

Selenium incorporation into sulphur amino acids and
glutathione and the stability of the incorporation
products

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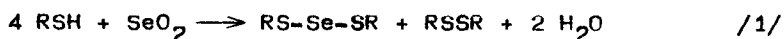
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Abstract:

The reaction of ^{75}Se -sodium selenite with cysteine, cystine and glutathione was studied. Selenodicysteine and selenodiglutathione were obtained as the incorporation products of inorganic selenium. The incorporation of selenium into these organic compounds is greatly affected by pH. The equilibrium constants of the selenodicysteine and selenodiglutathione formation were calculated. The stability of selenodicysteine in a strong acidic solution, in the hydrogen peroxide solution and in the presence of mercaptoethylamine were investigated. The mechanism of the decomposition of the investigated selenium compounds was suggested.

Introduction

It has been known that aqueous solutions of selenium dioxide react with sulphhydryl compounds to form relatively unstable derivatives. Painter /1/ proposed that the reaction between thiols and selenium dioxide took place in the following manner:



Compounds of the type of RS-Se-SR are analogues of the trisulphides in which one atom of sulphur is replaced by selenium and therefore they may be conveniently referred to as selenotrisulphides. Selenotrisulphides appear to participate far more readily than disulphides in reactions with thiols; the presence of selenium between two sulphur atoms confers a much greater sensitivity /compared to disulphides/ to attack by thiols. These compounds are likely to exhibit an activity in catalyzing oxidation-reduction reaction of thiols /2/. Dickson and Tappel /3/ have found interesting effects involving catalysis of disulphide interchange in proteins by seleno-amino acids. Ganther and Corcoran /4/ investigated the possibility of a sulphhydryl-selenotrisulphide exchange reaction in protein.

Because of the importance of selenium compounds in the biochemistry of selenium this paper reports a study of the action of selenite on sulphur amino acids: cysteine, cystine and glutathione.

Materials and Methods

The radionuclide ^{75}Se -selenium obtained from The Radioisotope Production and Distribution Centre Świerk, Poland, was used as $\text{Na}_2^{75}\text{SeO}_3$.

The specific activity was 322 MBq/mg Se. The purity was controlled by high voltage paper electrophoresis 70 V/cm and by the radiochromatogram scanner Packard Model 7201. Cysteine and cystine were obtained from Flucka AG Buchs SG; glutathione from E. Merck AG.

Other reagent-grade chemicals were obtained from commercial sources, satisfactory for use without further purification.

The reaction of selenium dioxide or other selenium compounds with sulphur amino acids was studied in aqueous, thermostated solutions, from which the oxygen was rigorously excluded by continuously flushing the system with argon $4 \text{ cm}^3/\text{s}$ [Figure 1].

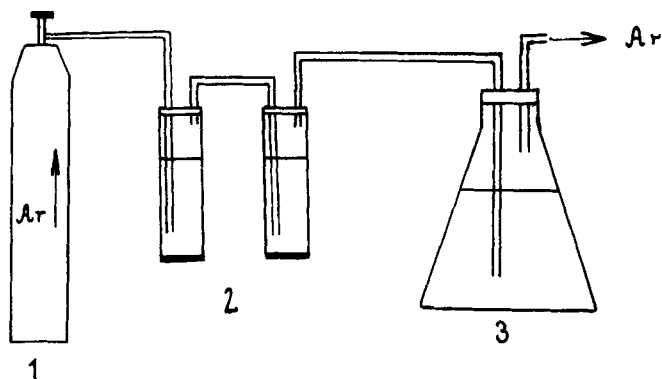


Figure 1. Apparatus for the investigation of the stability of selenium compounds:

1. argon cylinder
2. washers with pyrogallol
3. flask with the investigated sample

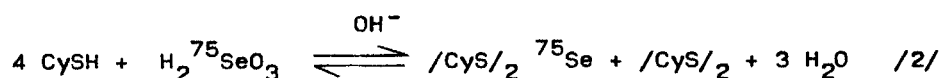
The reaction products were examined by high voltage paper electrophoresis in 0.02 M sodium acetate solution, by the thin-layer chromatography and spectrophotometrically with a "Spectromom 202" spectrophotometer. The radioactive products were detected with radiochromatogram scanner Packard.

Results

I. Incorporation of selenium into cysteine

The reaction of ^{75}Se -sodium selenite with cysteine was studied at 291°K in 0.1 M NaCl solutions at pH 1.4 - 9.5 in darkness. After 15 minutes the reaction products were separated

and identified. The selenodicysteine $/\text{Cys}/_2\text{Se}$ and cystine $/\text{Cys}/_2$ have been found as reaction products by the comparison with their standards. The same quality and quantity of reaction products were also identified after 48 hours of the action of ^{75}Se -sodium selenite with cysteine, at pH 1.4 - 2.4. This indicates that the equilibrium of the reaction of ^{75}Se -sodium selenite with cysteine has been established during 15 minutes. The following reaction is responsible for the incorporation of selenium into cysteine:



The apparent equilibrium constants for this reaction were calculated /Table 1/.

