2. Difference in mean values among 1st, 2nd, and 3rd observations of slipping force of tablet surface, and standard error are extremely small.

3. When tablet surface shows roughness in "excellent" tablets, it must be true that material itself has no smoothness.

Acknowledgement The authors are indebted to Mr. Yasuo Shimada, the President of Kikusui Seisakusho Co. Ltd., for his encouragement and cooperation. The authors are also grateful to Messrs. Masao Takada, Giichiro Kowada, Sakichi Ezaki, Toyojiro Koyama, and Kozo Kurokawa of the same Company, for their technical assistance.

[Chem. Pharm. Bull. 19(9)1956—1958(1971)]

UDC 547.233.04 : 547.269.1.04 : 546.23.04

Reduction of Selenocystamine by Thiols

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(Received July 25, 1970)

The role of the selenium compounds in the biological system has been discussed from various points of view,[2] and the reaction of selenium compounds with sulfur compounds has been considered as one of the important points to discuss the biological significance of the selenium compounds. In this connection, Dickson and Tappel[3] have shown that cysteine and glutathione are able to reduce selenocystine to selenocysteine, and Walter, *et al.*[4] have investigated the reductive scission of selenium-selenium bond of selenocystine by thiols and selenols by ion-exchange chromatography. Recently, the ability of selenium-containing amino acids to provide protection against radiation has been demonstrated in model chemical systems by Shimazu and Tappel,[5] and further Breccia, *et al.*[6] have reported that the radiation protection afforded by selenocystine, selenomethionine, colloidal selenium, selenoxanthene, selenoxathone, selenochromone and selenourea[7] at sublethal dose in rat is similar to, or sometimes even superior to that given by cysteine despite their low LD_{50} values. However, it was briefly reported the selenocysteamine[8] and selenocystamine[9] which are selenium-containing analogues of well known radioprotective agents, cysteamine and cystaamine, may not be regarded as favourable radiation protectors because of the considerable low LD_{50} values without further investigation of their protective effects. Information on the radiation protective activity of selenocysteamine and selenocystamine has not been reported so far.

On the basis of the increased interests in the biological significance of selenium compounds,[2] we have conducted the physico-chemical study on the property of selenocysteamine

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and selenocystamine.[10] The present paper deals with the reductive scission of selenocystamine (di-2-aminoethyl-diselenide) by biochemically significant thiols, namely glutathione, cysteine, cysteamine and penicillamine in physiological pH range.

Experimental

Materials——Selenocystamine dihydrochloride was prepared according to the previous report.[10] Glutathione, cysteine and cysteamine hydrochloride were purchased from Nakarai Chemicals. DL-Penicillamine was purchased from Sigma Chemical Company. All other compounds used were of the reagent grade.

Spectral Measurements——The ultraviolet spectra in the range from 220 mμ to 340 mμ were measured by a Hitachi recording spectrophotometer model EPS-2. The spectra of selenocystamine in the presence of various concentrations of thiols were recorded within three minutes after mixing. All absorbance measurements were made by a Shimadzu spectrophotometer model QV-50 at room temperature. All solutions were prepared in Michaelis buffer ($^1/_{30}$M KH_2PO_4–$^1/_{30}$M Na_2HPO_4, pH 7.4) with the saturation of nitrogen and the ionic strength was made to 0.13.

Reduction Equilibrium Constant——As selenocysteamine was found to be fully ionized[10] at physiological pH, reduction of selenocystamine by thiols is considered to be represented in the following reactions,

$$\mathrm{RSeSeR} + \mathrm{XSH} \overset{K_1}{\rightleftarrows} \mathrm{RSeSX} + \mathrm{RSe^-} + \mathrm{H^+} \quad (1)$$

$$\mathrm{RSeSX} + \mathrm{XSH} \overset{K_2}{\rightleftarrows} \mathrm{XSSX} + \mathrm{RSe^-} + \mathrm{H^+} \quad (2)$$

where $\overset{+}{\mathrm{N}}\mathrm{H_3CH_2CH_2}$ in selenocystamine and selenocysteamine is represented as R. The equilibrium constant K_1 was calculated for the solution of 3×10^{-4}M selenocystamine with each of four thiols of various concentrations. The absorbance of RSe^- is represented as Δ=A—(B+C), where A, B, and C represent the absorbance at 245 mμ of the mixture of selenocystamine and thiol, that of thiol and that of selenocystamine respectively. The amount of RSe^- produced by the reduction was determined from the results of the measurement of Δ and the calibration curve made by the reduction of selenocystamine with sodium borohydride which is able to reduce selenocystamine quantitatively at the same pH as in the reduction with thiols.

Result and Discussion

The reduction of selenocystamine can be achieved by hypophosphorous acid in strongly acid medium and sodium borohydride in neutral medium and the spectral change corresponding to the reduction can be clearly observed. Diselenides generally show a band of low absorptivity at about 300 mμ.[11] Addition of sodium borohydride to nitrogen-saturated solution of selenocystamine at pH 7.4 lowered the absorptivity at 300 mμ immediately, and a new band-appears at 245 mμ with molar extinction coefficient of 13000 which was calculated with respect to the concentration of selenocystamine dihydrochloride. The absorbance at 245 mμ was stable for about 20 minutes in the exposure of the solution to atmosphere. In the presence of excess amount of sodium borohydride at pH 7.4, the absorbance at 245 mμ correlates linearly with the concentration of selenocystamine as shown in Fig. 1. Hence selenocystamine is regarded to be reduced quantitatively by sodium borohydride and Fig. 1 can be used as a calibration curve for the determination of selenocysteamine produced by the reduction. When biochemically significant thiols such as glutathione, cysteine, cysteamine and penicillamine were added to the solution of selenocystamine, similar spectral change was observed to the case of the addition of sodium borohydride. Fig. 2 represents an example of the change in absorption spectra with the increase of the concentration of thiols. Complete reduction of 10^{-5}M selenocystamine with more than 4×10^{-3}M cysteine was observed as shown in Fig. 3, which represents the change in absorbance a 245 mμ as a function of the concentration of cysteine added. In Fig. 3, a small inflection was observed at the point where

