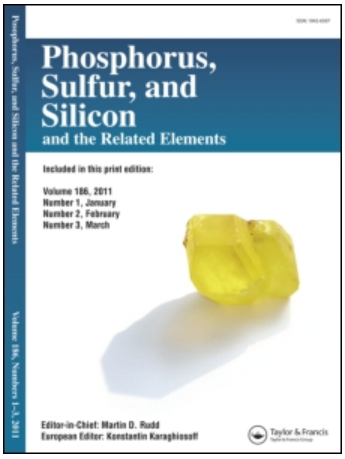


Phosphorus, Sulfur, and Silicon and the Related Elements

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

Chemistry in Thyroid Gland: Iodothyronine Deiodinases and Anti-Thyroid Drugs

Gouriprasanna Roy[a]; G. Mugesh[a]
[a] Department of Inorganic & Physical Chemistry, Indian Institute of Science, Bangalore, India

To cite this Article Roy, Gouriprasanna and Mugesh, G.(2005) 'Chemistry in Thyroid Gland: Iodothyronine Deiodinases and Anti-Thyroid Drugs', Phosphorus, Sulfur, and Silicon and the Related Elements, 180: 3, 891 — 902
To link to this Article: DOI: 10.1080/10426500590906391
URL: http://dx.doi.org/10.1080/10426500590906391

Phosphorus, Sulfur, and Silicon, 180:891–902, 2005

ISSN: 1042-6507 print / 1563-5325 online
DOI: 10.1080/10426500590906391

Taylor & Francis
Taylor & Francis Group

Chemistry in Thyroid Gland: Iodothyronine Deiodinases and Anti-Thyroid Drugs

Gouriprasanna Roy
G. Mugesh
Department of Inorganic & Physical Chemistry,
Indian Institute of Science, Bangalore, India

The monodeiodination of the prohormone thyroxine (T4) to the biologically active hormone 3,5,3′-triiodothyronine (T3) is the first step in thyroid hormone action and the type I iodothyronine deiodinase (ID-I), an enzyme containing selenocysteine in its active site, is responsible for most of this conversion. ID-I is an integral membrane protein present in highest amounts in liver, kidney, and thyroid. In the deiodinase cycle, the selenol group of the enzyme (E-SeH) first reacts with T4 to form a selenenyl iodide (E-SeI) with a release of the deiodinated iodothyronine. Subsequent reaction of the E-SeI with a thiol of other cofactors releases I^- and regenerates the active site. The thiourea drug, 6-n-propylthiouracil (PTU), reacts with the E-SeI intermediate to inhibit the enzyme active site regeneration. Owing to this property, PTU and related sulfur derivatives are often used in the acute treatment of severely hyperthyroid (Graves disease) patients and therefore commonly known as antithyroid drugs. Although the formation of a mixed selenenyl sulfide (ESe-S-PTU) adduct has been proposed to be a possible way of inhibition, it is still a matter of debate whether PTU reacts with a well-defined Se-I bond of it reacts with an equivalent species or directly with the enzyme active site. In view of this, the first successful model studies on the reactivity of PTU towards synthetic organoselenenyl iodides (RSeI) have been carried out and the results will be discussed as a basis for the deiodinase inhibition. On the basis of experimental data, a mechanism for the inhibition of ID-I by thiouracil drugs and possible amino acid residues responsible for the inhibition will be discussed.

Keywords Iodothyromine; selenenyl iodide; selenoysteine; thyronine

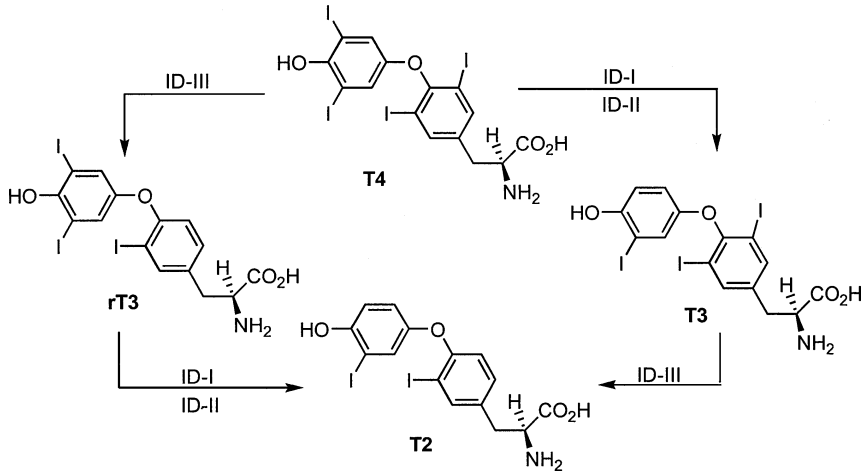
Received January 14, 2004; accepted September 29, 2004.