Table 1. Products from the reaction of $\text{Na}_2^{75}\text{SeO}_3$ with cysteine at pH 1.4 and 2.4

pH	$\text{Na}_2^{75}\text{SeO}_3$ mole dm^{-3}	CySH mole dm^{-3}	$/\text{Cys}/_2$ mole dm^{-3}	$/\text{Cys}/_2^{75}\text{Se}$ mole dm^{-3}	K_{pH} - the apparent equilibrium const.
1.4	$0.878 \cdot 10^{-4}$	$1.719 \cdot 10^{-4}$	$0.497 \cdot 10^{-4}$	$0.497 \cdot 10^{-4}$	$3.22 \cdot 10^{10}$
2.4	$0.830 \cdot 10^{-4}$	$1.540 \cdot 10^{-4}$	$0.545 \cdot 10^{-4}$	$0.545 \cdot 10^{-4}$	$6.36 \cdot 10^{10}$

When the influence of pH value for this reaction is taken into consideration the calculated equilibrium constant is $K = 1.93 \cdot 10^{14}$
 $/K = K_{\text{pH}} \cdot [\text{OH}^-]^{\delta}, \delta = -0.3/.$

The influence of pH value of the reaction on the selenium incorporation into cysteine has been observed /Table 2, Figure 2/. The yield of selenodicysteine and other selenium compounds were measured after 15 minutes of the reaction.

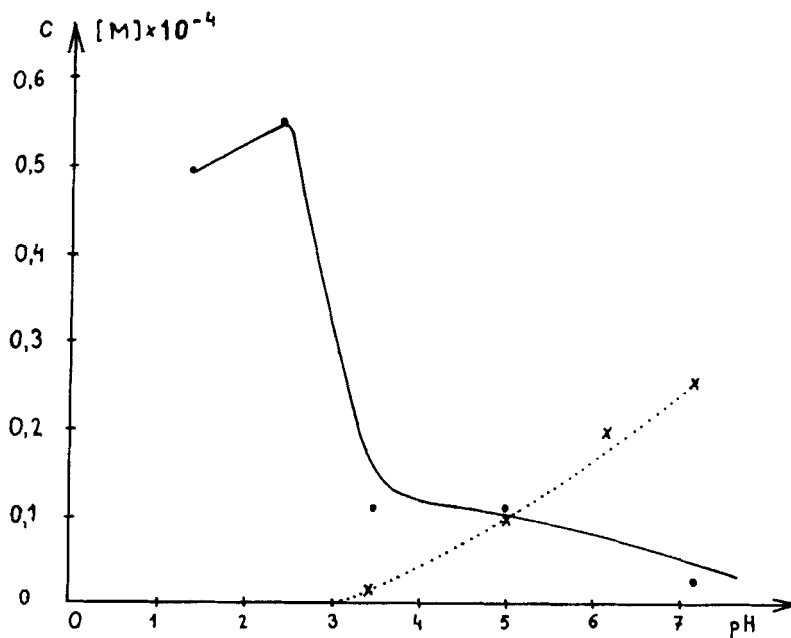


Figure 2. The influence of pH value on the selenium incorporation into cysteine. Reaction system: cysteine $4 \cdot 10^{-4}$ M and sodium selenite $1.4 \cdot 10^{-4}$ M

..... the colloidal selenium

—— selenodicysteine

Table 2. Selenium incorporation into cysteine in dependence of pH value. Reaction system: cysteine $4 \cdot 10^{-4}$ M and $\text{Na}_2^{75}\text{SeO}_3$ $1.4 \cdot 10^{-4}$ M.

pH	selenium in /Cys/ ₂ Se
	selenium in inorganic compounds
1.4	0.566
2.4	0.657
3.4	0.089
5.0	0.083
7.2	0.024

By increasing the pH value the yield of selenodicysteine decreased. At pH 3.4 - 5.0 the formation of an additional inorganic selenium compound has been observed, probably the unstable HSe_2O_3^- . The formation of the colloidal selenium /as a product of decomposition of $\text{Cys}/_2\text{Se}/$ has been studied at pH = 2 in dependence of the relative molecular concentrations of cysteine to selenium dioxide /Table 3/. The yield of the elementary selenium is measured after 100 hours of the reaction of selenium dioxide with cysteine.

Table 3. The formation of the elementary selenium in dependence of the relative molecular concentrations of cysteine to selenium dioxide.

Ratio of molecular concentration $\frac{[\text{cysteine}]^*}{[\text{SeO}_2]}$	amount of elementary Se [mg]
$\frac{1}{1000}$	30
$\frac{1}{120}$	27
$\frac{1}{30}$	9
$\frac{1}{15}$	7
$\frac{1}{4}$	0

* Reaction system: cysteine 10^{-3}M and the amount of selenium dioxide shown in a Table.

