Catalysis of Electron Transfer by Selenocysteine[†]

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ABSTRACT: Selenium is an essential element that is involved in biological redox processes. The electrode potentials of the selenocysteine half-reactions $RSe^{\cdot} + e^- \rightarrow RSe^-$, $(RSeSeR)^{\cdot -} + e^- \rightarrow 2\ RSe^-$, and $RSeSeR + 2\ e^- \rightarrow 2\ RSe^-$ [$E^{\circ\prime}$ (pH 7)] are +0.43, +0.18, and −0.38 V, respectively, at pH 7. The spectra of $RSe^{\cdot}$ and $(RSeSeR)^{\cdot -}$ are characterized by absorption maxima at 460 nm ($\epsilon = 560\ M^{-1}\ cm^{-1}$) and 455 nm ($\epsilon = 7100\ M^{-1}\ cm^{-1}$), respectively. The bond dissociation energy of RSe−H has been calculated, and the value of 310 kJ/mol is in agreement with literature values. In comparison with the sulfur analogue cysteine, the more facile accessibility of the radical oxidation state is striking and may have biological implications, such as in mediation of one-electron- and two-electron-transfer processes, as illustrated by catalysis by selenocysteine of the electron transfer between dithiothreitol and benzyl viologen.

Selenium is an essential trace element in the human body. It is present in several enzymes. Of some the function is known, of others it is still debated (*1*−*4*). Thioredoxin reductase is an example of a protein in which selenocysteine (Sec)[1] is critical for redox activity, which appears to be vital.[2] Selanyls and sulfanyls differ in two aspects, namely, pK_a and oxidizability. Selanyls [pK_a(Sec) = 5.24 (*6*)], being more acidic than sulfanyls [pK_a(Cys) = 8.53 (*7*)], are, under biological conditions, dissociated and, thus, better nucleophiles, which may be one reason that certain biological functions are mediated by Sec. Differences in another thermodynamic property, the electrode potential, may play a role in why certain reactions take place and others do not and could serve as the basis for testable hypotheses.

There have been four determinations of the two-electron electrode potentials of the diselenide (RSe−SeR)/selanide (RSe^-) couple. The reported values for pH 7 vary from −360 mV for selenocystamine to −490 mV for Sec (*4*, *6*−*9*); such a large difference for such similar species is counterintuitive. No information is available for the one-electron reduction couple $RSe^{\cdot}/RSe^-$. To explore the thermodynamics of the redox chemistry of Sec, we studied the electrode potential, bond dissociation energy (BDE), and spectral and kinetics properties of Sec and compared them with those of cysteine. We show that Sec catalyzes electron transfer between the two-electron reductant dithiothreitol (DTT) and the one-electron acceptor benzyl viologen (BV^{2+}).

EXPERIMENTAL PROCEDURES

Chemicals. Millpore MilliQ water (18.2 MΩ) was used in all experiments. Chemicals of the highest commercially available quality were used as received. Selenocystine (Sec_{ox}), 1,4-dimercaptobutane-2,3-diol (DTT), ammonia, and sulfuric acid were purchased from Fluka (Buchs SG, Switzerland); 1,1′-dibenzyl-4,4′-bipyridinium dichloride (BV^{2+}) was purchased from Aldrich (Buchs SG, Switzerland); and sodium tetrahydroborate was purchased from Fisher Scientific (Wohlen, Switzerland). Argon (5.0) and dinitrogen monoxide (5.0) were supplied by PanGas (Zürich, Switzerland).

Preparation of Sec. In a 3 mL glass vial, a slurry of 38 mg of yellow Sec_{ox} and 13 mg of $NaBH_4$ in 100 μL of NH_3 (13 M) and 100 μL of H_2O were stirred with a small magnetic stirrer until the yellow slurry became a transparent, almost colorless, solution. Then, 800 μL of H_2O and 50 μL of H_2SO_4 (18 M) were added, and the solutions were diluted to appropriate concentrations, transferred to Schlenk tubes, sealed with rubber stoppers with sleeves, and immediately degassed to prevent reoxidation of the selanyl. We avoided DTT and transition metals as reductants, because we assumed that these compounds would interfere with our measurements. Under acidic conditions, the oxidation of $NaBH_4$ to $B(OH)_3$ and H_2 is accelerated (*10*). Hydrogen gas is easily removed by degassing, and borate buffer is assumed to be unreactive under our experimental conditions.

