

# One-Electron Reduction of Selenomethionine Oxide

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Experimental evidence is provided that selenomethionine oxide (MetSeO) is more readily reducible than its sulfur analogue, methionine sulfoxide (MetSO). Pulse radiolysis experiments reveal an efficient reaction of MetSeO with one-electron reductants, such as  $e_{aq}^-$  ( $k = 1.2 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ ),  $\text{CO}_2^{\bullet-}$  ( $k = 5.9 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ ) and  $(\text{CH}_3)_2 \text{C}^{\bullet}\text{OH}$  ( $k = 3.5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ ), forming an intermediate selenium–nitrogen coupled zwitterionic radical with the positive charge at an intramolecularly formed  $\text{Se} : \text{N} \ 2\sigma/1\sigma^*$  three-electron bond, which is characterized by an optical absorption with  $\lambda_{\text{max}}$  at 375 nm, and a half-life of about 70  $\mu\text{s}$ . The same transient is generated upon  $\text{HO}^{\bullet}$  radical-induced one-electron oxidation of selenomethionine (MetSe). This radical thus constitutes the redox intermediate between the two oxidation states, MetSeO and MetSe. Time-resolved optical data further indicate sulfur–selenium interactions between the  $\text{Se} : \text{N}$  transient and GSH. The  $\text{Se} : \text{N}$  transient appears to play a key role in the reduction of selenomethionine oxide by glutathione.

**Keywords:** Selenomethionine oxide, glutathione, pulse radiolysis, odd-electron bonds

## INTRODUCTION

Selenomethionine (MetSe), an organoselenium compound of low abundance, replaces methionine unspecifically in proteins, i.e. hemoglobin.<sup>[1,2]</sup> Although closely chemically related to its sulfur analogue, the selenomethionine/selenomethionine oxide couple exhibits considerably higher reactivity in redox reactions, as can be concluded from radiation chemical investigations on free radical-induced oxidation,<sup>[3,4]</sup> in kinetic analyses of peroxyxynitrite-induced oxidation of methionine or selenomethionine,<sup>[5,6]</sup> as well as in protein oxidation.<sup>[7,8]</sup> Lower electronegativity of selenium implies facilitated oxidation reactions of organic selenides in comparison to analogous sulfur compounds.<sup>[9–11]</sup> The two-electron oxidation of MetSe by peroxyxynitrite, a potent biological oxidant formed by the reaction of nitrogen oxide with superoxide radical anions, occurs ten- to thousand-fold faster than the

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peroxynitrite-induced oxidation of its sulfur analogue methionine.<sup>[5]</sup> In contrast to the enzyme-dependent reduction of methionine sulfoxide by methionine sulfoxide reductases,<sup>[8,12]</sup> a rapid and efficient nonenzymatic reduction of selenomethionine oxide (MetSeO) by thiols, e.g. glutathione, has been reported.<sup>[13]</sup> In this context, a catalytic function in the protection against potent oxidants, i.e. peroxynitrite, sustained by thiols was assigned to MetSe and other organoselenium compounds including glutathione peroxidase, ebselen and corresponding carboxymethylated derivatives.<sup>[13–15]</sup>

Permitting reliable kinetic analysis of fast reactions, the use of the time-resolved technique of pulse radiolysis facilitates detailed investigation of free radicals and their chemical interactions.<sup>[16]</sup> In this work we study one-electron transfer reactions with MetSe and its corresponding oxide in order to characterize underlying molecular mechanisms in the reduction of MetSeO.

