Transition-State Stabilization by a Mammalian Reductive Dehalogenase

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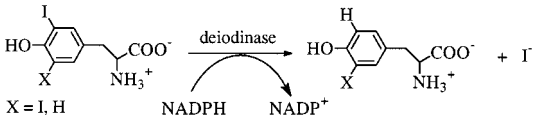
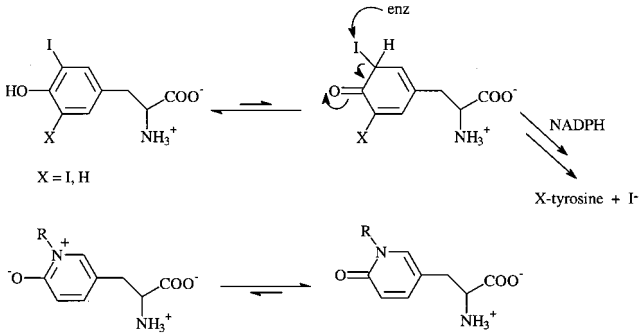
Enzyme-catalyzed dehalogenation is typically associated with xenobiotic metabolism and in particular with microorganisms that detoxify environmental pollutants.[1,2] Both aliphatic and aromatic substrates are susceptible to dehalogenation through mechanisms that include oxidation, reduction, and hydrolysis. Oxidative processes are usually associated with aerobic organisms, whereas reductive processes are usually associated with anaerobic organisms. Remarkably, mammals have the additional ability to promote reductive deiodination of the hormone thyroxine (3-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenylalanine), its metabolites, and related intermediates including iodotyrosine. A series of selenoenzymes found in tissues such as brown fat, liver, kidney, and the central nervous system are responsible for the reduction and deiodination of thyroxine and the concomitant oxidation of glutathione.[3] In contrast, an iodide salvage enzyme in the thyroid mediates reduction and deiodination of iodo- and diiodotyrosine with consumption of NADPH (Scheme 1).[4] Little mechanistic data has yet to be gathered on these mammalian reactions, and we now report compelling evidence for a key intermediate proposed in catalysis of iodotyrosine deiodinase.

Both direct aromatic substitution and halophilic reaction of a nonaromatic tautomer (see below) can be considered for this reductive process. However, aromatic substitution via nucleophilic addition and formation of an anionic Meisenheimer intermediate (S_NAR) is disfavored for electron-rich targets. The weak C−I bond might instead suggest a related homolytic process ($S_{RN}1$), and yet model conditions necessary for such a dehalogenation remain far from physiological.[5] Preliminary studies have also suggested that diiodotyrosine is stable to one-electron reductants including sulfite, metabisulfite, ferrocyanide, and dithionite.[4a] Electron-rich aryl halides are most commonly dehalogenated via Lewis acid-catalyzed two-electron processes.[6] Tautomerization (or possibly protonation) to form the nonaromatic intermediate illustrated in Scheme 2 facilitates halophilic attack and cleavage of the C−I bond. This type of process has been proposed for triphenylphosphine-dependent debromination of *o*-bromophenol and bromoaniline,[7] $AlCl_3$- and $GaCl_3$-catalyzed deiodination of diiodocresol,[8] hydriodic acid-dependent deiodination of aryl iodides,[9] and most recently, biomimetic coupling of two diiodotyrosines.[10] Similar mechanisms have been proposed for the biological deiodination of thyroxine by a selenocysteinyl residue[11] and dechlorination of tetrachlorohydroquinone by a cysteinyl residue or glutathione.[12] The active site of iodotyrosine deiodinase has not yet been characterized but may also include a cysteinyl residue.[13] The ability of this enzyme to stabilize the proposed[16] nonaromatic tautomer is described below by the binding affinity of pyridonyl-containing mimics of this intermediate.

Scheme 1



Scheme 2



The initial targets, D,L-3-(2-pyridon-5-yl)alanine **1** and D,L-3-(*N*-methyl-2-pyridon-5-yl)alanine **2**, were constructed using a standard condensation with diketopiperazine (Scheme 3).[17] The necessary aldehyde was prepared by sequential bromination, lithiation, and finally formylation of 2-methoxypyridine (see Supporting information for experimental details).[18] The intermediate diketopiperazine was reduced and hydrolyzed by HI and

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(13) Thiols exhibit a unique affinity for aryl and aliphatic iodo substituents.[14,15] For example, chloro- and bromomethyl ketones react with thiols to generate a mixture of substitution and reduction products, whereas iodomethyl ketones react under equivalent conditions to generate the reduced methyl ketone exclusively.[15]

