Bioinorganic Chemistry in Thyroid Gland: Effect of Antithyroid Drugs on Peroxidase-Catalyzed Oxidation and Iodination Reactions

Gouriprasanna Roy and G. Mugesh

Department of Inorganic and Physical Chemistry, Indian Institute of Science, Bangalore 560 012, India

Received 19 June 2006; Accepted 29 August 2006

Propylthiouracil (PTU) and methimazole (MMI) are the most commonly used antithyroid drugs. The available data suggest that these drugs may block the thyroid hormone synthesis by inhibiting the thyroid peroxidase (TPO) or diverting oxidized iodides away from thyroglobulin. It is also known that PTU inhibits the selenocysteine-containing enzyme ID-1 by reacting with the selenenyl iodide intermediate (E-SeI). In view of the current interest in antithyroid drugs, we have recently carried out biomimetic studies to understand the mechanism by which the antithyroid drugs inhibit the thyroid hormone synthesis and found that the replacement of sulfur with selenium in MMI leads to an interesting compound that may reversibly block the thyroid hormone synthesis. Our recent results on the inhibition of lactoperoxidase (LPO)-catalyzed oxidation and iodination reactions by antithyroid drugs are described.

INTRODUCTION

Thyroxine or 3,3′,5,5′-tetraiodothyronine (T4) is the major hormone secreted by the follicular cells of the thyroid gland. This hormone is produced on thyroglobulin by thyroid peroxidase (TPO)/hydrogen peroxide/iodide system. The synthesis of T4 by TPO involves two independent steps: iodination of tyrosine and phenolic coupling of the resulting iodotyrosine residues [1–5]. The prohormone T4 is then converted to its biologically active form T3 by an outer ring deiodination pathway. This particular reaction is catalyzed by a selenocysteine-containing enzyme called iodothyronine deiodinase (ID-I), which is present in highest amounts in liver, kidney, thyroid, and pituitary [6–16]. The thyroid gland also produces an inactive metabolite rT3 by an inner ring deiodination pathway. The triiodo derivatives T3 and rT3 are further metabolized by inner ring and outer ring deiodination, respectively, by ID-I, ID-II, and ID-III to produce the inactive metabolite T2 (3,3′-T2, 3,5′-T2, and 3′,5′-T2). The outer ring 5′-deiodination catalyzed by the ID-I enzyme is considered to be the first step in thyroid hormone action because this is the only deiodination pathway that leads to the formation of an active thyroid hormone. It is now widely accepted that the deiodination catalyzed by ID-I is a ping-pong, bisubstrate reaction in which the selenol (or selenolate) group of the enzyme (E-SeH or E-Se−) first reacts with thyroxine (T4) to form a selenenyl iodide (E-SeI) intermediate. Subsequent reaction of the selenenyl iodide intermediate with an as yet unidentified intracellular cofactor completes the catalytic cycle and regenerates the enzyme active site (Figure 1) [8, 14]. Although it is customary to use dithiothreitol (DTT, 1,4-dithiothreitol, Cleland’s reagent) as the second substrate in vitro experiments, the identity of the physiological second substrate is still uncertain. The tripeptide glutathione (GSH) can also act as a thiol cosubstrate, but GSH is a much less potent cofactor than DTT for ID-I [10, 17]. In addition to GSH, other native thiols such as dihydrolipoic acid or dihydrolipoamide may serve as cofactors for ID-I [17, 18]. Therefore, Figure 1 may be an incomplete or incorrect representation of the catalytic mechanism of ID-I since evidence for the cofactor systems mentioned above has only been presented for in vitro studies and not for in vivo analysis.

Although the deiodination reactions are essential for the function of thyroid gland, the activation of thyroid stimulating hormone (TSH) receptor by autoantibodies leads to an overproduction of thyroid hormones. As these antibodies are not under pituitary feedback control system, there is no negative influence on the thyroid activity and, therefore, the uncontrolled production of thyroid hormones leads...
Bioinorganic Chemistry and Applications

Figure 1: Proposed mechanism for the deiodination of thyroxine by ID-I and inhibition of ID-I by n-propyl-2-thiouracil (PTU) and gold thioglucose (GTG).