UV/Vis Spectrophotometry. Spectra were collected with a UVIKON 820 dual-beam spectrophotometer (Kontron AG, Zürich, Switzerland). Stock solutions of oxygen-sensitive compounds were prepared in argon-saturated water in Schlenk tubes and kept under argon at all times. Appropriate amounts were transferred to a 1 cm quartz fluorescence cell (Hellma GmbH and Co. KG, Müllheim, Germany) equipped

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[1] Abbreviations and Systmatic Names: BV^{2+}, 1,1′-dibenzyl-4,4′-bipyridinium^{2+} or benzyl viologen; DTT, 1,4-dimercapto-2,3-butanediol or dithiothreitol; DTT_{ox}, 1,2-dithiane-4,5-diol; Sec, selenocysteine (according to IUPAC/IUBMB, see ref *5*); H_2Se, selane; HSeSeH, diselane; $HSe^{\cdot}$, hydridoselenium$^{\cdot}$ (selanyl radical); $(HSeSeH)^{\cdot -}$, bis(hydridoselanide)$(Se-Se)^{\cdot -}$ or diselanuidyl (diselane radical anion); −SeH, selanyl; −SH, sulfanyl; $N_3^{\cdot}$, trinitrogen$^{\cdot}$.

[2] "Being the only cellular device to reduce thioredoxins, thioredoxin reductases should be of vital importance, because genetic disruption of thioredoxin causes embryonic lethality" (*1*).

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with a high-vacuum valve. After the addition of all solutions, the cell was evacuated (20 mbar) and flushed with argon repeatedly to minimize reductant consumption by dioxygen. Repeated spectra were recorded until equilibrium was reached.

Electrochemsitry. Cyclic voltammograms were recorded at a high sweep rate of 20 V/s with an AMEL 2049 Potentiostat with an AMEL 568 programmable waveform generator (AMEL SPA, Milan, Italy), controlled by a PC fitted with a 60 kHz 12 bit ADC card for data acquisition. The reaction is not fully reversible because of radical−radical recombination. At lower sweep rates, we were unable to reproducibly observe a peak in the reductive wave. The three-electrode system consisted of a graphite working electrode (Metrohm Ultra Trace, Metrohm AG, Herisau, Switzerland), a glassy carbon counter electrode (Metrohm AG), and an Ag/AgCl reference electrode (3 M KCl, +0.200 V versus NHE, Metrohm AG). The reference electrode made contact with a solution containing 1 mM Sec and 0.1 M phosphate buffer at pH 2.8 through a salt bridge fitted with low-leakage frits and filled with 1.5 M $(NH_4)_2SO_4$. The same experimental setup was found to be unsuitable for the study of the two-electron reduction of Sec_{ox} because of adsorption to the electrode; adsorption was even more problematic when metal (platinum or gold) electrodes were used. Errors are reported as standard deviations.

Laser-Flash Photolysis. An Applied Photophysics (Leatherhead, U.K.) LKS 50 instrument, equipped with a Quantel (Les Ulis, France) Brilliant B YAG laser, of which we used the 4th harmonic (266 nm, 5 ns pulses), fitted with a vacuum-tight fluorescence cell (see above) was used. The pulse energy was generally kept below 20 mJ/pulse to avoid water photolysis (*11*).

Pulse Radiolysis. A Febetron 705 (Titan Systems Corp., San Leandro, CA) 2.3 MeV accelerator with a pulse width (fwhm) of <50 ns was used as the radiation source. Doses between 10 and 50 Gy/Pulse were applied. The optical system consists of a 75 W Xe arc lamp (Hamamatsu, Schüpfen, Switzerland), a 1- or 2-cm-optical-path quartz cell (Hellma GmbH and Co. KG, Müllheim, Germany), and an Acton SP300i monochromator (Roper Scientific, Ottobrunn, Germany). For signal detection, we used either a R928 photomultiplier (Hamamatsu) with a DHPCA-200 amplifier (Femto Messtechnik GmbH, Berlin, Germany) with a DL7100 digital storage oscilloscope (Yokogawa Electric Corporation, Tokyo, Japan) for kinetics traces or a Princeton Instruments PI-MAX 512T gateable ICCD camera (Roper Scientific) for time-resolved spectra.