We thank Prof. W.-W. du Mont (Braunschweig, Germany), Prof. H. Sies (Duesseldorf, Germany), Prof. H. B. Singh (Mumbai, India), Prof. J. Koehrle (Wuerzburg, Germany), and Prof. K. Becker (Giessen, Germany) for helpful discussions and the Deutsche Forschungsgemeinschaft, the Alexander von Humboldt Foundation (Bonn, Germany) and the Department of Science and Technology (New Delhi, India) for financial support.

Address correspondence to G. Mugesh, Indian Institute of Science, Department of Inorganic & Physical Chemistry, Bangalore 560 012, India. E-mail: mugesh@ipc.iisc.ernet.in

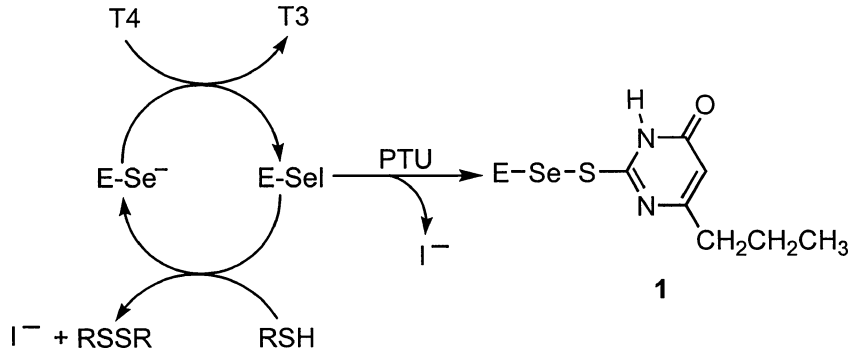
INTRODUCTION

Thyroxine (**T4**) is the main secretory product of the thyroid gland and the monodeiodination of this prohormone to the biologically active hormone, 3,5,3′-triiodothyronine (**T3**) is the first step in thyroid hormone action.[1] The thyroid gland also produces an inactive metabolite **rT3** by inner ring deiodination (Scheme 1). The triiodo derivatives **T3** and **rT3** are further metabolized by inner ring and outer ring deiodination, respectively, to produce the inactive metaboilite **T2**. Three selenoenzymes catalyzing these deiodinations have been identified, namely type I (ID-1), type II (ID-2), and type III (ID-3) iodothyronine deiodinases.[1–7]



SCHEME 1 Biochemical deiodination of thyroxine catalyzed by iodothyronine deiodinases.

The outer ring deiodination of thyroxine by ID-1, a selenocysteine-containing enzyme,[3] is considered to be the physiologically more important process since this reaction produces a biologically active hormone. The 5′-deiodination catalyzed by ID-1 is a ping-pong, bisubstrate reaction in which the selenol (or selenolate) group of the enzyme (E-SeH) first reacts with thyroxine (**T4**) to form an enzyme selenenyl iodide (E-SeI) complex with release of deiodinated iodothyronine (**T3**). Subsequent reaction of the selenenyl iodide with an unidentified cytoplasmic thiol cofactor releases I^- ions and regenerates the E-SeH active site (Scheme 2).[8] The anti-thyroid drug, 6-n-propyl-2-thiouracil (PTU), react with the selenenyl iodide intermediate to form a stable selenenyl sulfide (**1**).[8]

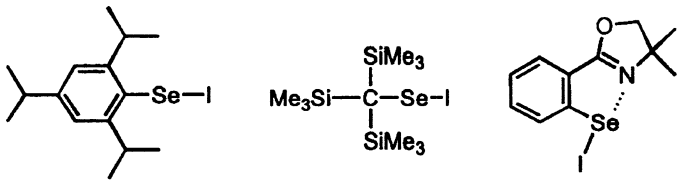


SCHEME 2 Proposed mechanism for the ID-I catalyzed deiodination and inhibition by PTU.

