Bioinorganic chemistry of anti-thyroid drugs: An unusual formation of a copper (II) complex

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Abstract

We report the first example of a metal complex derived from the selenium analogue of the anti-thyroid drug methimazole (MSeI). The treatment of the diselenide form of Se-methimazole with copper (II) perchlorate leads to an unexpected deselenation reaction resulting in the formation of a novel copper (II) complex (**7**). Theoretical studies are performed on compounds **5** and **6** to understand the structural changes that take place at the imidazole moiety upon deselenation. In addition, glutathione peroxidase (GPx) behavior and lactoperoxidase (LPO) inhibition activities of complex **7** are described.
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Keywords: Anti-thyroid drugs; Copper complex; DFT calculations; Lactoperoxidase inhibition activity; Methimazole; Selenium

Methimazole (MMI, **1**) and 6-*n*-propyl-2-thiouracil (PTU, **3**) are the most commonly employed drugs in the treatment of hyperthyroidism. These compounds block the formation of thyroid hormone thyroxine (**T4**) by inhibiting the first step of the biosynthetic pathway, which is the incorporation of iodides into tyrosine residues on thyroglobulin catalyzed by thyroid peroxidase (TPO) [1]. Although the detailed mechanism of their action is still not clear, the available information reveals that these drugs may block the thyroid hormone synthesis by coordinating to the metal center of TPO [2]. Recently, the selenium analogues of methimazole (**2**) and PTU (**4**) attracted considerable attention because these compounds also inhibit the TPO and related enzymes [3]. In contrast to methimazole (**1**), the selenium analogue **2** may not coordinate to TPO through selenium because the selone moiety in **2** is found to be unstable and oxidizes readily to the corresponding diselenide (**5**) [4], but may inhibit the TPO activity by coor-

dination of the heterocyclic nitrogen atoms. Because the therapeutic effects of anti-thyroid drugs have been linked to their donor properties (Lewis base character) [5], it is important to study the coordination ability of these compounds towards metal ions.

To the best of our knowledge, examples of metal complexes bearing methimazole pharmacophore have not been described to date and only some related metal complexes having tris(methimazolyl)borate moiety are reported in the literature [6]. Furthermore, the reactivity of the selenium analogues of anti-thyroid drugs towards metal ions is still not known. In continuation our work on the selenium analogues of anti-thyroid drugs [4], we report here an unexpected reaction of diselenide **5** with Cu(ClO$_4$)$_2$ in which **5** undergoes a deselenation reaction to form a stable octahedral Cu(II)-selenide complex **7** having the monoselenide **6** as the chelating ligand (Fig. 1) [7,8]. The complex [CuL$_2$(H$_2$O)$_2$](ClO$_4$)$_2$ · 2H$_2$O (**7**) is centrosymmetric and the two bis(imidazoline)selenides are coordinated to the metal through the nitrogen atoms and there are no significant inter or intramolecular interactions with the selenium

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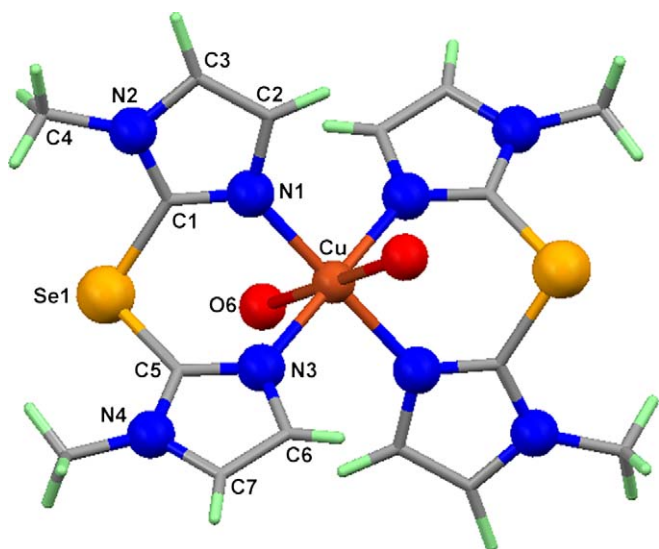 *E-mail address:* mugesh@ipc.iisc.ernet.in (G. Mugesh).