RESULTS

The one-electron electrode potential of the $NH_3^+CH(COO^-)CH_2Se^{\cdot}$/Sec couple was determined by cyclic voltammetry (data not shown). The half-wave potential for the one-electron oxidation of Sec at pH 2.8 is (405 ± 20) mV versus Ag/AgCl; therefore, E° = 770 mV and $E^{\circ\prime}$ (pH 7) = 430 mV versus NHE.

The mediation of one- and two-electron transfers by Sec_{ox} and Sec was investigated with BV^{2+}. The addition of a trace amount of Sec_{ox} to a 100 μM solution of colorless BV^{2+} at pH 9 in the presence of 2 mM DTT led to the development of a purple color, because of the formation of $BV^{\cdot+}$, after only 15 s (Figure 1), whereas color development in the absence of Sec_{ox} required ca. 2 min. The color development was not visibly accelerated by the addition of Cys. Development of a purple color was also observed in an anaerobic, slightly alkaline (pH 9) solution of 2 mM Sec and 200 μM BV^{2+}; upon acidification, the solution returned to the colorless state (i.e., purple $BV^{\cdot+}$ was reoxidized to colorless BV^{2+}). We determined the equilibrium constant $K = ([Sec_{ox}][BV^{\cdot+}]^2)/([Sec]^2[BV^{2+}]^2) \approx 0.6$ for a solution with initial concentrations of 2.5 mM Sec and 110 μM BV^{2+} by UV spectroscopy and obtained an estimate of approximately −350 mV for the two-electron electrode potential of the Sec_{ox}/Sec couple at pH 7. Additionally, we determined the equilibrium constant $K = ([Sec_{ox}][DTT])/([Sec]^2[DTT_{ox}])$ of 2.0 mM DTT/1.1 mM Sec_{ox} at different pH values (Table 1). From these values and on the basis of pK_a values of 9.26 and 10.34 for DTT (*12*), the electrode potential of DTT, $E^{\circ\prime}(DTT_{ox}/DTT, pH 7)$, is −332 mV (*13*),[3] and on a pK_a of 5.24 for the selanyl group in Sec (*6*), the electrode potential of the Sec_{ox}/Sec couple at pH 7 is −(386 ± 6) mV.

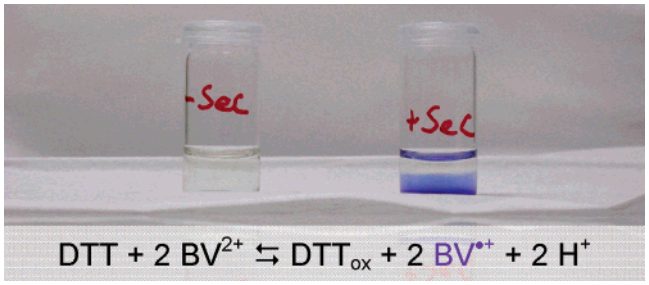


FIGURE 1: Development of a purple color upon reduction of BV^{2+} to $BV^{\cdot+}$ by DTT in the presence (right) and absence (left) of Sec_{ox}. In the absence of Sec_{ox}, color development required 2 min, compared to only 15 s with Sec_{ox}.

Table 1: Dependence on pH of the Equilibrium Constant ($K = [Sec_{ox}][DTT])/([Sec]^2[DTT_{ox}])$[a] and the Electrode Potential of the Sec_{ox}/Sec Couple

pH	K (M^{-1})	$E^{\circ\prime}$ [b] (mV)
1.6	10^5	−377
6.2	(330 ± 60)	−(387 ± 2)[c]
7.0	(90 ± 20)	−(388 ± 7)[c]

[a] Solutions initially contained 2 mM DTT and 1.1 mM Sec_{ox}. [b] Normalized to pH 7. [c] Errors reported as the standard deviation from three determinations.

In pulse-radiolysis experiments, we used the one-electron oxidation of Sec by trinitrogen$^{\cdot}$ to study Sec radical species. The intensity of the spectra obtained was dependent upon the selanide concentration (Figure 2). In experiments carried out at a low Sec concentration (670 μM) and where the selanyl is protonated (pH 5), we measured a spectrum with an extinction coefficient of 560 M^{-1} cm^{-1} at 460 nm that we attribute to the selenocysteinyl radical. In experiments at high Sec concentrations (23 mM) and where Sec is dissociated (pH 10), we recorded a spectrum attributed to the corresponding diselane radical anion $(RSe\therefore SeR)^{\cdot-}$, with an extinction coefficient of 7100 M^{-1} cm^{-1} at 455 nm (Table 2). To verify these observations, we subjected solutions of

[3] We found two literature values for E° (pH 7): −332 mV (*13*) and −323 mV (*7*), of which only the former was experimentally determined. We suspect that the latter value reflects a typographical error in the citation of the former.

