

Reaction Mechanism of Selenate(IV) Ions with Several Thiols in Aqueous Media

by Daniel O. Forastiere^{a)}, Elena B. Borghi^{a)}, and Pedro J. Morando^{*b)c)d)}

^{a)} Universidad Nacional de Luján, Rutas 5 y 7, Buenos Aires, Argentina

^{b)} Unidad de Actividad Química, Centro Atómico Constituyentes, Comisión Nacional de Energía Atómica, Avenida General Paz 1499, (1650) San Martín, Pcia de Buenos Aires, Argentina
(phone: 54 11 6772 7176; fax: 54 11 6772 7886; e-mail: morando@cnea.gov.ar)

^{c)} Instituto de Tecnología Prof. J. Sábató, Universidad Nacional de General San Martín, Buenos Aires, Argentina

^{d)} Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina

In several enzymatic systems, selenium (an essential element commonly incorporated as sodium selenite to the human diet) is bonded through a mercapto group of a cysteine residue. The reaction of Se^{IV} (as selenite) with different thiols in aqueous media was studied to increase the information about the mechanism of the enzyme reaction. The influence of the reagents' concentration on the reaction rate and temporal spectral changes was analyzed. A complex with a UV maximum between 270 and 290 nm was formed, which quickly decayed when the thiol concentration was increased. The proposed reaction mechanism includes a fast Se^{IV}–thiol association step followed by two parallel steps (one is first-order in the complex and the other one is globally second-order). Kinetics and equilibrium constants were obtained, and the rate dependence of the thiol oxidation on the Se^{IV} concentration was modelled for all thiols tested. Very good agreement between the experimental and calculated curves was observed.

Introduction. – Although recently doubts have appeared on its importance in medicinal use [1], it is an accepted fact that selenium has antioxidant properties [2], prevents the development of diverse cancer types [3][4], and inhibits certain hepatic necrosis in rats [5][6].

The role of selenium as an essential element was demonstrated in 1954 when it was observed that low ingestion levels were associated with cardiopathies, specially in children, and with diseases in cattle [7]. It was reported that this alterations were reverted by addition of sodium selenite [8] to the diet, and numerous commercial products exist today to supply this element.

Selenium is incorporated in the proteins [9] glutathione peroxidase (25%), selenoprotein P (68%), whose biological function is not yet completely understood [10][11], and albumin A (7%) [12]. Glutathione peroxidase [13] is the best-characterized enzymatic system containing selenium. It is formed by four subunits bonded to a Se-atom as a selenocysteine residue. This enzyme transforms glutathione into the oxidized form, with the corresponding reduction of hydrogen peroxide and peroxides formed during the lipid oxidation in membranes. Therefore, it is nowadays used in antioxidant formulations.

It is well known that the paths followed by selenate and selenite in the organism are different. Both can be used as a nutritional supplement, but whereas selenite,

administered to rats, is captured by red cells and reduced by glutathione to selenide (which is then selectively bound to proteins), selenate is not captured by erythrocytes but reduced during its passage through the liver [14]. Some bacteria like *Escherichia coli* [15] are capable of reducing selenite and selenate to Se^0 , which is then incorporated in proteins as part of the amino acids selenocysteine or selenomethionine. It is known that compounds containing a thiol moiety play an essential role in many biochemical and pharmacological reactions because they can be easily oxidized, and they are rapidly regenerated. Therefore, they are fundamental in cytoprotection against several contaminating agents [16].

After *Painter's* work [17] on the reaction of Se^{IV} with thiols, many papers analyzing different '*in vivo*' aspects of the Se^{IV} metabolism have been published [14][18–21], many of them dealing with the enzymatic reactions '*in vitro*' [22–25], but the information about the chemical mechanism of the reaction is scanty. In some cases, studies were performed in nonaqueous or mixed solvents [26–33].

In this work, the kinetics of the reaction of three different thiols, *i.e.*, of L-cysteine ($\text{HSCH}_2\text{CH}(\text{NH}_2)\text{COOH}$; Cys), thioglycolic acid (HSCH_2COOH ; TGA), and 3-mercaptopropanoic acid ($\text{HSCH}_2\text{CH}_2\text{COOH}$; MPA), with Se^{IV} in aqueous medium is explored. A reaction mechanism is proposed, and the corresponding rate constants are determined following two different methodologies: the time dependence of the thiol concentration and the spectral evolution of an intermediate thiol– Se^{IV} complex. The agreement between the values validates the proposed mechanism.

