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Acid Dissociation of Selenocysteamine (2-Aminoethaneselenol)

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Selenocysteamine (2-aminoethaneselenol) hydrochloride was prepared by three methods. Compositive acid dissociation constants of selenocysteamine were determined by potentiometric titration. Microscopic dissociation constants of cationic, anionic, zwitterionic and neutral forms, and tautomeric constant between zwitterionic and neutral forms were calculated, in selenocysteamine and cysteamine. Selenocysteamine was found to present completely as zwitter-ionic form at pH 7—10, whereas cysteamine was found to present about 60% as zwitter-ionic form at pH 10.

The protecting activity against radiation was first discovered in cysteamine(2-aminoethanethiol) by Bacq,[2] and it has been approved as one of the most effective protectors. The effect of cysteamine can fairly be explained by the various mechanisms, such as radical scavenging, anoxia effect, and mixed disulfide formation, which have been advocated up to now.[3]

Most of the compounds, including cysteamine, which have protecting activity possess the ability of complex formation with various metal ions to some extent. The character of the complex formation may effectively be related to the protecting activity in the following ways,[4] namely in the scavenging of metal ions which have the catalytic effect on oxidation, to prevent or interrupt cellular oxidations initiated by radiation, in the stabilization of the valence state of metal in metal containing enzymes, and in the protection of the metal constituents of enzymes from free radical or ionic attack through the transient complexation. In spite of the importance of the complex formation, a few informations have been known so far on the relationship between the complex formation and the radiation protection.[5] In general, physico-chemical properties of the protective agents should be investigated extensively to approach the elucidation of the mechanism of the radiation protection. From the above-mentioned stand point, we attempted the investigations of physico-chemical properties, especially the complex formation reaction of cysteamine and its related compounds.

In some of the amino acids containing selenium atom, stronger protective activity has been found than in the corresponding sulfur compounds,[6,7] but the protective activity of selenocysteamine(2-aminoethaneselenol), which is selenium containing analogue of cysteamine has not been investigated. In an attempt to investigate the complex formation of selenocysteamine and compare it with that of cysteamine, we studied the acid dissociation of selenocysteamine by mainly the pH titration method, as the first step of this study. Only a few reports, namely the spectrophotometric studies on 8-quinolineselenol[8] and selenocysteine,[9] have been found, concerning to the dissociation and the complex formation of the compounds containing selenohydryl group.

1) Location: *Shimoadachi-cho, Sakyo-ku, Kyoto.*
2) Z.M. Bacq, A. Herve, J. Lecomte, P. Fischer, J. Blavier, G. Dechamps, H. Le Bihan, and P. Rayet, *Arch. Intern. Physiol. Biochem.*, **59**, 442 (1951).
3) A. Hanaki and A. Akaboshi, *Bunseki Kagaku*, **15**, 518 (1966).
4) W.O. Foye and J. Mickles, *Progr. Biochem. Pharmacol.*, **1**, 152 (1965).
5) A.J. Vergroesen, F. Budke, and O. Vos, *Intern. J. Radiation Biol.*, **13**, 77 (1956).
6) F. Shimazu and A.L. Tappel, *Radiation Res.*, **23**, 210 (1964).
7) K.A. Caldwell and A.L. Tappel, *Arch. Biochem. Biophys.*, **112**, 196 (1965).
8) E. Sekido, Q. Fernando, and H. Freiser, *Anal. Chem.*, **36**, 1768 (1964).
9) R.E. Huber and R.S. Criddle, *Arch. Biochem. Biophys.*, **122**, 161 (1967).

A method of the preparation of selenocysteamine from ethylenimine and potassium selenosulfite was reported recently by Klayman[10] (method A), on the other hand Coblentz[11] obtained selenocystamine (di-2-aminoethyldiselenide) from 2-bromoethylphthalimide and potassium selenocyanate (method B). We obtained selenocystamine by both methods and reduced it to selenocysteamine. In addition, another method, in which ethylenimine is treated with hydrogen selenide in absolute ethanol was also examined (method C). In all cases, as it was difficult to isolate selenocysteamine directly, we obtained selenocystamine, which is easily purified, and reduced it by sodium borohydride to selenocysteamine. Method A was the best in yield and simplicity among these three methods (Chart 1).