Table 4. Products from the reaction of cystine with ^{75}Se -sodium selenite at pH = 2; 7.5 and 8.5 at 291 $^{\circ}\text{K}$. Reaction system: $2.3 \cdot 10^{-4}$ M cystine, $3.5 \cdot 10^{-4}$ M $\text{Na}_2^{75}\text{SeO}_3$.

reaction time [s] x 3600	pH 2			pH 7.5		pH 8.5	
	$\text{Na}_2^{75}\text{SeO}_3$	/Cys/ 2	$^{75}\text{Se}^0$	$\text{Na}^{75}\text{SeO}_3$	$^{75}\text{Se}^0$	$\text{Na}_2^{75}\text{SeO}_3$	$^{75}\text{Se}^0$
	$\frac{\text{mole} \cdot 10^{-4}}{\text{dm}^3}$	$\frac{\text{mole} \cdot 10^{-4}}{\text{dm}^3}$	$\frac{\text{mole} \cdot 10^{-4}}{\text{dm}^3}$	$\frac{\text{mole} \cdot 10^{-4}}{\text{dm}^3}$	$\frac{\text{mole} \cdot 10^{-4}}{\text{dm}^3}$	$\frac{\text{mole} \cdot 10^{-4}}{\text{dm}^3}$	$\frac{\text{mole} \cdot 10^{-4}}{\text{dm}^3}$
0.12	3.023	-	0.477	3.270	0.230	3.280	0.220
2	3.026	-	0.474	3.325	0.175	3.350	0.150
20	3.027	-	0.473	3.353	0.147	3.391	0.109
48	3.055	0.005	0.440	3.395	0.105	3.432	0.068
72	2.987	0.080	0.433	3.413	0.087	3.437	0.063
144	2.980	0.160	0.360	3.433	0.067	3.447	0.053
240	2.913	0.230	0.357	3.430	0.070	3.446	0.054

II. Incorporation of selenium into cystine

The action of ^{75}Se -sodium selenite on cystine was studied under the same conditions as cysteine. This reaction is more complex and depends of the equilibrium between cysteine and cystine in aqueous solutions. The detected and identified reaction products were the selenodicysteine and the elementary selenium. The pH value of the reaction, as well as the time and temperature of the process affect the formation of selenodicysteine /Tables 4 and 5/.

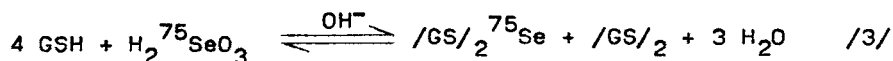
Table 5. Products from the reaction of cystine with ^{75}Se -sodium selenite at pH = 2, at 333^oK. Reaction system: $2.3 \cdot 10^{-4}\text{M}$ cystine, $3.5 \cdot 10^{-4}\text{M}$ $\text{Na}_2^{75}\text{SeO}_3$.

radioproduct [$\frac{\text{mole}}{\text{dm}^3}$] $\cdot 10^{-4}$	time of the reaction [s] x 3600				
	0.12	2.50	5.0	8.0	10.0
$\text{Na}_2^{75}\text{SeO}_3$	2.817	2.830	2.861	2.912	2.955
/Cys/ $_2^{75}\text{Se}$	0.543	0.500	0.439	0.395	0.335
$^{75}\text{Se}^0$	0.140	0.170	0.200	0.193	0.210

III. Incorporation of selenium into glutathione

A. The reaction of ^{75}Se -sodium selenite with glutathione GSH was studied under the same reaction conditions as with cysteine. The reaction was examined at pH = 1.4 - 7.2. The selenodigluthione /GS/ $_2\text{Se}$ has been identified as a reaction product by the comparison with the standard. The same quality and quantity of reaction products were also identified after 48 hours of the action of ^{75}Se -sodium selenite with glutathione at pH 1.4 - 2.4,

This indicate that the equilibrium of the reaction of glutathione with ^{75}Se -sodium selenite has been established during 15 minutes. The apparent equilibrium constant for the following reaction:



has been calculated /Table 6/.

Table 6. Products from reaction of $\text{Na}_2^{75}\text{SeO}_3$ with glutathione at pH 1.4 and 2.4

pH	$\text{Na}_2^{75}\text{SeO}_3$ mole · dm ⁻³	GSH mole · dm ⁻³	$/\text{GS}/_2$ mole · dm ⁻³	$/\text{GS}/_2^{75}\text{Se}$ mole · dm ⁻³	K_{pH} - the apparent equilibrium constant
1.4	$0.979 \cdot 10^{-4}$	$2.335 \cdot 10^{-4}$	$0.396 \cdot 10^{-4}$	$0.396 \cdot 10^{-4}$	$5.40 \cdot 10^9$
2.4	$0.858 \cdot 10^{-4}$	$1.919 \cdot 10^{-4}$	$0.517 \cdot 10^{-4}$	$0.517 \cdot 10^{-4}$	$2.30 \cdot 10^{10}$

When the influence of pH value for this reaction is taken into consideration the calculated equilibrium constant is $K = 4.68 \cdot 10^{17}$ / $K = K_{\text{pH}} \cdot [\text{OH}^-]^\beta$, $\beta = -0.63$ /. When the pH of the reaction mixture increased / $\text{pH} > 2.4$ / the yield of $/\text{GS}/_2 \text{Se}$ formation decreased and an additional reaction product has been observed /only at pH 3.4 - 5/. This is an inorganic selenium compound, probably HSe_2O_3^- which is formed in the first 15 minutes after the beginning of the reaction and disappears after 2 hours /Figure 3/. The influence of pH value of the reaction on the selenium incorporation into glutathione has been observed /Table 7, Figure 4/. The yield of selenodiglutathione and other selenium compounds are measured after 15 minutes of the reaction.