Experimental. – *General.* All the reagents used were of anal. grade, solids were used without recrystallization. The solns. were prepared with doubly distilled H_2O , and N_2 was bubbled through them to displace O_2 and, therefore, avoid the oxidation of the thiol. VIS and UV sequential spectra: *Shimadzu UV-240* spectrophotometer. Product analyses by IR spectrophotometry (*Nicolet Magna-IR-560* instrument) and by atomic-absorption spectrometry (*Perkin-Elmer 4 analyst-100* apparatus).

Kinetics. Kinetic analyses were performed by using a doubly walled glass cell, thermostated by means of a *Lauda MGWKR* thermostatic bath. Through holes made in the lid of the cell, a thermometer was introduced into the soln., and samples were extracted. The mixture was magnetically stirred. A typical kinetic run was started by adding a selenite soln. to the thiol soln. at the desired concentrations; the pH was maintained by the use of appropriate buffers. Both soln. had been previously thermostated and de-aired. At different times, samples of the soln. were taken, and the reaction was stopped by reaction with *Saville's* first reactive (HNO_2) [34]. Thiol concentration was determined by measuring the absorbance at 540 nm of the red complex formed, by using *Saville's* technique [34]. All experiments were performed at an ionic strength *I* of 0.3M and 10°.

Results. – 1. *Reaction Product Determination.* In all experiments, a reddish color and a colloidal suspension appeared at the end of the reaction between selenite and Cys, TGA, or MPA. It was determined that this suspension was formed by Se^0 . On the other hand, in experiments performed at smaller $[\text{Se}^{\text{IV}}]/[\text{Cys}]$ ratios, a white precipitate was separated. IR Spectroscopic analysis indicated that it corresponds exactly to a L-cysteine (Cys–Cys) reference sample.

2. *Influence of the pH Value.* At $\text{pH} < 6$, the reaction speeded up considerably, so stopped-flow techniques were applied. At high pH values, the reaction was very slow. For this experiment, a pH value of 9.4 was selected because it allowed a suitable thiol-concentration measure by both methods (*Saville* and UV/VIS); indeed, the reaction

proceeded within a reasonable times, and no spectra superposition of the reagents and the intermediary complex was observed.

3. Se^{IV} Dependence. Fig. 1 shows the profile of absorbance A (Saville's method) vs. time for several experimental conditions. Saville's method requires the determination of the total (free and Se^{IV} complexed) thiol concentration (see Discussion), and the time dependence establishes the products formation rates. From these profiles, the initial rates (v_0) can be obtained as the initial slopes.

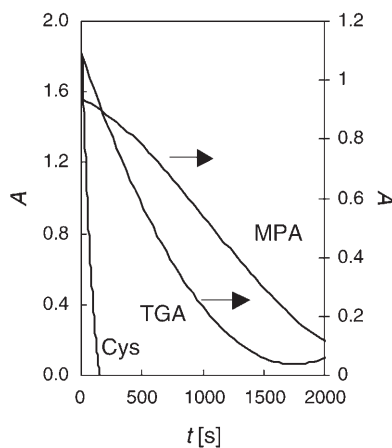


Fig. 1. Absorbance (Saville's method) vs. time profile for the different thiols. pH 9.4, T 10.0°, $[Se^{IV}]/[thiol]=50$. Arrows indicate the corresponding axes. Cys = L-cysteine, TGA = thioglycolic acid, and MPA = 3-mercaptopropanoic acid.

To analyze the dependence of the reaction on the Se^{IV} concentration, a series of experiments were performed at pH ca. 9.5 at different Se^{IV} concentrations but at constant thiol concentration ($[RSH]$). Fig. 2 shows the values of the initial rates for the three thiols studied vs. $[Se^{IV}]$.

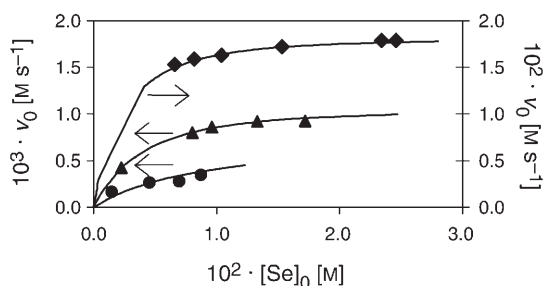


Fig. 2. Initial rate (Saville's method) as $[Se^{IV}]$ function. pH 9.4, T 10.0°, $[Cys]_0 = 5.0 \cdot 10^{-4}$, $[TGA]_0 = 2.93 \cdot 10^{-4}$, and $[MPA]_0 = 3.1 \cdot 10^{-4}$ M. Arrows indicate the corresponding axes. The solid line corresponds to the initial rate calculated by Eqn. 14 with the parameters presented in the Table. Experimental points: Cys \blacklozenge , TGA \blacktriangle , MPA \bullet .