Experimental

Preparation of Selenocystamine Dihydrochloride (Chart 1)——Method A, B: Selenocystamine dihydrochloride was prepared according to the methods reported by Klayman[10] and Coblentz,[11] respectively. mp 189°. Method C: To ice-cooled 70 ml of dehydrated EtOH in the three necked flask, the solution of 10 g of ethylenimine in dehydrated EtOH and H_2Se generated from Al_2Se_3 and dil. HCl were introduced simultaneously under continuous stirring. After the addition of ethylenimine, H_2Se was further introduced slowly into the reaction mixture for 3 hours. After the solution was exposed in air for one day, red selenium separated out, was removed. Dry HCl gas was led into the remaining solution under ice-cooling. EtOH was evaporated and the residue was recrystallized from mixture of EtOH and ether. mp 180°, yield about 10%.

Preparation of Selenocysteamine Hydrochloride——From 0.62 g of selenocystamine which was prepared by the above-mentioned methods, 0.63 g of selenocysteamine hydrochloride was obtained by the use of 0.055 g of $NaBH_4$, according to the method of Klayman.[10] The melting point (105—108°) of the compound obtained agreed with that reported by Klayman.[10] The purity of the compound was confirmed to be 99.7%, by the iodometric determination of the selenohydryl group.

Preparation of Se-Methyl-selenocysteamine Hydrochloride——To 10 ml of aqueous solution containing 1 g of selenocysteamine hydrochloride, 0.5 g of NaOH, and 0.8 g of CH_3I were added. The mixture was stirred for 3 hours by the use of magnetic stirrer at room temperature. The reaction mixture was shaken with ether 3 times to extract the reaction product into ether layer. The layer of ether was dried by anhydrous Na_2CO_3. Dehydrated HCl gas was bubbled into the ether solution for 30—40 minutes under ice-cooling, and white precipitate obtained was recrystallized from mixture of EtOH and ether. mp 149—151°. *Anal.* Calcd. for $C_3H_{10}NClSe$: C, 20.64; H, 5.70. Found: C, 20.34; H, 5.85.

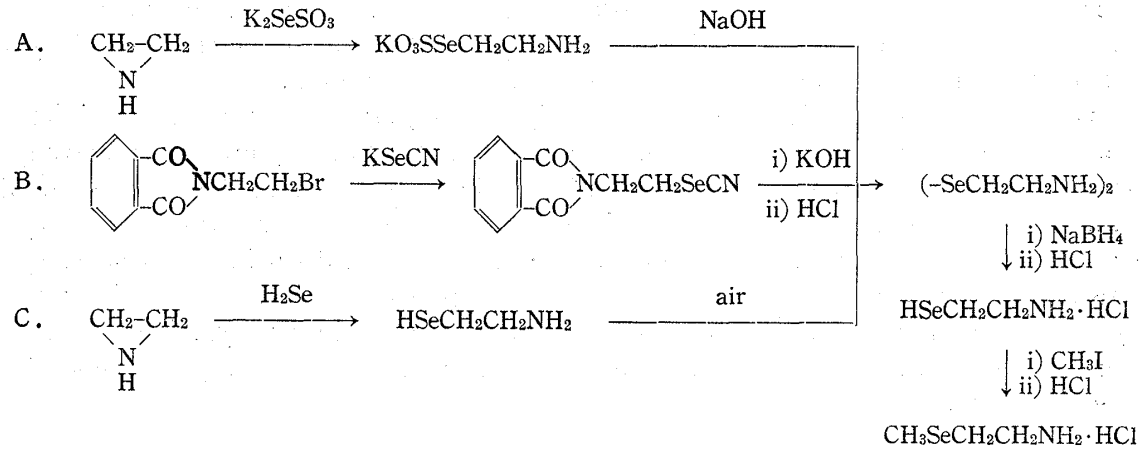


Chart 1

Measurement of Absorption Spectra——The absorption spectra of aqueous solutions in ultraviolet region were measured by a Hitachi Model EPS-211 recording spectrometer.

pH Titrations——The pH titrations were carried out by a Radiometer Titrator TTT 1 and Titrigraph equipped with a Radiometer G 202 B glass electrode and a K 401 saturated calomel electrode. The pH

10) D.L. Klayman, *J. Org. Chem.*, **30**, 2454 (1965).
11) V. Coblentz, *Chem. Ber.*, **24**, 2131 (1891).

meter was calibrated by standard buffer solutions (pH 4.01, 6.98, 9.18). The solution containing 5×10^{-3}M of the compound was titrated with carbonate-free 0.1N NaOH. Stirring was effected by a magnetic stirrer and CO_2 free nitrogen gas was introduced into the solution slowly during the titration. The ionic strength was made to 0.1 with $NaClO_4$. The temperature of the solution was maintained at $22\pm0.3°$.