## MATERIALS AND METHODS

### Reagents

MetSe and GSH were purchased from Sigma (Deisenhofen, Germany), MnO<sub>2</sub> was from Fluka (Buchs, Switzerland) and other chemicals from Merck (Darmstadt, Germany). MetSeO was synthesized from MetSe (10 mM) and hydrogen peroxide (100 mM) and incubated in aqua bidest for 2 h at room temperature. Remaining hydrogen peroxide was eliminated using MnO<sub>2</sub>, which was finally removed by filtration. H<sub>2</sub>O<sub>2</sub>-induced oxidation of scopoletin in the presence of horseradish peroxidase was applied in order to check for completion of this process.<sup>[17]</sup> The yield of MetSeO was measured with HPLC after derivatization with fluorescamine.<sup>[13]</sup>

### Pulse Radiolysis

Pulse radiolysis studies were performed by applying short (2 ns) pulses of 8 MeV electrons,

generated with the Notre Dame Titan Beta Model TBS-8/16-1S linear accelerator, to continuously flowing aqueous solutions. Deposition of the radiation energy leads to the formation of three highly reactive radical species: hydrated electrons ( $e_{\text{aq}}^-$ ), hydroxyl radicals (HO $\cdot$ ) and hydrogen atoms (H $\cdot$ ) at yields of 0.27, 0.28 and 0.06  $\mu\text{mol/J}$  absorbed energy, respectively.<sup>[18]</sup> Reactions of  $e_{\text{aq}}^-$  with MetSeO (up to 5.0 mM) were investigated in N<sub>2</sub>-saturated (i.e., oxygen-free) solutions containing 2-methyl-2-propanol, 0.1 M, in order to eliminate hydroxyl radicals. CO<sub>2</sub> $^{\bullet-}$  and (CH<sub>3</sub>)<sub>2</sub>C $\cdot$ OH were generated in N<sub>2</sub>O-saturated solutions of sodium formate, 0.1 M, or 2-propanol, 0.1 M, respectively. The nitrous oxide converts  $e_{\text{aq}}^-$  into HO $\cdot$ , which practically doubles the yield of the latter. In turn, HO $\cdot$  and H $\cdot$  react with formate or 2-propanol to generate, respectively, the two radicals of interest. In these experiments MetSeO was added in concentrations varying from 0.5 to 2.0 mM. Experiments concerning the oxidation of MetSe by hydroxyl radicals and interactions with GSH were carried out in N<sub>2</sub>O-saturated solutions, containing either MetSe at a fixed concentration of 5 mM in combination with GSH (up to 10 mM), or a fixed amount of GSH (20 mM) in combination with a varying concentration of MetSe (1–8 mM). The pH was generally kept at 7.2 unless specifically noted.

Details of the pulse radiolysis set-up, dosimetry (based on SCN $^-$  oxidation to (SCN)<sub>2</sub> $^{\bullet-}$ ) and evaluation of the data have been published.<sup>[16,19]</sup> Kinetic analysis of optical absorption vs. time traces was carried out with ORIGIN (Microcal). Error limits are  $\pm 10\%$ . All experiments have been carried out at room temperature.

## RESULTS AND DISCUSSION

MetSeO strongly accelerates the decay of hydrated electrons as illustrated in Figure 1A where the optical absorption of  $e_{\text{aq}}^-$  at 720 nm is shown as a function of time in a pulse irradiated, deoxygenated aqueous solution containing 0.1 M

