POLAROGRAPHY OF ALLITHIAMINE

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In the previous reports (1, 2) the authors described the polarographic behaviours of thiamine and clarified the properties of a redox system consisting of thiol and disulfide forms of thiamine. Similar investigations have been made with allithiamine and its homologs (3, 4), the results of which will be reported here.

EXPERIMENTAL

Materials and Procedures. The crystals of allithiamine (thiamine allyl disulfide, TAD) and thiamine propyl disulfide (TPD) used in this experiment have been prepared by Dr. T. Matsukawa of the Takeda Research Laboratory. The concentrations of the stock solutions of these compounds were 5×10^{-3} M in 0.1 N hydrochloric acid and for the experiment they were neutralized by adding the same volume of 0.1 N sodium hydroxide in the electrolytic solution to be examined.

The current-voltage curves were recorded at room temperatures of 20–22° by means of the usual polarographic procedures using a mirror galvanometer with a full sensitivity of 3.0×10^{-9} A/mm/m. The oxygen dissolved in solutions was removed by bubbling hydrogen gas. A normal calomel electrode (NCE) was used as the reference electrode which was connected to the dropping mercury electrode by a liquid junction of saturated potassium chloride solution.

RESULTS

1. Effect of pH. The solutions of TAD and TPD, respectively, in a concentration of 5×10^{-4} M in various buffer solutions covering the whole pH range containing 0.1 N potassium chloride were examined polarographically. Fig. 1 and Fig. 2 show some of the reduction waves of TAD and TPD, respectively, in these solutions.

At pH 1 to 3, both waves of TAD and TPD represented the maximum phenomena which were not suppressed by the ordinary addition of gelatin. The second gentle-sloping wave appeared when the pH was above 11 with TAD. The main wave of TPD at pH above 11 was also gentle-sloping. Table I shows the half-wave potentials ($\pi_{1/2}$) and the wave heights of 5×10^{-4} M TAD and TPD, respectively, at different pH's. The wave heights of both

Fig. 1.

Polarograms of 5×10^{-4} M TAD in Different Buffer Solutions.

pH: (1) 2.5, (2) 5.6, (3) 8.1, (4) 10.0, (5) 13.0.

Fig. 2.

Polarograms of 5×10^{-4} M TPD in Different Buffer Solutions.

pH: (1) 2.1, (2) 5.0, (3) 7.8, (4) 10.8, (5) 13.0.

the compounds decreased gradually with the increase of the pH. This was not seen in the case of the reduction wave of the disulfide form of thiamine (thiamine disulfide, B_1SSB_1). Fig. 3 shows the curves of $\pi_{1/2}$ vs. pH with the 5×10^{-4} M solutions of TAD and TPD and a 4.17×10^{-4} M solution of B_1SSB_1 ¹, respectively.

The half-wave potentials of TAD approximately coincided with those of

¹ Cited from the author's previous reports (1, 2).

Table I

The Relationships among the Half-wave Potential ($\pi_{1/2}$), the Wave Height (h), and the pH in the Buffer Solutions Containing 0.1 *N* Potassium Chloride and 5×10^{-4} *M* TAD and TPD, respectively.

TAD			TPD		
pH	$\pi_{1/2}$ <i>V vs. NCE</i>	<i>h</i> μA	pH	$\pi_{1/2}$ <i>V vs. NCE</i>	<i>h</i> μA
1.0	} with maximum		1.0	} with maximum	
2.5			2.1		
4.3			3.1		
5.6		2.061	4.0		2.17
7.0		1.800	5.0	-0.350	1.92
8.1	-0.365	1.890	6.0	-0.344	1.92
10.0	-0.455	1.632	7.0	-0.392	1.83
11.0	-0.536	1.632	7.8	-0.448	1.86
13.0	-0.572	1.515	9.0	-0.498	1.86
	-0.590		10.0	-0.550	1.83
	-0.881		10.8	-0.578	1.71
			12.1	-0.600	1.62
				-0.590	

Fig. 3 $\pi_{1/2}$ -pH Curves.

(1) TAD, (2) TPD, (3) B_1SSB_1

B_1SSB_1 and were more negative by only 0.1 volt than those of TAD at pH 4 to 7.

The differences of the half-wave potentials of the three compounds were not over 0.1 volt at any pH. This means that the waves of the three compounds should overlap with one another and the analysis of the individual compounds in mixtures would be impossible.