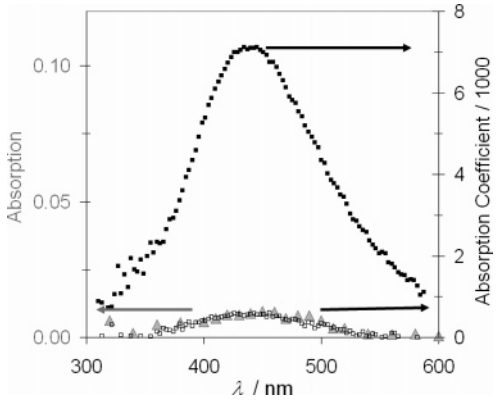


FIGURE 2: Spectra of Sec radicals. Pulse radiolysis: (■) alkaline solution (borate buffer at pH 10) of 23 mM Sec selanide and 100 mM N_3^- in N_2O-saturated solution; the spectrum was recorded 20 μs after the pulse, when the absorption maximum, attributed to the corresponding diselane radical anion $(RSeSeR)^{\cdot -}$, was reached; (□) N_2O-saturated acidic (pH 5) solution of 100 mM N_3^- and 670 μM Sec. Laser-flash photolysis: (gray triangles) spectrum of the product from 0.9 mM Sec_{ox} (RSe−SeR) flashed in 30 mM HCl and 900 mM *t*-BuOH aqueous solution saturated with N_2O.

Table 2: Absorption Maxima (λ_{max}) of Selenium- and Sulfur-Containing Radical Species

	X = Se		X = S	
	λ_{max} (nm)	reference	λ_{max} (nm)	reference
$HX^{\cdot}$	350	*24*	240	*45*
$(HXXH)^{\cdot -}$	410	*24*	380	*45* and *46*
$RX^{\cdot}$ [a]	340, 460	*26* and this paper	330	*47*
$(RXXR)^{\cdot -}$ [a]	455	this paper	410	*46*

[a] $R = H_3N^+-C(COO^-)-CH_2$.

0.9 mM Sec_{ox}, 30 mM HCl, and 900 mM *t*-BuOH saturated with dinitrogen monoxide to flash photolysis at laser energies < 20 mJ/pulse. The resulting spectrum (gray triangles in Figure 2) was identical to that obtained by oxidation of a dilute acidic Sec solution by trinitrogen$^{\cdot}$ (□ in Figure 2), and we are, therefore, confident that the λ_{max} of the selenocysteinyl radical is 460 nm, shifted to the red by 110 nm relative to that of selenanyl, $HSe^{\cdot}$. In laser-flash-photolysis experiments with water as the solvent, an absorption maximum smaller than the maximum at 460 nm was observed at 340 nm but only in acidic medium (30 mM HCl, results not shown); under alkaline conditions, the 340 nm band was only barely detectable (Figure 2).

Laser-flash-photolysis experiments were conducted on aqueous N_2O-saturated solutions containing 0.9 mM Sec_{ox} (RSe−SeR) in 30 mM HCl and 900 mM *t*-BuOH (Figure 3). The least-squares fit of the kinetics trace is consistent with $2k/\epsilon = 3.6 \times 10^6$ s^{-1} cm, which corresponds to a k_3 of ca. 1×10^9 M^{-1} s^{-1}. Identical results were obtained upon repeated flashing of the same solutions of Sec_{ox}.

In our investigations of the one-electron oxidation and reduction of the Sec diselane radical anion, $(RSe\therefore SeR)^{\cdot -}$, we assumed the equilibrium constant to be similar to that for the dissociation of the diselane radical anion, $(HSe\therefore SeH)^{\cdot -}$. We used the electrode potentials determined here to generate an oxidation-state diagram for Sec (Figure 4) for a comparison with that of cysteine; electrode potentials for couples involving $(RSSR)^{\cdot -}$ and $RS^{\cdot}$ were taken from Surdhar et al. (*14*), and the two-electron electrode potential of the RSSR/RSH couple was taken from Jocelyn (*15*).

