

Electrochemical Behavior of Copper(II)–Dipeptide Complexes in Mixed Solvents

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(Received December 30, 1985)

Abstract

Several copper(II)–dipeptide complexes were investigated in water–solvent (acetonitrile, *N,N*-dimethylformamide and dimethylsulfoxide) mixtures by electrochemical methods. The effects of the solvents on the reduction potentials of the copper(II) dipeptide complexes were discussed. The reduction potentials in mixed solvents were different from those in aqueous solution and the order of reduction potentials was found to be due to the functional groups of the amino acid residue of the peptides in spite of quasi-reversible electrode reactions for the complexes.

Introduction

Copper-containing biological molecules have been studied by a number of authors. As demonstrated by Gurd *et al.* for bovine serum albumin, metmyoglobin and small peptide fragments, the affinity of protein towards Cu(II) can be enhanced by the formation of chelate ring structures [1–4]. Oligopeptides form five-membered or six-membered chelates with copper(II) as demonstrated by potentiometric titration, spectrophotometric investigation and X-ray crystallography [2–8]. Nakahara *et al.* investigated the relative stability of the copper-dipeptide complexes with a 5-5-, 5-6- or 6-6-membered fused-ring [9]. The relative stabilities of the fused-ring chelates suggest an explanation of specificity or selectivity of enzymic reaction where metal ions are indispensable. In order to obtain a more detailed view of copper(II)–peptide complexes, the complex formation in water–solvent (acetonitrile (AN), *N,N*-dimethylformamide (DMF) and dimethylsulfoxide (DMSO)) mixtures was investigated by electrochemical methods.

The present paper deals with the electrochemical behavior of copper(II)–dipeptide complexes in water–solvent mixtures and discusses their structures and reduction potentials in water–solvent mixtures.

These findings indicate the solvent effect of the complex formation.

Experimental

Materials

Glycyl-glycine (abbreviated as Gly-Gly), Gly-Ala, Gly-Val, Gly-Leu, Gly-Ileu, Gly-Thr, Gly-Glu, Gly-Phe, Ala-Ala, Ala-Val, Ala-Leu, Ala-Ileu, Ala-Glu, Ala-Ser, and Ala-Tyr were obtained by coupling of benzyloxycarbonyl-amino acids and amino acid benzyl ester using a mixed anhydride method followed by deprotection by catalytic hydrogenation in the presence of palladium black. Each dipeptide was identified by thin layer chromatography and elemental analysis.

All other chemicals used were of reagent grade.

Electrochemical Measurements

All measurements were carried out in a 0.1 M (1 M = 1 mol dm⁻³) sodium perchlorate solution of water–solvent at 25 ± 0.1 °C. Britton–Robinson buffer was used. Apparent pH of each water–solvent mixture was measured by the usual glass electrode. A pH shift by addition of each solvent was approximate ±0.1 and the accuracy of pH was assumed to be ±0.1 pH. A Yanako Model p-1000 voltammetric analyzer and DME (or HMDE) were employed for polarographic and cyclic voltammetric measurements. The reference electrode was an SCE with a 0.1 M NaClO₄/agar salt bridge. The HMDE used was a Metrohm E 410 model.

Results and Discussion

Copper(II)–dipeptide Systems in Aqueous Solutions

Typical current-sampled d.c. polarograms of copper(II) in the presence of Ala-Ala at various pH are shown in Fig. 1. The two waves were observed at lower pH. The first wave disappeared at the higher pH region. The second wave was assumed to correspond to the reduction of the Cu(II)-Ala-Ala complex. The second wave was found to be diffusion-control-

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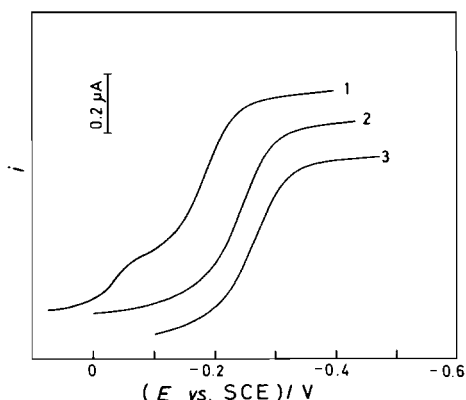


Fig. 1. Current-sampled d.c. polarograms of 0.1 mM copper(II) in the solutions of 1 mM Ala-Ala and 0.1 M NaClO₄ at the various pH. (1); pH = 6.5, (2): pH = 7.5, (3): pH = 9.4.

