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Selenenyl iodide: a new substrate for mammalian thioredoxin reductase †

Govindasamy Mugesh,[a] Lars-Oliver Klotz,[a] Wolf-Walther du Mont,[b] Katja Becker[c] and Helmut Sies *[a]

[a] *Institut für Biochemie und Molekularbiologie I, Heinrich-Heine-Universität Düsseldorf, Postfach 101007, D-40001 Düsseldorf, Germany. E-mail: sies@uni-duesseldorf.de; Fax: +49-211-811 3029; Tel: +49-211-811 2707*

[b] *Institut für Anorganische und Analytische Chemie der Technischen Universität, Postfach 3329, D-38023 Braunschweig, Germany*

[c] *Interdisziplinäres Forschungszentrum der Universität, Postfach 111440, D-35359 Giessen, Germany*

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Areneselenenyl iodide stabilised by internal chelation has been synthesized and evaluated as a substrate of thioredoxin reductase (TrxR). The reactivity of TrxR obtained from human placenta towards selenenyl iodide was found to be much higher than that of the *E. coli* enzyme, indicating the essential nature of a selenocysteine residue in the active site of the human enzyme. The addition of thioredoxin (Trx) significantly enhanced the TrxR-catalysed reduction of selenenyl iodide **1**. These studies on the reduction of a selenenyl iodide by the thioredoxin system suggest that stable selenenyl iodides could be new substrates for human TrxR. The Trx system could act as a cofactor for iodothyronine deiodinase by reducing the selenenyl iodide intermediate in the second-half of the deiodinase catalytic cycle to regenerate the active site. The TrxR-catalysed reduction of **1** was not inhibited by the anti-thyroid drug, PTU, suggesting that the involvement of the Trx system in the deiodinase cycle may be responsible for the insensitivity of certain deiodinases towards clinically useful thiourea drugs.

Introduction

Thyroxine (T4) is the main secretory product of the thyroid gland, and the monodeiodination of this hormone to the biologically more active form, 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 (Fig. 1). The triiodo derivatives T3 and rT3 are further metabolized by inner ring and outer ring deiodination, respectively, to produce the inactive metabolite T2. Three selenoenzymes catalyzing these deiodinations have been identified, namely type I (ID-I), type II (ID-II) and type III (ID-III) iodothyronine deiodinases.[2]

The outer ring deiodination of thyroxine (T4) by ID-I, a selenocysteine-containing enzyme present in highest amounts in liver, kidney, thyroid and pituitary,[3] is considered to be the physiologically more important process since this reaction produces a biologically active hormone (T3).[2] It has been proven that the 5′-deiodination catalyzed by ID-I is a ping-pong, bisubstrate reaction in which the selenol group of the enzyme first reacts with thyroxine (T4) to form a selenenyl iodide (E-SeI) with release of deiodinated iodothyronine (T3). Subsequent reaction of the selenenyl iodide with an unidentified cytoplasmic thiol cofactor (RSH) releases I^- and regenerates the active site E-SeH (Fig. 2).[4] The thiourea drug, 6-*n*-propylthiouracil (PTU), reacts with the enzyme–iodide complex (E–SeI), blocking enzyme regeneration. Thus, PTU inhibition of ID-I is uncompetitive with respect to T4 and competitive with respect to the thiol cofactor (RSH).

According to the mechanism shown in Fig. 2, the complete reaction requires two substrates: iodothyronine and a thiol or other cofactor to maintain the catalytic cycle. 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*

† Electronic supplementary information (ESI) available: additional data. See http://www.rsc.org/suppdata/ob/b3/b302220j/

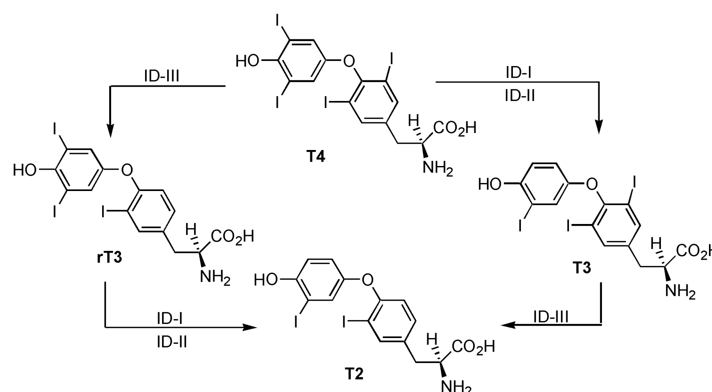

Fig. 1 Biochemical deiodination of thyroxine catalysed by ID.