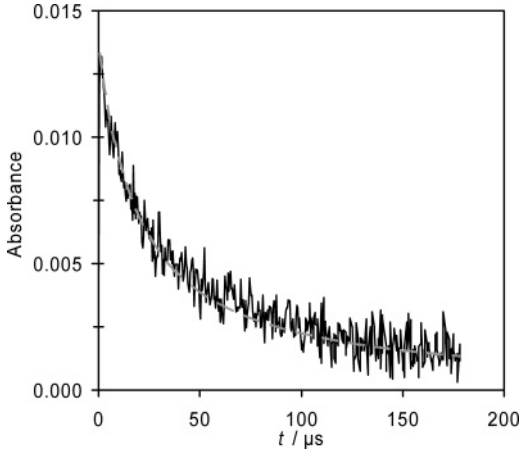


FIGURE 3: Laser-flash photolysis (pulse: 266 nm, 30 mJ, 5 ns) of 0.9 mM Sec_{ox} (RSe−SeR) in 30 mM HCl and 900 mM *t*-BuOH aqueous solution saturated with N_2O. The kinetics show a second-order decay of $Sec^{\cdot}$ radicals recorded at 460 nm with a least-squares fit (gray) and $2k/\epsilon = 3.6 \times 10^6$ s^{-1} cm, which corresponds to $k \approx 10^9$ M^{-1} s^{-1}.

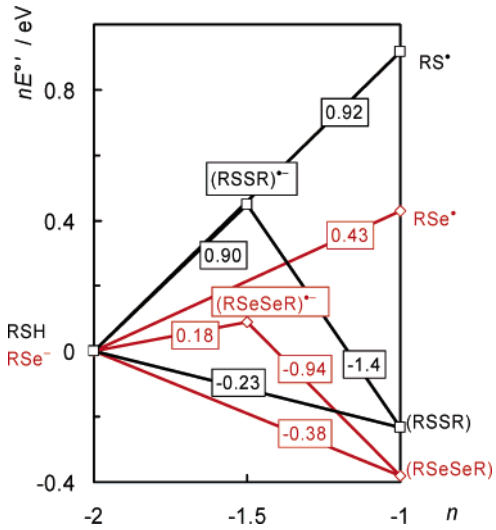
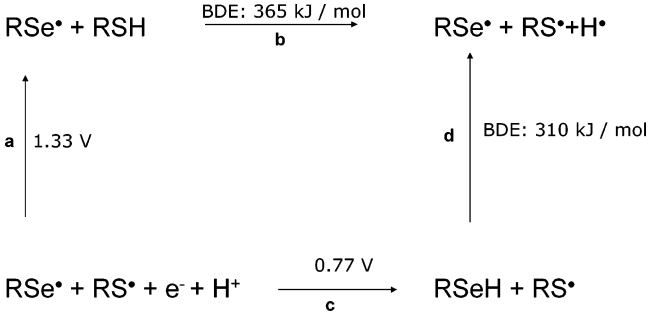


FIGURE 4: Oxidation-state diagram of Cys and Sec. Energies are given in electron volts (1 eV = 96.5 kJ/mol). The slope of a line joining two compounds, e.g., $RSe^{\cdot}$ and RSe^-, represents the reduction potential $E^{\circ\prime}(Sec^{\cdot}/Sec^-)$, 0.43 V at pH 7 versus NHE.

Scheme 1: Thermodynamic Cycle Used To Calculate the BDE of the Sec Se−H Bond



The BDE for the Se−H bond in cysteine was calculated according to the thermodynamic cycle shown in Scheme 1. Because the entropies of Sec and Cys in aqueous solution are only negligibly different, the difference in the Gibbs energies of formation are equal to the difference in the BDE between Se−H (reaction d in Scheme 1) and S−H (reaction b in Scheme 1). A measure of the difference in Gibbs

energies is the difference in standard electrode potentials (pH 0) of the couples Sec$^{\cdot}$/Sec (reaction c in Scheme 1, $E^{\circ} = +0.77$ V) and Cys$^{\cdot}$/Cys [reaction a in Scheme 1, $E^{\circ} = +1.33$ V (*14*)].

