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Why Selenocysteine Replaces Cysteine in Thioredoxin Reductase: A Radical Hypothesis

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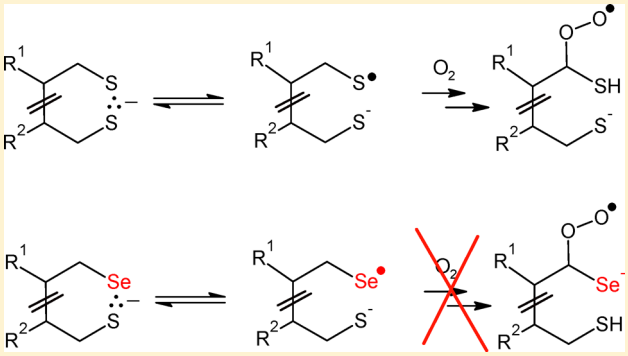
Supporting Information

ABSTRACT: Thioredoxin reductases, important biological redox mediators for two-electron transfers, contain either 2 cysteines or a cysteine (Cys) and a selenocysteine (Sec) at the active site. The incorporation of Sec is metabolically costly, and therefore surprising. We provide here a rationale: in the case of an accidental one-electron transfer to a S−S or a S−Se bond during catalysis, a thiyl or a selanyl radical, respectively would be formed. The thiyl radical can abstract a hydrogen from the protein backbone, which subsequently leads to the inactivation of the protein. In contrast, a selanyl radical will not abstract a hydrogen. Therefore, formation of Sec radicals in a GlyCys-SecGly active site will less likely result in the destruction of a protein compared to a GlyCysCysGly active site.



Selenium Can Replace Sulfur in Thioredoxin Reductase, But Why? Trx is a major cellular disulfide reductase and is, therefore, critical for the regulation of protein function and signaling via thiol redox control.[1] TrxRs are essential enzymes that catalyze the reduction of Trx by NADPH. The active site of the mammalian form of TrxR contains Sec in a conserved GlyCysSecGly quartet at the C-terminus, whereas TrxR in *Drosophila* and many other invertebrates contains the corresponding active-site residues SerCysCysSer. Although the activity of the *Drosophila* TrxR is comparable to that of the human enzyme,[2] the replacement of Sec with Cys in the mammalian TrxR drastically reduces (to ca. 5%) its catalytic efficiency.[3] Gromer et al. demonstrated that there is virtually no difference in activity between wild-type *Drosophila melanogaster* with the SerCysCysSer C-terminus and a mutant with the GlyCysSecGly C-terminus and that the presence of the Ser residues was important in the interaction with Trx of *Drosophila melanogaster*.[4] Since the insertion of Sec into a protein involves "costly and inefficient synthesis machinery",[4] and if there are no clear advantages to the use of Sec over Cys, why has selection favored Sec in the mammalian TrxR?

Experimentally, it has been found that Sec-containing proteins are more stable: glutathione peroxidase contains a single Se as an active site. Rocher et al.[5] found that this enzyme is easily inactivated by its natural substrate, peroxides, if the Se is replaced by S. Since this report, additional reports have appeared that show that selenoproteins are more resistant to oxidative stress than are their sulfur analogues.[6] In vivo and under normal conditions, that is, redox homeostasis, one might argue that such a protective effect of Se is not needed in the case of TrxR. For example, if in a GlyCysCysGly active site one of the cysteines reacts with H_2O_2, a sulfenic acid is formed next to the remaining thiol. Sulfenic acids are known to react rapidly with thiols to form disulfides, that is, the oxidized form of the protein and water. Hondal and co-workers[7] exposed different TrxRs to a variety of one-electron and two-electron oxidants in vitro. They observed that the two seleno varieties they examined were less inactivated by $HO^{\bullet}$ than were the sulfur ones.

