-Tyr, and Cl-Tyr are almost equivalent, and their sub- μM values demonstrate a very high affinity for IYD. Even F-Tyr binds quite tightly to IYD despite the diminutive size of fluorine vs the other halogen substituents. Still, the effect of all halogens is significant when comparing binding of the halotyrosines to that of Tyr or 3-methyltyrosine ($K_{\text{D}} > 150 \mu\text{M}$, Figure S6). This trend is likely influenced by the $\text{p}K_{\text{a}}$ of the phenolic proton (Table 1), but certainly sterics and perhaps noncovalent halogen bonding¹⁹ also contribute.

Table 1. Recognition and Processing of Halotyrosines by IYD

substrate	K_{D}^{a} (μM)	oxidation of reduced IYD	equivalents of tyrosine produced ^b	$\text{p}K_{\text{a}}$ (2-halophenol) ²⁴
I-Tyr	0.09 ± 0.04	yes	0.96 ± 0.02	8.53
Br-Tyr	0.11 ± 0.03	yes	0.92 ± 0.01	8.35
Cl-Tyr	0.15 ± 0.02	yes	0.94 ± 0.02	8.48
F-Tyr	1.3 ± 0.2	no	$\leq 0.02 \pm 0.03$	8.86
Tyr	>140	no	–	10.05

^a As determined by quenching of flavin fluorescence (see Figure S6). Uncertainties derive from the standard deviation of three or more independent measurements. ^b Generation of the Tyr product was quantified by reversed-phase C-18 HPLC (Figure S3).

Flavin may now be added to the pantheon of cofactors that are competent to promote reductive dehalogenation. Consequently, the flavoprotein IYD offers a fresh target to engineer for bioremediation. The significance of the debromination and dechlorination reactions catalyzed by IYD, however, is most immediate for human health and metabolism. Only one report had previously suggested the debromination reaction, and this relied on the release of [⁸²Br]-bromide from [⁸²Br]-Tyr in the presence of thyroid microsomes.²⁰ More recently, Cl-Tyr was thought not to act as a substrate for IYD,²¹ although this observation may have resulted from an instability of the endogenous reductase required for NADPH dependent deiodination. Both Cl-Tyr and Br-Tyr are indirectly generated by peroxidases in our body and have been used as markers for lung disease and asthma.^{21,22} Although IYD in the thyroid is unlikely to contribute to their metabolism, its reported presence in the liver and the kidney would provide a likely site for dehalogenation.^{21,23} The ability of IYD to promote aromatic

debromination and dechlorination expands its potential role in mammalian biology and provides a foundation for future mechanistic studies.

Acknowledgment. We thank Prof. Veronika Szalai for performing the EPR spectroscopy and Min Jia for help with the initial anaerobic titrations. This work was supported in part by the National Institutes of Health (DK 084186 to S.R.) and the Herman Kraybill Biochemistry Fellowship (P.M.).

Supporting Information Available: General materials and methods, complete reduction and oxidation titrations of IYD, HPLC analysis of halotyrosine dehalogenation, binding titrations of halotyrosines with IYD. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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JA906642N