DISCUSSION

Electrode Potentials. The electrode potential of the selenocysteinyl/Sec couple is +0.77 V at pH 0 and +0.43 V at pH 7 compared to $E^{\circ} = +1.33$ V (pH 0) and $E^{\circ\prime} = +0.92$ (pH 7) for the cysteinyl/cysteine couple (*14*). The difference of nearly 0.5 V allows Sec_{ox} to mediate the one-electron reduction of BV^{2+} by the two-electron reductant DTT. The electrode potential of DTT is pH-dependent because of the sulfhydryl groups; at pH 9, the two-electron electrode potential of the DTT_{ox}/DTT couple is −450 mV.[4] The electrode potential of the one-electron oxidant BV^{2+} is independent of pH (*16*); the $BV^{\cdot+}/BV^{2+}$ couple has an electrode potential of −370 mV. From a purely thermodynamic point of view, DTT should be able to reduce BV^{2+}, but because one- and two-electron processes are coupled, the activation energy may be high and the reaction rate may be correspondingly small.[5] When we introduce a suitable catalyst, in this case Sec, the activation energy is smaller and the process is faster. Our findings indicate that Sec, present in trace quantities, mediates the transfer of one electron from a two-electron donor to a one-electron acceptor and vice versa (see Figure 1). Therefore, the easily detectable equilibria involving the $BV^{2+}/BV^{\cdot-}$ and Sec_{ox}/Sec couples may be used to derive electrode potentials. At alkaline pH, Sec reduces BV^{2+} to $BV^{\cdot+}$, as evidenced by the development of the purple color, which, upon acidification, fades, indicating reoxidation of $BV^{\cdot+}$ by Sec_{ox}. Because the reduction of the BV^{2+} by Sec is reversible, we could use the equilibrium constant determined by UV spectroscopy to estimate a two-electron electrode potential for the Sec_{ox}/Sec couple of −0.35 V at pH 7. However, this result may not be precise, because $BV^{\cdot+}$ forms aggregates (*16*) and is extremely sensitive to oxygen. More reliable results were derived from measurements of equilibrium between DTT and Sec; we obtained an electrode potential $E^{\circ\prime}(Sec_{ox}/Sec)$ of −0.38 V at pH 7, similar to that published (−0.36 V) for aminoethylselenol (*8*), which was calculated on the basis of $E^{\circ\prime}(DTT) = -0.332$ V (*13*). Values of ca. −0.38 V have also been determined for diselane moieties in two different proteins (*7*, *9*). We question the value of −0.488 V published by Jacob et al., who used a mercury electrode to determine the two-electron electrode potential of Sec_{ox}/Sec (*4*) by cyclic voltammetry. The value of Huber et al., who, on the basis of polarographic measurements, estimated a potential of 233 mV lower than that of cystine/Cys (*6*) (i.e., ca. −0.47 V versus NHE), is similarly suspect. As mentioned under the Experimental Procedures, metal electrodes tend to adsorb selenium compounds; it is likely that both determinations (*4*, *6*) were influenced by adsorption to the mercury electrode, because mercury sulfides and mercury selenides are among the most stable complexes known in chemistry. The equilibrium constant for formation of the mercury−cysteine complex is $[HgCys]/([Hg^{2+}][Cys] > 10^{14}\ M^{-1}$; there appears to be no literature value for the K of formation of the Sec analogue (*17*), but, on the basis of a comparison of the solubility products of HgS ($\log K_s = -51$) and HgSe ($\log K_s = -64$), we assume the equilibrium constant to be even higher (*18*).

The ability of Sec to promote one-electron-transfer reactions could be beneficial in vivo. For example, we know from the radiation chemistry literature that the tyrosyl radical is very stable and usually decays by recombination; in ribonucleotide reductase, the tyrosyl radical lives quite long, even under aerobic conditions (*19*). The reaction with a selanyl would, further, be a feasible route for repair of radical damage in biomolecules, because the reaction with glutathione is comparatively much slower ($k \approx 2 \times 10^3\ M^{-1}\ s^{-1}$) (*20*). Preliminary data show that, indeed, Sec reduces the tyrosyl radical 5−6 orders of magnitude faster than glutathione.[6] Catalysis of one-electron reduction by Sec in GSH reduction of radicals complements the well-known catalysis of peroxide reduction by GSH by the Sec of glutathione peroxidase (believed to be a formally two-electron process, i.e., nucleophilic attack by the selanide on a peroxide) (*1*).[7]