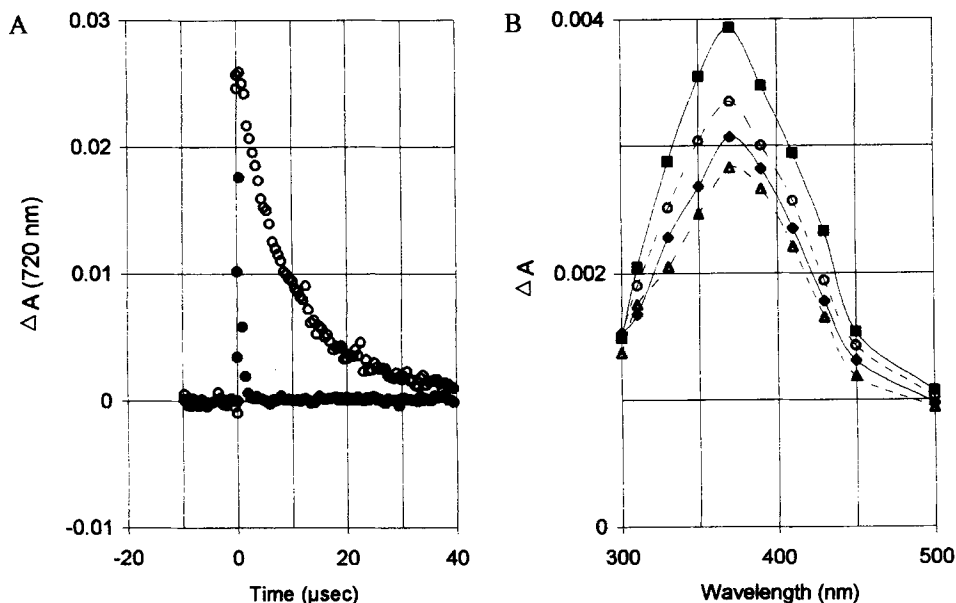


FIGURE 1 Reaction of selenomethionine oxide with hydrated electrons. (A) Decay of  $e_{aq}^-$  absorbance at 720 nm in pulse-irradiated oxygen-free ( $N_2$ -saturated) aqueous solution, pH 7.2, containing 0.1 M 2-methyl-2-propanol (open circles) and in the presence of selenomethionine oxide, 0.2 mM (full circles). Pulse width approx. 10 ns, dose approx. 6 Gy. (B) Optical absorption spectrum of Se:N coupled transient formed upon one-electron reduction of selenomethionine oxide, 0.2 mM, in pulse-irradiated oxygen-free ( $N_2$ -saturated) aqueous solution, pH 7.2, containing 0.1 M 2-methyl-2-propanol, measured at 55  $\mu$ s (full squares), 85  $\mu$ s (open circles), 145  $\mu$ s (full diamonds), 165  $\mu$ s (open triangles) after the pulse, dose approx. 5 Gy.

2-methyl-2-propanol at pH 7.2 in both the absence (open symbols) and presence of 0.2 mM MetSeO (closed symbols). Kinetic analyses of such traces at various concentrations of MetSeO, 0.03–0.20 mM, revealed a pseudo-first-order rate law with  $k_{obs}$  (in  $s^{-1}$ ) being linearly dependent on [MetSeO]. A bimolecular rate constant for the reaction of MetSeO with hydrated electrons of  $k = 1.3 \times 10^{10} M^{-1} s^{-1}$  was obtained. Concomitantly, formation of a transient product occurred, characterized by a broad absorption spectrum with a maximum at 375 nm (Figure 1B). The transient disappeared again by a first-order process with a half-life of about 70  $\mu$ s, corresponding to  $k \cong 10^4 s^{-1}$ . Kinetic analysis gave the best-fit bimolecular formation rate constant of  $k = 1.1 \times 10^{10} M^{-1} s^{-1}$ , corresponding to that obtained from the  $e_{aq}^-$  decay. The average value  $k_1 = (1.2 \pm 0.1) \times 10^{10} M^{-1} s^{-1}$  is listed in Table I. This clearly identifies the 375 nm transient to be the product of the reaction between hydrated elec-

TABLE I Second-order rate constants for the one-electron reduction of selenomethionine oxide