$RS^{\bullet}$ is Dangerous. Thiyl radicals can be formed in the protein either by a one-electron oxidation of a thiol, for example with $HO^{\bullet}$, or via a one-electron reduction of a disulfide. During the redox-cycle of TrxR, the active site disulfide or selenosulfide bond in TrxR is reduced following the reduction of the proximal disulfide bond by NADH, a reaction that takes place via a long-range, or outer-sphere, two-electron transfer. Such reactions take place as two consecutive one-electron transfers. The second transfer may occur very shortly after the first. In studies of a model compound for hydrogenase, such a rapid transfer of a second electron was demonstrated,[8] and other examples are known. Clearly, if the second electron is diverted, a one-electron reduction of the disulfide occurs. Another scenario is that if NADH bound to TrxR is oxidized by a mild one-electron oxidant, then $NAD^{\bullet}$ is formed, which is a strong reductant (−0.94 V,[9,10]) and may reduce to some extent the proximal disulfide by one electron. Thus, a one-electron reduction may occur, which in a SerCysCysSer active site, yields $RS^{\bullet}$ next to RSH, in equilibrium with $RSSR^{\bullet-}$ and H^+. The $RSSR^{\bullet-}$ radical is unlikely to damage an amino acid, while

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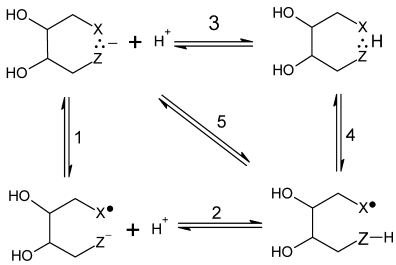
RS$^{\bullet}$ has been shown to abstract hydrogen intra- and intermolecularly.[11−16] The resulting C-centered radicals could reform the thiyl radical via the reverse reaction or react with dioxygen to yield peroxyl radicals, which react further and may ultimately destroy the protein.[11,17,18] Intramolecular "repair" of an α-C-centered radical by the thiol could lead to racemization and a mixture of the D- and L-forms of the amino acid.[17,19] Thus, a one-electron transfer may result in irreversible damage to the protein; therefore, even if a one-electron transfer is a rare event, the longevity of the protein may be considerably reduced.

With the help of the oxidation state diagrams derived in this work, Figures 1 and 2, we can calculate the equilibrium speciation of radical species that are likely to be involved in the TrxR reaction, see Figure 3. As shown, the diselanyl radical anion, RSeSeR$^{\bullet-}$, is better stabilized with respect to its reactants than the corresponding RSSR$^{\bullet-}$ radical anion. In the case of RSSR$^{\bullet-}$, even under conditions of high concentrations of Cys, there are considerable levels of free thiyl radicals present, which could be hazardous for a protein as pointed out above. In contrast, RSSR$^{\bullet-}$ and, most likely, RSeSeR$^{\bullet-}$ anions may transfer their electrons to dioxygen,[20] and the subsequent superoxide would be disposed of by superoxide dismutase.[21,22]

The following question now arises, why are harmful radical reactions avoided by the substitution of Cys by Sec? To answer that, we further characterize the reactivity of the SeS bond.

We combine the earlier experimental investigations of DTT and its mono- and diselenium analogues[23] with thermodynamic considerations to show that the very different reactivities of selenium and sulfur radicals confer upon the selenoprotein, the singular advantage of confining any radical formed at the active site of TrxR to the selenium, and that the selenyl radical cannot propagate damage.

Scheme 1. Reactions 1−5: Radical Equilibria of DTT$^{\bullet-}$ and Its Se Derivatives[a]



a: X = Se, Z = S (SeTT radicals)
b: X = S, Z = S (DTT radicals)
c: X = Se, Z = Se (DSeT radicals)

[a]The equilibria of interest are described by K_1. Experimentally reported in the literature is pK_{5b} = 5.2.[26] We determined the corresponding constants for DSeT$^{\bullet-}$ and SeTT$^{\bullet-}$; pK_{5a} = 2.4 and pK_{5c} = 7.4.[23]

