DECOMPOSITION OF SELENIUM-CYSTINE IN ELECTRODIALYSIS AND ACID HYDROLYSIS[1]

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Franke and associates[2] have shown that the selenium which occurs naturally in grains is present in an organic form intimately associated with the protein. Horn and Jones[3] have succeeded in isolating a crystalline selenium compound having the properties of an amino acid. Since there are numerous indications that selenium is perhaps present in an amino acid,[3,4] replacing the sulphur from cystine, methionine or similar compounds, it was thought of value to study the action of electrodialysis and acid hydrolysis on selenium-cystine. It has been noted that a large percent of the selenium in plant and animal extracts is lost when subjected to heat or strong acid hydrolysis. If selenium-cystine would resist acid hydrolysis and migrate into the anode or cathode chamber in electrodialysis these processes would be of great value in determining whether selenium-cystine occurred in plant and animal extracts. However, if selenium-cystine would decompose when subjected to these processes it would indicate that strong acid hydrolysis and electrodialysis would be of doubtful value in its separation and purification.

The selenium-cystine used in this work was obtained from Arne Fredga, Uppsala University, Uppsala, Sweden.

Experimental

The purpose of the first trial in electrodialysis was to determine whether selenium-cystine could be separated from cystine by fractional electrodialysis. Electrodialysis apparatus similar to that described by Loddesol[5] was used in this experiment. Equal amounts of cystine and selenium-cystine dissolved in 100 cc of .1N HCl were placed in the middle chamber. The cathode and anode fractions were completely drained at intervals during the dialysis. Selenium was determined by the method reported by Moxon,[6] and cystine was determined by the method of Folin and Marenzi.[7] The results of the first trial are shown in table 1.

[1]Approved for publication by the director of the South Dakota Agricultural Experiment Station as Journal Series No. 134.

[2]Franke, K. W. and Painter, E. P. Selenium in proteins from toxic foodstuffs. J. Nutr. 10:599-611 (1935).

[3]Horn, M. J. and Jones, D. Breese. Isolation of a crystalline selenium containing organic compound from plant material. J. A. C. S. 62:234 (1940).

[4]Moxon, Alvin L, Schaefer, A. E., Lardy, H. A., DuBois, K. P. and Olson, O. E. Increasing the rate of excretion of selenium from selenized animals by the administration of bromobenzene. J. Biol. Chem. 132:785-786 (1940).

TABLE 1

Electrodialysis of Selenium-Cystine and Cystine*

	Cystine	Se from Selenium-Cystine
% of Total in Anode	47.63%	43.189%
% of Total in Cathode	9.91%	39.58 %
% of Total in Middle	42.47%	17.22 %

*Electrodialyzed for 90 hours at 64-122 volts.

Since cystine and selenium were found in each chamber it appears impossible to separate selenium-cystine from cystine by the electrical transport method and it is possible that selenium-cystine was partially decomposed. Cystine migrates to the anode at a steady rate, but a portion of it is also found to migrate to the cathode. It was thought that perhaps the hydrochloric acid may be responsible for the migration of selenium-cystine to both poles. Therefore a second trial was attempted.

In the second trial selenium-cystine was dissolved in 3 cc of 2.5N H_2SO_4 and the experiment was repeated. The results are shown in table 2.

[5]Loddesol, Aasulv. A new modification of the 3-compartment electro-dialysis apparatus. J. Am. Soc. Agron., 24:74-81 (1931).

[6]Moxon, A. L. Alkali disease or selenium poisoning. S. Dak. Agr. Expt. Sta. Bulletin 311 (1937).

[7]Folin, Otto, and Marenzi, A. D. An improved colorimetric method for the determination of cystine in proteins. J. Biol. Chem. 63:103-108 (1929).

TABLE 2

Electrodialysis of Selenium-Cystine and Cystine*

	Cystine	Se from Se-Cystine
% of Total in Anode	49.98%	15.28%
% of Total in Cathode	39.45%	76.97%
% of Total in Middle	10.56%	7.74%

*Electrodialyzed for 120 hours at 72-120 volts.

The results of this trial tend to point out the same possibilities as trial 1. From these two trials there was an indication that the selenium from selenium-cystine tended to migrate to the cathode and then migrate to the anode.

In a third trial selenium-cystine in a water solution was placed in the middle chamber. Selenium-cystine is only slightly soluble in water, but as electrodialysis proceeded all of the selenium-cystine was dissolved. Twenty cc samples were withdrawn at various intervals from the anode and cathode chambers. Decomposition in the cathode chamber could be noted in that the samples removed contained precipitates of elemental selenium. The pH of the various samples was recorded.

TABLE 3

Electrodialysis of Selenium-Cystine in Water Solution*

Time of Electrodialysis	Total mg Se in Anode Region	Total mg in Cathode Region	Total mg Se in Middle	pH Anode	pH Cathode
10 hrs.	1.08 mg.	0.27		4.0	7.0
24 hrs.	2.76	2.23		3.8	7.2
48 hrs.	4.32	2.21		3.35	7.58
72 hrs.	5.04	2.61		3.25	7.80
96 hrs.	7.46	2.59		3.10	8.30
120 hrs.	8.15	1.89		3.05	9.01
144 hrs.	10.35	1.79	4.875 mg.	3.00	9.06
144 hrs.				pH of Middle=4.10	

*Electrodialyzed at 70 volts and .005 amperes.

