Interaction of anti-thyroid drugs with iodine: the isolation of two unusual ionic compounds derived from Se-methimazole[†]

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The inhibition of lactoperoxidase (LPO)-catalyzed iodination of L-tyrosine by the anti-thyroid drug methimazole (MMI) and its selenium analogue (MSeI) is described. MSeI inhibits LPO with an IC_{50} value of 12.4 μ M, and this inhibition could be completely reversed by increasing the peroxide concentration. In addition to the inhibition, MSeI reacts with molecular iodine to produce novel ionic diselenides, and the nature of the species formed in this reaction appear to be solvent-dependent. The formation of ionic species in the reaction is confirmed by single-crystal X-ray studies, FT-IR and FT-Raman spectroscopic investigations. This study provides the first experimental evidence that MSeI not only effectively inhibits the LPO-catalyzed iodination of tyrosine, but also reacts with I₂ to produce novel ionic diselenides. These results also suggest that MSeI reacts with iodine, even in its oxidized form, to form ionic diselenides containing iodide or polyiodide anions, which might be effective intermediates in the inhibition of thyroid hormones.

Introduction

The molecular interactions of anti-thyroid drugs (1 and 3, Scheme 1) with iodine have been subjected to many investigations because these drugs inhibit thyroid hormone synthesis by forming donor-acceptor complexes with iodine¹ or by interacting with an active iodine species of thyroid peroxidase (TPO), a heme enzyme, which catalyzes the iodination of tyrosine residues of thyroglobulin.² In addition, 6-n-propyl-2-thiouracil (PTU, 1) can also inhibit the deiodination reactions catalyzed by type I iodothyronine deiodinase (ID-I).3 Recently, the selenium analogues of anti-thyroid drugs 2 (PSeU) and 4 (MSeI) attracted considerable attention⁴ because these compounds may inhibit thyroid hormone synthesis by a mechanism different from that of the sulfur analogues.⁵ Recent experimental and theoretical studies suggest that the selenium compound 4 does not exist as a true selone or selenol, but it exists in a zwitterionic form.^{5b} Interestingly, this compound, in contrast to the sulfur analogue, is found to be unstable under aerobic conditions and is readily oxidized to the diselenide 5.⁵ Therefore, 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 of these drugs in vivo. In this paper, we describe the inhibition of lactoperoxidase (LPO)catalyzed iodination of L-tyrosine by 3 and 4, and the isolation of two novel cationic species from the reaction of 4 and 5 with molecular iodine.



Scheme 1 Chemical structures of some anti-thyroid drugs and their iodine complexes.

Results and discussion

The enzyme inhibition experiments were carried out with Fecontaining lactoperoxidase (LPO), since it is readily available in purified form. Furthermore, LPO has been shown to behave very similarly to TPO with respect to iodination of thyroglobulin, the natural substrate, and other iodide acceptors. Therefore, the iodination of tyrosine was studied by using an LPO/H2O2/Iassay, and the initial rates for the conversion of L-tyrosine to 3iodo-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, for which 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 obtained by injecting known concentrations of L-tyrosine. The effect of compound 4 on the iodination reaction

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[†] Electronic supplementary information (ESI) available: Details of theoretical calculations, UV-Vis spectra of **5** with iodine, and far-IR spectra for compounds **5–7**. See DOI: 10.1039/b604060h



Scheme 2 Iodination of L-tyrosine by the LPO/peroxide/iodide system.

was determined at various concentrations of **4** under identical experimental conditions (Fig. 1).



Fig. 1 Decrease in the amount of tyrosine with time: (a) control; (b) $6 \,\mu$ M of **4**; (c) $9 \,\mu$ M of **4**; (d) $12 \,\mu$ M of **4**; (d) $15 \,\mu$ M of **4** and (e) $20 \,\mu$ M of **4**.