Characterization of Radical Species. In pulse-radiolysis experiments, Sec$^{\cdot}$ radicals reacted with free selanides to generate diselane radical anions. The intensity of the spectrum observed during one-electron oxidation of Sec by trinitrogen$^{\cdot}$ was dependent upon the selanide concentration (see Figure 2); in the absence of free selanide, the mean absorption coefficient of the transients was lower by 1 order of magnitude. Similar observations have been made with thiols (*14*, *21*−*23*) and with selane (*24*). For the equilibrium in reaction 1, a $K_1 = 6 \times 10^5$ M has been determined (*24*)

$$(HSeSeH)^{\cdot-} = HSe^{\cdot} + HSe^{-} \qquad (1)$$

The positions of the absorption maxima of selenium and sulfur radical species (Table 2) differ markedly depending upon the nature of the substituent. Unexpectedly, the absorbance maxima of RSe$^{\cdot}$ and $(RSeSeR)^{\cdot+}$ are remarkably similar, and we have painstakingly ruled out other plausible assignments. With selenane, molar absorptivities for HSe$^{\cdot}$ and $(HSeSeH)^{\cdot-}$ of 300 $M^{-1}\ cm^{-1}$ at 350 nm and 8600 $M^{-1}\ cm^{-1}$ at 410 nm, respectively, have been determined (*24*). With Sec, we found molar absorptivities of similar magnitudes for RSe$^{\cdot}$ and $(RSeSeR)^{\cdot-}$ of 560 $M^{-1}\ cm^{-1}$ at 460 nm and 7100 $M^{-1}\ cm^{-1}$ at 455 nm, respectively. The results of pulse-radiolysis experiments were confirmed by laser-flash photolysis, where the transient was produced by homolysis rather than oxidation. Because identical results were obtained

[4] Calculation based on data in refs *12* and *13*.

[5] We propose the following reaction sequences, of which (a) and (d) reflect activation steps:

uncatalyzed:

$$BV^{2+} + DTT^{2-} \rightarrow BV^{\cdot+} + DTT^{\cdot-} \qquad (a)$$

$$BV^{2+} + DTT^{\cdot-} \rightarrow BV^{\cdot+} + DTT_{ox} \qquad (b)$$

catalyzed:

$$Sec_{ox} + DTT^{2-} \rightarrow 2Sec^{-} + DTT_{ox} \qquad (c)$$

$$Sec^{-} + BV^{2+} \rightarrow BV^{\cdot+} + Sec^{\cdot} \qquad (d)$$

$$Sec^{-} + Sec^{\cdot} \rightarrow (RSeSeR)^{\cdot-} \qquad (e)$$

$$(RSeSeR)^{\cdot-} + BV^{2+} \rightarrow BV^{\cdot+} + Sec_{ox} \qquad (f)$$

[6] Nauser, T., Steinmann, D., Schöneich, C., and Koppenol, W. H. Unpublished results.

[7] We thank a reviewer for this comment.

upon repeated flashing of the same solutions of Sec_{ox} in laser-flash-photolysis experiments, we conclude that the following reactions take place:

$$RSeSeR \xrightarrow{h\nu} 2RSe^{\cdot} \quad (2)$$

$$2RSe^{\cdot} \rightarrow RSeSeR \quad (3)$$

where $R = [H_3N^+CH(COOH)CH_2]$ and $k_3 \approx 1 \times 10^9$ M^{-1} s^{-1} (see Figure 3). This rate constant is almost identical to that determined in pulse-radiolysis experiments by Tamba and Badiello (*25*), who found, upon reduction of Sec_{ox}, a species with $\lambda_{max} = 460$ nm and $\epsilon = 760$ M^{-1} cm^{-1} (attributed to the Sec$^{\cdot}$ radical) and measured a recombination rate of $2k_3 = 1.5 \times 10^9$ M^{-1} s^{-1}. Kolano et al. (*26*), who performed laser-flash-photolysis experiments on carboxylate- and amine-protected Sec derivatives in acetonitrile, reported absorption maxima of 340 and 460 nm for the selanyl radical. We also observed a weak absorbance band at 340 nm for the Sec$^{\cdot}$ radical in aqueous solution, which became even weaker at low pH. We have considered three-electron two-center bond models for the band at 460 nm, e.g., Se$\therefore$N; however, the absorption was unchanged by protonation of the amino group. We cannot explain the similarity in the absorption maxima of RSe$^{\cdot}$ and $(RSeSeR)^{\cdot-}$ but point out that the absorptivities of the two species are radically different.