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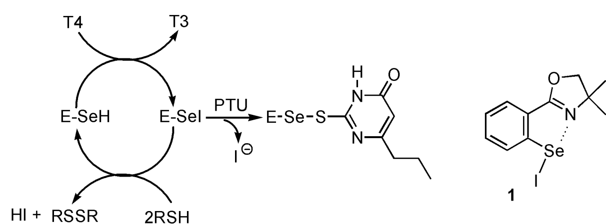


Fig. 2 Proposed catalytic mechanism of deiodination and inhibition.

experiments,[2c] the identity of the physiological second substrate is still uncertain. The tripeptide glutathione (GSH) can also act as a co-substrate, but GSH is a much less potent cofactor for ID-I than DTT.[1,2c] In addition to GSH, other native thiols such as dihydrolipoic acid or dihydrolipoamide may serve as cofactors for ID-I.[5] Fig. 2 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.

Thioredoxin reductase (TrxR) is a dimeric flavoenzyme that catalyzes the reduction of thioredoxin (Trx) using NADPH as a cofactor.[6] This reaction is the basis for a number of further processes such as enzymatic synthesis of deoxyribonucleotides, defense against oxidative stress, redox regulation of gene expression or signal transduction.[7] The reduced form of Trx can reduce a number of disulfide bonds in proteins (Fig. 3). Mammalian TrxRs are selenoenzymes[8] 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, ascorbic acid, *S*-nitroso-glutathione, hydroperoxides and peroxynitrite.[9] The human TrxR has also been proposed to be a major target for antitumor platinum complexes.[10]

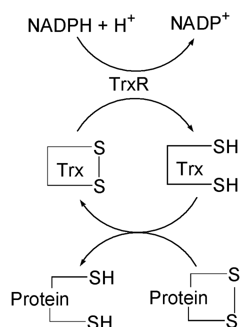


Fig. 3 The thioredoxin system: Trx, TrxR, NADPH.

The objective of the present study was to investigate whether the Trx–TrxR system can reduce selenenyl iodides to selenols, since this could be an important alternative pathway for the *in vivo* regeneration of the active site of certain deiodinases.[11] Interestingly, there is evidence that a non-GSH, but NADPH-dependent, cofactor system activates the deiodination,[11] and the Trx–TrxR system has been proposed to account for such an activation.[12] However, it is still a matter of debate whether the Trx redox system acts together with some other thiol cofactor or whether it interacts directly with the intermediates in the deiodinase cycle. In this paper, we report the first observation on the reactivity of a selenenyl iodide (**1**) towards the Trx–TrxR system and propose that the system could be a suitable cofactor for the reduction of selenenyl iodide in the deiodinase cycle.

Experimental

NADPH, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), *E. coli* thioredoxin (Trx), and *E. coli* TrxR were obtained from Sigma. Human TrxR was purified from human placenta as described by Gromer *et al.*[13] In the presence of 100 μM NADPH and 100 μM compound **1** and assuming that all subunits were saturated with selenium, a specific activity of 0.8 U mg^{-1} was calculated for HP-TrxR at 25 °C. This corresponds to 2–3% of the DTNB reducing activity, which is 35 U mg^{-1} for freshly prepared enzyme.[3b] K_M and V_{max} values for **1** were determined in 50 mM Tris–HCl (pH 7.4), 1 mM EDTA, 80 μM NADPH and 460 nM HP-TrxR at 23 °C.