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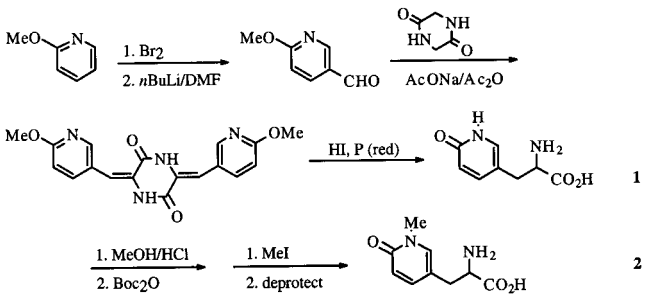
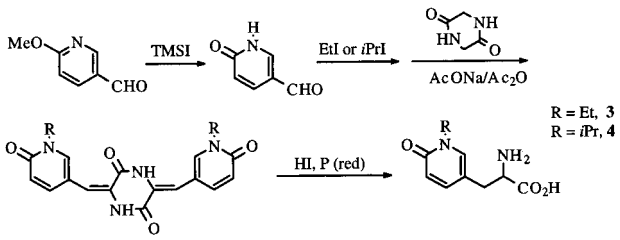
Scheme 3



Scheme 4

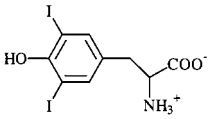


phosphorus (red) in acetic anhydride. The resulting product was neutralized with ammonia and crystallized to provide the desired pyridonyl amino acid in an overall yield of 17%. Selective methylation of this material with CH_3I required initial formation of its N^{α}-BOC methyl ester derivative and then subsequent deprotection to generate the product in a 57% yield from **1**. Our initial success with this compound as described below encouraged us further to prepare the *N*-ethyl and *N*-isopropyl analogues (**3** and **4**, respectively) as well. The final protection and deprotection steps were avoided when preparing these compounds (29% and 13% overall yields, respectively) by alkylating the intermediate 5-formyl-2-pyridone rather than the final pyridonyl amino acid (Scheme 4).

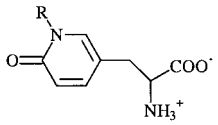
Iodotyrosine deiodinase was solubilized from thyroid microsomes (porcine) using 1.5% Triton X-100 and assayed according to the literature[19] by $^{125}I^-$ release from [^{125}I]-diiodotyrosine. This preparation of enzyme could be stored at 4 °C for a month without significant loss of activity and exhibited a K_m for diiodotyrosine (9.3 μM, porcine) that was a little greater than that (2.5 μM, bovine) measured after solubilizing the enzyme with a lipase/protease combination.[4b]

The pyridonyl amino acids (**1**−**4**) were all observed to be reversible and competitive inhibitors of diiodotyrosine turnover under standard assay conditions. Time-dependent inactivation of the enzyme was neither anticipated nor detected during incubation with **2** (200 nM) for 120 min at 25 °C. Analogue **1** was an efficient but unremarkable inhibitor with a K_I that was approximately 9-fold lower than the K_m for diiodotyrosine (Table 1). However, methylation of the pyridonyl ring generated an analogue **2** with a highly significant 46-fold increase in enzyme affinity relative to **1**. The K_I of this *N*-methyl derivative is thus more than 380-fold lower than the K_m of diiodotyrosine and 2100-fold lower than the K_m reported for iodotyrosine (50 μM, bovine).[4b] Such alkylation was expected to enhance binding since an iodo group would normally occupy this site. Ethyl and isopropyl analogues of **2** were subsequently examined since these larger alkyl substituents were previously shown to act as even better mimics of the crucial 3′-iodo group in 3,3′,5-triiodothyronine.[20] For the deiodinase, the large alkyl groups were less effective inhibitors and bound 3.6- and 17-fold weaker, respectively, than the methyl derivative **2** (Table 1). Still, the N-substituted amino acids exhibited substantial affinity as expected for intermediate or transition-state analogues. In comparison, substrate analogues such as 3-methyl- and 3-isopropyltyrosine are very weak inhibitors of the enzyme with K_I values greater than 880 μM.[21]

Table 1. Summary of Kinetic and Binding Constants for Iodotyrosine Deiodinase[a]



K_m 9,300 (±1,000) nM



inhibitor	R	K_I (nM)
1	H	1,100 (±100)
2	Me	24 (±8)
3	Et	87 (±13)
4	*i*Pr	400 (±33)

[a] All inhibitors bound competitively with respect to diiodotyrosine, and hence, K_I values were calculated accordingly from their concentration-dependent effect on the apparent K_m of diiodotyrosine.

The pyridonyl analogues described here are particularly useful for mimicking the nonaromatic tautomer of iodotyrosine, a critical intermediate proposed for the enzyme-catalyzed deiodination of this amino acid (Scheme 2).[16] The π-electrons of this heterocyclic system are substantially localized and overwhelmingly form the pyridone rather than hydroxypyridine tautomer.[22] The pyridonyl-containing inhibitors also present structures that contrast to the delocalized radical and anionic intermediates envisioned for alternative[23] homolytic ($S_{RN}1$) and heterolytic (S_NAR) mechanisms. Additional analogues are currently under development for further active-site characterization of iodotyrosine deiodinase and related enzymes. The use of pyridonyl amino acids should also be generally applicable to a variety of enzymes that catalyze other aromatic substitution reactions including tyrosine-phenol lyase.[24]

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Supporting Information Available: Synthesis and characterization of the transition-state (or intermediate) analogues, 1−4, preparation and assay of iodotyrosine deiodinase (porcine) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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