

Comparison of the Polarographic Behavior of the Cd^{2+} –Glycine, N-Acetyl- and N-Benzoyl-Glycine Systems in Aqueous and Ethanolic Solution

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The complexation reactions of glycine, N-acetyl- and N-benzoyl-glycine with the Cd^{2+} ion were studied as a model for metal-protein interactions, using polarographic techniques in aqueous and ethanolic solution at 0.1 M NaClO_4 , as base electrolyte, to identify the number and type of solution complex species and to calculate their stability constants. In aqueous solution three complexes of the forms CdL^+ , CdL_2 and CdL_3^- coexist in the pH range 7–10 for the glycine, while the N-protected amino acids do not react under any pH conditions. In ethanolic solution four complexes of the forms CdL^+ , CdL_2 , CdL_3^- and CdL_4^{2-} coexist for the N-protected amino acids, while for the glycine the CdL_4^{2-} complex prevails. A Hammett relationship is observed between the reversible $E_{1/2}$ or β_4 (stability constant) values versus the σ constants for the substituents on the amino group. A coordinative hypothesis for the complexes in solution is also suggested.

Introduction

In a series of papers numerous examples are reported of the *in vivo* interaction of transition metal ions with amino acids and peptides and these interactions are of considerable biological importance [1]. Interest has been principally focused on the simplest amino acids, glycine and alanine, and on the biologically important compounds containing histidine and related species.

We have till now paid particular attention to the study of the solid state behavior of the metal(II) complexes of N-protected amino acid coordination [2–4], showing that N-protected amino acids coordinate solely through the carboxylate group, while the simple amino acids coordinate through the amino and carboxylate group [1].

Since these differences in the amino acid coordination may also play an important role in the solution biological systems, we thought it of interest to study the type and number of complexes which are present in solution, the properties connected with the electron transfer process and the solvent effect of the systems previously examined in the solid state.

In this paper the reduction polarographic behavior of the cadmium(II)–glycinate, N-acetyl- and N-benzoyl-glycinate systems are investigated in aqueous and ethanolic solutions.

Experimental

All the complexes used were prepared as reported in ref. 5 and 6. All chemical products used were of the type C. Erba R.P.E. and the ethyl alcohol was of the type C. Erba R.S.E. Cadmium nitrate tetrahydrate and sodium perchlorate were recrystallized from ethanol before use.

Sodium perchlorate (0.1 M) was used as base electrolyte and the ionic strength of solution was kept constant ($\mu = 0.1$).

Accurately weighed amounts of the amino acids were dissolved in deionized doubly-distilled water or anhydrous ethyl alcohol. Polarographic measurements were performed with 5×10^{-4} M cadmium(II) nitrate tetrahydrate. Polarographic waves were recorded at 25 ± 0.1 °C with an Amel Multipolarograph Model 471 with a saturated calomel electrode as reference electrode. The dropping mercury electrode used in these experiments has a drop time of 2.0 sec.

Results and Discussion

Cadmium(II)–Amino Acid Systems in Water

The study of the polarographic waves obtained in aqueous solutions of the solid complexes (2.5×10^{-4}

M) shows that their half-wave potential ($E_{1/2}$) and current (i_d) values correspond to those of the cadmium(II) ion recorded in the same experimental conditions. This suggests the absence of any complexes. For increasing values of the pH (pH greater than 7) the $E_{1/2}$ values of the cadmium(II)-glycinate systems decrease until they level off at pH greater than 10, while the $E_{1/2}$ values of the cadmium(II)-N-protected amino acid systems do not change under any experimental conditions of pH (Fig. 1). This suggests that in aqueous solution only the cadmium(II) ion and the glycine give rise to complexation.