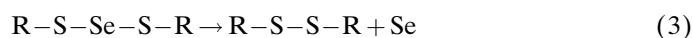
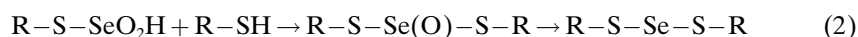
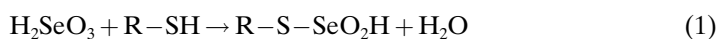
Table. Constants Used in Eqns. 10 and 14 to Model Experimental Data (Fig. 2)

Thiol ^{a)}	K [M ⁻¹]	k_2 [s ⁻¹]	k_3 [M ⁻¹ s ⁻¹]	ϵ [M ⁻¹ cm ⁻¹]
Cys	200	$8.2 \cdot 10^{-3}$	7.9	$4.4 \cdot 10^3$
TGA	120	$9.0 \cdot 10^{-4}$	2.0	$4.6 \cdot 10^3$
MPA	100	$7.0 \cdot 10^{-4}$	0.1	$4.0 \cdot 10^3$

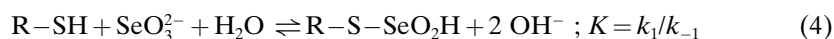
^{a)} Cys = L-cysteine, TGA = thioglycolic acid; MPA = 3-mercaptopropanoic acid.

4. *Spectral Changes as a Function of Time.* Fig. 3, a–c show the time evolution of the spectra for a reaction mixture with an initial [Se^{IV}]/[RSH] ratio of 50 and pH 9.5. They reveal the rapid appearance of a maximum at *ca.* 256 nm which disappears between 4 and 30 min for the different thiols. Fig 3, c includes the spectra of MPA and Se^{IV} at initial concentrations. Both have a negligibly absorbance at this wavelength. The same behavior is observed with Cys and TGA. The absorbance at 256 nm should be assigned to the formation of a complex RSH–Se^{IV} (see below). In the cases of Cys and TGA, an isosbestic point at 290 and 298 nm, respectively, is observed.

Discussion. – Kice and co-workers [29] studied the reaction of Se^{IV} with butane-1-thiol and 1,1-dimethylethanethiol in dioxane/water (6:4) mixtures and excess of thiol. They reported the spectra of the reaction mixtures and observed two maxima and two isosbestic points. These authors assigned these absorbances to the intermediates I₁ and I₂, proposing a complex mechanism for the reaction, illustrated by Eqns. 1–3. The decomposition of R–S–Se–S–R according to Eqn. 3 was accelerated in alkaline media [31].



In our case, the spectra show the formation of a single intermediate I and its subsequent decrease. This intermediate is assigned to the adduct R–S–SeO₂H. A second intermediate could intervene in the case of Cys and TGA, for which isosbestic points at 290 and 298 nm were detected (Fig. 3, a and b), respectively. The second intermediate (with a maximum at *ca.* 290 nm) can be reasonably attributed to R–S–Se–S–R [31][33], and its fast decomposition (involving two new molecules of thiol) to Se⁰ and R–S–S–R cannot be measured under our experimental conditions. On the other hand, in the presence of an excess of Se^{IV}, the decrease of the intermediate I did not follow a first-order kinetic; therefore, a new mechanism must be proposed, as illustrated in Eqns. 4–8, where Eqns. 7 and 8 summarize several fast steps.



