salt concentrations on the two sides of the membrane was found to be proportional to the albumin concentration, and approximately inversely proportional to the salt concentration. Such a relation might result from a tight binding of a few, about six, salt ions to each albumin molecule.

The membrane potentials and other independent measurements indicate that it is the chloride ion which is bound.

The osmotic pressure was found to be a quadratic function of the albumin concentration. The first term corresponds to a molecular weight of 69,000. From the second term, the salt distribution and the valence of the albumin, the effect of albumin on its own activity coefficient was calculated. This varies from a large positive value for neutral albumin to a larger negative value for albumin in acid solutions. Some of the implications are discussed.

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Polarographic Determination of Cupric Glycinate and Cupric Alaninate Complex Ions

BY R. M. KEEFER

Many investigators[1] have studied the complex ions formed between cupric ion and glycinate (G^-) or alaninate (A^-) ion. Boorsook and Thimann[1] concluded from spectrophotometric and electrometric data that the complex ions formed in basic solutions were CuG_2 and CuA_3^-. Gould and Vosburgh[2] using the method of continuous variation determined from spectrophotometric data that with equal molal quantities of cupric ion and glycinate ion CuG^+ was formed and that with glycinate ion more than twice the concentration of cupric ion that CuG_2 was formed. Riley and co-workers[3,4] used a cupric ion concentration cell and state that CuG_3^- and CuA_3^- are the main complex ions formed. Only the last investigation gave values for the dissociation constants of the complex ions. This investigation was undertaken using the dropping mercury electrode to determine the formula of the complex ions formed[5] and their dissociation constants.

Experimental

Glycine and alanine were purified by recrystallization. Potassium nitrate and potassium hydroxide were of Merck reagent quality. Stock solutions of potassium glycinate or potassium alaninate were prepared from the amino acids and potassium hydroxide using boiled distilled water. Potassium nitrate was used as a supporting electrolyte. At low glycinate or alaninate concentrations the electrode reaction was irreversible if the solutions were prepared using equivalent amounts of amino acid and potassium hydroxide. To obtain values at low glycinate or alaninate concentrations runs were made with solutions containing less than the equivalent amount of potassium hydroxide. The *p*H of all solutions was determined using a Coleman Model 3D *p*H Electrometer. To minimize errors in calculating glycinate or alaninate concentrations from the *p*H the pK_2 for glycine and alanine was determined at the same ionic strength using carbonate free sodium hydroxide.

All solutions to be analyzed by the dropping mercury electrode were made up to 5×10^{-4} *M* cupric nitrate. Sufficient potassium nitrate was added to keep the ionic strength constant at $\mu = 1.0$ or 0.1. Methyl red (0.003%) and brom cresol green (0.002%) were used as a maximum suppressor. The solutions were prepared and analyzed at 25.00 ± 0.05°. The solutions were analyzed using a Fisher Elecdropode modified as follows: (1) A cell was constructed[6] so that oxygen could be eliminated by bubbling nitrogen through the solution. (2) Since the potential of the quiet electrode varied during the electrolysis, the potential of the dropping electrode was checked at every voltage against a saturated calomel electrode using a Leeds and Northrup student's potentiometer. The currents were corrected by subtracting the current due to the supporting electrolyte. The reversibility of the electrode reaction was tested for each analysis by plotting log $i/(i_d - i)$ against $E_{d.e.}$. The analysis was discarded unless a straight line[5] was obtained with $\Delta E/\Delta \log i/i_d - i = 0.035 \pm 0.002$. 5×10^{-4} *M* cupric nitrate in 0.1 *M* potassium nitrate solutions gave a slope of 0.035 (calcd. = 0.0296). The value of $E_{1/2}$ was obtained from the plot of $E_{d.e.}$ *vs.* log $i/(i_d - i)$ at log $i/(i_d - i) = 0$. $E_{1/2}$ values could be duplicated, in the solutions up to 0.1*M*, to ±1 mv. At higher glycinate ion concentrations the slope tended to increase till at 1 *M* glycinate ion the slope was 0.039 ± 0.003. The $E_{1/2}$ values at 1 *M* glycinate were reproducible to ±5 mv.

Discussion

The reduction to a metallic state (amalgam) of a complex ion of copper may be represented by

$$CuX_p^{(a-pb)+} + ne + Hg \rightleftarrows Cu(Hg) + X^{-b} \quad (1)$$

(1) Summary in Boorsook and Thimann, *J. Biol. Chem.*, **98**, 671 (1932).

(2) Gould and Vosburgh, THIS JOURNAL, **64**, 1630 (1942).

(3) Riley and Gallafent, *J. Chem. Soc.*, 2029 (1931).

(4) Ferrel, Ridgion and Riley, *ibid.*, 1440 (1934).

(5) Lingane, *Chem. Rev.*, **29**, 1 (1941).