The cadmium(II)-glycine systems were therefore studied by polarographic analysis in the pH range

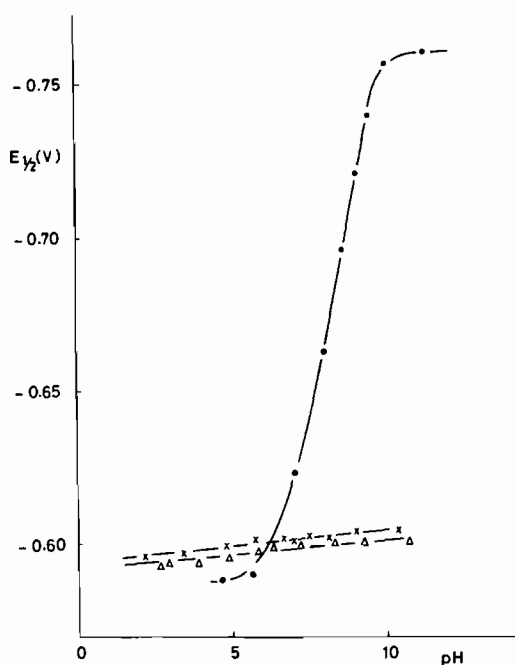


Fig. 1. Dependence of the $E_{1/2}$ values on the pH of the CdX_2 complexes in aqueous solution (X = glycine (•••), N-acetylglycine (xxx) and N-benzoylglycine ($\Delta\Delta\Delta$)).

6.0–11.0. Half-wave potentials decrease when either ligand concentration or pH increase (Table I).

The analysis of the polarographic waves shows that the reduction process is reversible, diffusion controlled, and involves two electrons, under any experimental conditions. Therefore the method of DeFord-Hume [7, 8] may be used to identify the number and type of solution complex species and to calculate their stability constants.

Cadmium(II) ion and glycine form three complexes of the type $CdGly^+$, $CdGly_2$ and $CdGly_3^-$, whose overall stability constants, reported in Table I, are in agreement with the literature data [9–11].

The different coordination behavior toward the cadmium(II) ion of the glycine with respect to the N-protected amino acids may be attributed to the different coordination ability of these amino acids. In effect glycine acts as bidentate, coordinating through the amino and carboxylate groups, forming stable five-membered chelate rings with the metal ions [1]; while, since the introduction of a substituent, such as an acetyl- or benzoyl-group, directly on the amino group, reduces the ligand field of the in-plane donor and diminishes the affinity of the amino group, the other amino acids under study can only coordinate through the carboxylate group.

Cadmium(II)-Amino Acid Systems in Ethanol

The same systems examined in aqueous solution were also investigated in ethanol to evaluate the solvent effect on the number of complexes, that may be formed, and on their coordination type and stability.

The $E_{1/2}$ values of ethanolic solutions of the bis(N-acetylglycinate)cadmium(II) tetrahydrate and bis(N-benzoylglycinate)cadmium(II) monohydrate complexes (5×10^{-4} M) show the existence of ligand complexation in ethanolic solution. On increasing amounts of ethanolic NaOH solution the $E_{1/2}$ values remain constant, while the current (i_d) decreases until it disappears, for the formation of cadmium(II) hydroxide, which is electrochemically inactive and

TABLE I. Polarographic Data of the Cd^{2+} -Glycine Systems in Aqueous Solution. Cd^{2+} 2.5×10^{-4} M in $NaHCO_3/NaOH$ buffer solution (pH 9); X = glycine concentrations.

$[X] \times 10^{-4}$ M	$-E_{1/2}$ (V)	$E_{1/2}$	F°	$F' \times 10^{-3}$	$F'' \times 10^{-6}$	$F''' \times 10^{-9}$
0	0.59					
4.96	0.614	0.024	6.49	11.07		
9.95	0.618	0.028	8.87	7.90	1.91	0.914
39.70	0.639	0.049	45.55	11.22	1.31	0.078
78.60	0.665	0.075	345.60	43.84	4.81	0.485
97.30	0.678	0.088	951.90	97.73	9.43	0.866
189.00	0.704	0.114	7221.80	382.05	19.90	1.000
278.00	0.714	0.124	15744.20	516.30	20.15	0.689
$\beta_1 = 6 \times 10^3$		$\beta_2 = 10^6$		$\beta_3 = 6.5 \times 10^8$		

TABLE II. Polarographic Results for the Cd²⁺-N-acetylglycine System in Ethanolic Solution. Cd²⁺ 5 × 10⁻⁴ in NaClO₄ 0.1 M solutions and NaOH with NaOH/N-acetylglycine ratio of 2:5; X = N-acetylglycine concentration.