Results and Discussion

Stability of Selenocysteamine Hydrochloride

An aqueous solution of selenocysteamine hydrochloride was exposed in air, and the change of the absorption with time was measured at 300 mμ, where an absorption maximum of selenocystamine is observed.[12] When the solution was allowed to stand for 10 hours the absorption at 300 mμ became almost constant and selenocysteamine was completely oxidized into selenocystamine by standing for 12 hours. An aqueous solution of selenocysteamine hydrochloride is fairy stable against air-oxidation within 1 hour (Fig. 1).

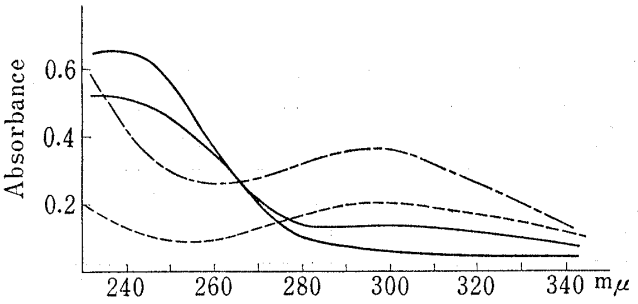


Fig. 1. Stability of Selenocysteamine Hydrochloride in Aqueous Solution

——: 10 min ——: 1 hr ·····: 14 hr
———: selenocystamine•2HCl (2×10^{-3}M)
concentration of selenocysteamine•HCl: 1×10^{-3}M

Dissociation of Selenocysteamine in Aqueous Solution

Acid dissociation constants were calculated from the titration curve, and the values were compared with those of corresponding oxygen and sulfur analogues.

From these values, the dissociation phenomenone of selenocysteamine are supposed to be considerably different from that of cysteamine. In aqueous solution, neutral molecule and various ionic forms, namely cationic, anionic, and zwitter-ionic forms may exist in the state of equilibrium[8,14] (Chart 2). The microscopic acid dissociation constants in each equilibrium were determined by the use of the relationship which is considered among the species shown in the equations 1, 2 and 3 in Chart 2, and the tautomeric constant between the zwitter-ionic form and the neutral form of selenocysteamine was calculated and the value was compared with

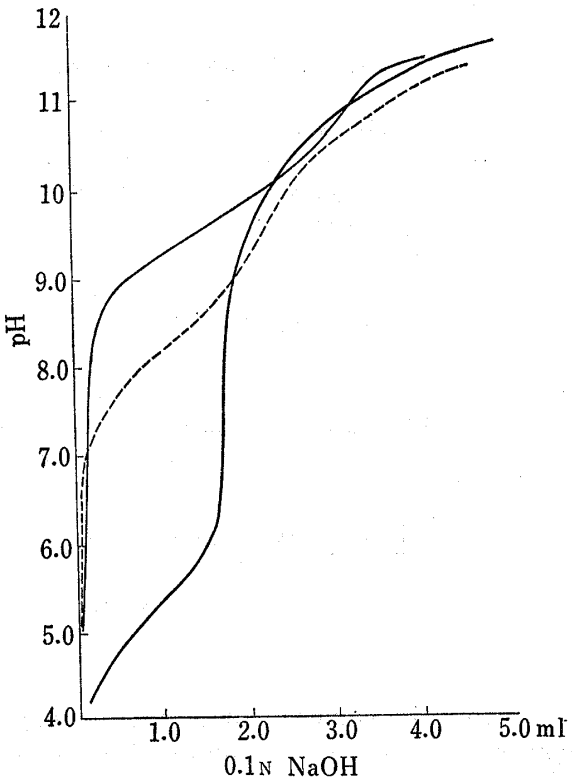


Fig. 2. Titration Curves of Cysteamine, Selenocysteamine, and Se-Methylselenocysteamine

——: selenocysteamine•HCl
——: Se-methylselenocysteamine•HCl
------: cysteamine•HCl
concentration: 5×10^{-3}M
μ=0.1
temperature: $22°\pm0.3°$