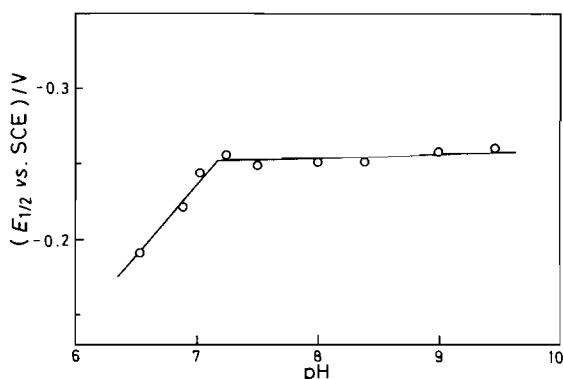
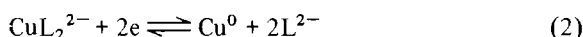
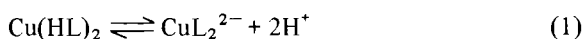


Fig. 2. Relation between the half-wave potentials for Cu(II)-Ala-Ala system in aqueous solutions vs. pH.

led and a quasi-reversible two electron reduction process.

Dependence of half-wave potentials of the Cu(II)-Ala-Ala complex on pH is shown in Fig. 2. The plots indicate a reflection point at about pH = 7.0. The half-wave potential of the complexes was not affected by pH in basic regions above pH = 7.0. The system did not involve protonation in the potential determination steps at these regions.

Copper(II) complexes with peptides were found to exist in the 1:2 ratio at the basic regions from spectrophotometric and potentiometric studies [10–11]. Therefore, the equilibrium of the complex and the electrode reaction at the basic region are assumed to be as follows



where H₂L is dipeptide.

Other systems indicated the same polarographic behavior as this system. Polarographic data for copper(II)-dipeptide (Gly-Gly, Gly-Glu, etc. (Gly-system) and Ala-Ala, Ala-Val, etc. (Ala system)) complexes are summarized in Table I.

Copper(II)-dipeptide Systems in DMF-water Mixtures

Figure 3 shows the typical current-sampled d.c. polarograms for the Gly-Gly systems at various pH in 20% v/v DMF and 80% v/v water solutions (abbreviated as 20% DMF solution). The two-stepped wave was observed at the lower region above pH = ca. 8. The electrode process is assumed to be as follows

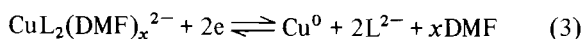


TABLE I. Polarographic Data for Cu(II)-dipeptide Complexes in Aqueous and 20% DMF Solutions. pH = 8.5

Dipeptide	Aqueous solution			20% DMF solution		
	$E_{1/2}$ (V vs. SCE)	i_d (μA)	Slope (mV)	$E_{1/2}$ (V vs. SCE)	i_d (μA)	Slope (mV)
Gly-Gly	-0.206	0.44	46	-0.399	0.32	46
Gly-Ala	-0.270	0.31	40	-0.438	0.39	44
Gly-Glu	-0.250	0.39	38	-0.512	0.29	52
Gly-Val	-0.255	0.43	42	-0.410	0.37	50
Gly-Leu	-0.220	0.42	40	-0.374	0.37	48
Gly-Ileu	-0.236	0.40	40	-0.377	0.37	48
Gly-Phe	-0.225	0.40	28	-0.293	0.38	28
Gly-Tyr	-0.213	0.41	32	-0.267	0.23	40
Ala-Ala	-0.273	0.42	43	-0.444	0.39	46
Ala-Ser	-0.281	0.43	41	-0.452	0.37	47
Ala-Glu	-0.275	0.43	46	-0.511	0.34	52
Ala-Val	-0.268	0.44	42	-0.417	0.38	42
Ala-Leu	-0.237	0.44	38	-0.385	0.38	42
Ala-Ileu	-0.251	0.42	38	-0.410	0.42	42
Ala-Tyr	-0.216	0.39	27	-0.273	0.35	50