[X] × 10 ⁻⁴ M	-E _{1/2} (V)	-E _{1/2} ^r (V)	F°	F' × 10 ⁻³	F'' × 10 ⁻⁷	F''' × 10 ⁻⁹	F'''' × 10 ⁻¹¹
0	0.500	0.474					
5	0.536	0.526	45.55	89.1	3.8		
10	0.550	0.536	84.98	83.89	1.4	2.0	16.0
50	0.580	0.565	1200.70	240.0	3.4	4.4	20.0
100	0.610	0.595	12434.8	1243.4	11.7	10.4	10.2
β ₁ = 7 × 10 ⁴		β ₂ = 1.2 × 10 ⁷		β ₃ = 5 × 10 ⁸		β ₄ = 10 ¹²	

TABLE III. Polarographic Results for the Cd²⁺-N-benzoylglycine System in Ethanolic Solution. Cd²⁺ 5 × 10⁻⁴ in NaClO₄ 0.1 M solutions and NaOH with NaOH/N-benzoylglycine ratio of 2:5; X = N-benzoylglycine concentration.

[X] × 10 ⁻⁴ M	-E _{1/2} (V)	-E _{1/2} ^r (V)	F°	F' × 10 ⁻⁴	F'' × 10 ⁻⁷	F''' × 10 ⁻⁹	F'''' × 10 ⁻¹¹
	0.500	0.474					
5	0.530	0.513	21.00	4.00	0.20		
10	0.536	0.525	42.10	4.10	0.10	0.40	0.8
20	0.543	0.531	84.98	4.20	0.15	0.45	2.1
50	0.560	0.546	273.20	5.40	0.29	0.46	2.8
100	0.585	0.573	2239.50	22.40	1.84	1.78	1.7
200	0.610	0.596	13472.00	67.36	3.17	1.55	0.6
β ₁ = 3.9 × 10 ⁴		β ₂ = 6 × 10 ⁵		β ₃ = 3 × 10 ⁸		β ₄ = 1.6 × 10 ¹¹	

more stable than the N-protected amino acid complexes. This is in agreement with the experimental conditions of the preparation of these complexes, as pure compounds were obtained when the amino acid/sodium hydroxide ratio is 1:1 [5, 6].

Since the bis(glycinato)cadmium(II) complex is slightly soluble in ethanol, it presents a weak reduction wave and its E_{1/2} value is about ~-0.75 V. On increasing the amounts of ethanolic sodium hydroxide solution added, the E_{1/2} and current values do not change; this demonstrates the great stability of this complex also in sodium hydroxide excess.

To determine the number, type and stability constants of the complexes present in ethanolic solution, we investigated the trends of the E_{1/2} and i_d values for the Cd²⁺-N-acetyl- and -N-benzoylglycinate systems in ethanolic solution on increasing amino acid and sodium hydroxide concentrations. E_{1/2} values decrease when sodium hydroxide concentration increases, until they reach constant values when the sodium hydroxide/amino acid ratio is 2:5. These systems were therefore studied in this sodium hydroxide/amino acid ratio and their E_{1/2} values are reported in Tables II and III.

As glycine is not soluble in ethanolic sodium hydroxide solution, to investigate the Cd²⁺-glycine systems aqueous sodium hydroxide-glycine solutions, having constant sodium hydroxide/glycine ratio

TABLE IV. E_{1/2} Values of the Cd²⁺-Glycine System in Ethanolic Solution. Cd²⁺ 5 × 10⁻⁴ M in NaClO₄ 0.1 M and NaOH with a NaOH/glycine ratio of 2:4; X = glycine concentration.