REDUCTION OF SELENENYL IODIDES BY THIOLS

According to the mechanism shown in Scheme 2, the complete cycle requires two substrates: thyroxine (**T4**) and a cellular thiol or another cofactor.[8,9] A number of substrates have been proposed as suitable cofactors for the reduction of the E-SeI intermediate. Although it is customary to use dithiothreitol (DTT) as the second substrate in *in vitro* experiments,[4] the identity of the physiological second substrate still is uncertain. The tripeptide glutathione (GSH) also can act as a thiol cosubstrate, but GSH is a much less potent cofactor than DTT for ID-1.[1,4] In addition to GSH, other native thiols such as dihydrolipoic acid or dihydrolipoamide may serve as cofactors for ID-1.[1,10] Therefore, Scheme 2 may be an incomplete or incorrect representation of the catalytic mechanism of ID-1, since evidence for the cofactor systems mentioned above only has been presented for *in vitro* studies and not for *in vivo* analysis. It is well known in the case of glutathione peroxidase (GPx) that the active site selenol is regenerated from the oxidized selenium species (E-SeOH) through the formation of selenenyl sulfide (E-Se-S-G) by reaction with GSH.[11] Surprisingly, no such selenenyl sulfide intermediate has been proposed for the *in vivo* ID-1 mechanism due to the lack of sufficient information about the physiologically relevant thiol or other cofactors that can reduce the E-SeI intermediate.

Organoselenenyl iodides such as PhSeI are generally unstable and disproportionate in solution.[12] Even the sterically hindered areneselenenyl iodides such as **2** (Scheme 3) have been found to exist in equilibrium with iodine and the corresponding diselenide in solution.[13–15] The "non-existence" of stable binary Se—I compounds is associated with the very similar electronegativities of Se and I, that is, the lack of ionic



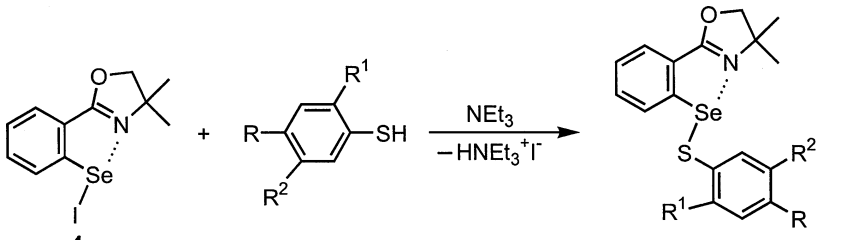
2 4

SCHEME 3 Monosyano selenium iodides.

contribution to the resonance energy in the covalent Se—I bond.[16] However, the recent observations suggest that the covalent Se—I bond could be stabilized against dismutation (disproportionation) reactions by introducing sterically, highly demanding alkyl substituents[12–15] (**3**) or internally chelating groups[17–20] (e.g. **4**).

Recent model studies on the deiodinase cycle have been directed toward the reactivity of selenenyl iodides with thiols to probe the mechanism of the reduction of E-SeI intermediate.[21] The reactivity of the Se—I bond in compound **4** has been studied in detail since (1) this compound does not involve in dismutation equilibria in solution, (2) the Se—I bond in this compound is kinetically activated by a strong Se···N interaction (Se···N distance: 2.133(4)),[17] (3) the Se···N interaction would be expected to stabilize the resulting selenenyl sulfides against dismutation reactions, and (4) the five-membered oxazoline moiety in compound **4** resembles one of the active site features of ID-1, as the basic histidine residues present near the selenium atom have been shown to play important roles in the deiodination.[22–24] To investigate the possible intermediacy of a selenenyl sulfide (E-Se-S-R) in the deiodinase cycle experimentally, model reactions have been carried out with various aromatic thiols. The reaction **4** with PhSH (**5**) in the presence of NEt_3 afforded the expected selenenyl sulfide **10**. Similarly, reactions between **4** and p-thiocresol (**6**), p-chlorobenzenethiol (**7**), p-bromobenzenethiol (**8**), and 2,5-dimethylbenzenethiol (**9**) in the presence of NEt_3 afforded the corresponding selenenyl sulfides **11–14** in good yields (Scheme 4). In the absence of NEt_3, compound **4** reacted with all of the above-mentioned thiols to afford the corresponding diselenide, indicating that HI plays a major role in these reactions.