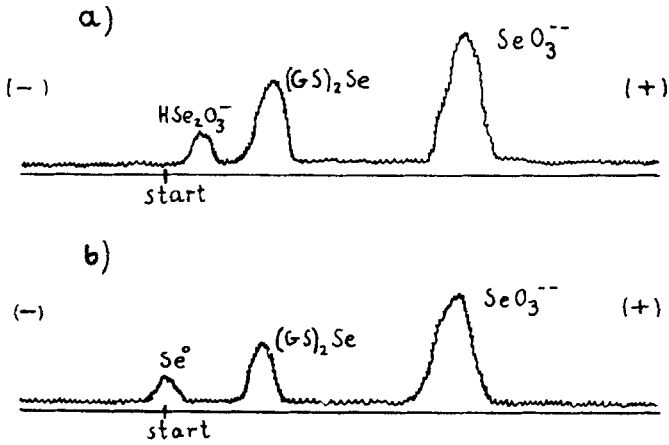


Figure 3. Radioelectrophoregramme of the reaction products in the system: glutathione $4 \cdot 10^{-4}$ M and sodium selenite $1.4 \cdot 10^{-4}$ M at pH 5 in time dependence

a/ after 15 minutes

b/ after 2 hours

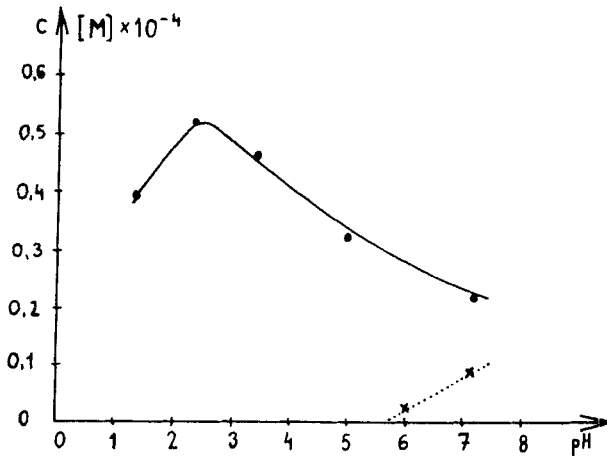


Figure 4. The influence of pH value on the selenium incorporation into glutathione. Reaction system: glutathione $4 \cdot 10^{-4}$ M and sodium selenite $1.4 \cdot 10^{-4}$ M

..... the colloidal selenium

———— selenodiglutathione

Table 7. Selenium incorporation into glutathione in dependence of pH value. Reaction system: glutathione $4 \cdot 10^{-4}$ M and $\text{Na}_2^{75}\text{SeO}_3$ $1.4 \cdot 10^{-4}$ M

pH	selenium in /GS/ $_2\text{Se}$
	selenium in inorganic compounds
1.4	0.405
2.4	0.603
3.4	0.490
5.0	0.307
7.2	0.206

B. The action of radioactive selenodicysteine /Cys/ $_2^{75}\text{Se}$ with glutathione was examined in deaerated 0.01 M sodium acetate solution at pH 4.1, at temperature 293⁰K in darkness. The reaction products were separated and identified spectrophotometrically and by the use of high voltage paper electrophoresis /in 0.02 M sodium acetate, at pH 4.1, during 50 minutes at voltage 70 V/cm and current intensity 7 mA/. The following reaction products with incorporated selenium were obtained: selenodiglutathione, selenocysteinoglutathione /GS/-Se... /Cys/ and the colloidal selenium. The formation of above products is a time dependent process /Figures 5 and 6/.

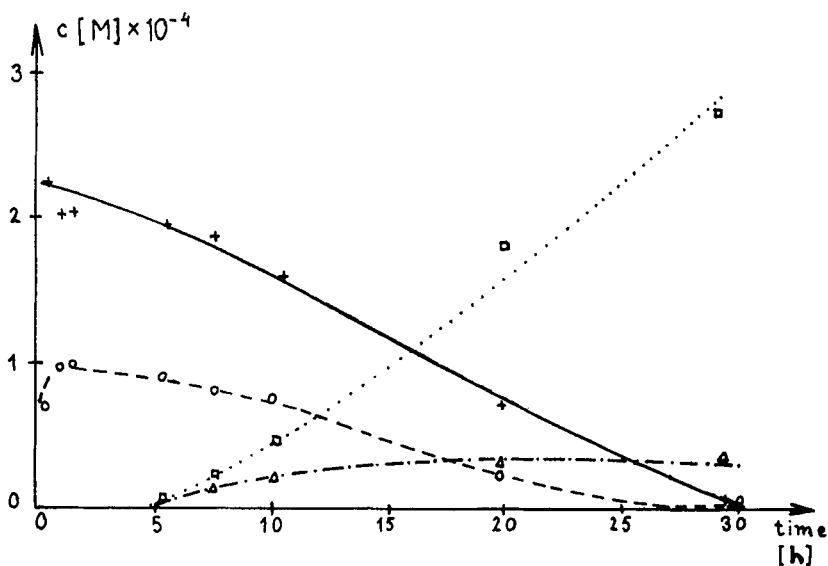


Figure 5. The reaction products in the system: selenodicysteine $3 \cdot 10^{-4}$ M and glutathione $1.2 \cdot 10^{-4}$ M