[X] × 10 ⁻⁴ M	-E _{1/2} (V)	-E _{1/2} ^r (V)
0	0.496	0.474
5	0.738	0.706
10	0.754	0.740
50	0.836	0.820
100	0.858	0.845
β ₄ = 1.7 × 10 ²⁰		

(1:1), were added to ethanolic Cd²⁺ solutions. The E_{1/2} values determined with several concentrations of glycine are reported in Table IV.

The logarithmic analysis applied to all the polarographic waves shows that the reduction process is quasi-reversible and diffusion controlled. Therefore for any ligand concentration it is possible to obtain the reversible half wave potentials (E_{1/2}^r) [12]. These E_{1/2}^r values may be used to identify the successive complex species and their stability constants by the DeFord-Hume method [7, 8, 12]. In Fig. 2 the plot of E_{1/2}^r vs. log[X] for the three systems is reported. The linear plot obtained for the Cd²⁺-glycine system

TABLE V. Comparison of the $E_{1/2}$ and $\ln\beta_4$ Values versus the Substituent Polar Constants (σ) [14].

$\text{Cd}[X]_n$	$-E_{1/2}$ (V)	$\ln\beta_4^a$	$\ln\beta_4^b$	σ_p
$\text{Cd}(\text{Gly})_2$ $\text{Cd}(\text{Gly})_4^{2-}$	0.738	46.58		$-\text{NH}_2 = -0.66$
$\text{Cd}(\text{AcGly})_2$ $\text{Cd}(\text{AcGly})_4^{2-}$	0.567	27.73	27.63	$\text{NH}-\text{COCH}_3 = -0.015$
$\text{Cd}(\text{BzGly})_2$ $\text{Cd}(\text{BzGly})_4^{2-}$	0.534	26.25	25.77	$\text{NH}-\text{COC}_6\text{H}_5 = 0.08$

^a Calculated by the DeFord-Hume method [7, 8]. ^b Extrapolated from the $E_{1/2}$ vs. $\lg[X]$ (see plot of Fig. 3).

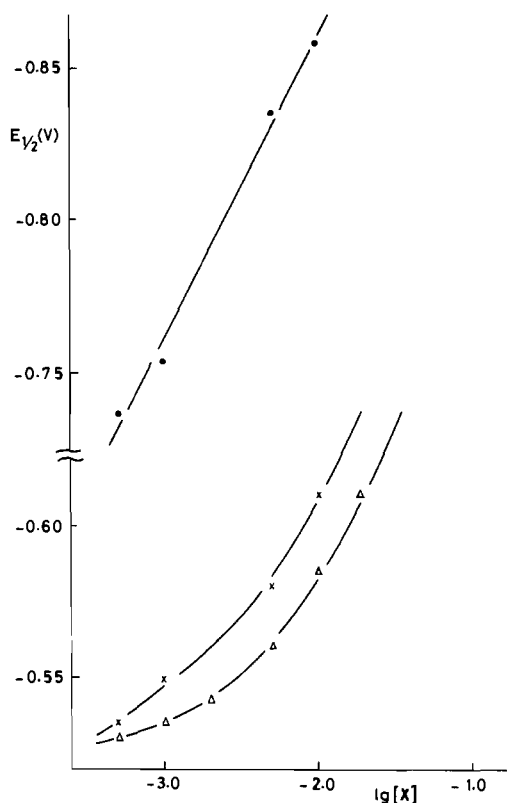


Fig. 2. Plot of $E_{1/2}$ vs. $\lg[X]$ of the Cd^{2+} -glycine (\cdots), -N-acetyl-(xxx) and -N-benzoyl-glycine ($\Delta\Delta\Delta$) systems in ethanolic solution.

suggests that in ethanolic solution one complex only prevails, corresponding to a Cd^{2+} -glycine ratio of 1:4, with a stability constant value of 1.7×10^{-20} . These data are obtained from the following equation:

$$E_{1/2}^r = (E_{1/2}^r)_M + \frac{RT}{ZF} \ln \left(\frac{D_M}{D_{\text{ox}}} \right)^{1/2} - \frac{RT}{ZF} \ln \beta_n - \frac{RT}{ZF} \ln [X]^n$$

where n is the number of ligands coordinated with the metal ion, β is the stability constant of the complex and D_M/D_{ox} is kept = 1 [12].