Fig. 1. The X-ray crystal structure of complex **7**. The ClO_4^- anions and additional H_2O molecules are omitted for clarity. Selected bond lengths [Å] and angles [°]: Cu—N1 2.000(2); Cu—N3 2.015(2); C1—N1 1.316(4); C5—N3 1.320(3); C1—Se1—C5 92.93(11); N1—Cu—N3 88.99(9); N1—Cu—N1 180.00(13); N1—Cu—N3 180.00(13).
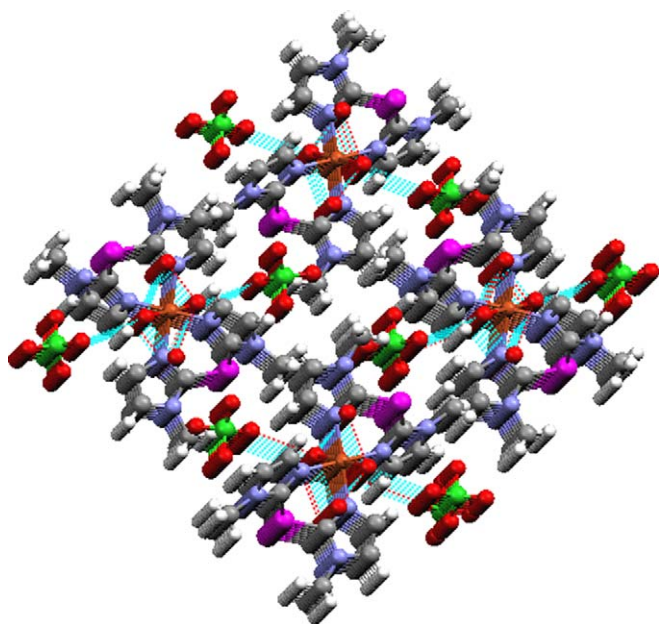

Fig. 2. The 3D network showing intermolecular hydrogen bonding.

atom. However, the two coordinated H_2O molecules are involved in strong hydrogen bonding interactions with the perchlorate ions, forming a 3D network (Fig. 2). Interestingly, this deselenation reaction is accompanied by the formation of $CuSeO_3 \cdot 2H_2O$ as a byproduct. The structure of this compound was confirmed by single crystal X-ray studies, which show that the Cu atom is located in the square pyramid of oxygen atoms as shown by Asai and Kiriyama [9].
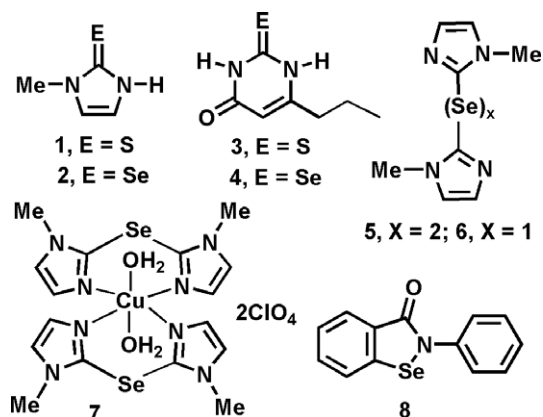
To get further insight into the structural changes that take place at the imidazole moiety, we carried out theoretical calculations on compounds **5** and **6** [10–12]. The DFT optimized geometry for **6** at the B3LYP/6-31+G(d) level of theory is shown in Fig. 3. The significant bond lengths and angles obtained from HF, DFT and MP2 level calculations and those obtained from the crystal structure of **7** are summarized in Table 1.

These studies reveal that the strain introduced on the diselenide bond of **5** upon coordination of the imidazole nitrogens to the copper center may be responsible for the deselenation reaction. A comparison of the bond lengths and angles of the free ligand with that of the complex shows that the C—Se, C—N and C=N bond lengths are not altered much upon coordination. On the other hand, a considerable change is observed in the C—Se—C bond angle and C—Se—C—N dihedral angle (Table 1). The
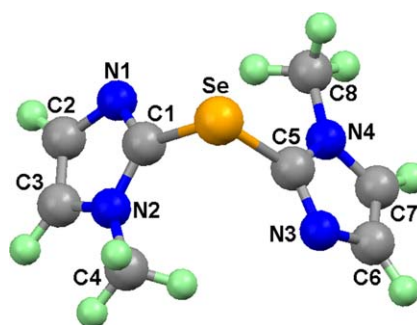

Fig. 3. The DFT-optimized structure of the free ligand **6** at B3LYP/6-31+G(d) level showing that the relative orientation of the chelating nitrogen atoms is different from that of the X-ray structure.