Synthesis of [2-(4,4-dimethyl-2-oxazolinyl)phenyl]selenenyl iodide (1)

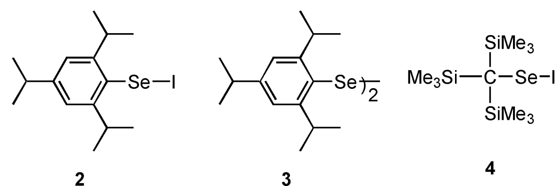
This compound was synthesized by the following method.[14] A solution of bis[2-(4,4-dimethyl-2-oxazolinyl)phenyl]diselenide (0.51 g, 1 mmol) in CCl_4 (25 mL) was added to a solution of iodine (0.254 g, 1 mmol) in CCl_4 at 0 °C. The addition was carried out dropwise over a period of 1 h and the mixture was allowed to come to room temperature. The stirring was continued for an additional 2 h. The solution obtained was concentrated to give a red, crystalline product. This was recrystallised from a chloroform–hexane mixture to give brick-red crystals of **1**. Yield: 0.68 g (90%). Mp 124–126 °C (lit. 122–124 °C); $C_{11}H_{12}NOSeI$: calcd C 34.76, H 3.16, N 3.69; found C 34.36, H 3.08, N 3.54%. ^1H, ^{13}C and ^{77}Se NMR data are in accordance with the literature values.

TrxR assay

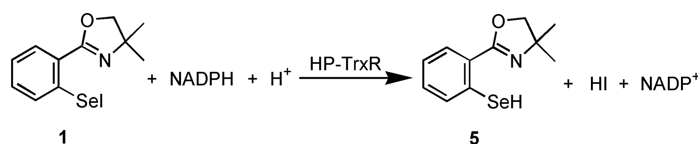
The reactions were performed in 50 mM Tris–HCl buffer, 1 mM EDTA, pH 7.5, with HP-TrxR or *E. coli* TrxR, *E. coli* Trx and NADPH. The samples were added to the test mixture at room temperature and the reaction was started by the addition of a stock solution of TrxR. The initial reduction rates were calculated from the rate of NADPH oxidation at 340 nm. Each initial rate was measured at least 3 times and was calculated from the first 5–10% of the reaction by using 6.22 mM^{-1} cm^{-1} as the molar absorption coefficient for NADPH. The rates were corrected for the background reaction between TrxR and NADPH without test compounds.

Results and discussion

The reactions of organoselenenyl iodides with an enzyme have not been studied previously as areneselenenyl iodides such as PhSeI are themselves generally unstable and disproportionate in solution.[16] Even the sterically hindered areneselenenyl iodides such as **2** have been found to exist in equilibrium with iodine and the corresponding diselenide (**3**) in solution.[17] 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 contribution to the resonance energy in the covalent Se ··· I bond.[18] However, the recent observations that the covalent Se ··· I bond could be stabilized against dismutation (disproportionation) reactions by introducing sterically highly demanding alkyl substituents[16,17] (**4**) or internally chelating groups[14,19] (*e.g.* **1**) have given opportunities for studying the reactivity of pure Se ··· I compounds.[20]



Compound **1** was chosen for our studies due to its remarkable stability against dismutation in solution. The Se ··· I bond in compound **1** can be considered as a result of Se ··· N interactions as part of a 3c–4e system. The reduction of selenenyl iodide **1** by TrxR was followed spectrophotometrically at 340 nm as consumption of NADPH. Incubation of TrxR from



Scheme 1 Reduction of compound **1** by HP-TrxR.

Table 1 Initial rate for the reduction of **1** by HP- and *E. coli* TrxR [a]

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

human placenta (HP-TrxR) and **1** without Trx resulted in a fast initial oxidation of NADPH, observed as a rapid loss of absorbance after addition of **1** to the reaction mixture (at 4 min in Fig. 4). This spontaneous reaction is consistent with the observation that NADPH exhibits a stimulatory effect on microsomal 5′-deiodination.[12a] This initial reaction is followed by a steady rate of reaction (after 4 min in Fig. 4), where **1** is reduced to the corresponding selenol (**5**) by HP-TrxR with a concomitant oxidation of NADPH (Scheme 1).

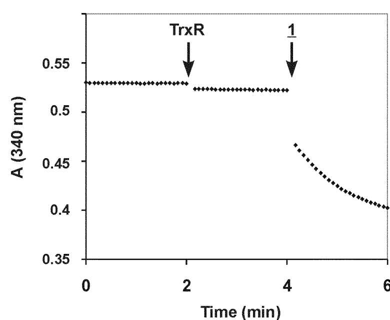


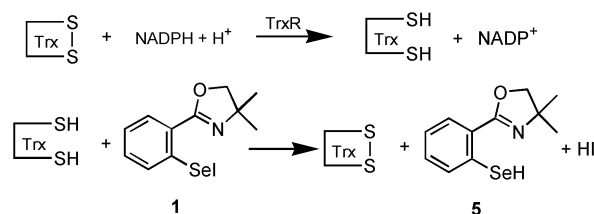
Fig. 4 Reduction of compound **1** by hTrxR. TrxR (460 nM) was added to 80 μM NADPH in 50 mM Tris–HCl, pH 7.4, 1 mM EDTA. After addition of **1** (33 μM, see arrows), a pronounced reaction occurred in the presence of NADPH.