RESULTS AND DISCUSSION

The Model. We chose SeTT, the Se/S analogue of DTT, as a model compound to study the Se−S bond as well as DSeT, compounds that are well-defined and soluble in water.[23] To help us understand the stabilization versus destabilization of one-electron oxidized DTT-analogue species, we consider the equilibria of the two-center three-electron-bonded species of DTT (S,S), SeTT (Se,S), and DSeT(Se,Se) (Figure 2, left panel). Proton-binding equilibria of open and closed forms of the radicals of DTT and its selenium derivatives DSeT and SeTT are shown in Scheme 1. The thermodynamic properties of the species that we derive and discuss below are shown in Table 1, together with values from the literature. We determined that pK_{5a} = 2.4 and pK_{5c} = 7.4 for DSeT$^{\bullet-}$ and SeTT$^{\bullet-}$.[23] Given that E° (RS$^{\bullet}$, H$^+$/RSH) > E° (RSe$^{\bullet}$/H$^+$/RSeH) (Figure 1), the species (RSeSR)$^{\bullet-}$ is in equilibrium (K_{1a}) with RSe$^{\bullet}$ and RS$^-$, Scheme 1. We assume that the pK_{2a} for SeTT, which describes the dissociation of H$^+$ from RSH, is equal to 9.1, as is the first pK_a of DTT,[24] p$K_{2b,\ DTT}$ = p$K_{2a,\ SeTT}$ = 9.1. We determined the pK_a values for DSeT (6.1 and 7.1) and the first pK_a of SeTT (6.2) (see ref 25 and also the Supporting Information). Because the first pK_a of DSeT is 6.1, we use pK_{2c} = 6.1; further, pK_5 = pK_1 + pK_2 = pK_3 + pK_4; thus, $\log_{10}(K_{1a,\ SeTT})$ = p$K_{2b,\ DTT}$ − p$K_{5a,\ SeTT}$ = 1.7.

The values for K_1 are then K_{1a} (SeTT$^{\bullet-}$) = 50, K_{1b} (DTT$^{\bullet-}$) = 7.9 × 10^3, and K_{1c} (DSeT$^{\bullet-}$) = 5.0 × 10^3. Note that in our model compounds (Figure 2) the bonds are *intra*molecular, whereas in the reaction between Cys and Sec (Figure 1) the bonds are *inter*molecular. In the former case, there is a higher probability for the formation of the Se−S, Se−Se, and S−S two-center three-electron bonds, and the resulting equilibrium constants are not directly comparable to the *inter*molecular equilibrium constants. However, the ratio K_{1a}/K_{6a} should be equal to K_{1b}/K_{6b} and K_{1c}/K_{6c}, provided that the difference between the ring strain of DTT and its Se-analogues is negligible (see later):

$$RSe^{\bullet} + RS^{-} = (RSeSR)^{\bullet-} \quad (6a)$$

$$RS^{\bullet} + RS^{-} = (RSSR)^{\bullet-} \quad (6b)$$

$$RSe^{\bullet} + RSe^{-} = (RSeSeR)^{\bullet-} \quad (6c)$$

***Inter*molecular S−S and S−Se Bonds.** With pK_{5b} = 5.2,[26] pK_{5c} = 2.4,[23] K_{6b} = 1.2 × 10^3 M^{-1} [27] (K_{6b} (pH 7) = 45 M^{-1}),[27] and K_{6c} = 2 × 10^3 M^{-1} [28] we find that the $\log_{10}(K_1/K_6)$ = pK_2 − pK_5 − $\log_{10}(K_6)$ = 0.8 and 0.4 for (S,S) and (Se,Se), respectively, which confirms our assumption that the difference in ring strain for the three models is negligible. This result is important because it implies that the considerable difference in bond lengths between S−S and Se−Se, 2.00 and 2.33 Å, respectively,[29] appears not to influence our thermodynamic considerations. We, therefore, assume that the different lengths of (S.∴S)$^{\bullet-}$, (S.∴Se)$^{\bullet-}$, and (Se.∴Se)$^{\bullet-}$ three-electron bonds also have no influence. With the $\log_{10}(K_1/K_6)$ = 0.6 ± 0.2, K_{6a} equals 8−20 M^{-1} from the $\log_{10}(K_{6a})$ = pK_{2b} − pK_{5a} − (0.6 ± 0.2) = 1.1 ± 0.2, which is 2 orders of magnitude lower than the values of K_{6b} or K_{6c}.

The two-center three-electron bond between sulfur and selenium (K_{6a} = 20 M^{-1}, ΔG° = −7.4 kJ/mol) is much weaker than the corresponding (S.∴S)$^{\bullet-}$ (−19.8 kJ/mol) or (Se.∴Se)$^{\bullet-}$ (−18.8 kJ/mol) bond.