Reductant	$k$ ( $M^{-1} s^{-1}$ )	Reduction potential (V vs. NHE) <sup>[23]</sup>	
$e_{aq}^-$	$1.2 \times 10^{10}$	$E(aq/e_{aq}^-)$	-2.9
$CO_2^{\bullet-}$	$5.9 \times 10^8$	$E(CO_2/CO_2^{\bullet-})$	-2.0
$(CH_3)_2C^{\bullet}OH$	$3.5 \times 10^7$	$E(Me_2CO; H^+/Me_2C^{\bullet}OH)$	-1.5

trons and MetSeO, formed in a near diffusion-controlled reaction. In contrast, no accelerated decay of  $e_{aq}^-$  in the presence of 0.2 mM methionine sulfoxide was observed, suggesting that the reaction of methionine sulfoxide with hydrated electrons occurs with  $k < 2 \times 10^8 M^{-1} s^{-1}$ . In fact, hydrated electrons, the most powerful chemical reducing entity, are able to reduce aliphatic sulfoxides. However, this process is relatively slow occurring at rate constants several orders of magnitudes below the limit for diffusion controlled reactions.<sup>[18]</sup>

Reduction of MetSeO associated with the formation of the same transient with  $\lambda_{\max}$  at 375 nm was also observed for  $\text{CO}_2^{\bullet-}$  and  $(\text{CH}_3)_2\text{C}^{\bullet}\text{OH}$ . Bimolecular rate constants of  $k = 5.9 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$  and  $k = 3.5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$  were obtained for the two reducing radicals, respectively, by monitoring the formation kinetics at 375 nm. These rate constants indicate an efficient, although not diffusion-controlled, one-electron reduction of MetSeO by  $\text{CO}_2^{\bullet-}$  and  $(\text{CH}_3)_2\text{C}^{\bullet}\text{OH}$ . In contrast, no evidence has been found for reduction of aliphatic sulfoxides by either of those radicals. Table I summarizes the bimolecular rate constants for the reaction of MetSeO with  $e_{\text{aq}}^-$ ,  $\text{CO}_2^{\bullet-}$  and  $(\text{CH}_3)_2\text{C}^{\bullet}\text{OH}$  and the reduction potentials of the applied reducing species showing that the decrease of the rate constants follows the trend in reduction potential. Corroborating data on the reduction of diaryl chalcogenide oxides have been published recently.<sup>[20]</sup> Interestingly, one-electron

oxidation of MetSe by  $\text{HO}^{\bullet}$  or  $\text{N}_3^{\bullet}$  radicals leads to the formation of the same transient species with  $\lambda_{\max}$  at 375 nm (Figure 2A; Bonifacic, M., Asmus, K.-D., unpublished work). An absorbing transient with a similar spectrum was observed in a previous study.<sup>[3]</sup> We suggest the formation of a selenium–nitrogen coupled species in which the two heteroatoms have established a stabilizing  $2\sigma/1\sigma^*$  three-electron bond in a cyclic 5-membered ring structure generated via a process summarized in Scheme 1. For the reason of simplicity the species will be further denoted as  $\text{Se}:\text{N}$ . This radical species represents an intermediate in the selenide/selenoxide system. Formation of an analogous sulfur–nitrogen coupled three-electron bonded intermediate has been suggested for hydroxyl radical-induced one-electron oxidation of methionine, showing an optical absorption with  $\lambda_{\max}$  at 390 nm.<sup>[4]</sup> However, the  $\text{Se}:\text{N}$  radical is much longer lived ( $t_{1/2} \approx 70 \mu\text{s}$ )