This is consistent with a recent report that the Se–N bond in ebselen could be cleaved by mammalian TrxR to produce ebselen–selenol.[21] 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 **1** with NADPH alone showed considerable spectral changes when compared with the individual spectra of **1** and NADPH. Similar changes were also observed with NADH. However, while direct reduction of **1** was observed with both NADPH and NADH, the HP-TrxR-induced reduction of **1** was – as expected – only observed with NADPH (Fig. 4).

The rate of the reduction of **1** in the presence of HP-TrxR (Table 1. entry b, 1.45 μM min^{-1}) is higher than the rate of **1** with NADPH in the absence of HP-TrxR (0.48 μM min^{-1}), suggesting a catalytic role for HP-TrxR. Determination of the steady-state kinetic parameters, K_M and k_{cat} values, for the reaction of **1** with HP-TrxR was complicated by the low solubility of compound **1** at higher concentrations. When an excess amount of **1** was used, the reaction mixture precipitated, probably due to the low solubility of **1** in aqueous medium or the reaction of selenol **5** with selenenyl iodide **1**, leading to the formation of the corresponding diselenide. However, the V_{max} and K_M values for the reduction of **1** by HP-TrxR were determined from the initial rates (after addition of **1** at 4 min in Fig. 4) and are 14.8 ± 2.6 μM min^{-1} and 17.5 ± 5.7 μM, respectively. The apparent K_M value observed for this reaction is higher than that of human Trx (~2.5 μM), but smaller than the value for the reaction of HP-TrxR with *E. coli* Trx (25 μM).[13]

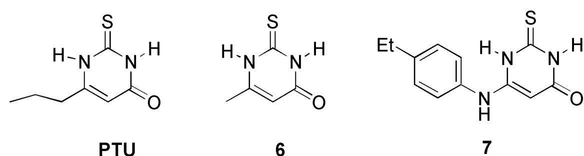
The TrxR from *E. coli* was less efficient (Table 1, entry e) in the reduction compared with the HP-TrxR (entry b). In the absence of Trx, the reduction rate for *E. coli* TrxR was found to be 6-fold lower than that of HP-TrxR. The higher activity of HP-TrxR as compared with the *E. coli* enzyme can be ascribed to the presence of a selenocysteine residue in the active center of the human enzyme. HP-TrxR (M_r approx. 55 kDa) belongs to a class of "high M_r TrxRs" which are more related to glutathione reductases (GRs) than to *E. coli* TrxR (M_r approx. 35 kDa).[8b,22] 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 M_r 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 is catalytically more favorable than the redox-active cysteines of the bacterial enzymes.[22,23] The selenol (or selenolate) group in HP-TrxR is, therefore, 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. Addition of 2 μM Trx only slightly enhanced the rate (Table 1, 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.[24] 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.[12b,25] This indicates that the reduced form of Trx may have a direct effect on the reduction as shown in Scheme 2.

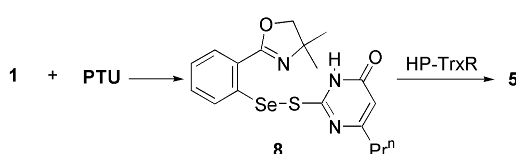


Scheme 2 Reaction of compound **1** with reduced Trx.