Table 1
A comparison of the bond lengths (Å) and angles (°) in the free ligand **6** (theoretical calculations) and in the complex **7** (X-ray)

Method[a]	C1—Se	C1—N1	C1—N2	C1—Se—C5	C1—Se–C5—N3
HF	1.897	1.296	1.352	97.5	−100.0
DFT	1.908	1.323	1.373	97.1	−101.0
MP2	1.894	1.339	1.371	94.0	−102.9
X-ray	1.897	1.316	1.344	93.0	−44.8

[a] Hartree–Fock (HF), density functional theory (DFT) and MP2 theoretical calculations were performed at B3LYP and MP2 levels and with 6-31+G(d) basis set.
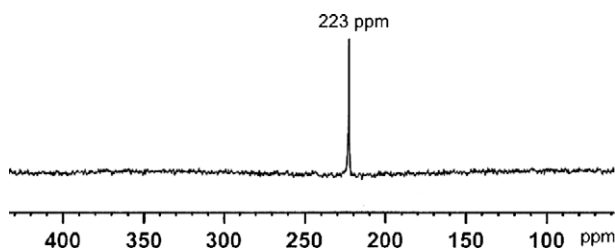
Fig. 4. The ^{77}Se NMR spectrum of complex 7 after reduction with ascorbic acid.

calculations performed at the B3LYP level of theory by using 6-311++G(d,p) basis set show that the structure with a C1—Se—C5—N3 dihedral angle of −101° is the most stable conformation. The variation in the relative stabilization energy of 6 by changing the position of Se—C5—N3 with respect to the N1—C1—Se plane is shown in Fig. S2 (Supporting Information).

As expected, the paramagnetic Cu(II) complex 7 could not be examined by ^{77}Se NMR spectroscopy. However, the reduction of the Cu(II) complex by ascorbic acid in CH_3CN/D_2O produced a sharp ^{77}Se NMR signal at 223 ppm with respect to Me_2Se, indicating the formation of a Cu(I) species (Fig. 4). To compare the NMR chemical shift of this compound with that of the free ligand, we carried out ^{77}Se NMR calculations on 6 by using GIAO method [13]. The structure was optimized at DFT level with B3LYP/6-31+G(d) basis set and the NMR calculations were performed at the same level of theory with B3LYP/6-311++G(2d,p) basis set. These calculations predict a ^{77}Se NMR chemical shift of 110 ppm with respect to Me_2Se, which is shifted upfield as compared with that of the complex, suggesting that the electron density around the selenium atom is considerably reduced upon coordination of the nitrogen atoms to the metal. The interconversion between the two oxidation states was observed to be reversible in solution. The oxidation of the reduced species by air resulted in the regeneration of complex 7, which was confirmed by single crystal X-ray studies.

The crystal structure shows that the two selenium atoms are well exposed above and below the Cu—N$_4$ plane. These observations led us to investigate the glutathione peroxidase (GPx) activity of 7, because the starting compound

5 and a number of selenium compounds have been shown to act as GPx mimics by reducing H_2O_2 and other harmful peroxides [4,15]. In a GSH–GSSG reductase assay, complex 7 exhibited significant GPx activity and the initial rate obtained for the reduction of H_2O_2 by 7 was found to be comparable with that of ebselen (8), a well-known GPx mimic [15] (Table 2). This is in agreement with a recent report that the attachment of a selenide moiety to an Mn-porphyrin complex leads to a bifunctional enzyme model with GPx activity [16]. However, the GPx activity of 7 was found to be lower than that of 5, indicating that the deselenation and coordination to the copper center reduces the anti-oxidant activity of the parent compound. This also indicates that the coordination of the imidazole nitrogen atoms to copper may also alter the electronic properties of selenium. In addition to the GPx activity, the inhibition of lactoperoxidase (LPO)-catalyzed oxidation reactions by 7 was also tested. These studies reveal that complex 7, in contrast to 2, does not inhibit the LPO activity even at higher concentrations (up to 100 μM).