12) G. Bergson, G. Claeson, and L. Schotte, *Acta. Chem. Scand.*, **16**, 1159 (1962).
13) L.G. Sillen and A.E. Martel, "Stability Constants of Metal-Ion Complexes," Special Publication No. 17, London, The Chemical Society, Burlington House, W. 1, 1964, pp. 382—384, 402—404.
14) J.T. Edsall and M.H. Blanchard, *J. Am. Chem. Soc.*, **55**, 2337 (1933).

that of cysteamine (Table II). In the calculations, dissociation constants of the S-methyl or Se-methyl derivatives obtained were expediently used as the values of pK_{aB} of both the cysteamine and selenocysteamine, since in these methyl derivatives, participations of the zwitter-ionic forms can be excluded.

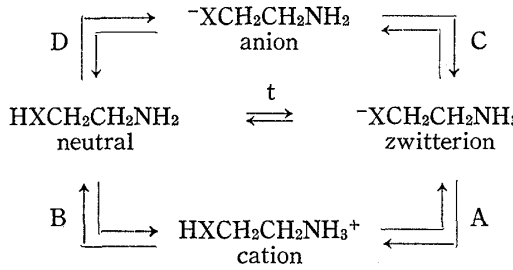
Table I. Compositive Acid Dissociation Constants

Compound	pK_a		
	O[a]	S[a]	Se
$HXCH_2CH_2NH_2$		8.27	5.01
	9.60	10.53	10.99
$CH_3XCH_2CH_2NH_2$	9.45	9.45	9.53

a) data from ref. 13

Table II. Tautomeric Constants and Microscopic Acid Dissociation Constants

Compounds	Kt	pK_{aA}	pK_{aB}	pK_{aC}	pK_{aD}
Selenocysteamine	$10^{4.52}$	5.01	9.53	10.99	6.47
Cysteamine	6	8.68	9.45	10.98	10.21



$$K_{a_1}=K_{a_A}+K_{a_B} \quad (1)$$

$$\frac{1}{K_{a_2}}=\frac{1}{K_{a_C}}+\frac{1}{K_{a_D}} \quad (2)$$

$$K_t=\frac{K_{a_A}}{K_{a_B}}=\frac{K_{a_D}}{K_{a_C}} \quad (3)$$

Chart 2

From the values of these microscopic constants, relative concentrations and total anionic concentrations were calculated from equation 4.[15]

$$(\%)\ [RS^-] \text{ or } [RSe^-]=\frac{K_{a_A}/K_{a_B}+K_{a_D}/H^+}{H^+/K_{a_B}+K_{a_A}/K_{a_B}+K_{a_D}/H^++1} \quad (4)$$

As seen in Fig. 3, in the case of selenocysteamine, the concentration of the zwitter-ionic form is more than 50% in the range from pH 5.5 to 11.5, and it becomes almost 100% in the range from pH 7 to 10. On the other hand, in the case of cysteamine (Fig. 4), the maximum concentration of the zwitter-ionic form is about 50% in the range from pH 8.8 to 10.8, and the zwitter-ionic form exists in very limited pH range. The relative concentrations of the anionic forms of cysteamine and selenocysteamine agree comparatively with those of selenocysteine and cysteine which were measured spectrophotometrically[9] (Fig. 5).

From these results, it is obvious that selenohydryl group begins to dissociate at pH 5, and at physiological pH (7.4), it dissociates completely. Sekido and co-workers[8] have pointed out that the difference of the dissociation between the selenohydryl and sulfhydryl groups

15) R.E. Benesch and R. Benesch, *J. Am. Chem. Soc.*, **77**, 5877 (1955).