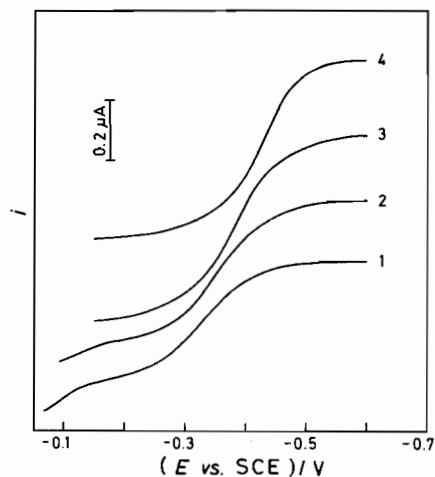


Fig. 3. Current-sampled d.c. polarograms of 0.1 mM copper(II) in the solutions of 1 mM Gly-Gly, 20% DMF and 0.1 M NaClO₄ at various pH. (1): pH = 7.1, (2): pH = 7.5, (3): pH = 8.0, (4): pH = 9.4.

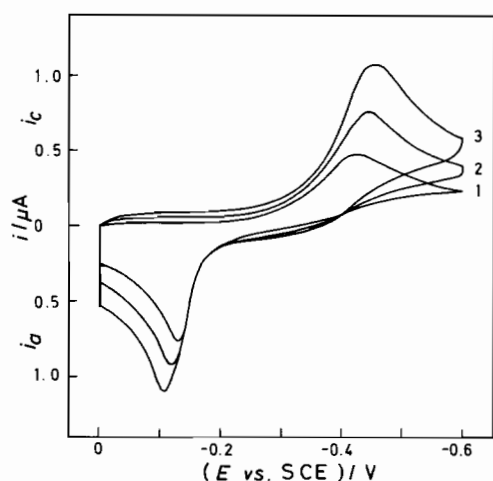


Fig. 4. Cyclic voltammograms of 0.1 mM copper(II) in the solutions of 1 mM Gly-Gly, 20% DMF and 0.1 M NaClO₄ at pH = 9. Scan rate; (1): 50, (2): 100, (3): 200 mV/s.

Table I summarizes the results of measurements with the current-sampled d.c. polarography at 20% DMF systems. The half-wave potentials were shifted to the more negative values and the reciprocal slopes of these complexes were somewhat larger in 20% DMF solutions when compared with those in water.

The typical cyclic voltammograms of copper(II) in the presence of Gly-Gly, 20% DMF and 0.1 M NaClO₄ at pH = 9 is shown in Fig. 4. The reduction wave of the Gly-Gly complex was observed at the cathodic branch but reoxidation of the complex was not observed at the anodic branch. The reoxidation wave was observed at the anodic branch for Cu produced in the reduction process. These results support the electrode process described above.

Effect of Concentration of DMF

The effect of the concentration of DMF on the differential pulse polarograms for the Cu(II)–Gly-Phe and Gly-Glu systems as the representative systems was investigated. Both waves were shifted to more negative potentials and the reversibility of these systems became lower with increasing DMF concentration as shown in Fig. 5. This fact indicates that molecules of DMF may solvate strongly to the bis(dipeptide)copper(II) complex. The solvation of DMF on the complex is assumed to cause the stable complex formation and over potential for reduction.