- selenodicysteine
- selenodiglutathione
- selenocysteinoglutathione
- the colloidal selenium

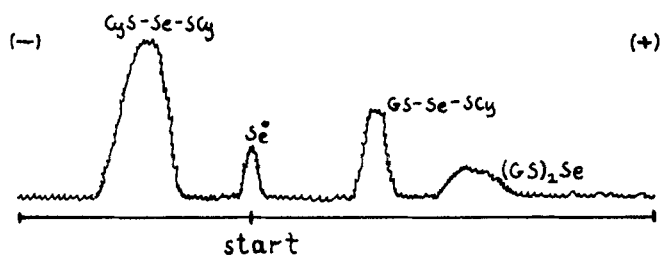


Figure 6. Radioelectrophoregramme of the reaction products in the system: selenodicysteine $3 \cdot 10^{-4}$ M and glutathione $1.2 \cdot 10^{-4}$ M.

IV. The stability of selenium incorporated compounds

A. In the reaction mixture:

The reactions of ^{75}Se -sodium selenite $1.8 \cdot 10^{-4}$ M with cysteine $/4 \cdot 10^{-4}$ M/ and with glutathione $/4 \cdot 10^{-4}$ M/ were investigated.

The reactions products were separated and identified after 15 minutes and 2 hours, respectively. The results obtained are shown in Table 8.

Table 8. Selenium incorporation into cysteine and glutathione after 15 minutes and 120 hours in 0.01 M sodium acetate, at pH = 5.0 at 291 K.

reaction time [s]. 3600	selenium in /CyS/ ₂ Se	selenium in /GS/ ₂ Se
	selenium in inorganic compounds	selenium in inorganic compounds
0.25	0.073	0.290
120	0.059	0.286

B. In strong acidic solution:

The formation of colloidal selenium /as a product of decomposition of /CyS/₂Se /has been observed in strong acids solutions/ H_2SO_4 , HCl/.

The rate of decomposition reaction of /CyS/₂Se decreases with decreasing concentration of the acid. The rate of decomposition reaction of /CyS/₂Se decreases to zero in 2 M HCl /Table 9/.

Table 9. The decomposition of $/\text{Cys}/_2\text{Se}$ in strongly acid medium i.e. HCl. Initial concentration of $/\text{Cys}/_2\text{Se}$ - 10^{-3} M at 293°K .

concentration of HCl [M]	total decomposition of $/\text{Cys}/_2\text{Se}$ [s]
12	instantly
10	instantly
7	300
5.5	720
4	32400

C. In the presence of mercaptoethylamine MEA:

The reaction of ^{75}Se -sodium selenite with cysteine in the presence of mercaptoethylamine was investigated using the following molecular concentration: ^{75}Se -sodium selenite $1.8 \cdot 10^{-3}$ M, cysteine $6.0 \cdot 10^{-3}$ M, MEA $7 \cdot 10^{-3}$ M in 0.01 M sodium acetate at pH 3.2 and temperature 291°K in darkness. The formation of the colloidal selenium was instantly observed. In the reaction mixture the following selenium compounds were identified: selenodicysteine and seleno-mercaptoethylaminecysteine $/\text{MEA}/-\text{Se}-/\text{Cys}/$. The results are shown in Table 10.

Table 10. Selenium incorporation into cysteine and mercaptoethylamine. Reaction system: ^{75}Se -sodium selenite $1.8 \cdot 10^{-3}$ M, cysteine $6 \cdot 10^{-3}$ M, mercaptoethylamine $7 \cdot 10^{-3}$ M; at pH 3.2; at 291°K

radioproduct [mole·dm ⁻³].10 ⁻³	time of the reaction [s].3600		
	0.2 [mole·dm ⁻³].10 ⁻³	3.0 [mole·dm ⁻³].10 ⁻³	72.0 [mole·dm ⁻³]. 10 ⁻³
$\text{Na}_2^{75}\text{SeO}_3$	1.489	1.430	1.395
$^{75}\text{Se}^0$	0.244	0.297	0.399
/Cys/ ₂ ⁷⁵ Se	0.074	0.073	0.007
/MEA/ _{-Se-} /Cys/	0.002	0.001	-

D. In the presence of mercaptoethylamine and glutathione:

The reaction of ^{75}Se -sodium selenite with cysteine and mercaptoethylamine in the presence of glutathione was investigated using the following molecular concentrations: ^{75}Se -sodium selenite $1.8 \cdot 10^{-3}$ M, cysteine $6 \cdot 10^{-3}$ M, mercaptoethylamine $7 \cdot 10^{-3}$ M, glutathione $5.5 \cdot 10^{-3}$ M in 0.01 M sodium acetate at pH 3.2 and temperature 291°K in darkness. Selenium was incorporated mainly into glutathione and cysteine as: /GS/₂Se, /GS/_{-Se-}/Cys/, /GS/_{-Se-}/MEA/, /Cys/₂Se and /MEA/_{-Se-}/Cys/. The yields both of the colloidal selenium and of the unreacted selenite remained

unchanged during 72 hours of observations. The results are shown in Table 11.