Figure 2: Chemical structures of some commonly employed antithyroid drugs and their selenium analogues.

In recent years, the selenium analogues 2 (MSeI), 4 (PSeU), and 6 (MSeU) attracted considerable attention because these compounds are expected to be more nucleophilic than their sulfur analogues and the formation of an \( \text{Se}^-\text{Se}^- \) bond may occur more readily than the formation of an \( \text{S}^-\text{S}^- \) bond with the ID-I enzyme [25–29]. However, the data derived from the inhibition of TPO by selenium compounds show that these compounds may inhibit the TPO activity by a different mechanism. We have recently shown that the unexpected behavior of the selenium compound MSeI as compared to that of its sulfur analogue may be due to the existence of this compound in the zwitterionic form and its facile oxidation to the corresponding diselenide [30, 31]. In this paper, we summarize our recent results on the effect of antithyroid drugs on peroxide-catalyzed oxidation and iodination reactions. In addition, we show that the replacement of sulfur with selenium in MMI leads to an interesting compound (MSeI) that exhibits significant glutathione peroxidase (GPx)-like antioxidant activity.

INHIBITION OF LACTOPEROXIDASE-CATALYZED OXIDATION BY ANTITHYROID DRUGS

The effect of antithyroid drugs on peroxide-catalyzed oxidation was studied in vitro by using spectroscopic
techniques. The enzyme inhibition experiments were carried out with iron-containing lactoperoxidase (LPO) since it is readily available in purified form. Furthermore, LPO has been shown to behave very similarly to TPO with respect to oxidation of organic substrates and iodination of thyroglobulin and other iodide acceptors [32]. We have employed 2,2’-azio-bis-3-ethyl-benthiazoline-6-sulfonic acid (ABTS) and H$_2$O$_2$ as substrates [33] to determine the half-maximal inhibitory concentration (IC$_{50}$) of test compounds. The IC$_{50}$ values for the inhibition of LPO-catalyzed oxidation of ABTS by 1–3 and 5 are summarized in Table 1 [31]. The sulfur compound MMI inhibited the LPO activity with an IC$_{50}$ value of 7.0 ± 1.1 μM, which is much lower than those observed with PTU and MTU. The selenium analogue (2) also inhibited LPO activity and the IC$_{50}$ value was found to be almost 2-3 times lower than those of PTU and MTU. The higher activity of MMI as compared with those of PTU and MTU is in agreement with the previous studies on the inhibition of TPO. Since the activation of the iron center in TPO must proceed through an interaction of Fe(III) with ABTS versus concentration of H$_2$O$_2$: (a) control activity; (b) 40 μM of 2; (c) 40 μM of 8; (d) 80 μM of PTU; (e) 80 μM of MTU; (f) 40 μM of MMI. Conditions: LPO: 6.5 nM; H$_2$O$_2$: 22.9 μM/a. 

### Table 1: Inhibition of LPO activity by 1–3, and 5 [31].

<table>
<thead>
<tr>
<th>No</th>
<th>Compound</th>
<th>IC$_{50}$ (μM)</th>
<th>a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MMI (1)</td>
<td>7.0 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>MSeI (2)</td>
<td>16.4 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>PTU (3)</td>
<td>45.0 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>MTU (5)</td>
<td>47.8 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

* Concentration of the compound causing 50% inhibition. Each IC$_{50}$ value was calculated from at least three independent experiments.