The IC₅₀ values for the inhibition of LPO-catalyzed iodination of L-tyrosine by the test compounds were also determined by following the same procedure. The initial rates for the iodination reaction were determined at various concentrations of inhibitors. The inhibition curves obtained by plotting the percentage control activity against the concentration of inhibitors are shown in Fig. 2. As expected, MMI exhibited a strong inhibition, with an IC₅₀ value of 5.2 μ M, which is comparable with the IC₅₀ value obtained for the LPO-catalyzed oxidation reaction.⁵ The selenium analogue (4) also showed a strong inhibition, with an IC₅₀ value of 12.4 μ M, which is consistent with the effect of this compound on peroxidasecatalyzed oxidation reactions.⁵ Similarly to the LPO-catalyzed oxidation of 2,2'-azino-bis(3-ethylbenzathiazoline sulfonic acid)



Fig. 2 Inhibition of LPO-catalyzed iodination of tyrosine by MMI (3) and MSeI (4).

(ABTS), this suggests that the selenium analogue may inhibit the LPO by a different mechanism. The diselenide **5**, on the other hand, did not show any significant inhibition under identical conditions. Although weak inhibition of LPO by **5** was observed during the initial period of the reaction, a reliable IC_{50} value could not be obtained.

To understand the effect of the peroxide substrate on the reaction rate and the inhibition, the LPO activity was determined at various concentrations of hydrogen peroxide. In addition, the effect of peroxide on the inhibition of LPO-catalyzed iodination by anti-thyroid drugs **3** and **4** was evaluated by carrying out the experiments at various concentrations of H_2O_2 . The initial rates (v_0) derived from various concentrations of H_2O_2 were plotted against the concentration of H_2O_2 . Although the LPO activity was inhibited by **4** at lower concentrations of H_2O_2 , the enzyme's activity could be completely recovered by increasing the H_2O_2 concentration (Fig. 3). These results suggest that the concentration of H_2O_2 has a dramatic effect on the inhibition of iodination reaction by compound **4** (Fig. 1).



Fig. 3 Effect of H_2O_2 on the inhibition of LPO by 4: (a) 0 μ M; (b) 20 μ M; (c) 30 μ M; (d) 40 μ M.

Because the oxidation of MMI to the corresponding disulfide by TPO/H₂O₂/I⁻ system is associated with the reaction of MMI with I₂,⁶ we have investigated the interaction of 4 and 5 with iodine. It has been reported that I₂ chemically oxidizes MMI to produce ionic disulfides that exist in two different protonated forms.^{6a} It is not known whether the selenium analogue of MMI, in its reduced form, also undergoes such oxidation by I₂ to produce ionic species. Therefore, we carried out the experiments with the reduced species (4), which exists in its zwitterionic form.^{5b} The reaction of 4 with I₂ in CH₂Cl₂ produced red-brown crystals. Interestingly, the X-ray crystal structure shows the formation of compound 6, which consists of a monocation containing a diselenide and I₃⁻ as counterion (Fig. 4). This is in contrast to the reaction of MMI with I₂ in CH₂Cl₂, which afforded a disulfide-containing dication and I₈⁻ as counterions.

The formation of **6** 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.⁷ 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 that it produces a 1 : 1 thione–I₂ charge-transfer



Fig. 4 ORTEP diagram of compound **6** (with 50% probability ellipsoids), showing two monocations stabilized by triiodide anions.

adduct.⁸ The *N*-methylated derivative of **4** (1,3-dimethylimidazole-2-selone), on the other hand, produces a hypervalent "T-shaped" compound containing an I–Se–I moiety.⁹ Therefore, the existence of **4** in its selenolate (zwitterionic) 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 anti-thyroid drugs with iodine.

The chemical oxidation of 4 by I_2 suggests that compound 5, which exists in the oxidized form of 4, may not produce any ionic species. To test this, compound 5 was treated with I_2 in a 1 : 2 molar ratio in CH_2Cl_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 4 with I_2 (Fig. 4). 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 consisting of diselenide–molecular iodine adducts.¹⁰ However, the biological significance of the reaction between 5 and molecular iodine is still not clear, as compound 5 does not show any significant effect on the LPO-catalyzed iodination reaction.