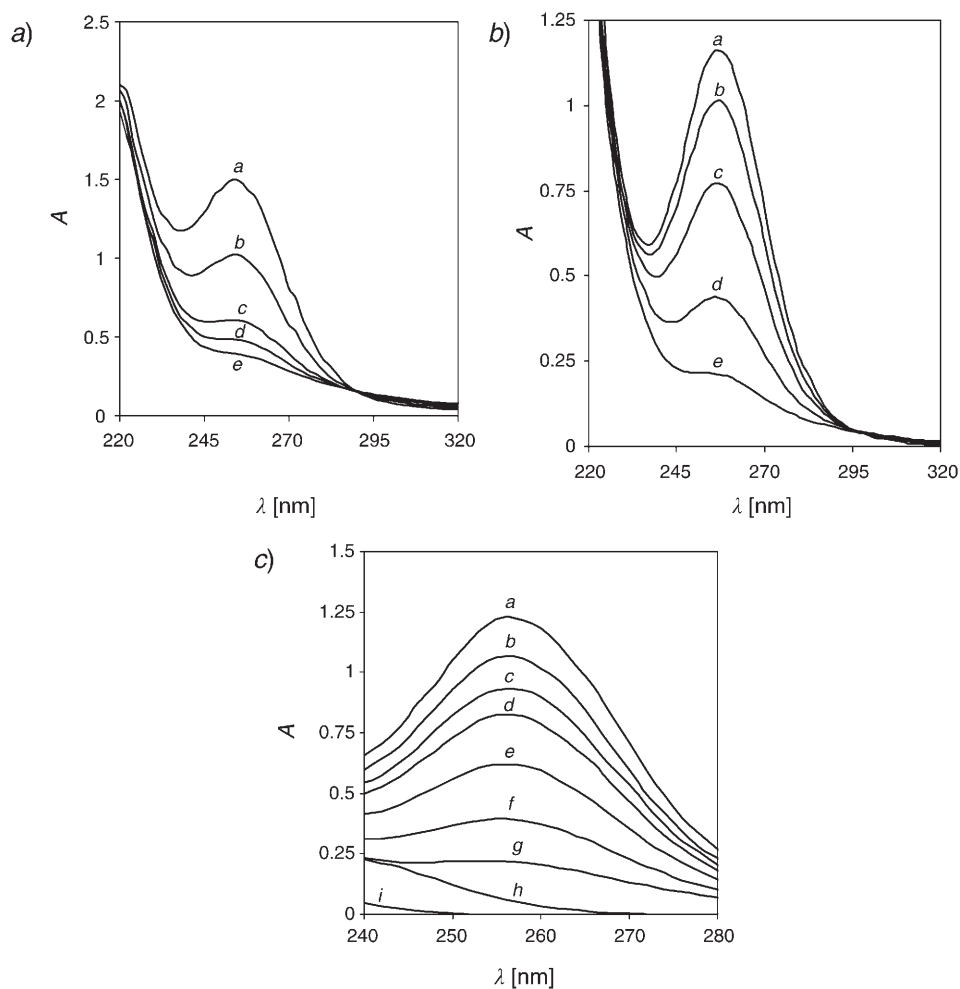
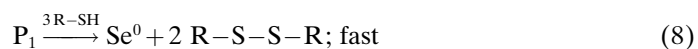
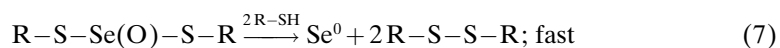
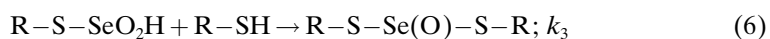


Fig. 3. Intermediate Spectrum at 256 nm for different times: a) Cys (a 45, b 90, c 131, d 155, and e 180 s); b) TGA (a 12, b 150, c 420, d 900, and e 1500 s); c) MPA (a 20, b 120, c 240, d 360, e 600, f 900, and g 1500 s; h $[MPA]_0 = 3.1 \cdot 10^{-4}$ M; i $[Se^{IV}]_0 = 1.5 \cdot 10^{-2}$ M)



Then, the reaction rate of disappearance of intermediate I can be expressed by Eqn. 9. From the integration of Eqn. 9, the instantaneous $R-S-SeO_2H$ concentration is obtained as shown by Eqn. 10 where K is given by Eqn. 11. Since the pH was constant throughout the experiments, the dependency on $[OH^-]$ is included in the value of K .

$$-\frac{d[\text{R-S-SeO}_2\text{H}]}{dt} = k_2[\text{R-S-SeO}_2\text{H}] + k_3[\text{R-S-SeO}_2\text{H}][\text{R-SH}] \quad (9)$$

$$[\text{R-S-SeO}_2\text{H}] = \frac{k_2[\text{R-S-SeO}_2\text{H}]_0}{k_2 e^{k_2 t} + k_3 K^{-1} [\text{SeO}_3^{2-}]^{-1} [\text{R-S-SeO}_2\text{H}]_0 e^{k_2 t} - k_3 K^{-1} [\text{SeO}_3^{2-}]^{-1} [\text{R-S-SeO}_2\text{H}]_0} \quad (10)$$

$$K = \frac{[\text{R-S-SeO}_2\text{H}]}{[\text{SeO}_3^{2-}][\text{R-SH}]} \quad (11)$$

By using the adequate molar absorptivity coefficient, absorbance values can be calculated and compared with the experimental values obtained from the spectra. *Fig. 4* shows the experimental data of the absorbance values at 256 nm vs. time for the different thiols. Values calculated with *Eqn. 10* and parameters shown in the *Table* are also included; as can be seen, the correlation obtained is excellent. The relative values of K follow the same correlation as that observed for the $\text{p}K_a$ values of the thiols (8.2, 9.9, and 10.0 for Cys, TGA, and MPA, resp.) [35].