Remarkably, MSeI (2) inhibited the enzyme within few seconds even at lower concentrations, which can be ascribed to the facile oxidation of the reactive selenolate group in 2 (MSeI) by H$_2$O$_2$ or by the oxidized enzyme. Because MMI also inhibits the enzyme very efficiently, we have carried out further experiments to prove that the mechanisms by which MMI and MSeI exert their inhibitory action are different. The initial rates ($v_0$) derived from various concentrations of H$_2$O$_2$ were plotted against the concentration of H$_2$O$_2$. The LPO activity was completely inhibited by 40 μM MMI, and the enzyme’s activity could not be recovered by increasing the H$_2$O$_2$ concentration (Figure 3f) [31]. The LPO activity could not be recovered even at lower concentration of MMI (10 μM) and higher concentration of H$_2$O$_2$ (230 μM). This

---

**Scheme 1:** The possible tautomeric structures of compound 2. The compound exists predominantly in its zwitterionic form 2c, which may have a partial C-Se double bond character.
suggests that MMI does not act on H$_2$O$_2$ but acts on the enzyme itself, leading to an irreversible inhibition as previously proposed. On the other hand, 2 also inhibited the LPO activity as efficiently as MMI, but in this case, the enzyme's activity could be completely recovered by increasing H$_2$O$_2$ concentration (Figure 3b). These observations may support the assumption that MSeI, in contrast to MMI, does not interfere with the native enzyme directly but it inhibits the LPO activity by reducing the H$_2$O$_2$, which is required for the oxidation of the iron center in LPO. The reduction of H$_2$O$_2$ by 2 may become more efficient in the presence of suitable thiols such as GSH because this process may constitute a redox cycle involving a catalytic reduction of H$_2$O$_2$ (glutathione peroxidase (GPx) activity) [46]. Thus, compound 2 mimics the action of GPx, a selenoenzyme that protects the cellular components from oxidative damage by reducing H$_2$O$_2$ with the help of GSH [16]. Recently, the GPx enzyme present in thyroid gland has been shown to inhibit the iodination reactions by degrading the intracellular H$_2$O$_2$ [47, 48]. The high GPx activity of the key compound 2 leads to an assumption that the antithyroid drugs may act as antioxidants in addition to their inhibition behavior.

**INHIBITION OF LACTOPEROXIDASE-CATALYZED IODINATION BY ANTITHYROID DRUGS**

The interesting results that we obtained from the inhibition of LPO-catalyzed oxidation reactions by MSeI (2) prompted us to study the effect of this compound and related derivatives on the LPO-catalyzed iodination reactions [49]. In addition, we have studied the reactivity of MSeI toward iodine because the effect of the selenium compounds on the iodination of tyrosine and the identification of the products formed in the reactions of these compounds with iodine are crucial in understanding the mechanism of action in vivo of these drugs. The iodination of tyrosine was studied by using LPO/H$_2$O$_2$/I$^{-}$/assay and the initial rates for the conversion of L-tyrosine to 3-iodo L-tyrosine (Scheme 2) were determined by an HPLC method.

As the formation of 3,5-diiodo-L-tyrosine was also observed in the reaction, only the initial 5%–10% of the conversion was followed where only a trace amount of the diiodo compound was produced. The decrease in the concentration of L-tyrosine was followed by measuring the peak area at 277 nm and the amount of tyrosine present in the solution at a given time was calculated from the calibration plot.

![Scheme 2: Iodination of L-tyrosine by LPO/peroxide/iodide system.](image)

**Figure 4: Inhibition of the LPO-catalyzed iodination of L-tyrosine by MSeI.** The decrease in the amount of tyrosine with time was followed by HPLC: (a) control; (b) 6 μM of 2; (c) 9 μM of 2; (d) 12 μM of 2; (d) 15 μM of 2; and (e) 20 μM of 2 (see [49]).
Similarly to the LPO-catalyzed oxidation of 2,2'-azino-bis-3-ethylbenz-thiazoline sulfonic acid (ABTS), this suggests that the selenium analogue may inhibit the LPO by electron donor-acceptor complexes with diiodine, which can effectively reduce the thyroid hormone biosynthesis [19, 20]. Because the oxidation of MMI to the corresponding disulfide (7) by TPO/H2O2/I− system is associated with the reaction of MMI with I2 [55–59], we have investigated the interaction of 2 and 8 with iodine. It has been reported that I2 chemically oxidizes MMI to produce ionic disulfides that exist in two different protonated forms [55]. It is unknown whether the selenium analogue of MMI, in its reduced form, also undergoes such oxidation by I2 to produce ionic species (Figure 6). Therefore, we carried out the experiments with the reduced species (2), which exists in its zwitterionic form [31, 35]. The reaction of 2 with I2 in CH2Cl2 produced red-brown crystals. Interestingly, the X-ray crystal structure shows the formation of compound 9, which consists of a monocation containing a diselenide and I3− as counterions (Figure 7) [49]. This is in contrast to the reaction of MMI with I2 in CH2Cl2, which afforded a disulfide-containing dication and I8− as counterions [55].