Since the K_{6b} value for Cys is somewhat larger than the corresponding value for glutathione,[22] we may safely assume that the values we derived above are also representative for *inter*molecular equilibria found between peptides and proteins, specifically between Trx and TrxR.

To place (Cys.∴Sec)$^{\bullet-}$ in Figure 1, we derived $E^{\circ\prime}$ for the couple ((Cys.∴Sec)$^{\bullet-}$,H$^+$/CysH,Sec$^-$) by combining eqs 7, 8, and 9:

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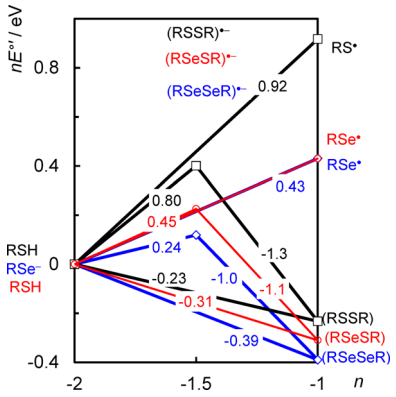


Figure 1. Oxidation state diagram of Sec and Cys at pH 7[30] (with additional data from ref 28). With n representing the oxidation state, the slope connecting two points in the diagram represents the electrode potential $\Delta E^{\circ\prime}$ of the corresponding reaction. For example, $E^{\circ\prime}(RS^{\bullet}/RSH) = 0.92$ V.[33] Since $nE^{\circ\prime}$ is proportional to $-\Delta G^{\circ\prime}$, favorable products lie below the line connecting the reactants. For example, $(RSeSeR)^{\bullet-}$ is favored over $RSe^{\bullet}$ and RSe^{-}. Likewise, $(RSeSR)^{\bullet-}$ is disfavored over RSe^{-} and RSeSR.

$$Cys^{-} + H^{+} \rightarrow CysH \text{ (from pH 7, p}K = 8.5) \quad -8.6 \text{ kJ/mol} \quad (7)$$

$$Sec^{\bullet} + e^{-} \rightarrow Sec^{-} \quad E^{\circ} = +0.43 \text{ V } (30) \quad -41.5 \text{ kJ/mol} \quad (8)$$

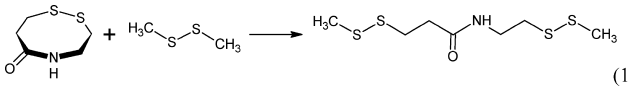
$$(Cys.\therefore Sec)^{\bullet-} \rightarrow Sec^{\bullet} + Cys^{-} \quad \text{(obtained here)} \quad +5.2 - 7.4 \text{ kJ/mol} \quad (9)$$

$$(Cys.\therefore Sec)^{\bullet-} + e^{-} + H^{+} \rightarrow Sec^{-} + CysH \quad -44 \pm 1 \text{ kJ/mol}, \quad (10)$$

$$\text{or } E^{\circ\prime}(\text{pH 7}) = +0.45 \pm 0.01 \text{ V}$$

In Figure 1, $(Cys.\therefore Sec)^{\bullet-}$ lies above the line that joins RSH, RSe^{-} with RSH, $RSe^{\bullet}$ and is thus unstable with respect to decomposition to Cys and the selanyl radical.

***Intra*molecular S−S and S−Se Bonds.** When we consider the situation in a protein that contains a disulfide, diselanide, or a mixed S−Se bond; the radical anions $(S.\therefore S)^{\bullet-}$, $(Se.\therefore Se)^{\bullet-}$, or $(Se.\therefore S)^{\bullet-}$ may be formed with an *intra*molecular bond. This more resembles our model compounds, except for the ring size. In TrxR, an eight-membered ring will be formed, which will result in strain that decreases the stability of the three-electron bond. Similarly, a covalent bond between sulfur atoms of two neighboring Cys's results in an eight-membered ring that has some strain: the ab initio calculations for reaction 11 suggest that this strain amounts to 18 kJ/mol.[23] We therefore expect a strain of about 18 kJ/mol to affect three-electron two-centered bonds (Figure 2, right panel).