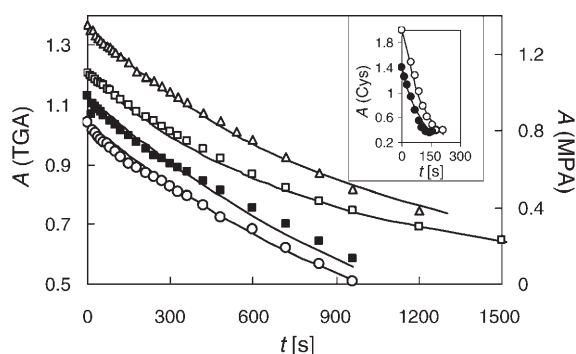


Fig. 4. Intermediate absorbance (A) decrease with time for the three thiols studied. \triangle $[\text{Se}^{\text{IV}}]/[\text{TGA}] = 50$; \square $[\text{Se}^{\text{IV}}]/[\text{TGA}] = 30$; \blacksquare $[\text{Se}^{\text{IV}}]/[\text{MPA}] = 50$; \circ $[\text{Se}^{\text{IV}}]/[\text{MPA}] = 40$; Inset: \circ $[\text{Se}^{\text{IV}}]/[\text{Cys}] = 50$; \bullet $[\text{Se}^{\text{IV}}]/[\text{Cys}] = 20$. The solid line corresponds to the initial rate calculated with *Eqn. 10* by using the parameters presented in the *Table*.

Dependence of the Initial Rate on $[\text{Se}^{\text{IV}}]$. In *Fig. 3, b*, the maximum absorbance of the complex formed from TGA at 12 s is 1.17. By using the K and ε values shown in the *Table*, the calculated complex concentration is ca. 80% of the initial thiol concentration. It can be seen from *Fig. 1* that, at this time, *Saville's* value has only decreased by ca. 3%, and the same behavior is observed for the other thiols. Therefore, *Saville's* method measures the total concentration of thiol ($[\text{R-SH}]_{\text{free}}$ and $[\text{R-S-SeO}_2\text{H}]$).

Then, this dependence of the total concentration of thiol according to *Saville's* method can be expressed by *Eqn. 12*, and, taking into account *Eqns. 4–8*, by *Eqn. 13*. At initial conditions, *Eqn. 14* holds.

$$\frac{-d[\text{R-SH}_{\text{tot}}]}{dt} = \frac{-d[\text{R-SH}_{\text{free}}]}{dt} + \frac{-d[\text{R-S-SeO}_2\text{H}]}{dt} \quad (12)$$

$$\frac{-d[\text{R-SH}_{\text{tot}}]}{dt} = k_2[\text{R-S-SeO}_2\text{H}] + 2k_3[\text{R-S-SeO}_2\text{H}][\text{R-SH}_{\text{free}}] \quad (13)$$

$$\begin{aligned} \frac{-d[\text{R-SH}_0]}{dt} &= \frac{k_2K[\text{SeO}_3^{2-}][\text{R-SH}_0]}{1 + K[\text{SeO}_3^{2-}]} \\ &+ 2k_3K[\text{SeO}_3^{2-}] \left\{ [\text{R-SH}_0] - [\text{R-SH}_0] \left(\frac{K[\text{SeO}_3^{2-}]}{1 + K[\text{SeO}_3^{2-}]} \right) \right\}^2 \end{aligned} \quad (14)$$

Fig. 2 shows the values of the initial rate (obtained as the initial slope from experimental curves of absorbance (*Saville's* method) vs. time) for different selenite concentrations. The solid line corresponds to the initial rate calculated by Eqn. 14 with the parameters presented in the Table. The excellent correlation observed validates the proposed model.

Conclusions. – At alkaline pH and with an excess of Se^{IV} , the reaction of thiols with Se^{IV} in aqueous media requires an additional pathway as compared with the *Kice* mechanism. The mechanism presented in this work allows modeling the rate of disappearance of the intermediate (followed by spectral changes) as the dependency of the initial rate of disappearance of thiol on the initial Se concentration. The values of thermodynamic and kinetics constants obtained in aqueous media should potentially be more convenient to characterize the biological process.

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