The DeFord-Hume analysis of the cadmium(II)-N-acetyl- and -N-benzoyl-glycine systems shows the simultaneous presence of four step-wise equilibria in solution. As the experimental behavior suggests that the complexes are formed between the cadmium(II) ion and the anionic form of the amino acids, they may be represented as follows: CdL^+ , CdL_2 , CdL_3^- and CdL_4^{2-} .

In all cases the electrode reduction involves the CdL_2 complex [13]. In Tables II, III and IV the electrochemical parameters and the overall stability constants of the complexes are reported.

To quantify the substituent effects in order to suggest a coordinative hypothesis, we have correlated in Fig. 3 the $E_{1/2}$ values, resulting from the reduction

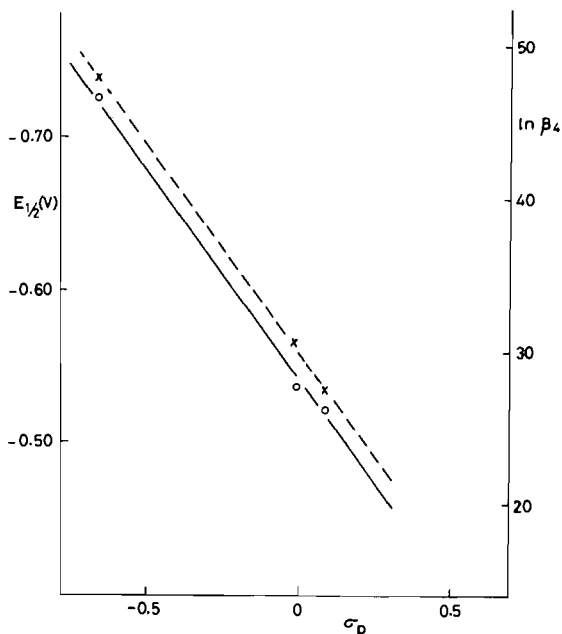


Fig. 3. Plot of $E_{1/2}$ vs. σ (—) and $\ln\beta_4$ vs. σ (---) for the Cd^{2+} -amino acid systems in ethanolic solution.

of the complexes in ethanol, and the $\ln\beta_4$ values versus the substituent polar constants [14] with the Hammett relationships:

$$\Delta E_{1/2} = \rho\sigma_x \text{ and } \Delta \ln\beta_4 = \rho\sigma_x$$

in which σ_x , a substituent constant, measures the electron-donating or electron-withdrawing characteristics of X, and ρ , the 'reaction constant' measures the sensitivity of the reaction to the characteristics of substituents [15].

The linear plots, obtained in both cases, show that $E_{1/2}$ and β_4 values depend on the substituents and especially confirm that $E_{1/2}$ values correspond to the same electrode reduction process described by the following equation [14]:



in agreement with the suggestion reported above. This also suggests that in the coordination of the cadmium(II) ion the amino acids use the same coordination sites.

For the cadmium(II)-N-acetyl- and -N-benzoyl-glycinate complexes the coordination of the amino acid toward the carboxylate group may reasonably be proposed, since in the peptide complexes the N(peptide) atom binds a metal only when the process is accompanied by the dissociation of the peptide proton and the formation of metal-N(peptide) bonds are limited to the d⁶-d⁹ first row transition metals [16].

In particular, since for the CdL₄²⁻ complexes a linear correlation between $\Delta \ln\beta_4$ and σ is obtained, we may propose that they have the same configuration type, probably tetrahedral for steric reasons, and that the glycinate ions in ethanol also coordinate the metal ion through the carboxylate group.

Acknowledgments

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