From the results of table 3 the migration of a fraction of the selenium can be traced from the cathode to the anode. After a period of 72 hours the concentration of selenium in the cathode chamber was gradually reduced while the concentration in the anode chamber steadily increased. A portion of the selenium-cystine is decomposed at the cathode to give elemental selenium and another portion of that which migrates to the cathode is apparently decomposed and then migrates to the anode. Cystine has been shown to be decomposed in alkaline solutions.[8] It is possible therefore that the selenium-cystine could be decomposed in the cathode chamber because of the alkalinity of the colution. At the point where the selenium apparently migrates out of the cathode chamber the pH of the cathode solution is 7.8 and it gradually increases to 9.06.

It was of interest to note whether the reduction of selenium-cystine to selenium-cysteine is similar to the reduction of cystine to cysteine. Selenium-cystine was dissolved in a small amount of 6N H_2SO_4; 2 cc of sodium sulfite was added and the procedure carried out for cystine determination as described by Folin and Marenzi.[9] A blue color developed. However, a blue color was also obtained when the procedure was repeated without the addition of sodium sulfite. This indicated that selenium-cystine was reduced to selenium-cysteine in sulfuric acid solution. To further substantiate our observations selenium cystine was added to water without making it acidic. Cystine determination was made and without the addition of sodium sulfite no color was developed, however, upon addition of sodium sulfite a blue color was produced. This indicated that in sulfuric acid solution selenium-cystine is reduced to the cysteine form, while in water solution reduction does not occur.

In order to determine whether selenium-cystine could withstand acid hydrolysis, and to note the effect of the time of hydrolysis on selenium-cystine, the compound was hydro-

[8]Jones, D. Breese and Gersdorff, Charles E. E. Studies on digestibility of proteins in vitro. VII. Liberation of cystine on tryptic digestion of casein, with observations on the instability of cystine toward alkali. J. Biol. Chem. 129:207-223 (1939).

[9]Op. cit.

lyzed for various periods in 33% H_2SO_4 or in 20% HCl solutions and the hydrolysates were then analyzed for selenium. The results of this experiment are given in table 4A.

TABLE 4A

Decomposition of Se-Cystine During Acid Hydrolysis

Sample	Time of Hydrolysis	Hydrolyzing Agent	Mg Se in Sample of Se-Cystine	Mg Se Analyzed in Hylrolysate	% Se Lost
1	6 hrs.	33% H_2SO_4	1.1596	0.96	17.22%
2	12 hrs.	33% H_2SO_4	1.1596	0.72	37.91%
3	12 hrs.	33% H_2SO_4	0.4725	0.32	32.27%
4	14 hrs.	33% H_2SO_4	5.795	2.80	51.71%
5	24 hrs.	33% H_2SO_4	0.4725	0.20	57.88%
6	24 hrs.	33% H_2SO_4	1.1596	0.40	65.51%
7	36 hrs.	33% H_2SO_4	1.1596	0.424	63.44%
8	48 hrs.	33% H_2SO_4	1.1596	0.416	64.13%
9	6 hrs.	20% HCl	1.1596	0.08	93.11%
10	20 hrs.	20% HCl	1.1596	0.278	76.00%
11	30 hrs.	20% HCl	1.1596	0.08	93.11%
12	60 hrs.	20% HCl	1.1596	0.26	77.57%

TABLE 4B

Elemental Selenium Recovered After Acid Hydrolysis

Sample	Time of Hydrolysis	Hydrolzing Agent	Mg Se in Sample of Se-Cystine	Mg Se Analyzed in Precipitate	% Se in ppt.
9	6 hrs.	HCl 20%	1.1596	.22	18.97%
10	20 hrs.	HCl 20%	1.1596	.196	16.9 %
11	30 hrs.	HCl 20%	1.1596	.44	37.94%
12	60 hrs.	HCl 20%	1.1596	.01	0.86%

Six hours of sulfuric acid hydrolysis caused a loss of 17.22% of selenium from the selenium-cystine solution. Hydrolysis for twelve hours and longer showed a loss of selenium from 32.37% to 65.51%.

Hydrochloric acid hydrolysis proved to be much more destructive in its action, causing a loss of selenium of from 76% to 93.11%. In the hydrochloric acid hydrolysis a precipitate was formed resembling elemental selenium. The precipitate was analyzed for selenium and was found to contain a large share of the selenium lost from the solution (see Table 4B).

It can therefore be concluded that acid hydrolysis decomposes a large portion of the selenium-cystine, causing a considerable loss of selenium.

Summary

Cystine and selenium-cystine cannot be separated by the electrical transport method. Selenium-cystine migrates to the cathode and anode chambers. In the electrodialysis of a water solution a portion of the selenium-cystine is decomposed to give elemental selenium. After a period of time the selenium concentration in the cathode chamber decreases while the selenium concentration in the anode chamber increases.

In sulphuric acid solution selenium-cystine is apparently decomposed to the cysteine form, giving a blue color with uric acid reagent without the use of sodium sulfite. In water solution selenium-cysteine is not formed until sodium sulfite is added.

Selenium-cystine is decomposed on acid hydrolysis causing a large loss of selenium. Elemental selenium was recovered in the precipitate formed during hydrochloric hydrolysis.