The synthesis and characterization of selenenyl sulfides (R-Se-S-R) are of great interest from the biological point of view, as illustrated by a number of reports that postulate the intermediacy of these species in the catalytic cycle of GPx and its synthetic model compounds.[25] More recently, it has been reported that the selenocysteine active site of mammalian thioredoxin reductase forms a selenenyl



SCHEME 4 Reactions between areneselenenyl iodide **4** and various thiols.

sulfide intermediate during the catalytic cycle.[26–28] Similar to the selenenyl iodides, certain selenenyl sulfides also are known to be unstable compounds that undergo disproportionation reactions to give the corresponding diselenides (R-Se-Se-R) and disulfides (R-S-S-R).[29] Compounds **10–14** were found to be very stable in solution, and no dismutation reactions were observed. The ^{77}Se NMR chemical shifts indicate that compounds **10–14** also exhibit intramolecular Se···N interactions in solution. In general, the ^{77}SeNMR signals of arylselenenyl sulfides are known to appear downfield from those of the corresponding diaryl diselenides and upfield from those of the corresponding arylselenenyl halides.[30,31] As was to be expected, a large deshielding of the signals was observed for the selenenyl sulfides **10–14** (557–633 ppm), with respect to the corresponding diselenide (454 ppm), and a large shielding of the signals was observed with respect to the selenenyl iodide **4** (762 ppm). The facile reaction of selenenyl iodides with thiols suggests that the concept of a selenenyl sulfide intermediacy is more reasonable than a one-step, two-electron reduction of E-SeI to E-SeH when a thiol co-substrate is used for the reduction.

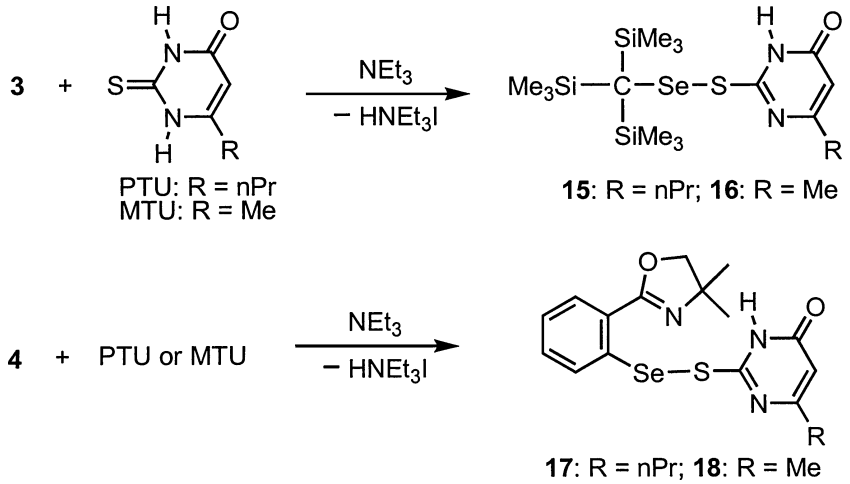
REACTIONS OF SELENENYL IODIDES WITH THIOUREA DRUGS

It has been proposed that the anti-thyroid drug 6-n-propyl-2-thiouracil (PTU) inhibits ID-1 probably by reacting with the E-SeI intermediate to form a selenenyl sulfide (Scheme 2).[8] The selenenyl sulfide **1** is considered to be a dead-end product since this compound does not react with thiols under physiological conditions. Owing to this property, PTU often is used in the treatment of severely hyperthyroid (Graves disease) patients and is therefore well known as an anti-thyroid drug. The

formation of a mixed selenenyl sulfide adduct (**1**, Scheme 1) in the reaction of the selenenyl iodide with PTU has been proposed mainly on the basis of the following assumptions. (1) The PTU inhibition is noncompetitive with respect to thyroxine and competitive with respect to thiol cofactor, which suggests that PTU and cofactor react with the same enzyme intermediate.[1] (2) The thiouracil derivatives are particularly reactive towards protein sulfenyl iodide (S-I) groups[1] and presumably even more reactive towards selenenyl iodide (Se—I) groups. In contrast to ID-1, the other two deiodinases (ID-2 and ID-3) are insensitive to PTU.[2–7]

The reactivity of selenenyl iodides towards the anti-thyroid drugs has been experimentally verified, for the first time, with synthetic selenenyl iodides.[32] When "PhSeI" (0.5 $Ph_2Se_2.I_2$) and **2**, which are known to disproportionate to diselenide and iodine or their adducts,[12] independently were treated with stoichiometric amounts of PTU or 6-methyl-2-thiouracil (MTU) in the presence of triethylamine, both the reactions afforded the corresponding diselenides, rather than the selenenyl sulfides, as the only products. This indicates that the unstable selenenyl iodides PhSeI and **2** are reduced by PTU and MTU to the corresponding diselenides (and not the PTU/MTU derivatives). These properties of PhSeI and **2** therefore resemble the inhibitory action of PTU-insensitive deiodinases. In the absence of triethylamine, selenenyl iodide **3** reacted with PTU and MTU much more slowly than the internally chelated compound **4**. Although compound **4** reacted rapidly with PTU and MTU under similar experimental conditions, it unexpectedly afforded the corresponding diselenide as the major product. This result indicates that the HI produced during the reaction may act as a catalyst for the diselenide formation. However, in the presence of triethylamine, no diselenide is formed and both **3** and **4** reacted rapidly with PTU and MTU to give the desired selenenyl sulfides **15–18** (Scheme 5).[32]