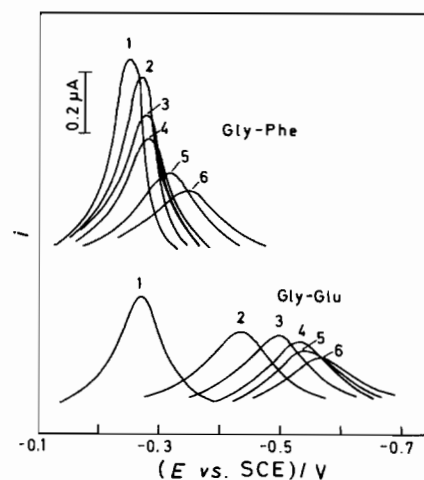


Fig. 5. Differential pulse polarograms of 0.1 mM copper(II) in the solutions of 1 mM dipeptide and 0.1 M NaClO₄ at pH = 8.5. Concentration of DMF; (1): 0, (2): 5, (3): 10, (4): 15, (5): 20, (6): 30%.

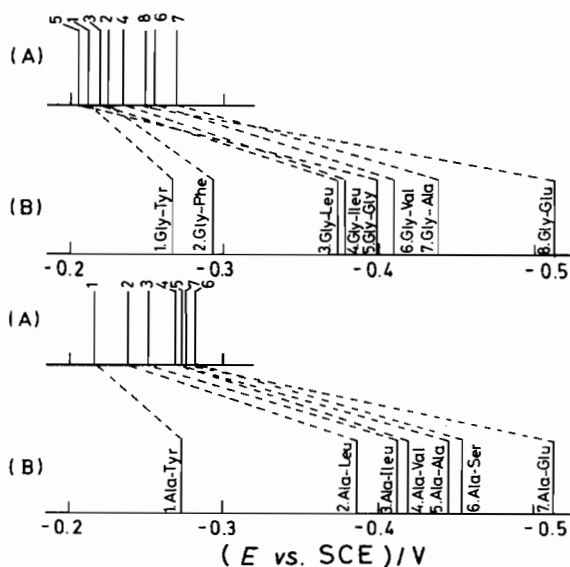


Fig. 6. Comparison of the half-wave potentials for copper(II) dipeptide complexes in aqueous (A) and 20% DMF (B) solutions at pH = 8.5.

Solvent Effects on the Half-wave Potentials of the Complexes

Figure 6 shows the comparison of the half-wave potentials of the current-sampled d.c. polarograms in aqueous and 20% solvent-water solutions. The differences of the half-wave potentials of these systems were very narrow range (ca. 65 mV) in aqueous solutions but the differences in 20% DMF solutions were ca. 240 mV. The order of the half-wave potentials in 20% DMF solutions is as follows:

Gly-systems

Gly-Try > Gly-Phe > Gly-Leu > Gly-Ileu > Gly-Gly > Gly-Val > Gly-Ala > Gly-Glu

Ala-systems

Ala-Tyr > > Ala-Ileu > > Ala-Val > Ala-Ala > Ala-Glu

The order of the half-wave potentials for the Gly-systems coincides with the Ala-systems at the variation of the other amino acid residue. The order of the half-wave potentials at the variation of the substituent groups was $-\text{CH}_2\text{C}_6\text{H}_4\text{OH}(\text{Tyr}) > -\text{CH}_2\text{C}_6\text{H}_4(\text{Phe}) > -\text{CH}_2\text{CH}(\text{CH}_3)_2(\text{Leu}) > -\text{CH}(\text{CH}_3)_3(\text{Val}) > -\text{CH}_3(\text{Ala})$. The electron-donating effect of the phenyl group was observed in spite of the overpotential for the quasi-reversible process.

Other Solvent Effects

The polarographic behavior in 20% AN or 20% DMSO solutions was nearly the same as that in 20% DMF solution. The comparison of the half-wave potentials for the Ala-system in water, 20% AN, 20% DMSO and 20% DMF is shown in Fig. 7. The half-wave potential of each system is in the order of water > 20% AN > 20% DMSO > 20% DMF. The solvent which has a large donor number gives the large nega-

