THE REACTION OF SELENOUS ACID WITH CYSTEINE[1,2]

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Attempts to purify and characterize a selenium-glutathione complex led to the study of selenium-cysteine compounds because better analytical methods for the substances in the reactions were available. Previous tests indicated that when selenous acid reacted with glutathione, a selenium tetra- or hexa- glutathione was formed depending on the conditions but the possibility of the following reactions taking place was difficult to eliminate due to inadequate analytical methods.

$$\text{(a)}\quad 2RSH + SeO_2 \longrightarrow RS - \overset{\overset{O}{\Vert}}{Se} - SR + H_2O$$

$$\text{(b)}\quad 4RSH + SeO_2 \longrightarrow RS - Se - SR + RSSR + 2H_2O$$

$$\text{(c)}\quad 4RSH + SeO_2 \longrightarrow 2\ RSSR + Se + 2H_2O \qquad (1)$$

In this paper, the data support equation (b) instead of the production of a tetracysteine of selenium as reported in the literature (2).

Experimental

Selenium tetracysteine, as made in this laboratory according to Stekol (2), and a sample of selenium dicysteine furnished by Dr. E. P. Painter, were analyzed for cysteine and cystine by the Kassell and Brand method (3). A check on the method as shown by recoveries of these two amino acids from aqueous solutions is shown in Table I.

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TABLE I

Recoveries of Cysteine and Cystine from pure solutions by the Kassel and Brand method.

All values in micrograms

Expt. No.	Cystine Added	Cysteine Added	Cystine Found	Cysteine Found
1.	29.4	none	29.6	none
2.	none	29.4	none	29.0
3.	29.4	29.4	30.6	27.0

Each value represents averages of at least three determinations.

This indicates good recoveries although cysteine values may be slightly low. The application of the method to the compounds gave the following results. No cysteine was found in a 50 ug. sample of selenium tetracysteine but the analysis showed 22.8 ug. of cystine. This would indicate a mixture of a dicysteine of selenium and cystine instead of the selenium tetracysteine compound. This was also shown with the iodate titration (4) which showed no cysteine in the samples but on reduction with Zn dust indicated 36.7 ug. of cysteine. This is approximately the theoretical amount of cysteine that should be found in 50 ug. of selenium dicysteine instead of the tetracysteine, but it is not conclusive because cysteine also comes from the reduction of cystine and is also measured by this method on reduction with Zn. Similar tests were made on the sample of selenium dicysteine and the data for both compounds are summarized in Table II.

The disturbing factor in Table II is the presence of cystine as shown by the Kassel and Brand method. Other data pertinent to the problem are shown in Table III. The inability to get well defined melting points made this data less helpful, but the elemental analysis appeared to be satisfactory.

TABLE II

Cysteine and Cystine Contents of Selenium-Cysteine Complexes

Fifty micrograms of each compound were used

Amino Acid	Method	Selenium dicysteine		Selenium tetracysteine		Mixture of Selenium Dicysteine and Cystine	
		Theoretical	Found	Theoretical	Found	Theoretical	
I. Cysteine (a) Before reduction	(3)	none	none	none	none	none	none
	(4)						
(b) After Zn reduction	(4)	37.9	36.7	43.3	36.7	50	
II. Cystine	(3)	none	21.0	none	22.8	24.8	

TABLE III

Characterizing data of Selenium Cysteine complexes

	Selenium Dicysteine		Selenium Tetracysteine This Laboratory	
	Theoretical	Found	Theoretical	Stekol
I. Melting Point	colors 178° decomposes 209°		164° 195°	162° 212°
II. Selenium Percentage	24.76	21.5	14.16	14.00	14.00
III. Total Nitrogen Percentage	8.79	8.35	10.01	9.87	9.83

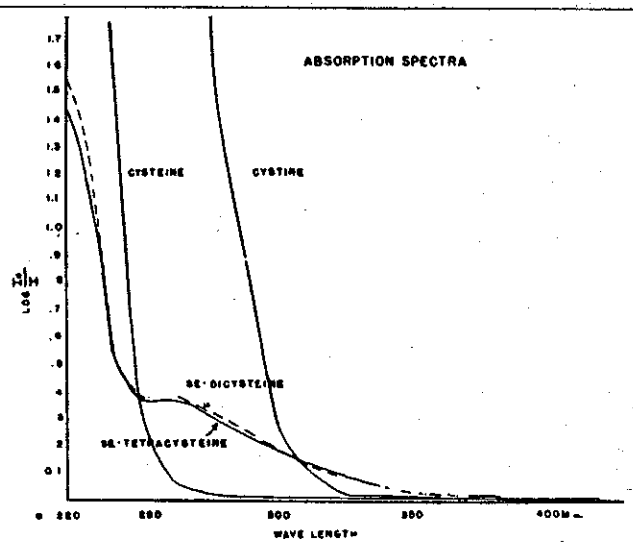

Fig. 1. Absorption spectra of Cysteine, Cystine, Selenium-Dicysteine, and Selenium-Tetracysteine.

Selenium-dicysteine and selenium-tetracysteine were 0.01 per cent aqueous solutions. The cystine was 0.3 per cent in 2 N HCl and the cysteine was a 1 per cent solution of cysteine hydrochloride. Beckman spectrophotometer.

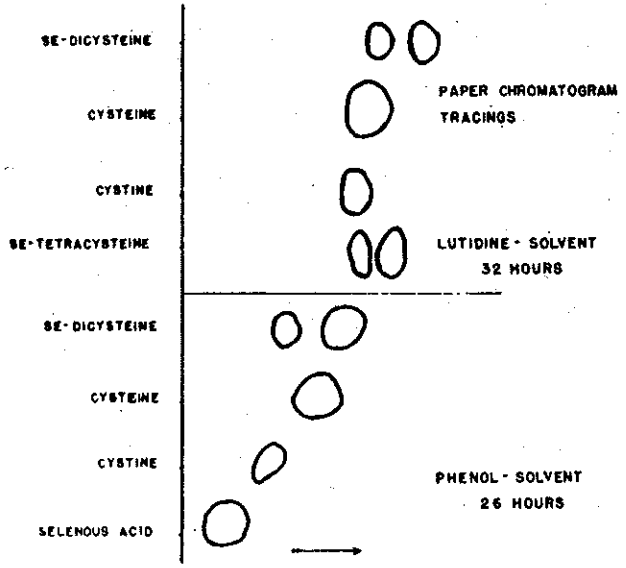

Fig. 2. Paper chromatogram tracings.

Ninhydrin, nitroprusside and ascorbic acid were used as developers.

Spectrographic data as shown in Figure 1 however demonstrated that the two selenium-cysteine complexes were the same entity regardless of the close elemental analyses to the theoretical and to Stekol's values. That this entity consisted of two substances was shown by paper partition chromatography. The tracings of two determinations with different solvents are shown in Figure 2.

According to these data, Stekol's selenium tetracysteine as made in this laboratory and Painter's selenium dicysteine are really mixtures of cystine and probably selenium dicysteine. The separation and positive identification of these two compounds as well as their physiological significance in selenium poisoning still remains to be determined. Whether a similar condition is also true of the selenium-glutathione complexes is not known but the analogous products are to be expected on the basis of the reactions as outlined by Painter.

Conclusion

Synthesized complexes resulting from the reactions of selenous acid with cysteine have been shown to be mixtures of at least two components. It is concluded that selenium oxidizes two moles of cysteine to cystine and further, it probably binds two moles of cysteine to form selenium dicysteine. This work confirms equations published by Painter in 1941 as typical of sulfhydryl-selenium reactions.

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