(6) Kolthoff and Lingane, "Polarography," Interscience Publishers, Inc., New York, N. Y., 1941, p. 245.

where Cu(Hg) represents the amalgam formed on the dropping mercury electrode, and X^{-b} is the complex forming substance. Lingane[5] has shown that if the above reaction is rapid and reversible at the dropping electrode that at 25°

$$\frac{\Delta E_{1/2}}{\Delta \log(X^{-b})} = -p\frac{0.0591}{n} \quad (2)$$

where $E_{1/2}$ is the half wave potential. Thus from the slope of the curve obtained by plotting $E_{1/2}$ *vs.* the activity of the complex forming substance the coördination number, p, may be determined. The dissociation constant

$$K_p = \frac{(Cu^{++})(X^{-b})^p}{(CuX_p^{(n-pb)+})} \quad (3)$$

may be obtained from

$$(E_{1/2})_c - (E_{1/2})(s) = \frac{0.0591}{n}\log\frac{K_p f_c k_s}{f_s k_c} - p\frac{0.0591}{n}\log(X^{-b}) \quad (4)$$

where $E_{1/2}$ is the half wave potential, f is the activity coefficient and k is proportional to the square root of the diffusion coefficient of the ion. The subscript c refers to the complex ion while the subscript s refers to the simple cupric ion in the absence of any complex forming material.

The results of the analyses of solutions containing cupric nitrate ($5 \times 10^{-4} M$) and varying concentrations of glycinate or alaninate ion are given in Tables I and II. In all analyses the slope of the $\log i/i_d - i$ *vs.* E line indicated that two electrons are involved in the electrode reaction. Thus under these conditions the cuprous glycinate or alaninate complexes are not stable whereas in the case of the ammonia complexes the cuprous complex is stable.[7]

TABLE I

HALF WAVE POTENTIAL OF THE COPPER GLYCINATE COMPLEX AS A FUNCTION OF THE GLYCINATE ION CONCENTRATION

Total glycine, moles/l.	pH	$-E_{1/2}$ *vs.* S. C. E.	$-\log[G^-]$
μ = 1.0		pK_2 = 9.84	
1.000	11.75	0.465	0.006
0.800	12.02	.458	.098
1.000	10.31	.449	.128
0.600	12.00	.446	.224
.500	11.80	.441	.302
.500	10.37	.423	.415
.200	11.90	.402	.705
.150	11.80	.394	.824
.200	10.15	.392	.877
.100	10.25	.371	1.148
.080	9.93	.357	1.360
.040	10.18	.344	1.573
.040	9.90	.338	1.682
.020	9.86	.318	2.014
.010	9.80	.298	2.368
.000	...	−.014	...
μ = 0.10		pK_2 = 9.69	
0.095	10.01	0.364	1.197
.050	9.99	.345[5]	1.486
.000	...	−.013[5]	...

TABLE II

HALF WAVE POTENTIAL OF THE COPPER ALANINATE COMPLEX AS A FUNCTION OF THE ALANINATE ION CONCENTRATION

Total alanine moles/l.	pH	$-E_{1/2}$ *vs.* S. C. E.	$-\log[A^-]$
0.250	10.44	0.390	0.706
.100[a]	10.40	.365	1.116
μ = 0.100		pK_2 = 9.86	
0.100	10.44	0.366	1.106
.080	10.50	.360	1.193
.040	10.46	.341	1.506
.020	10.39	.322	1.834
.010	10.28	.303	2.186

[a] 0.09 M potassium nitrate as S. E.

The concentration of free amino acid was obtained by subtracting the concentration of amino acid combined with cupric ion (assumed CuX_2) from the total amino acid present. Then the concentration of glycinate or alaninate ion was calculated from the concentration of uncombined amino acid and the pH using the appropriate value of pK as given in Table I or Table II.

The ionic strength of the solutions was kept constant at either 1.0 or 0.10. Since μ was constant, the activity coefficient of the glycinate or alaninate ion would remain nearly constant and the coördination number, p, may be determined from equation 2 by plotting $E_{1/2}$ *vs.* log $[X^-]$. This has been done in Fig. 1. The slopes obtained from Fig. 1 are for 0.01 to 0.08 M glycinate ion (μ = 0.10)0.0597, (μ = 1.0)0.0597, and for 0.01 to 0.08 M alaninate ion (μ = 0.10)0.0598. From equation 2 the theoretical slope for p = 2 is 0.0591. The agreement is good. At higher concentrations of glycinate ion (above 0.10 M) the slope increases due to the formation of higher complexes. In the range (0.2 to 1.0 M) the slope is approximately 0.090. The exact slope is difficult to determine because of the larger experimental error in $E_{1/2}$ values at these glycinate ion concentrations. This slope indicates that p = 3 or the complex ion in this range would be CuG_3^-.

Since the activity coefficients of the ions are not known, values of the concentration dissocia-

TABLE III

CONCENTRATION DISSOCIATION CONSTANTS FOR THE GLYCINATE AND ALANINATE CUPRIC COMPLEX IONS

$-\log[G^-]$	μ	$K_2 \times 10^{16}$	$K_3 \times 10^{17}$
1.486	0.10	7.4	...
2.014	1.0	5.3	...
0	1.0	...	5.4
0	1.0	...	12[a]
$-\log[A^-]$			
1.506	.10	9.8	...