The reactions of **4** with PTU and MTU were found to be faster than those of **3**, probably because of an increase in the electrophilic reactivity of selenium atom in compound **4** by Se···N interactions. Whereas the Se—I bond in compound **3** is a 2-center 2-electron (2c–2e) covalent bond, the Se—I bond in compound **4** can be considered as a result of Se···N interactions as part of a 3c–4e system. Therefore, the Se—I bond in **4** thermodynamically is stabilized in isolated form and kinetically activated towards reactions with nucleophilic reagents. As suggested for the natural enzyme,[4] the hydrogen atom attached to N1 of PTU and MTU plays an important role in the reactions. The replacement of the hydrogen atom at the N1 position by a methyl group normally produces inactive substances. For example, it has been reported that the 6-anilino-2-thiouracil exhibits 85% inhibition towards human placenta



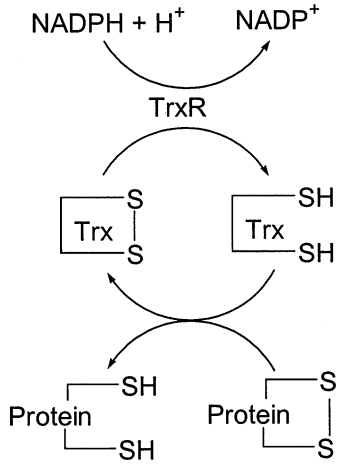
SCHEME 5 Synthesis of the thiouracil derivatives.

deiodinase activity, but the corresponding N-methylated derivative exhibits only 2% inhibition.[33]

SELENENYL IODIDE: A NEW SUBSTRATE FOR THIOREDOXIN REDUCTASE

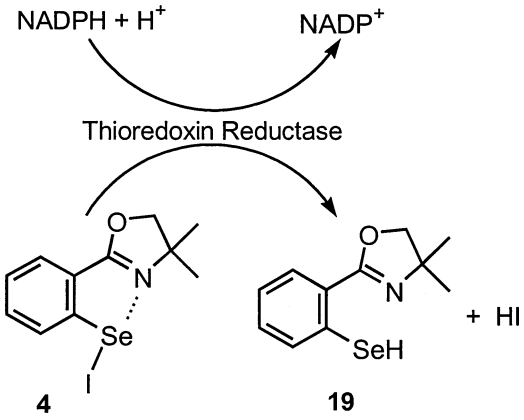
As pointed out by several research groups, Scheme 2 may be an incomplete or incorrect representation of the catalytic mechanism of ID-1, since there is no evidence for the *in vivo* cofactor system of deiodination reactions. Therefore, recently some model experiments have been carried out to investigate whether there is any link between deiodinases and other selenoenzymes such as thioredoxin reductases.[34] Thioredoxin reductase (TrxR) is a dimeric flavoenzyme that catalyzes the reduction of thioredoxin (Trx) using NADPH as a cofactor.[35,36] The reduced form of Trx can reduce a number of disulfide bonds in proteins (Scheme 6). Mammalian TrxRs are selenoenzymes[37] and have a broad substrate specificity, reacting not only with Trx from different species but also with a variety of non-disulfide substrates, such as selenoglutathione, selenite, *S*-nitrosoglutathione, hydroperoxides and peroxynitrite.[38] There is evidence that a non-GSH, but NADPH-dependent, cofactor system activates the deiodination,[39] and the Trx-TrxR system has been proposed to account for such an activation.[40–42]

The reduction of selenenyl iodide **4** by TrxR was followed spectrophotometrically at 340 nm as consumption of NADPH. Incubation of TrxR from human placenta (HP-TrxR) and **4** without Trx resulted in a fast



SCHEME 6 The thioredoxin system: Trx, TrxR, NADPH.

initial oxidation of NADPH, observed as a rapid loss of absorbance after addition of **4** to the reaction mixture. This initial reaction is followed by a steady rate of reaction, where **4** is reduced to the corresponding selenol (**19**) by HP-TrxR with a concomitant oxidation of NADPH (Scheme 7). This is consistent with a recent report that the Se–N bond in ebselen could be cleaved by mammalian TrxR to produce ebselen–selenol.[43] In this particular case, human and bovine TrxR-catalysed the reduction of ebselen by NADPH with an apparent K_M value of 2.5 μM. Reaction of **4** with NADPH alone showed considerable spectral changes when compared with the individual spectra of **4** and NADPH. Similar



SCHEME 7 Reduction of compound **4** by HP-TrxR.