The formation of the monocationic species 9 is interesting from a chemical point of view as only one of the imidazole rings undergoes oxidation. It should be mentioned that the N-methylation on MMI has been shown to abolish its TPO inhibitory activity [60]. Freeman et al have shown that the reaction of the N-methylated derivative (1,3-dimethylimidazole-2-thione) with I2 does not produce any disulfide, but it produces a 1 : 1 thione : I2 charge-transfer adduct [61]. The N-methylated derivative of 2 (1,3-dimethylimidazole-2-selone), on the other hand, produces a hypervalent “T-shaped” compound having I−Se−I moiety [62, 63]. It should be noted that the reaction of the methylated analogue of 2, 1,3-dimethylimidazole-2-selone, with one equivalent bromine affords a hypervalent compound having Br−Se−Br moiety, whereas the corresponding reaction utilizing a half-equivalent bromine leads to the formation of a diselenide dication having two Br− as counterions [64]. Therefore, the existence of 2 in its zwitterionic (or selanolate) form is probably responsible for its different reactivity toward iodine. Stable open-chain cationic diselenide species are very uncommon in the literature and to the best of our knowledge no structural information is available for complexes derived from the reactions of selenium analogues of antithyroid drugs with iodine. The chemical oxidation of 2 by I2 suggests that compound 8, which exists in the oxidized form of 2, may not produce any ionic species. To test this, the diselenide 8 was treated with I2 in a 1 : 2 molar ratio in CH2Cl2.

**Figure 5:** Inhibition of the LPO-catalyzed iodination of L-tyrosine by MSeI. Effect of H2O2 on the inhibition by 2; (a) 0 μM; (b) 20 μM; (c) 30 μM; (d) 40 μM; inset: inhibition of tyrosine iodination by (e) 1; (f) 2 at a fixed H2O2 concentration.

**Figure 6:** Chemical structures of compounds 9 and 10 derived from the compound 2.
This reaction yielded a brown solution from which dark-brown crystals were obtained on standing at room temperature. Surprisingly, the X-ray crystal structure shows the formation of a monocationic species, which is identical with that obtained from the reaction of 2 with I₂ (Figure 7). The formation of a cationic species in this reaction is quite unexpected because the reactions of iodine with diselenides generally produce selenenyl iodide species or charge-transfer complexes having diselenide-molecular iodine adducts [65]. It is also known that some of the selenenyl iodides may undergo disproportionation to give diselenide-iodine complexes.

The far-IR spectrum of complex 9 shows a distinct band at 135 cm⁻¹ for the ν(I—I) stretching vibration mode. This is in agreement with the fact that I₂ gives a strong band at 180 cm⁻¹ in the solid state, which shifts to lower wavenumbers upon coordination to a donor atom, reflecting a reduction in the I—I bond order [56]. The FT-Raman spectrum of the complex in the ν(I—I) region shows intense peaks at 164 cm⁻¹, 143 cm⁻¹, and 110 cm⁻¹. In addition, a weak band is observed around 67 cm⁻¹ (Figure 8). The band at 110 cm⁻¹ can be certainly assigned to the ν₁ symmetric stretching of I₃⁻, which being a symmetrical ion normally exhibits only one Raman active band. However, when a distortion of I₃⁻ occurs, the antisymmetric stretching may become Raman active and additional bands at higher (140 cm⁻¹–130 cm⁻¹) and at lower frequencies (80 cm⁻¹–70 cm⁻¹) may be observed [56, 66]. Therefore, the relatively weak bands at 143 cm⁻¹ and 67 cm⁻¹ can be attributed to the antisymmetric stretching and deformation motions, respectively, for the I₃⁻ ion (Figure 8a).