(11)

The following scenarios are possible (Figure 3): (i) In the RCysCysR active site, where $(S.\therefore S)^{\bullet-}$ is formed initially, the probability is high that the thiyl radical, which is in equilibrium with $(S.\therefore S)^{\bullet-}$ but will be present in 99% yield, abstracts a hydrogen from a carbon of the protein backbone, which may ultimately lead to the destruction of the protein backbone. (ii) In a RSecSecR active site, the formation of the strongly reducing diselanide radical anion, $(Se.\therefore Se)^{\bullet-}$, is favored (56%). Depending on the local oxygen concentration, the radical anion could reduce oxygen to the superoxide radical. At low oxygen concentrations, the radical is so reducing (Figure 2) that it may pose a currently unknown threat to the protein. (iii) One electron reduction of RCysSecR active site residues gives rise to selenocysteinyl radicals (>99.9%) with the unpaired electron residing at the Se. In this case, we will now show that the $-Se^{\bullet}$ does not attack the protein backbone and might eventually be "repaired" by ascorbate. Alternatively, like the thiyl radical, selanyl radicals might react reversibly with dioxygen to form $SeOO^{\bullet}$.

Given a simple relation between electrode potentials and bond energy,[31] the Sec radical can abstract a hydrogen from a C−H bond with a BDE of 311 kJ/mol or less. Experimentally, these C−H BDEs are >370 kJ/mol; thus, the abstraction of an α−hydrogen is unfavorable by about 60 kJ/mol. By comparison, the abstraction of such a hydrogen by a cysteinyl radical is roughly thermoneutral. Although unfavorable by approximately 60 kJ/mol, an α-hydrogen abstraction could, in principle, be forced by the rapid removal of the product. An example could be the reaction of dioxygen with the α−C radical (see also ref 17). In the case of thiyl hydrogen abstraction, the forward reaction is of the order of 10^4 to 10^5 s^{-1}, with an equilibrium constant $K_S \approx 0.1$.[18] The abstraction by a selanyl radical is expected to be much slower. To estimate this rate constant, let us consider *intra*molecular hydrogen abstractions at a carbon:

$$H{-}CR'R''RS^{\bullet} = {}^{\bullet}CR'R''RS{-}H \quad (12)$$

$$H{-}CR'R''RSe^{\bullet} = {}^{\bullet}CR'R''RSe{-}H \quad (13)$$

The difference in Gibbs energies of reactions 12 and 13 is governed by the difference in the BDEs of the −S−H and −Se−H bonds, since the entropy terms are very similar. Thus, $\Delta G^{\circ}_{12} - BDE(RS{-}H) \approx \Delta G^{\circ}_{13} - BDE(RSe{-}H)$, and the equilibrium constant for the hydrogen abstraction by selanyl radicals is $K_{13} \approx K_{12} \times e^{-(\Delta BDE/RT)} \approx 10^{-11}$, with $\Delta BDE = BDE(RS\text{-}H) - BDE(RSe\text{-}H) = +55$ kJ/mol.[30] There is no simple equation that would link k_{12} to k_{13}. To derive an upper limit for k_{13}, we make the assumption that k_{-13}, the abstraction of a hydrogen from the selenol by a carbon-centered radical, happens at every encounter during an intramolecular rotation. The rate constant k_{-13} would then be close to the internal rotation speed, which is on the order of 10^{11} s^{-1}.[32] It follows that the abstraction of a hydrogen by a selanyl radical proceeds with a rate of k_{13} that is maximally on the order of 1 s^{-1}, which is essentially negligible. Thiyl radicals may, in principle, oxidize tyrosine and tryptophan while selanyl radicals will not. At pH 7, the $RS^{\bullet}$, H^{+}/RSH couple has an electrode potential of 0.92 V (Figure 1)[33], while the potentials of the tyrosyl and the tryptophanyl radicals are around 0.93 and 1.0 V,[9] respectively. Therefore, in equilibrium, one would expect around 10%−50% of the radicals to be located on the aromatic amino acid. The intramolecular redox equilibration of thiyl radicals with aromatic amino acids, however, is much slower than reaction 12 (Nauser, T.; Carreras, A.; Koppenol, W. H., 2014, unpublished work). Because of its low electrode potential of 0.43 V (Figure 1),[30] the selanyl radical will not oxidize aromatic amino acids. Indeed, selenols are efficient scavengers of tyrosyl radicals.[28]