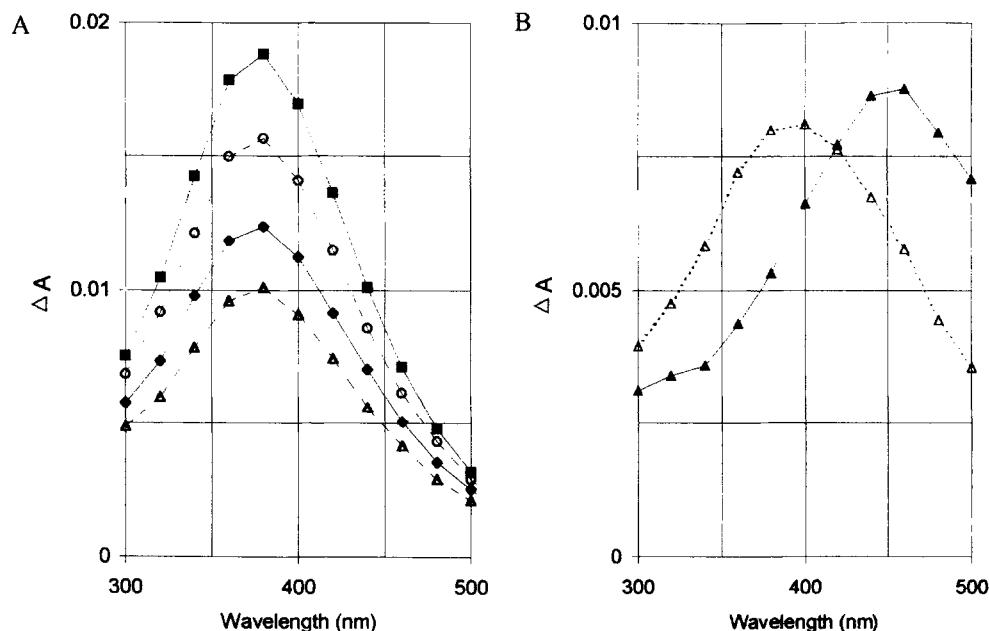
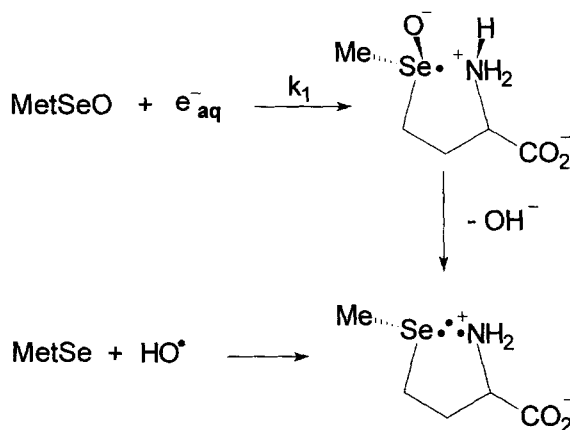


FIGURE 2 Reaction of selenomethionine with hydroxyl radicals alone and in the presence of glutathione. (A) Optical absorption spectrum of  $\text{Se}:\text{N}$  coupled transient formed upon one-electron oxidation of selenomethionine, 5.0 mM, after pulse irradiation of aqueous, oxygen-free,  $\text{N}_2\text{O}$ -saturated solution, pH 7.2, measured at 55  $\mu\text{s}$  (full squares), 85  $\mu\text{s}$  (open circles), 145  $\mu\text{s}$  (full diamonds), 165  $\mu\text{s}$  (open triangles) after the pulse, dose approx. 7 Gy. (B) Absorption spectrum obtained at 12  $\mu\text{s}$  (open triangles) and 30  $\mu\text{s}$  (full triangles) after pulse irradiation of aqueous, oxygen-free,  $\text{N}_2\text{O}$ -saturated solution, pH 3.2, containing selenomethionine, 5.0 mM, and glutathione, 9.2 mM, dose approx. 7 Gy.



Scheme 1 Reaction between selenomethionine oxide and hydrated electrons leading to the formation of a  $\text{Se}\cdot\text{:N}$  coupled transient ( $\lambda_{\text{max}}$  at 375 nm). The same transient is formed upon one-electron oxidation of selenomethionine.

than the corresponding  $\text{S}\cdot\text{:N}$  species which was shown to decay with  $t_{1/2} \approx 0.2 \mu\text{s}$  via irreversible expulsion of  $\text{CO}_2$ .<sup>[4]</sup> Therefore, the  $\text{Se}\cdot\text{:N}$  transient is available for involvement in truly reversible redox processes for a much longer time and may thus be recycled more efficiently than the sulfur–nitrogen analogue. The  $\text{Se}\cdot\text{:N}$  intermediate being generated not only by one-electron oxidation of MetSe, but also in contrast to its sulfur analogue in a fast one-electron reduction of MetSeO, confirms the idea of the MetSe/MetSeO couple exhibiting redox properties which render seleno derivatives much more sensitive and versatile substrates than the corresponding sulfur compounds.