2. *The Effect of Concentration.* The concentration of TAD in the buffer solution of pH 7.1 containing 0.1 *N* potassium chloride was varied in the range from 0.5 to 5×10^{-4} *M* and that of TPD in the buffer solution of pH 7.0 containing 0.1 *N* potassium chloride from 2 to 7.5×10^{-4} *M*. Table II shows the relationships among the wave heights, the half-wave potentials,

and the concentrations of TAD and TPD, respectively. The relationship between the wave-heights and the concentrations of each compound was linear and thus they could be determined polarographically. The half-wave potentials of TAD and TPD shifted progressively to the more negative potential with the increase of their concentrations. Such a behaviour has been observed also with B_1SSB_1 and oxidized glutathione (5,6), probably in common with disulfide compounds in general. It is therefore to be concluded from this fact that the electrolytic reductions of TAD and TPD are of the general type of disulfide compounds: $RSSR + 2 H^+ + 2 e \rightarrow 2 RSH$.

Table II

The Relationships among the Concentration (c), the Wave Height (h), and the Half-wave Potential ($\pi_{1/2}$) of TAD, and TPD, respectively, in a pH 7.0 McIlvaine Buffer Containing 0.1 N Potassium Chloride.

TAD			TPD		
c	h	$\pi_{1/2}$	c	h	$\pi_{1/2}$
10^{-4} M	μA	V vs. NCE	10^{-4} M	μA	V vs. NCE
5	1.986	-0.511	7.5	2.16	-0.534
4	1.392	-0.485	5	1.57	-0.527
3	1.044	-0.460	4	1.23	-0.490
2	0.630	-0.460	3	0.93	-0.480
1	0.296	-0.440	2	0.57	-0.465
0.75	0.213	-0.447			
0.50	0.123	-0.461			

3. *Catalytic Wave in Cobalt Salt Solution.* Previously, we found that thiamine and some of its related compounds represented a catalytic wave in divalent or trivalent cobalt salt solutions, while thiamine disulfide represented it only at temperatures above 60°. A 3×10^{-4} M TAD solution in 10^{-3} M CoCl_2 plus 0.1 N NH_4Cl plus 0.1 N NH_3 as well as the same solution in 10^{-3} M Luteo salt plus 0.1 N NH_4Cl plus 0.1 N NH_3 represented a catalytic wave as given in Fig. 4.

The catalytic wave was found to be the same, regardless of the valency of cobalt ions. But, the catalytic wave disappeared when ammonia was absent in each of the above solutions. No catalytic wave appeared with 3×10^{-4} M TPD in 10^{-3} M CoCl_2 plus 0.1 N NH_4Cl plus 0.1 N NH_3 and in 10^{-3} M CoCl_2 plus 0.1 N NH_4Cl plus 0.1 N NH_3 at 20°. Whenever the Luteo salt was used instead of cobaltous chloride in the above solutions, no catalytic wave appeared at 20°. These phenomena of TPD corresponded with those of thiamine disulfide.

DISCUSSION

From the above observation, it may be concluded that the TAD and TPD, respectively, construct a redox system of the type of thiol-disulfide such as glutathione and thiamine disulfide, *i.e.* :

Fig. 4.
Polarograms of 3×10^{-4} M TAD in (1) 10^{-3} M CoCl_2 + 0.1 N NH_4Cl + 0.1 N NH_3 and (2) 10^{-3} M Luteo Salt + 0.1 N NH_4Cl + 0.1 N NH_3 .

(R : allyl- or propyl group)

The half-wave potential ($E_{1/2}$) of the reduction wave of the general reversible redox system should be equal to the standard redox potential (E_o') of its system, while $E_{1/2}$ of the disulfide compound is not equal to its E_o' and depends on the limiting diffusion current of reduction wave and on the characteristics of dropping mercury electrode. Therefore, it is difficult to determine the standard redox potential of the thiol-disulfide system by the polarographic method. However, it is probable that the redox potential of TAD is approximately the same with that of thiamine disulfide and that of TPD will be more positive by 0.1 volt than those of the former two at pH 7.0.

According to the authors' experiments, the redox potentials of thiamine disulfide and of glutathione are -0.358 volt *vs.* NCE at pH 7.08 and -0.480 volt *vs.* NCE at pH 7.0, respectively. Therefore, reduced glutathione and cysteine, which has a more negative redox potential than that of glutathione, should reduce thiamine disulfide, TAD and TPD. This has been verified experimentally by other authors.

J. Cavalitto *et al.* (7) observed a reduction wave of allixin which reacts on thiamine to form allithiamine. In a preliminary experiment, we observed also a reduction wave at the nearly equal potential to allithiamine with the extract of garlic. This fact may be noted in the biochemical study of allithiamine formation and these polarographic observations will prove of account in the field of biochemistry.

SUMMARY

We investigated allithiamine and thiamine propyl disulfide polarographically. Both represented the polarographic reduction wave at the whole pH range, having the properties of the general disulfide compound. The former showed a catalytic wave in cobalt salt solution, while the latter no catalytic wave at 20°.

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