It is generally assumed that O_2 will oxidize the two-center three-electron radical anions. We have shown here that such an anion is formed in a substantial yield only between $Se^{\bullet}$ and Se^{-}. O_2 is expected to oxidize the diselenide radical anion quickly and quantitatively. In the corresponding (S,S) species, thiyl

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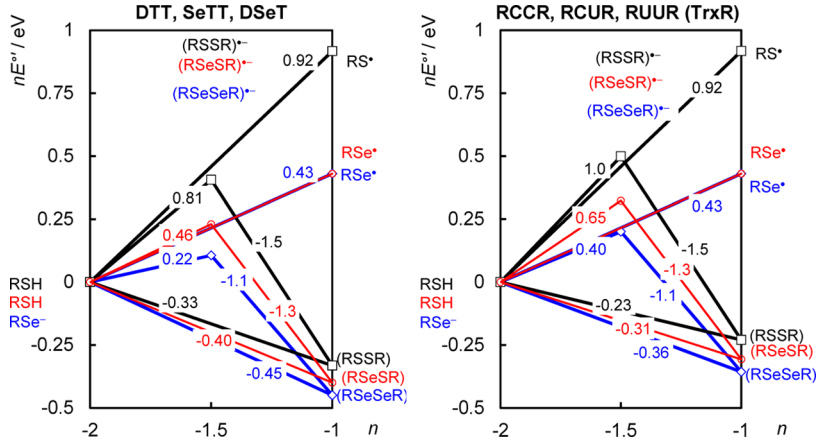

Figure 2. Oxidation state diagram for S/Se in ring structures. Left panel: DTT (black), SeTT (red), and DSeT (blue). Right panel: in a protein with consecutive CysCys (RCCR), CysSec (RCUR), or SecSec (RUUR) an eight membered ring would be formed with a ring strain of 18 kJ/mol, which changes the location and thus the potentials for the oxidation states $n = -1$ (+93 mV) and $n = -1.5$ (+187 mV). Under these conditions, both $(S^{\therefore}S)^{\bullet-}$ and $(Se^{\therefore}S)^{\bullet-}$ are not stable since they lie above the line that connects (RSH + RSe^-) and ($RSe^\bullet$ + RSH).

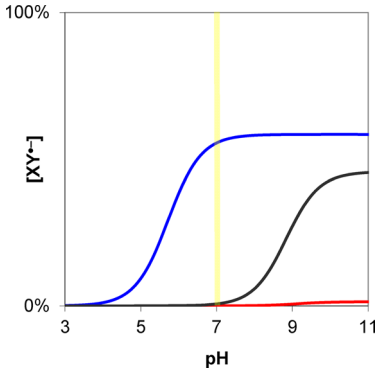

Figure 3. Speciation diagram for sulfur- and selenium-centered radicals of CysCys, CysSec, or SecSec. The calculated yields of $RSeSeR^{\bullet-}$ (blue, $K_{6c} = 1.4$), $RSSR^{\bullet-}$ (black, $K_{6b} = 0.84$), and $RSeSR^{\bullet-}$ (red, $K_{6a} = 1.4 \times 10^{-2}$) are plotted. Under all conditions, significant amounts of thiyl or selanyl radicals are formed. In contrast to the case of unstrained compounds (DTT, SeTT, DSeT),[23] which are protected by the formation of three electron–two center bonded radical anions, such species will not protect a peptide sequence with two successive cysteines. Because dissulfide anion formation in CysCys is negligible at pH 7, hydrogen abstraction from the peptide backbone and from the side chains by thiyl radicals is expected.[12] Selanyl radicals in CysSec will not attack the peptide backbone.

radicals will be formed quantitatively, which will damage the protein as discussed above. In TrxR, which has a GlyCysSecGly active site, a selanyl radical could be repaired by the reduction or by the addition of O_2 to a $SeOO^\bullet$ radical. We suspect that this radical might react with the adjacent thiol to form a Se–S bond, $O_2^{\bullet-}$, and H^+. Alternatively, selanyl radicals could be directly repaired by ascorbate with a rate constant of $k = (8 \pm 2) \times 10^7\ M^{-1}\ s^{-1}$.[23] In the presence of physiological amounts of ascorbate, 1 mM, and when the selanyl radical is accessible, we calculate a half-life for repair of ~10 μs.