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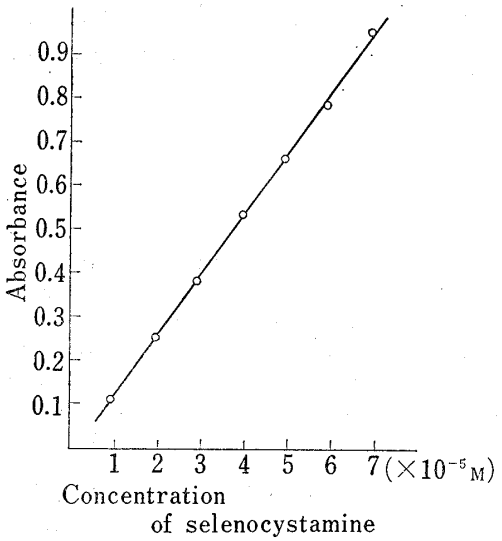

Fig. 1. Calibration Curve for Selenocysteamine Produced from Selenocystamine by Sodium Borohydride

concentration of $NaBH_4$: 5×10^{-4}M
pH: 7.4 (μ=0.13)
wavelength: 245 mμ

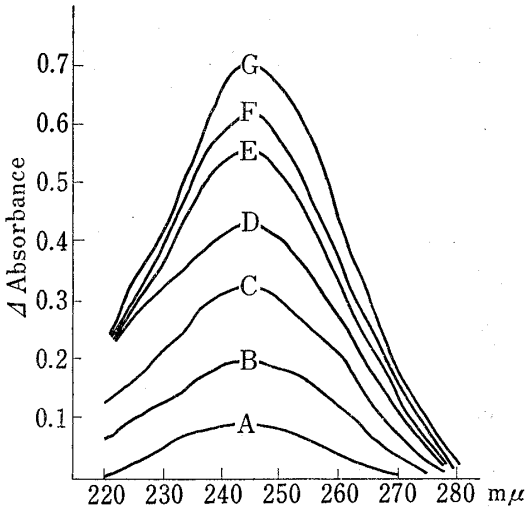

Fig. 2. Absorption Spectra of Selenocystamine with Cystein

concentration of selenocystamine: 3.0×10^{-4}M
concentration of cysteine: (A) 1.5×10^{-4}M, (B) 3.0×10^{-4}M, (C) 6.0×10^{-4}M, (D) 9.0×10^{-4}M, (E) 1.2×10^{-3}M, (F) 1.5×10^{-3}M, (G) 1.8×10^{-3}M
pH: 7.4 (phosphate buffer) μ=0.13

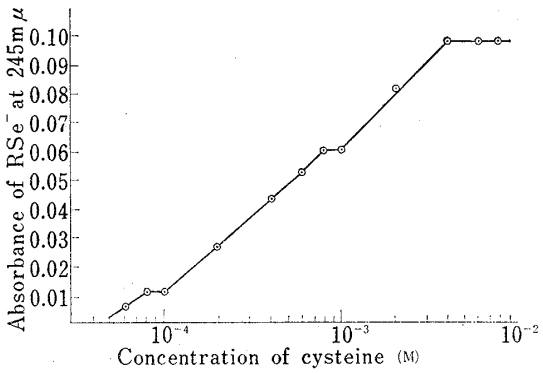

Fig. 3. Absorbance of RSe^- (245 mμ) with the Various Concentration of Cysteine

selenocystamine: 10^{-5}M
pH: 7.4 (μ=0.13)

the absorbance at 245 mμ becomes about half. From this observation, it may be reasonable to assume that the reduction proceeds in two steps according to the reactions (1) and (2). Similar results to Fig. 3 were obtained as to the reductions with other thiols. On the basis of the reaction (1), the apparent equilibrium constants K_1 for 3×10^{-4}M selenocystamine in the presence of various concentrations of thiols are calculated and the values obtained are shown in Table I.

These data indicate that when the concentrations of thiols are approximately 10^2 times greater than the concentration of selenocystamine, the complete reduction of diselenide to selenol can be achieved. The value of K_1 decreases in the order, glutathione>cysteine>cysteamine>penicillamine. Glutathione which exists abundantly in living body was found to be the most favorable reducing agent for selenocystamine among the biochemically significant thiols examined. From these observations, and the results of the study on the acid dissociation of selenocysteamine,[10] selenocystamine is considered to be reduced to selenocysteamine and present in zwitterionic form in the living body.

Table I. Calculated Equilibrium Constants for Reduction of Selenocystamine

Thiol	K_1 ($\times10^2$)[a]	Thiol	K_1 ($\times10^2$)[a]
Glutathione	5.3±0.1	Cysteamine	2.0±0.1
Cysteine	2.6±0.1	Penicillamine	1.3±0.2

a) average of data from five experiments