TABLE I Initial Rate for the Reduction of 4 by HP- and *E. coli* TrxR[a]

Entry	Source enzyme	NADPH oxidation (μM min^{-1})
a	HP-TrxR	0.063
b	+**4**	1.452
c	+Trx +**4**	1.608
d	*E. coli* TrxR	0.082
e	+**4**	0.241
f	+Trx +**4**	0.376

[a]The reactions were performed in 50 mM Tris-HCl buffer, 1 mM EDTA, pH 7.5, with 100 μM, 1, 50 nM HP-TrxR or *E. coli* TrxR, 2 μM Trx and 100 μM NADPH.

changes also were observed with NADH. However, while direct reduction of **4** was observed with both NADPH and NADH, the HP-TrxR-induced reduction of **4** only was—as expected—observed with NADPH.[34]

The rate of the reduction of **4** in the presence of HP-TrxR (Table I entry b, 1.45 μM min^{-1}) is higher than the rate of **4** with NADPH in the absence of HP-TrxR (0.48 μM min^{-1}), suggesting a catalytic role for HP-TrxR. The TrxR from *E. coli* was less efficient (Table I, entry e) in the reduction compared with the HP-TrxR (entry b). In the absence of Trx, the reduction rate for *E. coli* TrxR was found to be 6-fold lower than that of HP-TrxR.[34]

The higher activity of HP-TrxR as compared with the *E. coli* enzyme can be ascribed to the presence of a selenocysteine residue in the active center of the human enzyme. HP-TrxR (*Mr* approx. 55 kDa) belongs to a class of "high *Mr* TrxRs" which are related more to glutathione reductases (GRs) than to *E. coli* TrxR (*Mr* approx. 35 kDa).[37,44] There is a significant difference between the human and *E. coli* enzymes in terms of their reactivities. In addition to the FAD and a cysteine pair, the high *Mr* TrxRs contain a third redox active group at the *C*-terminal extension of the protein, which transfers reducing equivalents to their substrates. In HP-TrxR, this center is represented by Cys^{495}–SeCys^{496}. Previous studies on mammalian and bacterial TrxRs revealed that the Cys–SeCys pair near the *C*-terminus in the mammalian enzymes catalytically is more favorable than the redox-active cysteines of the bacterial enzymes.[44–48] The selenol (or selenolate) group in HP-TrxR is expected to be more reactive towards highly electrophilic species such as selenenyl halides.

To investigate whether Trx can enhance the rate of the reduction, the reaction mixture was incubated with *E. coli* Trx. The addition of

2 μM Trx only slightly enhanced the rate (Table I, entry c). This is consistent with the observation of Björnstedt et al. that the presence of Trx only marginally increases the peroxidase activity of HP-TrxR in a selenocysteine-coupled reaction.[49] The addition of Trx to the *E. coli* TrxR significantly enhanced the reduction rate. The increase in the reduction rate by Trx is consistent with the studies on Trx-dependent rat hepatic and renal ID-I activity which showed that the degree of activation of the ID-I activity by the Trx system depends on the concentration of reduced Trx available for the reduction of the oxidized form of deiodinase.[41,50] This study shows that a stable selenenyl iodide can serve as a substrate for mammalian TrxR by reacting with the selenocysteine residue. This study also suggests that the TrxR–Trx system may act as a cofactor for the IDs by acting on the Se—I intermediates of the deiodinase cycle. Since deiodinases are relatively small in size and the active site cavity of the TrxR is relatively large, it is possible that the TrxR identified in human thyreocytes[51,52] can serve as a cofactor for reactions catalysed by ID-1.