BDEs and Implications. Although thiols, e.g., GSH, are commonly viewed as repair molecules capable of rereducing oxidized peptides via the equilibrium in reaction 4

$$GSH + {}^{\alpha}C^{\cdot} = GS^{\cdot} + {}^{\alpha}C{-}H \quad (4)$$

most carbon-centered radicals in protein backbones are more stable than thiyl radicals; thus, the equilibrium lies to the left (*27*). It has been experimentally demonstrated that thiyl radicals abstract hydrogen from $^{\alpha}C-H$ bonds in peptides (*28*, *29*), lipids (*30*), and DNA (*31*, *32*). Could selanyl radicals react in an analogous way? We need to examine the relevant BDEs to answer this question. The BDE of the S−H bond in cysteine is approximately 365 kJ/mol (*27*), and therefore, the BDE of the Se−H bond in Sec, derived from electrode potentials, is 310 kJ/mol (Scheme 1). Our estimate is in good agreement with reported values for the BDE of Se−H of 330 kJ/mol for H_2Se (*33*) and 325 ± 16 kJ/mol (*34*) and 300 kJ/mol (*35*) for C_6H_5Se-H determined in the gas phase. Similar values, obtained by ab initio calculations of 324 kJ/mol (*36*) and 340−360 kJ/mol (*37*) for H_2Se and 340 kJ/mol for CH_3Se-H (*37*), have been published. These BDEs are about 20−55 kJ/mol lower than the average BDE of 330−365 kJ/mol of a $^{\alpha}C-H$ bond in peptides (*27*). In principle, the formation of a selanyl radical requires less energy than the formation of a thiyl or a carbon-centered radical in a protein backbone and would provide a natural radical sink. However, reaction 5 becomes important when the equilibrium is rapidly attained and pulled to the right by removal of products, i.e., via the formation of peroxyl radicals (*29*).

$$RSe^{\cdot} + {}^{\alpha}C{-}H = RSeH + {}^{\alpha}C^{\cdot} \quad (5)$$

In the case of hydrogen abstraction by thiyl radicals, the reaction rate is not particularly sensitive to the difference in bond energy; rather, orbital overlap or the polarity of the transition state plays an important role (*38*−*42*). We assume that this is also true for reactions with selanyls (*43*). Because intramolecular hydrogen transfer between thiyl radicals and $^{\alpha}C-H$ in peptides can be as fast as 10^6 s^{-1},[8] there is the possibility that substantial reaction rates could occur also with selanyl radicals.

Summary. The experiments reported here reveal similarities between the chalcogens, sulfur and selenium. The major differences between sulfanyls and selanyls are in acidity and the accessibility of the radical oxidation state (see Figure 4). Because selanyls are distinctly more acidic ($pK_{thiol} \approx 8.5$ and $pK_{selanyl} \approx 5.2$), selanyls but not sulfanyls are deprotonated at physiological pH and are more reactive than sulfanyls in nucleophilic substitution reactions. The electron-rich selanide is surely also a target for oxidants, and the ready accessibility of the radical oxidation state may also point to additional reactions facilitated by selenoproteins. We showed that the diselane radical anion has a strong absorption band at 455 nm with an absorptivity $\epsilon(RSe\therefore SeR)^{\cdot-} = 7100$ M^{-1} cm^{-1}, comparable to that reported for selanyl (reaction 1) (*24*). This species is strongly reducing with an $E^{\circ\prime}$ (pH 7) of −0.94 V, albeit less so than analogous disulfane radical anions (*22*) for which an $E^{\circ\prime}(GSSG/GSSG^{\cdot-})$ of −1.5 V has been reported (*44*). Similarly, the electrode potential of the selenocysteinyl/Sec couple at pH 7 is 0.5 V smaller than that of the cysteinyl/cysteine couple (Figure 4). As demonstrated in Figure 1, Sec catalyzes electron transfer between one- and two-electron donors and acceptors. The ability to couple one electron with two-electron processes may be an additional function of selanyls in vivo.

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