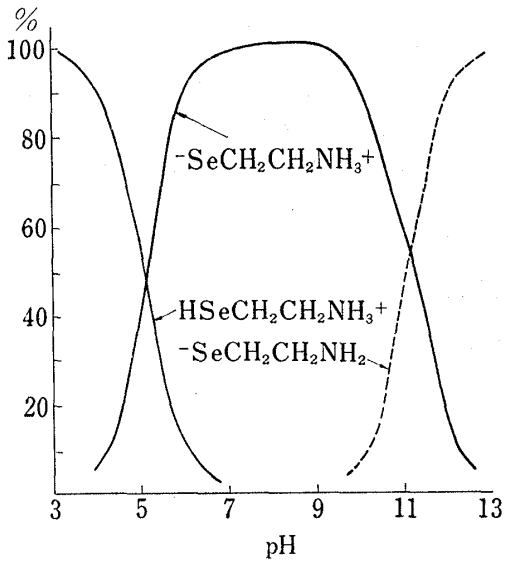


Fig. 3. Relative Concentration of Various Ionic Forms of Selenocysteamine

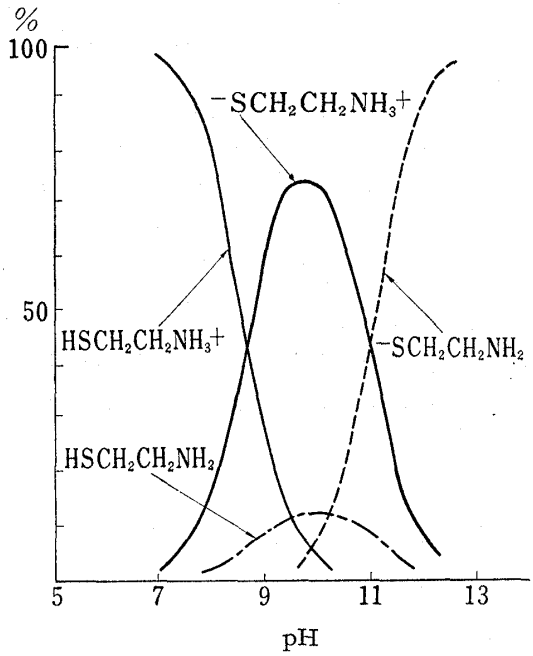


Fig. 4. Relative Concentration of Various Ionic Forms of Cysteamine

correspond to that of ionization constants between hydrogen selenide and hydrogen sulfide, in the investigation on 8-quinolinethiol and 8-quinolineselenol.[8] In our result, the similar relationship was observed between cysteamine and selenocysteamine. However, it may be difficult to explain the difference of ionization constants between hydrogen selenide and hydrogen sulfide only from their electronegativities, because selenium and sulfur do not differ largely from each other in their electronegativities. We need more data on the various types of sulfur and selenium containing compounds to inquire in general about the difference between selenohydryl and sulfhydryl compounds in their acid dissociations.

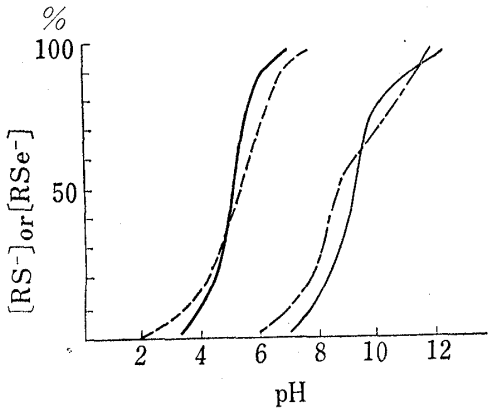


Fig. 5. Relative Concentrations of Total Anionic Forms

Table III. Reaction of Selenocysteamine with Metal Ions

Metal ions	Color		
	pH=6	pH=8	pH=11
Ni^{2+}	yellow	pink	brown
Co^{2+}	brown	green	green
Cu^{2+}	yellow	green	green
Pb^{2+}	yellow	yellow	yellow
Hg^{2+}	colorless	colorless	colorless
Ag^{2+}	yellow	yellow	orange
Pt^{2+}	orange	orange	orange
V^{5+}	gray	gray	gray
Pd^{2+}	yellow	yellow	yellow
UO_2^{2+}	yellow	yellow	yellow

In cysteamine, the large contribution of the zwitter-ionic form in its dissociation equilibrium has been regarded as a remarkable character[3] and it is assumed that this property may relate to radiation protective activity to some extent. It is very interesting that in selenocysteamine, the contribution of the zwitter-ionic form in its dissociation equilibrium is far greater than in cysteamine. The relationship between the character in the dissociation and the reactivity towards the various reactions, namely radical scavenging, redox reaction, and mixed disulfide formation, may be an interesting problem, in connection with the radiation protective activities of these compounds.

The reaction of selenocysteamine with various metal ions were investigated by spot tests in acid, neutral, and alkaline solutions. Some significant color changes or formations of intensely colored precipitate were observed as shown in Table III. The complex formations of selenocysteamine will be discussed in the succeeding papers.