SUMMARY

Although a number of questions related to the mechanism of iodothyronine deiodinases is still unsolved, the current knowledge in the area suggest the following: (1) The facile reactions of selenenyl iodides with thiols and the formation of stable selenenyl sulfides indicate that the deiodinase cycle must proceed through a selenenyl sulfide intermediate when a thiol cosubstrate is used for the regeneration of the active site. (2) PTU and related drugs do not react with the native enzyme but only with an E-SeI intermediate containing a covalent Se—I bond. (3) Some basic amino acid residues such as histidine near the active center may kinetically activate the Se—I bond, or these residues may act as general bases for the abstraction of HI during the inhibition. (4) The possible disproportionation of the E-SeI intermediate may, at least partially, account for the insensitivity of certain selenium-containing deiodinases towards thiourea drugs. (5) The high reactivity of the selenocysteine towards the Se—I bond suggests that Trx or other related systems may act as a cofactor for the IDs by acting on the Se—I intermediates of the deiodinase cycle.

REFERENCES

[1] J. L. Leonard and J. J. Visser, Biochemistry of Deiodination, in *Thyroid Hormone Metabolism*, G. Heinemann (Ed.) (Marcel Dekker, New York, 1986), pp. 186–229.

[2] D. Behne, A. Kyriakopoulos, H. Meinhold, and J. Köhrle, *Biochem. Biophys. Res. Commun.*, **173,** 1143 (1990).
[3] M. J. Berry, L. Banu, and P. R. Larsen, *Nature*, **349,** 438 (1991).
[4] P. R. Larsen and M. J. Berry, *Annu. Rev. Nutr.*, **15,** 323 (1995).
[5] D. L. St. Germain and V. A. Galton, *Thyroid*, **7,** 655 (1997).
[6] A. C. Bianco, D. Salvatore, B. Gereben, M. J. Berry, and P. R. Larsen, *Endocr. Rev.*, **23,** 38 (2002).
[7] J. Köhrle, *Methods Enzymol.*, **347,** 125 (2002).
[8] M. J. Berry, J. D. Kieffer, J. W. Harney, and P. R. Larsen, *J. Biol. Chem.*, **266,** 14155 (1991).
[9] G. Lian, L. Ding, M. Chen, Z. Liu, D. Zhao, and J. Ni, *J. Biol. Chem.*, **276,** 28037 (2001).
[10] A. Goswami and I. N. Rosenberg, *Endocrinology*, **112,** 1180 (1983).
[11] L. Flohé, Glutathione Peroxidase Brought into Focus, In *Free Radicals in Biology*, W. A. Pryor (Ed.), Academic Press, 1982.
[12] W.-W. du Mont, *Main Group Chemistry News*, **2(3),** 18 (1994).
[13] W.-W. du Mont, S. Kubiniok, K. Peters, and H.-G. Schnering, *Angew. Chem. Int. Ed. Engl.*, **26,** 780 (1987).
[14] W.-W. du Mont, A. Martens von Salzen, S. Pohl, and W. Saak, *Inorg. Chem.*, **29,** 4848 (1990).
[15] W.-W. du Mont, A. Martents-von Salzen, F. Ruthe, E. Seppälä, G. Mugesh, F. A. Devillanova, V. Lippolis, and N. Kuhn, *J. Organomet. Chem.*, **623,** 14 (2001).
[16] T. M. Klapötke and J. Passmore, *Acc. Chem. Res.*, **22,** 234 (1989).
[17] G. Mugesh, A. Panda, H. B. Singh, and R. J. Butcher, *Chem. Eur. J.*, **5,** 1411 (1999).
[18] G. Mugesh, H. B. Singh, and R. J. Butcher, *Tetrahedron: Asymmetry*, **10,** 237 (1999).
[19] A. Panda, G. Mugesh, H. B. Singh, and R. J. Butcher, *Organometallics*, **18,** 1986 (1999).
[20] Z. Majeed, W. R. McWhinnie, and P. R. Lowe, *Acta Cryst.*, **C56,** e105 (2000).
[21] G. Mugesh, W.-W. du Mont, C. Wismach, and P. G. Jones, *Chem. Bio. Chem.*, **3,** 440 (2002).
[22] J. A. Mol, R. Docter, G. Hennemann, and T. J. Visser, *Biochem. Biophys. Res. Commun.*, **120,** 1149 (1984).
[23] J. Köhrle and R.-D. Hesch, in *Hormone Metabolic Research (Suppl.)* Georg Thieme Verlag, Stuttgart, Vol. 14, pp 42–55 (1984).
[24] M. J. Berry, *J. Biol. Chem.*, **267,** 18055 (1992).
[25] G. Mugesh and H. B. Singh, *Chem. Soc. Rev.*, **29,** 347 (2000).
[26] S.-R. Lee, S.-B. Noy, J. Kwon, R. L. Levine, T. C. Stadtman, and S. G. Rhee, *Proc. Natl. Acad. Sci. USA*, **97,** 2521 (2000).
[27] L. Zhong, E. S. J. Arnér, and A. Holmgren, *Proc. Natl. Acad. Sci. USA*, **97,** 5854 (2000).
[28] C. H. Williams Jr., L. D. Arscott, S. Müller, B. W. Lennon, M. L. Ludwig, P.-F. Wang, D. M. Veine, K. Becker, and R. H. Schirmer, *Eur. J. Biochem.*, **267,** 6110 (2000).
[29] A. Ahrika, J. Auger, and J. Paris, *New. J. Chem.*, **23,** 679 (1999).
[30] H. Duddeck, *Prog. Nucl. Magn. Reson. Spectrosc.*, **27,** 1 (1995).
[31] M. Broschag and T. M. Klapötke, *Compilation of Reported*^{77} *Se NMR Chemical Shifts*, J. Wiley, Chichester (1996).
[32] W.-W. du Mont, G. Mugesh, C. Wismach, and P. G. Jones, *Angew. Chem. Int. Ed.*, **40,** 2486 (2001).
[33] T. Nogimori, C. H. Emerson, L. E. Braverman, C.-F. Wu, J. Gabino, and G. E. Wright, *J. Med. Chem.*, **28,** 1692 (1985).