In summary, we have shown that the reaction of the oxidized form of Se-methimazole with copper (II) perchlorate leads to an unexpected deselenation to produce a copper complex containing monoselenides as the ligands. This complex reduces H_2O_2 by using glutathione (GSH) as cosubstrate and thus mimics the antioxidant selenoenzyme, glutathione peroxidase (GPx) in vitro. However, complex 7 does not show any significant LPO inhibition, indicating that the LPO inhibitory activity of 2 is lost upon complexation. We are extending our research to other metal complexes of anti-thyroid drugs and further systematic investigation will focus on the biological activity of metal chalcogenides.

Acknowledgements

Appendix A. Supplementary data

Details of theoretical calculations, CV and EPR studies for 7 (PDF format), X-ray crystallographic file in CIF format for the structure determination of complex 7. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.inoche.2006.02.018.

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Table 2
GPx activity and LPO inhibition of compounds 2, 5, 7 and 8

No.	Compound	GPx activity v_0 (μM .min^{-1})[a]	LPO inhibition[b]
1	2	–[c]	Active
2	5	102.3 ± 2.1	Inactive
3	7	50.3 ± 1.6	Inactive
4	8	85.0 ± 1.1	Inactive

[a] Conditions: GSH, 1 mM; EDTA, 1 mM; GSSG reductase, 0.6 unit/ml; NADPH, 0.2 mM; H_2O_2, 1 mM; selenium catalysts, 0.02 mM; in 0.1 M phosphate buffer, pH 7.3.
[b] LPO Inhibition Assay: LPO: 6.5 nM; H_2O_2: 22.9 μM; pH 7.4.
[c] Because the reduction of 5 by GSH produces 2, compound 5 was used for the GPx activity.

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[7] To a solution of diselenide 5 (100 mg, 0.32 mmol) in MeOH (15 ml) was added Cu(ClO$_4$)$_2$.6H$_2$O (116 mg, 0.31 mmol) at room temperature and the reaction mixture was stirred for 30 min. A precipitate formed in the reaction was filtered and dried to give a greenish blue solid. Recrystallization of the product in acetonitrile afforded two types of crystals. Blue crystals: Complex 7. Green crystals: CuSeO$_3$.2H$_2$O. Anal. Calcd. For C$_{16}$H$_{20}$CuN$_8$O$_{12}$Cl$_2$Se$_2$ (7): C, 23.76: H, 3.46: N, 13.84. Found: C, 24.10; H, 3.61; N, 13.07. FTIR (KBr, cm^{-1}): 3525 (br, —OH), 3429 (s), 2922 (m, ≡C—H), 1739 (w), 1624 (m, —C≡N), 1535 (w), 1481 (s), 1410 (w), 1338 (w), 1284 (s), 1147 (s), 1091 (vs, ClO$_4^-$), 952 (w), 764 (m), 694 (m), 625 (m).

[8] Crystal data for 7: (C$_{16}$H$_{20}$N$_8$O$_{12}$Cl$_2$Cu$_1$Se$_2$): M$_r$ = 808.8, monoclinic, space group P2$_1$/n, a = 7.8167(16), b = 14.3243(30), c = 13.2729(28)

Å, β = 98.750(3)°, V = 1468.85(9) Å3, Z = 2, ρ$_{calcd}$ = 1.83 Mg/m^3, MoKα radiation (λ = 0.71073 Å), T = 293(2) K; orange prism, crystal dimensions 0.5 × 0.22 × 0.2 mm. Intensity data was collected by using the ω method in the 2θ range 2.2–56°. Of 12,454 reflections collected on a Bruker SMART APEX CCD diffractometer, 3484 were unique (R$_{int}$ = 0.0289) and used for all calculations. The structure was solved by direct methods. All non-hydrogen atoms were refined anisotropically on F^2 using full-matrix least-squares to give R$_1$ = 0.036, wR$_2$ = 0.103 (I > 2σ(I)); R$_1$ = 0.044, wR$_2$ = 0.109 (all data). All hydrogen atoms were in fixed position and refined using a riding model. CCDC-295904, contain the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: (internet) +44 1223 336 033; Email: deposit@ccdc.cam. ac.uk].

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