Interestingly, the anti-thyroid drug, 6-*n*-propyl-2-thiouracil (PTU), had no effect on the reduction of selenenyl iodide **1** by HP-TrxR although this drug has been shown to be an efficient inhibitor of Type I iodothyronine deiodinase.[26] PTU and related drugs (*e.g.* **6**,**7**) have been shown to inhibit the deiodinase activity by reacting with the selenenyl iodide intermediate (E–SeI) of the deiodinase cycle to form dead-end products (Fig. 2).[26,27]

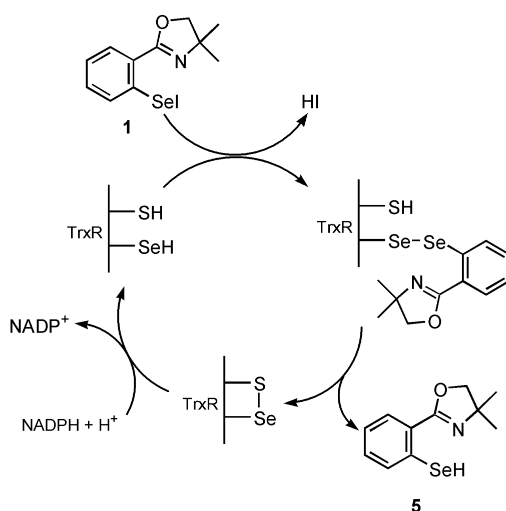


When 50 μM PTU was added to the mixture containing 50 nM HP-TrxR and 100 μM NADPH, there was no considerable change in the rate of the reaction as the initial rate observed in the absence of PTU was comparable to that of the reaction between HP-TrxR and **1** in the presence of PTU. These observations suggest two possibilities: i) the selenolate form of the enzyme may react with selenenyl iodide **1** much faster than PTU; ii) the reaction of selenenyl iodide **1** with PTU produces selenenyl sulfide **8** which could be reduced by HP-TrxR as shown in Scheme 3. Preliminary experiments on the reaction of an authentic sample of **8**[20a] with HP-TrxR indicate that the inhibition of deiodinase by PTU may become reversible in the presence of the Trx system.



Scheme 3 Reaction of **1** with HP-TrxR in the presence of PTU.

Based on our experimental data, a proposed reaction mechanism of HP-TrxR with selenenyl iodide **1** can be illustrated as shown in Scheme 4. According to this mechanism, the reduced form (selenol or selenolate) of HP-TrxR first reacts with selenenyl iodide **1** to form a diselenide. The internally available thiol group (Cys-495) of the enzyme attacks the selenium atom to produce an inter-selenenyl sulfide linkage by releasing selenol **5**. The Se–S bond in the selenenyl sulfide intermediate could be cleaved by the additional cysteine pair (not shown) and NADPH to regenerate the active site.



Scheme 4 Proposed mechanism for the reduction of **1** by TrxR.

Similar mechanisms have been proposed for the hydrogen peroxide reduction by mammalian TrxR involving selenenic acid and selenenyl sulfide intermediates. The *E. coli* TrxR, which does not have a selenocysteine in the active site, is expected to produce a disulfide intermediate instead of selenenyl sulfide during the catalysis. Since the mixed selenenyl sulfide bond can be reduced more easily by a thiol group than the disulfide bond, the activity of HP-TrxR is expected to be much higher than that of the *E. coli* enzyme.

In summary, 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[28] can serve as a cofactor for reactions catalysed by ID-I.

Acknowledgements

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References

1 J. L. Leonard and J. J. Visser, Biochemistry of Deiodinationin *Thyroid Hormone Metabolism*, ed. G. Heinemann, Marcel Dekker, New York, 1986, pp. 186–229.

2 (*a*) D. Behne, A. Kyriakopoulos, H. Meinhold and J. Köhrle, *Biochem. Biophys. Res. Commun.*, 1990, **173**, 1143; (*b*) M. J. Berry, L. Banu and P. R. Larsen, *Nature*, 1991, **349**, 438; (*c*) P. R. Larsen and M. J. Berry, *Annu. Rev. Nutr.*, 1995, **15**, 323; (*d*) D. L. St. Germain and V. A. Galton, *Thyroid*, 1997, **7**, 655; (*e*) J. Köhrle, *Biochimie*, 1999, **81**, 527; (*f*) J. L. Leonard and J. Köhrle, Intracellular Pathways of Iodothyronine Metabolism, in*The Thyroid*, eds L. E. Braverman, and R. D. Utiger, Lippincott-Williams & Wilkins, Philadelphia, 2000, pp. 136–173.