[34] G. Mugesh, L.-O. Klotz, W.-W. du Mont, K. Becker, and H. Sies, *Org. Biomol. Chem.*, **1,** 2848 (2003).
[35] C. H. Williams, Jr., in *Chemistry and Biochemistry of Flavoenzymes*, F. Müller, (Ed.) (CRC Press, Inc., Boca Raton, FL, 1992), vol. 3, pp. 121–211.
[36] A. Holmgren and M. Björnstedt, *Methods Enzymol.*, **252,** 199 (1995).
[37] T. Sandolova, L. Zhong, Y. Lindqvist, A. Holmgren, and G. Schneider, *Proc. Natl. Acad. Sci., U.S.A.*, **98,** 9533 (2001) and references therein.
[38] G. E. Arteel, K. Briviba, and H. Sies, *Chem. Res. Toxicol.*, **12,** 264 (1999).
[39] A. K. Das, C. W. Hummel, and P. G. Walfish, *Biochem. J.*, **240,** 559 (1986).
[40] A. Goswami and I. N. Rosenberg, *Endocrinology*, **121,** 1937 (1987).
[41] G. B. Bhat, K. Iwase, C. W. Hummel, and P. G. Walfish, *Biochem. J.*, **258,** 785 (1989).
[42] J. Sharifi and D. L. St. Germain, *J. Biol. Chem.*, **267,** 12539 (1992).
[43] R. Zhao, H. Masayasu, and A. Holmgren, *Proc. Natl. Acad. Sci. U.S.A.*, **99,** 8579 (2002).
[44] J. Kuriyan, T. S. R. Krishna, L. Wong, B. Guenther, A. Pahler, and C. H. Williams, Jr., *Nature*, **352,** 172 (1991).
[45] S. Gromer, J. Wissing, D. Behne, K. Ashman, R. H. Schirmer, L. Flohé, and K. Becker, *Biochem. J.*, **332,** 591 (1998).
[46] S.-R. Lee, S. Bar-Noy, J. Kwon, R. L. Levine, T. C. Stadtman, and S. G. Rhee, *Proc. Natl. Acad. Sci. U.S.A.*, **97,** 2521 (2000).
[47] L. Zhong, E. S. J. Arnér, and A. Holmgren, *Proc. Natl. Acad. Sci. U.S.A.*, **97,** 5854 (2000).
[48] S. Kanzok, A. Fechner, H. Bauer, J. K. Ulschmid, H. M. Müller, J. Botella-Munez, S. Schneuwly, R. H. Schirmer, and K. Becker, *Science*, **291,** 643 (2001).
[49] M. Björnstedt, M. Hamberg, S. Kumar, J. Xue, and A. Holmgren, *J. Biol. Chem.*, **270,** 11761 (1995).
[50] T. J. Visser, D. Fekkes, M. H. Otten, J. A. Mol, R. Docter, and G. Hennemann, *Horm. Cell. Regul.*, **8,** 179 (1984).
[51] J. Scharifi and D. L. Germain, *J. Biol. Chem.*, **267,** 12539 (1992).
[52] A. F. Howie, J. R. Arthur, F. Nicol, S. W. Walker, S. G. Beech, and G. J. Beckett, *J. Clin. Endocrinol. Metabol.*, **83,** 2052 (1998).