The far-IR spectrum of complex 6 shows a distinct band at 135 cm⁻¹ for the v(I–I) stretching vibration mode (Fig. S1, ESI^{\dagger}). 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.^{1b} The FT-Raman spectrum of the complex in the v(I–I) region shows intense peaks at 164, 143, and 110 cm⁻¹. In addition, a weak band is observed around 67 cm⁻¹ (Fig. 5). The band at 110 cm⁻¹ can be certainly assigned to the v_1 symmetric stretching of I_3^- , which being a symmetrical ion normally exhibits only one Raman-active band. However, when a distortion of I_3^- occurs, the antisymmetric stretching may become Raman-active, and additional bands at higher (140–130 cm⁻¹) and at lower frequencies (80-70 cm⁻¹) may be observed.^{1b,11} Therefore, the relatively weak bands at 143 cm⁻¹ and 67 cm⁻¹ can be attributed to the antisymmetric stretching and deformation motions (respectively) of the I_3^- ion (Fig. 5a).



Fig. 5 FT-Raman spectra of (a) compound 6, (b) compound 5, and (c) compound 7.

The single-crystal X-ray studies confirm the proposed structure of **6** (Fig. 4), 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 an overall charge of +2 (Fig. S3, ESI†). 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 almost equal (C–Se: 1.886–1.890 Å), although only one of the five-membered rings in each subunit is protonated. However, the I–I bond lengths observed 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 **6** range from 2.888 Å to 2.925 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 (*vide supra*).

In the reaction between **5** and I_2 in dichloromethane, the concentrations of I_2 do not appear to change the nature of products. During our attempts to oxidize the second ring using various concentrations of I_2 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 **5** with I_2 in a 1 : 2 molar ratio in water produced a mixture containing both monocation (**6**) and dication (**7**) as confirmed by single-crystal X-ray studies (Fig. 6). In contrast to the monocation, the charge balance in a crystal of the dication is achieved by two I⁻ anions. In compound **7**, the average C–Se bond length of 1.895 Å is comparable with that of the diselenide **5** (1.880 Å),^{5b} but this is significantly longer than the average C–Se bond length (1.848 Å) found in compound **4**, which exists in a zwitterionic form.¹² The FT-Raman spectrum of compound **7**



Fig. 6 ORTEP diagram of compound **7** (with 50% probability ellipsoids), showing a dication stabilized by two iodide ions.

shows no peaks in the region of lower wavenumbers (Fig. 5c), indicating the absence of any triiodide or polyiodide species in the crystals.

To understand further the nature of the interaction between the diselenide and iodine, we carried out density functional theory (DFT) calculations on compounds **6** and **7**.¹³⁻¹⁵ The bond lengths and angles are in good agreement with the experimental data.¹³ The Kohn–Sham HOMO and LUMO calculations¹⁴⁻¹⁷ show that the energy gap between the HOMO and LUMO in **7** is much higher than that of **6** (Fig. 7), indicating that the dication is more stable than the monocation. However, these results cannot be correlated directly with our experimental observations because the formation of the monocation and/or dication appears to be highly solvent-dependent. The free energy calculations (Table S5, ESI†) for the formation of complexes **6** and **7** in the gas phase and in water suggest that the solvent effect is more predominant for the monocation **6** than for the dication **7**.



Fig. 7 Kohn–Sham HOMO–LUMO diagrams of monocation **6** (a and b) and dication **7** (c and d).

Conclusions

In summary, the first experimental evidence reported here suggests that the selenium analogue of MMI not only effectively inhibits the LPO-catalyzed iodination of tyrosine, but also reacts with I_2 to produce novel ionic diselenides. The inhibition of LPO by 4 could be completely reversed by increasing the H_2O_2 concentration This study reveals that MSeI reacts with iodine, even in its oxidized form, to form ionic diselenides containing iodide or polyiodide anions, which might be effective intermediates in the inhibition of thyroid hormones.

