

PRO EXPERIMENTIS

A Differential Polarographic Study on Radioprotective Substances Containing Sulfhydryl

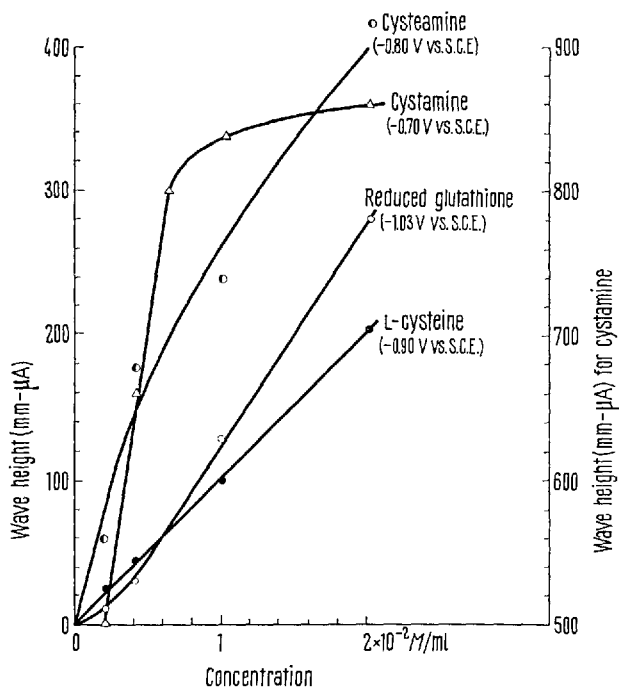
Polarographic behaviour of radioprotective substances containing sulfhydryl was observed by a differential polarography method, which was established under the same principles as one of alternating current polarography and was valuable for the detection of organic substances^{1,2}, in the present study. The chemicals reported here were L-cysteine, reduced and oxidized glutathiones, cysteamine hydrochloride, cystamine dihydrochloride, S- β -aminoethylisothiuronium bromide hydrobromide (AET) and bovine serum albumine. All chemicals were dissolved into 5 ml of 0.5 N acetate buffer (pH 5.2) in the concentration of $0.2 \times 10^{-2} M/ml$ to be detected. The differential polarography was performed at $25 \pm 1^\circ C$ after bubbling in N_2 gas for 10 min. The apparatus was a commercial polarograph (Shimadzu) type RP-2 with a dropping mercury electrode and Shimadzu autocorrector AIC-1. The condition of dropping was 3.05 mg/sec. The usable potential range was +0.3 to -1.5 V vs. S.C.E. All measured values responsible to wave position and to wave height were the means of results from 5 series of repeated experiments. The standard deviations were kept less than 3% of means of volts vs. S.C.E. and of wave height.

Under the condition of the buffer and 0.1% solution of bovine serum albumine, the wave of differential polarography was not detected. Three waves at -0.45, -0.65 and -0.90 V vs. S.C.E. were detected using the cysteine solution with concentration of $2 \times 10^{-2} M/ml$. Three waves at -0.35, -0.65 and -1.03 V vs. S.C.E. using the reduced glutathione solution, 3 waves at -0.65, -0.76 and -1.18 V vs. S.C.E. using the cystamine solution, 1 wave at -0.80 V vs. S.C.E. using the cysteamine solution, no wave using the AET solution was detected respectively. The detected top potentials of waves were summarized in the Table.

The dose dependency of wave height was studied in each solution. The increase in wave height of cysteine at -0.90 V vs. S.C.E. was parallel to the increase in the concentration in the range $0.2 \times 10^{-2} M/ml$, 1 in wave height of reduced glutathione at -1.03 V vs. S.C.E. was parallel to the increase in the concentration in the range 0.4×10^{-2} to $2 \times 10^{-2} M/ml$, 1 in wave height of cystamine at -0.70 V vs. S.C.E. was parallel to the increase in the concentration in the range 0.2×10^{-2} to $0.6 \times 10^{-2} M/ml$. The increase in wave height of cysteamine at -0.80 V vs. S.C.E. was almost parallel to the increase in the concentration in the range $0.2 \times 10^{-2} M/ml$. The height of other waves shown in the Table did not

show such a linear relationship as mentioned above. The results were illustrated in the Figure.

In the study of the mechanism of radioprotection, it is very important to differentiate sulfhydryl of glutathione or cysteine in tissues from other sulfhydryls. It appears to be possible to use the differential polarography on the base of the present report to determine quantitatively and qualitatively L-cysteine, reduced glutathione, cysteamine or cystamine. AET being unable to be detected by the present method, the method might be a valuable one to detect other kinds of sulfhydryl in tissues or fluids in mammals after the administration of AET³.



The relationship between wave height and concentration of radioprotective substances containing sulfhydryl in the differential polarography.

Zusammenfassung. Es wurden Strahlenschutzsubstanzen mit einer Differentialpolarographie in Acetatpuffer bei pH 5,2 gemessen und die Wellen der Differentialpolarographie aufgezeichnet. Die Wellenhöhe stieg parallel mit der Zunahme der Konzentration, wenn Cystein, reduziertes Glutathion, Cysteamin hydrochlorid und Cystamin dihydrochlorid verwendet wurden.

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1. M. ISHIBASHI and T. FUJINAGA, Bull. chem. Soc., Japan 23, 15 (1950).
2. M. MARUYAMA and M. NISHIYAMA, in *Polarography I* (Eds. T. FUJINAGA and M. MARUYAMA; Nanko-do, Tokyo 1962), p. 111.
3. The author wishes to thank Miss S. IWASE for her technical assistance.

The waves of radioprotective substances containing sulfhydryl in the differential polarography

Radioprotective substances	Top potential (V vs. S.C.E.)
L-cysteine	-0.45, -0.65, -0.90*
Reduced glutathione	-0.35, -0.65, -1.03*
Oxydized glutathione	-0.65, -0.76, -1.18*
Cysteamine hydrochloride	-0.80*
Cystamine dihydrochloride	-0.70*
S- β -Aminoethylisothiuronium bromide hydrobromide	-
Bovine serum albumine	-
0.5 N Acetate buffer (pH 5.2)	-

* Top potential, at which the linear relationship between wave height and concentration was given.