[a] Calculated from Riley's (ref. 4) data.

(7) M. v. Stackelberg and H. v. Freyhold, *Z. Elektrochem.*, **46**, 120 (1940).

tion constants were determined from equation 4 by assuming all activity coefficients to be unity. The ratio of k_c/k_s was determined experimentally and for glycinate or alaninate ion up to 0.1 M was 1.00 with 1%. For glycinate ion between 0.2 and 1.0 M $k_c/k_s = 0.94$. As an error of 1 mv. in $E_{1/2}$ would produce an error of 8% in the equilibrium constant, k_c/k_s was taken as unity in all cases. Table III gives the values of K_2 and K_3 obtained for glycinate and alaninate ion substituting the appropriate values of $E_{1/2}$ in equation 4.[8]

Riley and co-workers[3,4] used a cupric ion concentration cell to determine the equilibrium constants of the complex ions. If equation 3 is substituted into the equation for the e.m.f. of a concentration cell an equation very similar to equation 4 is obtained.

$$E = + \frac{0.0591}{n} \log \frac{K_p f_c}{f_s} - \frac{p}{n} 0.0591 \log (G^-)$$

where f_s is the activity coefficient of cupric ion in the reference solution (0.01 M cupric sulfate[3,4]). Thus if E is plotted *vs.* log $[G^-]$ the data of Riley readily may be compared with the results of this investigation. This has been done in Fig. 1. The glycinate concentrations were corrected for the glycinate ion combined with cupric ion by assuming CuG_2 up to 0.100 M glycinate ion and CuG_3^- above 0.200 M glycinate ion. The results up to 0.100 M do not give a definite indication of CuG_2 being formed in contrast to the results obtained with the dropping mercury electrode. From 0.200 to 1 M the slope is 0.092 indicating that CuG_3^- is the stable complex ion in this range which is in agreement with the results obtained with the dropping mercury electrode. It is interesting to note that above 1 M glycinate ion Riley's[4] results indicate that $CuG_4^=$ may be formed (slope 0.124). The data for alaninate solutions reported in the two papers of Riley[3,4] do not agree. Taking the data of his first paper[3] as correct the same deviations are obtained from the results of this investigation as with the glycinate results up to 0.20 M.

The value of K_3 calculated from Riley's[4] data is given in Table III. This value of K_3 differs by a factor of two from this investigation. This is not surprising considering that different liquid junctions are involved, that activity coefficients have been neglected, and the difference in temperature involved.

(8) Using the values for K_2 and K_3, then $CuG_2 + G^- = CuG_3^-$, $K = 10$ and if $G^- = 0.1$ M the concentration of CuG_2 is equal to that of CuG_3^-. Smaller concentrations of G^- would favor the formation of CuG_2 while larger concentrations of G^- would favor the formation of CuG_3^-.

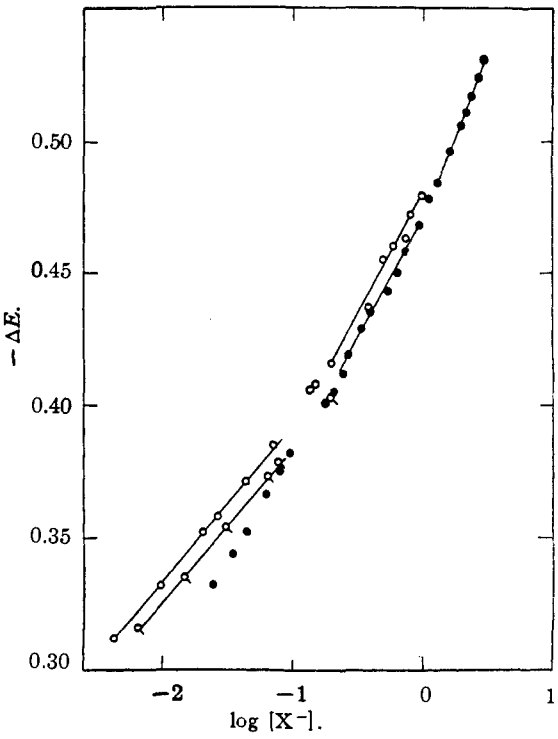

Fig. 1.—Half wave potential of the cupric complex ion in glycinate and alaninate solutions: O, glycinate solutions; ♂, alaninate solutions; ●, glycinate solutions.[4]

Summary

1. The cupric complex ions formed where excess glycinate or alaninate ion is present have been studied using the dropping mercury electrode.

2. In the presence of 0.01 to 0.08 M excess glycinate ion or alaninate ion the complex ion is mainly CuG_2 or CuA_2. From 0.2 to 1.0 M excess glycinate ion the complex ion is CuG_3^-. The concentration dissociation constants of the above complex ions have been determined.

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