3 (*a*) T. J. Visser, *Mol. Cell. Endocrinol.*, 1978, **10**, 241; (*b*) J. L. Leonard and I. N. Rosenberg, *Endocrinology*, 1980, **106**, 444; (*c*) T. J. Visser, E. Kaptein, O. T. Terpstra and E. P. Krenning, *J. Clin. Endocrinol. Metab.*, 1988, **67**, 17; (*d*) N. Byamungu, K. Mol and E. R. Kuhn, *Gen. Comp. Endocrinol.*, 1992, **88**, 199; (*e*) For a recent review on thyroid hormone metabolism in brain and pituitary, see: J. Köhrle, *Acta Med Austriaca*, 2000, **27**, 1.

4 (*a*) M. J. Berry, J. D. Kieffer, J. W. Harney and P. R. Larsen, *J. Biol. Chem.*, 1991, **266**, 14155; (*b*) G. Lian, L. Ding, M. Chen, Z. Liu, D. Zhao and J. Ni, *J. Biol. Chem.*, 2001, **276**, 28037.

5 A. Goswami and I. N. Rosenberg, *Endocrinology*, 1983, **112**, 1180.

6 (*a*) C. H. Williams, Jr. in *Chemistry and Biochemistry of Flavoenzymes*, ed.F. Müller, CRC Press Inc., Boca Raton, FL, 1992, vol. 3, pp. 121–211; (*b*) A. Holmgren and M. Björnstedt, *Methods Enzymol.*, 1995, **252**, 199.

7 (*a*) H. Nakamura, K. Nakamura and J. Yodoi, *Ann. Rev. Immunol.*, 1997, **15**, 351; (*b*) E. S. Arnér and A. Holmgren, *Eur. J. Biochem.*, 2000, **267**, 6102.

8 (*a*) V. N. Gladyshev, K. T. Jeang and T. C. Stadtman, *Proc. Natl. Acad. Sci. U. S. A.*, 1996, **93**, 6146; (*b*) L. D. Arscott, S. Gromer, R. H. Schirmer, K. Becker and C. H. Williams, Jr., *Proc. Natl. Acad. Sci., U. S. A.*, 1997, **94**, 3621; (*c*) L. Zhong, E. S. J. Arnér, J. Ljung, F. Åslund and A. Holmgren, *J. Biol. Chem.*, 1998, **273**, 8581; (*d*) L. Zhong and A. Holmgren, *J. Biol. Chem.*, 2000, **275**, 18121; (*e*) 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.*, 2000, **97**, 2521; (*f*) S. M. Kanzok, R. H. Schimer, I. Türbachova, R. Iozef and K. Becker, *J. Biol. Chem.*, 2000, **275**, 40180; (*g*) T. Sandolova, L. Zhong, Y. Lindqvist, A. Holmgren and G. Schneider, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 9533.

9 (*a*) D. Nikitovic and A. Holmgren, *J. Biol. Chem.*, 1996, **271**, 19180; (*b*) J. M. May, S. Mendiratta, K. E. Hill and R. F. Burk, *J. Biol. Chem.*, 1997, **272**, 22607; (*c*) J. M. May, C. E. Cobb, S. Mendiratta, K. E. Hill and R. F. Burk, *J. Biol. Chem.*, 1998, **273**, 23039; (*d*) K. Becker, S. N. Savvidas, M. Keese, R. H. Schirmer and P. A. Karplus, *Nat. Struct. Biol.*, 1998, **5**, 267; (*e*) S. Gromer, R. H. Schirmer and K. Becker, *Redox Report*, 1999, **4**, 221; (*f*) G. E. Arteel, K. Briviba and H. Sies, *Chem. Res. Toxicol.*, 1999, **12**, 264; (*g*) H. E. Ganther, *Carcinogenisis*, 1999, **20**, 1657; (*h*) K. Becker, S. Gromer, R. H. Schirmer and S. Müller, *Eur. J. Biochem.*, 2000, **267**, 6118; (*i*) D. Mustacich and G. Powis, *Biochem. J.*, 2000, **346**, 1.