Table 11. Selenium incorporation into: cysteine, mercaptoethylamine and glutathione. Reaction system: sodium selenite $1.8 \cdot 10^{-3}$ M, cysteine $6 \cdot 10^{-3}$ M, mercaptoethylamine $7 \cdot 10^{-3}$ M, glutathione $5.5 \cdot 10^{-3}$ M; at pH = 3.2; at 291^oK.

radioproduct	time of the reaction [s] 3600	
	0.2 /mole·dm ⁻³ / · 10 ⁻³	72.0 /mole·dm ⁻³ / · 10 ⁻³
Na ₂ ⁷⁵ SeO ₃	0.824	0.825
⁷⁵ Se ⁰	0.100	0.104
⁷⁵ Se incorporated into organic compounds	0.876 *	0.871 *

* selenium in: /Cys/₂ Se - $0.131 \cdot 10^{-3}$ M

selenium in: /MEA/ -Se- /Cys/ - trace

selenium in: /GS/₂Se, /GS/-Se- /Cys/, /GS/-Se- /MEA/ -
 $0.740 \cdot 10^{-3}$ M

E. In presence of hydrogen peroxide:

The stability of $/\text{Cys}/_2\text{Se}$ in the presence of hydrogen peroxide in 0.01 M sodium acetate solution /deaerated and protected from the action of light/was investigated. The decomposition rate of $/\text{Cys}/_2\text{Se}$ in various concentrations of hydrogen peroxide at pH 7.4 and 292^oK was examined. The results are shown in Figure 7.

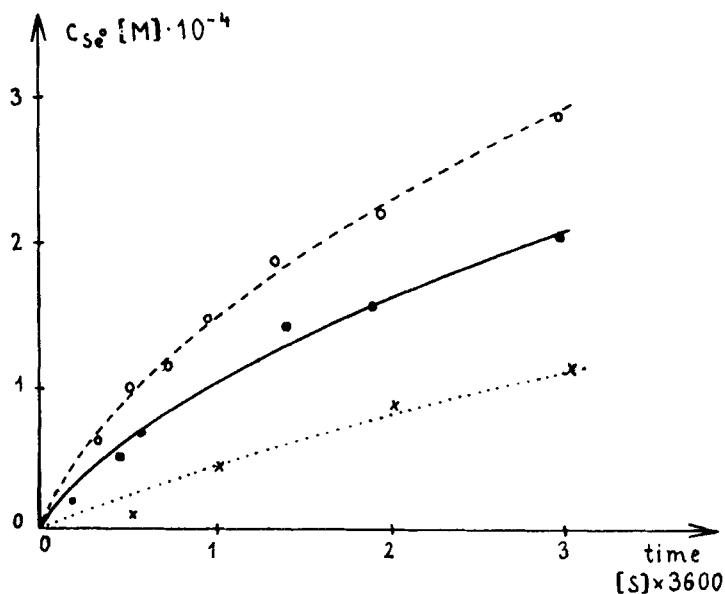


Figure 7. The decomposition rate of selenodicysteine in the presence of hydrogen peroxide at pH 7.4

- selenodicysteine $3 \cdot 10^{-4}$ M
- selenodicysteine $3 \cdot 10^{-4}$ M and hydrogen peroxide $3 \cdot 10^{-4}$ M
- selenodicysteine $3 \cdot 10^{-4}$ M and hydrogen peroxide $3 \cdot 10^{-3}$ M

Cysteine and the colloidal selenium were obtained. The presence of cystine was established using thin-layer chromatography and paper chromatography /Whatman 3MM, in 1-butanol-acetic acid-water 4 : 1 : 1/.

It was also observed that the decomposition rate of $/\text{Cys}/_2\text{Se}$ is a pH dependent process /Figure 8/. The decomposition reaction of $/\text{Cys}/_2\text{Se}$ in hydrogen peroxide is a first order reaction for $/\text{Cys}/_2\text{Se}$ and hydrogen peroxide /Tables 12 and 13/. The rate constant for the reaction of decomposition of $/\text{Cys}/_2\text{Se}$ in the presence of H_2O_2 is: $k = 3.6 \cdot 10^6 \left[\frac{\text{mole}}{\text{dm}^3} \right]^{-\frac{7}{4}} \cdot \text{s}^{-1}$.

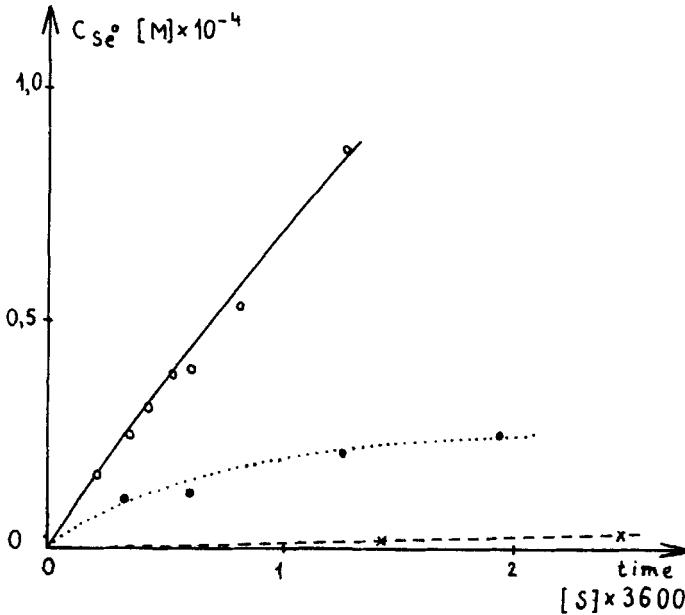


Figure 8. The decomposition rate of selenodicysteine $3 \cdot 10^{-4}$ M in the presence of hydrogen peroxide at 292°K in dependence of pH value