The single crystal X-ray studies confirm the proposed structure of 9 (Figure 7), which consists of two independent diselenide monocations [Se—Se: 2.382 Å; 2.364 Å]. These diselenide cations interact with their symmetry equivalents through N—H···N hydrogen bonds to form dimeric units with overall charge 2+. The charge balance in the crystals is achieved by the presence of two I₃⁻ anions. The two C—Se bond lengths in each subunit are unequal due to the monoprotonation of the one of the five-membered rings [C—Se: 1.886–1.890 Å]. The I—I bond lengths observed also differ significantly from the corresponding I—I bond length of I₂ in the solid state (2.715 Å). The two I—I bond lengths of the I₃⁻ species in complex 9 range from 2.888 Å to 2.919 Å, indicating a slight distortion of the I₃⁻ moiety. This distortion is probably responsible for additional bands in the FT-Raman spectrum of the complex.

In the reaction between 8 and I₂ in dichloromethane, the concentrations of I₂ do not appear to change the nature of products. During our attempts to oxidize the second
ring using various concentrations of I₂ up to an excess, only the monocation was obtained as a stable product. However, the choice of solvent has been found to have a large influence on the nature of products formed. The reaction of 8 with I₂ in a 1:2 molar ratio in water produced a mixture containing both monocation (9) and dication (10) as confirmed by single-crystal X-ray studies (Figure 7). In contrast to the monocation, the charge balance in the crystal of dication is achieved by two I⁻ anions. In compound 10, the average C–Se bond length of 1.895 Å is comparable with that of the diselenide 8 (1.880 Å) [30], but this is significantly longer than the average C–Se bond length (1.848 Å) found in compound 2 that exists in a zwitterionic form [35]. As expected, the FT-Raman spectrum of compound 10 shows no peaks in the region of lower wavenumbers (Figure 8c), indicating the absence of any polyiodide species in the crystals.

CONCLUSION AND OUTLOOK

Our recent results show that the selenium analogue of methimazole (MSeI) exists predominantly in its zwitterionic form, in which the selenium atom carries a negative charge and the five-membered heterocyclic ring carries a positive charge. In contrast to the sulfur analogue, the zwitterionic form of MSeI is unstable and oxidizes in air to the corresponding diselenide. The resulting diselenide can be easily reduced by reducing agents such as NaBH₄ or glutathione (GSH). In its reduced form (zwitterionic or selenolate), MSeI effectively and reversibly inhibits the lactoperoxidase (LPO)-catalyzed oxidation reactions. These results suggest that MSeI may not interfere with the native enzyme directly, but it may inhibit LPO either by reducing the H₂O₂ that is required for the oxidation of the iron center in LPO or by interfering with the oxidized enzyme. In the presence of GSH, MSeI may constitute a redox cycle involving a catalytic reduction of H₂O₂ and thereby mimics the glutathione peroxidase (GPx) activity in vitro. In addition, MSeI effectively inhibits the LPO-catalyzed iodination of L-tyrosine and the inhibition could be completely recovered by increasing the H₂O₂ concentration. These studies reveal that the degradation of the intracellular H₂O₂ by the selenium analogues of antithyroid drugs may be beneficial to the thyroid gland as these compounds may act as antioxidants and protect thyroid cells from oxidative damage. In addition to its antioxidant activity, MSeI reacts with I₂ to produce novel ionic diselenides containing iodide or polyiodide anions, which might be effective intermediates in the inhibition of thyroid hormones. However, further studies with TPO are required to derive some firm conclusions regarding the mode of action of the antithyroid drugs. Our future work will focus on the design and synthesis of novel sulfur and selenium compounds and study of their antithyroid and antioxidant activities.

ACKNOWLEDGMENT

This study was supported by the Department of Science and Technology (DST), New Delhi, India.

REFERENCES