The ability of thiols to reduce selenoxides has been demonstrated in several studies. After addition of glutathione, the organoselenium compounds MetSe, ebselen and glutathione peroxidase exhibited synergistically enhanced protection against oxidizing agents.<sup>[13–15]</sup> In order to assess the redox properties of the  $\text{Se}\cdot\text{:N}$  transient and its interactions with glutathione with respect to one-electron transfer processes, preliminary investigations were performed using pulse radiolysis technique. The aim was to find out if  $\text{Se}\cdot\text{:N}$  transient could be reduced to MetSe by thiols, i.e. glutathione. An analogous reaction has been

discussed for oxidation of thiols by sulfur-centered radical cations available in  $(\text{R}_2\text{S}\cdot\text{:SR}_2)^+ \rightleftharpoons (\text{R}_2\text{S})^{\cdot+} + \text{R}_2\text{S}$  equilibria:<sup>[21]</sup>



The mechanism of reaction (1) may generally involve a hydrogen atom abstraction from the thiol. Alternatively, the first step could be an electron transfer, leading to separation of selenium from nitrogen, followed by formal proton exchange. In either case the  $\text{Se}\cdot\text{:N}$  transient would be reduced to MetSe.

For reasons of thermal instability of MetSeO/GSH solutions, the  $\text{Se}\cdot\text{:N}$  transient was generated via hydroxyl radical-induced oxidation of MetSe (Figure 2A). In the presence of GSH in concentrations similar to that of MetSe, i.e.  $[\text{GSH}] \geq 5 \text{ mM}$ , an accelerated decay of the initial absorption at 375 nm corresponding to the  $\text{Se}\cdot\text{:N}$  transient was observed. Moreover, as shown in Figure 2B, a delayed formation of another secondary transient with a maximum of absorption at 450 nm was observed in the presence of glutathione. This shift in absorption to higher wavelengths, occurring in the presence of both MetSe and GSH only, may be caused by the formation of mixed  $>\text{Se}\cdot\text{:S}-$  intermediate species. However, stabilization of heteroatom-centered radicals into three-electron  $2\sigma/1\sigma^*$  bonds can occur *intramolecularly* as well as *intermolecularly*.<sup>[11,22]</sup> Thus, several transient species may be present including  $>\text{Se}\cdot\text{:S}-$ ,  $-\text{S}\cdot\text{:S}-$  and  $>\text{Se}\cdot\text{:Se}<$ . Further experiments are required to quantitatively resolve the underlying mechanism. From the results presented in this study it can however, be concluded that the  $\text{Se}\cdot\text{:N}$  transient does react with glutathione, although in a complex way. With regard to the capacity of glutathione to reduce MetSeO in an overall two-electron reduction forming MetSe and GSSG,<sup>[13]</sup> it seems a reasonable suggestion that the  $\text{Se}\cdot\text{:N}$  odd-electron bonded transient plays a key role in the overall reduction process.

In conclusion,  $e_{\text{aq}}^-$ ,  $\text{CO}_2^{\cdot-}$  and  $(\text{CH}_3)_2\text{C}\cdot\text{OH}$  reduce MetSeO, but not methionine sulfoxide,

in a one-electron transfer process with high rate constants up to near the diffusion limit. Thereby, an intramolecularly selenium–nitrogen coupled intermediate is formed. Being also formed by hydroxyl radical-induced one-electron oxidation of MetSe, the S...N transient constitutes the redox-intermediate between two oxidation states, MetSeO and MetSe.

The transient establishment of odd-electron bonds between different heteroatoms, including nitrogen, sulfur, and selenium, may consequently represent an important general aspect in reduction reactions involving thiols and organoselenium compounds.

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