10 K. Becker, C. Herold-Mende, J. J. Park, G. Lowe and R. H. Schirmer, *J. Med. Chem.*, 2001, **44**, 2784.

11 A. K. Das, C. W. Hummel and P. G. Walfish, *Biochem. J.*, 1986, **240**, 559.
12 (*a*) A. Goswami and I. N. Rosenberg, *Endocrinology*, 1987, **121**, 1937; (*b*) G. B. Bhat, K. Iwase, C. W. Hummel and P. G. Walfish, *Biochem. J.*, 1989, **258**, 785; (*c*) J. Sharifi and D. L. St. Germain, *J. Biol. Chem.*, 1992, **267**, 12539.
13 S. Gromer, L. D. Arscott, C. H. Williams, Jr., R. H. Schirmer and K. Becker, *J. Biol. Chem.*, 1998, **273**, 20096.
14 G. Mugesh, A. Panda, H. B. Singh and R. J. Butcher, *Chem. Eur. J.*, 1999, **5**, 1411.
15 W.-W. du Mont and I. Wagner, *Chem. Ber*, 1988, **121**, 2109.
16 W.-W. du Mont, *Main Group Chemistry News*, 1994, **2**(3), 18.
17 (*a*) W.-W. du Mont, S. Kubiniok, K. Peters and H.-G. Schnering, *Angew. Chem.*, 1987, **99**, 820 (*Angew. Chem., Int. Ed. Engl.*, 1987, **26**, 780); (*b*) W.-W. du Mont, A. Martens von Salzen, S. Pohl and W. Saak, *Inorg. Chem.*, 1990, **29**, 4848.
18 T. M. Klapötke and J. Passmore, *Acc. Chem. Res.*, 1989, **22**, 234.
19 (*a*) G. Mugesh, H. B. Singh and R. J. Butcher, *Tetrahedron: Asymmetry*, 1999, **10**, 237; (*b*) A. Panda, G. Mugesh, H. B. Singh and R. J. Butcher, *Organometallics*, 1999, **18**, 1986; (*c*) Z. Majeed, W. R. McWhinnie and P. R. Lowe, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.*, 2000, **56**, e105.
20 (*a*) W.-W. du Mont, G. Mugesh, C. Wismach and P. G. Jones, *Angew. Chem.*, 2001, **113**, 2547 (*Angew. Chem., Int. Ed.*, 2001, **40**, 2486); (*b*) G. Mugesh, W.-W. du Mont, C. Wismach and P. G. Jones, *ChemBioChem*, 2002, **3**, 440.
21 R. Zhao, H. Masayasu and A. Holmgren, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 8579.
22 J. Kuriyan, T. S. R. Krishna, L. Wong, B. Guenther, A. Pahler and C. H. Williams, Jr., *Nature*, 1991, **352**, 172.
23 (*a*) S. Gromer, J. Wissing, D. Behne, K. Ashman, R. H. Schirmer, L. Flohé and K. Becker, *Biochem. J.*, 1998, **332**, 591; (*b*) 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.*, 2000, **97**, 2521; (*c*) L. Zhong, E. S. J. Arnér and A. Holmgren, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 5854; (*d*) S. Kanzok, A. Fechner, H. Bauer, J. K. Ulschmid, H. M. Müller, J. Botella-Munez, S. Schneuwly, R. H. Schirmer and K. Becker, *Science*, 2001, **291**, 643.
24 M. Björnstedt, M. Hamberg, S. Kumar, J. Xue and A. Holmgren, *J. Biol. Chem.*, 1995, **270**, 11761.
25 T. J. Visser, D. Fekkes, M. H. Otten, J. A. Mol, R. Docter and G. Hennemann, *Horm. Cell. Regul.*, 1984, **8**, 179.
26 (*a*) T. Nogimori, C. H. Emerson, L. E. Braverman, C.-F. Wu, J. Gambino and G. E. Wright, *J. Med. Chem.*, 1985, **28**, 1692; (*b*) J. Köhrle, *Methods Enzymol.*, 2002, **347**, 125.
27 (*a*) G. Lian, L. Ding, M. Chen, L. Liu, D. Zhao and J. Ni, *Biochem. Biophys. Res. Commun.*, 2001, **283**, 1007; (*b*) G. Lian, L. Ding, M. Chen, Z. Liu, D. Zhao and J. Ni, *J. Biol. Chem.*, 2001, **276**, 28037.
28 (*a*) J. Scharifi and D. L. Germain, *J. Biol. Chem.*, 1992, **267**, 12539; (*b*) A. F. Howie, J. R. Arthur, F. Nicol, S. W. Walker, S. G. Beech and G. J. Beckett, *J. Clin. Endocrinol. Metabol.*, 1998, **83**, 2052.