Experimental

General

All reactions were carried out under a N_2 atmosphere using Schlenk techniques. Melting points were determined in open tubes on a Buchi melting point B-540 apparatus and are uncorrected. Infrared spectra were obtained as KBr discs with a JASCO FT/IR-410 spectrometer in the 4000–400 cm⁻¹ region and with a Perkin– Elmer spectrometer GX (FT-IR system) in the 400–50 cm⁻¹ region. A Perkin–Elmer Lambda 5 UV/Vis spectrophotometer was used to measure the electronic absorption spectra. ¹³C (100.5 MHz) and ¹H (400 MHz) NMR spectra were recorded on Bruker Avance 400 (400 MHz NMR) spectrometers. Elemental analyses were performed on a ThermoFinigan FLASH EA 1112 CHNS analyser.

Synthesis of 3-methyl-2-((1-methyl-1*H*-imidazol-2-yl)diselanyl)-1*H*-imidazol-3-ium triiodide (6). To a solution of 5 (1.00 g, 3.12 mmol) in CH₂Cl₂ (30 mL) was added a solution of I₂ (1.58 g, 6.25 mmol) in CH₂Cl₂ (70 mL) dropwise under nitrogen at 0 °C. The red-brown solution was stirred at room temperature for 3 h. The resulting solution was concentrated to give a red-brown solid product in quantitative yield. The product was recrystallized from CH₂Cl₂ to give black crystals. Yield: 98%, mp 146–148 °C, IR (KBr) (cm⁻¹): 3440vs, 3103m, 2922s, 2848w, 1618w, 1456s, 1404w, 1308w, 1267s, 1122s, 1018w, 945w, 762vs, 681s, 669s. ¹H NMR (400 MHz, CDCl₃): δ 7.39 (s, 2H), 7.24 (s, 2H), 3.91 (s, 6H). ¹³C NMR (100.5 MHz, CDCl₃): δ 30.3, 35.5, 124.1, 127.5, 133.6, 161.67. Anal. Calcd for C₈H₁₁N₄Se₂I₃: C, 13.69; H, 1.58; N 7.98. Found: C, 13.96; H, 1.82; N, 8.04.

Synthesis of 2,2'-diselandiylbis(3-methyl-1*H*-imidazol-3-ium) iodide (7). To a solution of 5 (1.00 g, 3.12 mmol) in H₂O (100 mL) was added solid I₂ (1.58 g, 6.25 mmol) in H₂O (70 mL) dropwise. The red brown solution was stirred at room temperature for 3 h. After filtration, the black filtrate was separated and concentrated. The compound was recrystallized in CH₂Cl₂. Yield 1.6 g (88%), mp 193–195 °C, IR (KBr) (cm⁻¹): 3418w, 3148m, 3101vs, 3057vs, 3026vs, 2963s, 2822m, 2730w, 2686w, 2520w, 1726w, 1638w, 1568s, 1479vs, 1361m, 1294vs, 1149s, 1104m, 1023w, 919m, 863.91w, 771vs, 759s, 675s, 630vs. ¹H NMR (400 MHz, CDCl₃): δ 7.39 (s, 2H), 7.24 (s, 2H), 3.91 (s, 6H). Anal. Calcd for C₈H₁₂N₄Se₂I₂: C, 16.68; H, 2.10; N 9.73. Found: C, 17.02; H, 2.64; N, 10.01.