The biosynthetic energy expenditure to incorporate selenium in the RSecSecR active site is significantly higher than that to construct the RCysCysR-type site. The cost of building an RCysSecR C-terminal active site is intermediate. Our findings predict a strong protective effect of Se, should radicals be formed in or near such an enzyme, which is in agreement with experimental data.[7] Our thermodynamic considerations allow us to speculate that the longevity of TrxR can be significantly enhanced by the presence of Se, enough so as to overcome the disadvantage conferred by the higher biosynthetic costs. Our hypothesis applies to chalcogen–chalcogen bonds with significant strain, as in TrxR. In the absence of such a strain, a three-electron two-centered radical anion is quantitatively formed, and our considerations may not apply. It should be noted that Sec will not only protect from oxidative damage, but

Table 1. Thermodynamic Properties of the Species Discussed

						$E^{\circ\prime}$ (pH 7)/V			
compound	(X,Z)	pK_2	pK_5	K_1	K_6	$X^\bullet + 1e^-$	$[X^{\therefore}Z]^{\bullet-} + 1e^-$	$[X\text{-}Z] + 1e^-$	$[X\text{-}Z] + 2e^-$
SeTT	Se,S	6.1[a,c]	7.4[b]	50[c]		0.43[d]	0.46[c]	−1.3[c]	−0.39[b]
DTT	S,S	9.1[h]	5.2[g]	7.9 × 10^3[c]		0.92[i]	0.81[c]	−1.5[c]	−0.33[k]
DSeT	Se,Se	6.1[a,c]	2.4[b]	5.0 × 10^3[c]		0.43[d]	0.22[c]	−1.1[c]	−0.46[b]
Sec + Cys	Se,S	5.3[f]			20[c]	0.43[d]	0.45[c]	−1.1[c]	−0.31[l]
Cys + Cys	S,S	8.4[f]			1.2 × 10^3[e]	0.92[i]	0.80[c]	−1.3[c]	−0.23[j]
Sec + Sec	Se,Se	5.3[f]			2.0 × 10^3[m]	0.43[d]	0.24[c]	−1.0[c]	−0.39[d]
SecCys	Se,S	5.3[f]		1.4 × 10^{-2}[c]		0.43[d]	0.65[c]	−1.3[c]	−0.30[c]
CysCys	S,S	8.4[f]		0.84[c]		0.92[i]	1.0[c]	−1.5[c]	−0.24[c]
SecSec	Se,Se	5.3[f]		1.4[c]		0.43[d]	0.40[c]	−1.1[c]	−0.37[c]

[a]See the Supporting Information. [b]Nauser et al., 2012.[23] [c]This work. [d]Nauser et al., 2006.[30] [e]Mezyk et al., 1996.[27] [f]Huber et al., 1967.[35] [g]Akhlag et al., 1987.[26] [h]Whitesides et al., 1983.[24] [i]Madej et al., 2007.[33] [j]Jocelyn et al., 1967.[36] [k]Cleland et al., 1964.[37] [l]Steinmann et al., 2010.[34] [m]Steinmann et al., 2008.[28]

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it has also kinetic advantages over Cys,[34] and it can be used to tune electrode potentials and the solvent exposure of an active site.[23] Therefore, the dominant mechanistic benefit of using Sec instead of Cys will vary from protein to protein.

■ ASSOCIATED CONTENT

Supporting Information

Diagrams of the pH-titration of DSeT and SeTT data published in ref 25. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

Trx, thioredoxin; TrxR, thioredoxin reductase; DTT, dithiothreitol, 2,3-dihydroxy-1,4-dimercaptobutane; oxidized DTT, 3,4-dihydroxy-1,2-dithiacyclohexane; DSeT, diselenothreitol, 2,3-dihydroxy-1,4-diselanylbutane; SeTT, selenothiothreitol, 2,3-dihydroxy-4-mercapto-1-selanylbutane; Sec, selenocysteine, U; Cys, cysteine, C; Ser, serine, S; Gly, glycine, G; BDE, bond dissociation energy; $Hasc^-$, monohydrogenascorbate

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