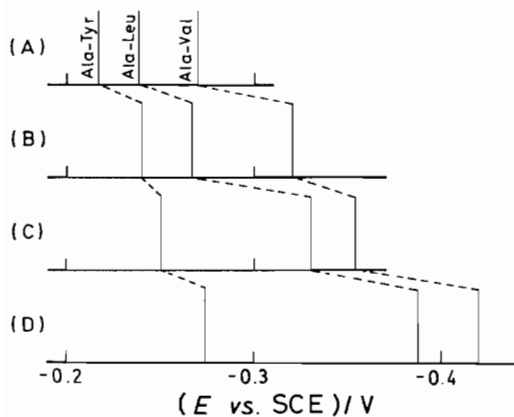


Fig. 7. Comparison of the half-wave potentials for typical copper(II)-Ala-Ala systems in water (A), 20% AN (B), 20% DMSO (C) and 20% DMF (D) solutions at pH = 8.5.

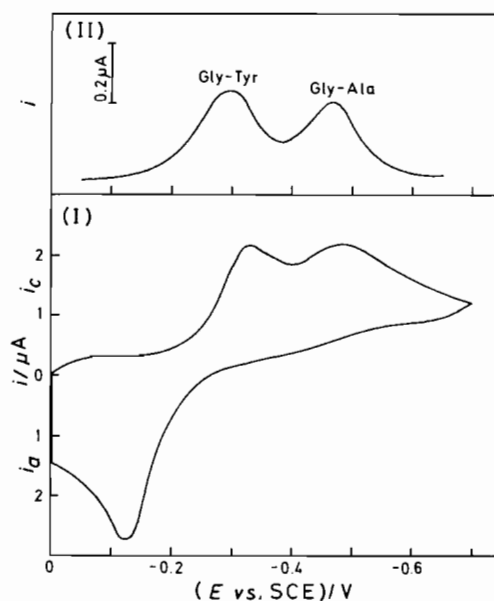


Fig. 8. Cyclic voltammogram (I) and differential pulse polarogram (II) of 0.2 mM copper(II) in the solutions of 0.1 mM Gly-Try, 0.1 mM Gly-Ala, 20% DMF and 0.1 M NaClO₄ at pH = 9.0.

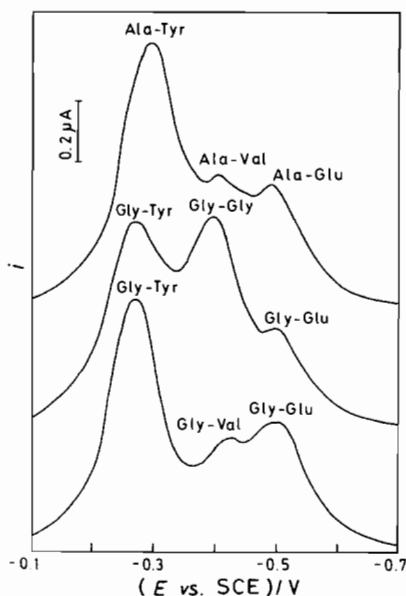


Fig. 9. Differential pulse polarograms of 0.3 mM copper(II) in the solution of 0.1 mM three dipeptides, 20% DMF and 0.1 M NaClO₄ at pH = 9.0.

tive shift of the half-wave potential. The dipeptide complexes are solvated by the solvent which has a large donor number. This may cause the stable complex formation and the overpotential for reduction of the complex.

Copper(II)–dipeptide Systems Containing Two or Three Peptides in 20% DMF Solution

A typical differential pulse polarogram and cyclic voltammogram of Cu(II) in the mixture of Gly-Try and Gly-Ala at pH = 9.0 are shown in Fig. 8. The separation of the waves of the Gly-Try and Gly-Ala complexes was observed at the differential pulse polarogram. On the other hand, the two peaks on the cathodic branch and one peak on the anodic branch were observed at the cyclic voltammogram. This fact indicates that the reduction product of these peptide complexes was copper(0) metal.

The typical differential pulse polarograms of copper(II) in the mixtures of the three dipeptide solutions are shown in Fig. 9. Good separations of the waves were observed when the value of difference of half-wave potentials was *ca.* 150 mV. These data may give information for identification of the dipeptides in biological systems.

Acknowledgement

We are grateful to the Nakanura Jishiro Foundation for partial financial support of this work.

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