- pH 4.9
- pH 6.0
- pH 7.4

Table 12. Decomposition rate of $\text{Cys}/_2\text{Se}$ in the presence of H_2O_2 .
 Reaction system: $\text{H}_2\text{O}_2 = 3.1 \cdot 10^{-3} \text{ M}$; $\text{Cys}/_2\text{Se} = 3.1 \cdot 10^{-4} \text{ M}$
 and $1.55 \cdot 10^{-4} \text{ M}$ respectively at pH = 6.5 at 295^oK.

concentration of reagents: $\text{C}^0 \text{Cys}/_2\text{Se} = 3.1 \cdot 10^{-4} \text{ M}$; $\text{C}^0 \text{H}_2\text{O}_2 = 3.1 \cdot 10^{-3} \text{ M}$		concentration of reagents: $\text{C}^0 \text{Cys}/_2\text{Se} = 1.55 \cdot 10^{-4} \text{ M}$; $\text{C}^0 \text{H}_2\text{O}_2 = 3.1 \cdot 10^{-3} \text{ M}$	
reaction time [s]	concentration of $\text{Cys}/_2\text{Se}$ mole $\cdot \text{dm}^{-3}$	decomposition rate $V = -\frac{\Delta C}{\Delta t}$ mole $\cdot \text{dm}^{-3} \cdot \text{s}^{-1}$	reaction time [s]
1020	$2.936 \cdot 10^{-4}$	$1.62 \cdot 10^{-8}$	1020
1300	$2.880 \cdot 10^{-4}$	$1.70 \cdot 10^{-8}$	1300
1680	$2.812 \cdot 10^{-4}$	$1.71 \cdot 10^{-8}$	1680
			decomposition rate $V = -\frac{\Delta C}{\Delta t}$ mole $\cdot \text{dm}^{-3} \cdot \text{s}^{-1}$
			$8.65 \cdot 10^{-9}$
			$8.14 \cdot 10^{-9}$
			$8.05 \cdot 10^{-9}$

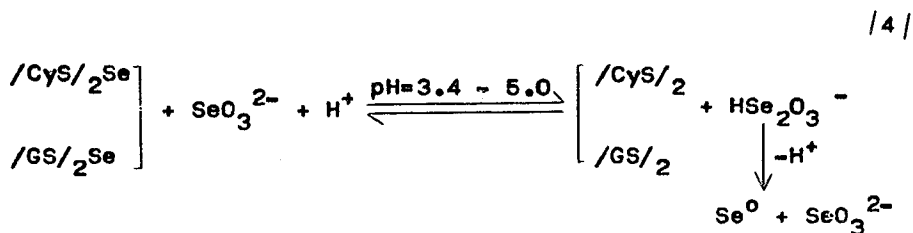
The activation energy the decomposition reaction of $/\text{Cys}/_2\text{Se}$ in the presence of H_2O_2 is: $E = 69.0 \frac{\text{kJ}}{\text{mole}}$.

The decomposition of hydrogen peroxide in the reaction of hydrogen peroxide $/3 \cdot 10^{-3} \text{ M}/$ with selenodicysteine $/3 \cdot 10^{-4} \text{ M}/$ at pH 7.5 was $6 \cdot 10^{-4} \text{ M}$. Hydrogen peroxide was titrated /iodine - thiosulphate method/ after complete decomposition of selenodicysteine.

Discussion and Conclusion

The formation of selenodicysteine and selenodiglutathione in the reaction of cysteine and glutathione with selenite confirms earlier results of Painter [1].

The incorporation of inorganic selenium into cysteine, cystine and glutathione is greatly affected by pH. The yield of incorporation products decreased with increasing pH in the reaction mixture. Two radioactive selenium compounds were observed: the colloidal selenium /product of decomposition of selenodicysteine and selenodiglutathione/ and the unidentified inorganic compound /most likely HSe_2O_3^- , Figure 3/. The formation of supposed HSe_2O_3^- product is a time dependent process. After 2 hours after the reaction beginning HSe_2O_3^- disappears and the yield of colloidal selenium and unreacted selenite increased. It seems that the following reaction is responsible for the decomposition of selenodicysteine and selenodiglutathione in the reaction mixture

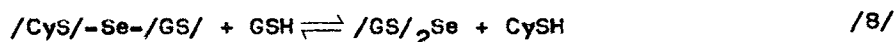
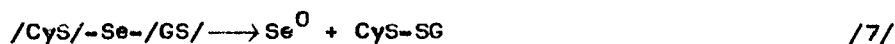
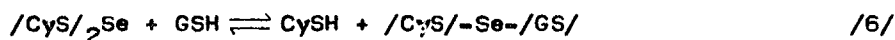


[4]