X-Ray Crystallography

X-Ray crystallographic studies were carried out on a Bruker CCD diffractometer with graphite-monochromatized Mo-K α radiation ($\lambda = 0.71073$ Å) controlled by a Pentium-based PC running on the SMART software package. (SMART, version 5.05; Bruker AXS: Madison, WI, 1998). Single crystals were mounted at room temperature on the ends of glass fibers, and data were collected at room temperature. The structures were solved by direct methods and refined using the SHELXTL software package.¹⁸ In general, all non-hydrogen atoms were refined anisotropically. Hydrogen atoms were assigned idealized locations. Empirical absorption corrections were applied to all structures using SADABS.^{19,20}

Crystal data for 6. C₈H₁₁N₄Se₂I₃, $M_r = 701.8$, monoclinic, space group $P2_1/n$, a = 15.484(6), b = 12.017(5), c = 19.604(8)Å, $\beta = 105.509(6)$; V = 3515(2) Å³, Z = 8, $\rho_{calcd} = 2.65$ Mg m⁻³, Mo-Kα radiation ($\lambda = 0.71073$ Å), T = 293(2) K, GOF = 1.019, $R_1 = 0.036$, $wR_2 = 0.085$ ($I > 2\sigma(I)$); $R_1 = 0.049$, $wR_2 = 0.093$ (all data). The structure was solved by a direct method (SIR-92)¹⁸ and refined by a full-matrix least-squares procedure on F^2 for all reflections (SHELXL-97).^{19,20} CCDC reference number 256230. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b604060h. **Crystal data for 7.** C₈H₁₂N₄Se₂I₂, $M_r = 575.9$, triclinic, space group $P\bar{1}$, a = 6.6979(1), b = 10.3741(2), c = 12.158(3) Å, a = 68.916(3); $\beta = 82.135(3)$; $\gamma = 78.816(3)^\circ$, V = 771.16(1) Å³, Z = 2, $\rho_{calcd} = 2.48$ Mg m⁻³, Mo-Kα radiation ($\lambda = 0.71073$ Å), T = 293(2) K, GOF = 1.03; $R_1 = 0.023$, $wR_2 = 0.056$ ($I > 2\sigma(I)$); $R_1 = 0.026$, $wR_2 = 0.058$ (all data). The structure was solved by a direct method (SIR-92)¹⁸ and refined by a full-matrix least-squares procedure on F^2 for all reflections (SHELXL-97).^{19,20} CCDC reference number 293611. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b604060h.

Lactoperoxidase (LPO)-catalyzed iodination of L-tyrosine

Inhibition of LPO-catalyzed iodination of L-tyrosine. This procedure, using various concentrations of MSeI (4) was carried out by an HPLC method. The incubation mixtures for the HPLC analysis contained KI (1×10^{-3} M), L-tyrosine (1×10^{-3} M), hydrogen peroxide (1×10^{-3} M) and LPO enzyme ($1 \mu g$) in 0.05 M phosphate buffer, pH 7.4. The mixture was incubated at room temperature and aliquots ($10 \mu L$) injected onto the HPLC column and eluted with a gradient solvent system (0.1% TFA in water–MeCN). The decrease in the amount of tyrosine (μg) was calculated from the calibration plot. The chromatograms were extracted at 277 nm.

The effect of hydrogen peroxide concentration on the inhibition (Fig. 3). In this HPLC assay, the incubation mixtures contained KI (1×10^{-4} M), L-tyrosine (9×10^{-4} M) and LPO enzyme ($1.5 \mu g$) in 0.05 M phosphate buffer, pH 7.4. The mixtures were incubated at room temperature and aliquots ($10 \mu L$) were injected onto the HPLC column and eluted with a gradient solvent system (0.1% TFA in water–MeCN). The formation of the monoiodotyrosine was followed at 295 nm.

Theoretical calculations

All calculations were performed using the Gaussian98 suite of quantum chemical programs.¹³ The hybrid Becke 3–Lee–Yang–Parr (B3LYP) exchange correlation functional was applied for DFT calculations.¹⁴ Geometries were fully optimized at the B3LYP level of theory using 6-31G(d) basis sets. All stationary points were characterized as minima by the corresponding Hessian indices. The HOMO calculations were done at the B3LYP/6-31G(d) level. The solvent effect was included in the calculations at the same level using Tomasi's polarizable continuum model (PCM) in aqueous solution.¹⁵ All structures were characterized as potential energy minima at the B3LYP level by verifying that all vibrational frequencies were real.

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