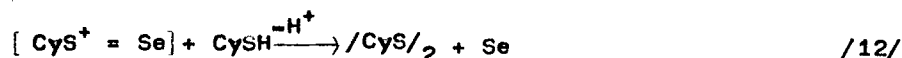
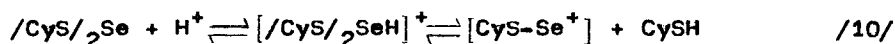
The formation of selenodicysteine in the reaction of cysteine with selenite is due to the equilibrium reached in the water solution between cysteine and cystine:



In the presence of other thiols the selenodicysteine and selenodiglutathione are unstable. The reaction of selenodicysteine with glutathione or mercaptoethylamine leads to the formation of unsymmetric organic selenium compounds: /GS-/Se-/MEA/ /CyS-/Se-/GS/ and /CyS-/Se-/MEA/ and symmetric ones: /GS/₂Se, /CyS/₂Se and /MEA/₂Se. Simultaneously colloidal selenium was formed as a product of decomposition of the organic selenium compounds:

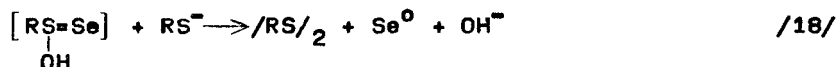
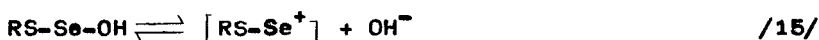
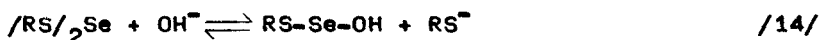


The presence of cysteine in the reaction mixture is firmly established in our experiment using thin layer chromatography and high voltage electrophoresis. The decomposition reaction of selenodicysteine occurred both in the strong acidic and the alkaline solutions. We proposed the following mechanism for the decomposition reaction of selenodicysteine in the strong acidic solution:

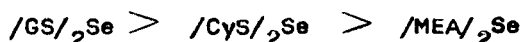




The supposed mechanism for the decomposition reaction of selenodicysteine or selenodiglutathione in the alkaline solutions is:



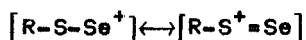
The stability of this organic selenium compound was found to decrease in the following range:



It follows from the literature data that the dissociation constants of SH groups of glutathione, cysteine and mercaptoethylamine are found to obey the same order:

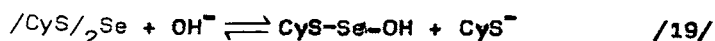


The dissociation constants of SH groups of glutathione, cysteine and mercaptoethylamine are: $2.24 \cdot 10^{-10}$, $2.82 \cdot 10^{-11}$ and $1.55 \cdot 10^{-11}$ respectively /5, 6/. If the decomposition reaction of selenodicysteine, selenodiglutathione and selenodimercaptoethylamine occurs with the formation of the intermediate product:

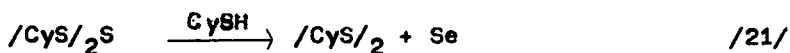
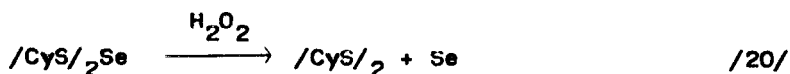


the electron density at the sulphur atom affect the dissociation of the [S-Se] bond. In glutathione the sulphur atom has the smallest electron density. The sulphur atom in cysteine has greater electron density than that in glutathione, therefore the formation of mesomeric structure $[R-S^+ = Se]$ is energetically more favourable. Hence, the rate for the decomposition of selenodicysteine is faster than that of selenodiglutathione and that for selenodimercaptoethylamine is the fastest of all three selenium compounds.

Organic sulphur compounds of selenium /undergoing decomposition to the elemental selenium and sulphur compounds/ affect greatly the decomposition of peroxide. According to the suggestion of Tappel and Caldwell /7/ decomposition of hydrogen peroxide is involved by the action of selenium atom of selenocysteine. Selenium is known to have more nucleophilic character than sulphur; therefore, under the same experimental conditions, its compounds may be predicted to react faster with peroxides than their sulphur analogs. The experimental results show that the decomposition rate of selenodicysteine in the presence of hydrogen peroxide is markedly accelerated. For the molecular ratio of hydrogen peroxide to selenodicysteine 1 : 1, the values for the decomposition rates of $(CyS)_2Se$ suggest that cysteine influences the decomposition of selenodicysteine /8, 9/. Cysteine is formed presumably according to the reaction:



and is partially oxidized by hydrogen peroxide to cystine. Thus the decomposition of selenodicysteine results from two reactions:



In the presence of excess H_2O_2 /for the molecular ratio of hydrogen peroxide to selenodicysteine 10 : 1/ cysteine is indifferent to the decomposition rate of selenodicysteine. Total cysteine is oxidized to cystine by excess hydrogen peroxide. The decomposition of selenodicysteine is due to hydrogen peroxide only. So with the molecular concentration of hydrogen peroxide /from $3.1 \cdot 10^{-4}$ M to $3.1 \cdot 10^{-3}$ M /increased tenfold the decomposition rate of selenodicysteine / $3.1 \cdot 10^{-4}$ M/ does not increase in proportion to increased hydrogen peroxide concentration /Figure 7/. The chief conclusion is that selenodicysteine has the chemical capacity to decompose the peroxides.

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