Reactions of Cu(II) with Glycine and Glycylglycine in Aqueous Solution at High Concentrations of Sodium Chloride

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Abstract

The complex equilibria between the Cu(II) ion and the amino acids glycine and alanine and the peptide glycylglycine are studied in aqueous solution at high concentrations of sodium chloride, where condensation of glycine to peptide molecules has been observed. Comparison of the species distribution at the conditions where this reaction takes place leads to the conclusion that Cu(II) is already complexed by chloride and glycine at very low pH values. The presence of chloride in the detected species, and its influence on their stability are discussed.

Introduction

Recent investigations have shown that the formation of peptides from their amino acid constituents takes place in aqueous solution in the presence of 3–5 M NaCl and 0.4–0.8 M Cu(II) ion [1]. In order to be able to interpret the reaction mechanism for the condensation of amino acid molecules, it is of maximum interest to know the reactions occurring between the constituents of the solutions, the amino acids, $CuCl_2$ and NaCl, at similar conditions to the ones observed for the peptide formation. This study of the conditions where peptide formation takes place is of further interest, since this process has been proposed as a possible way of how peptides could have formed in the early steps of prebiotic chemical evolution [1, 2].

As a first step of this study, species distributions in the system had to be considered. The aim of the present work was, therefore, to develop a model for the description of complex equilibria present in solutions containing high concentrations of sodium chloride (5.0 M), Cu(II) and amino acids such as glycine (gly) or alanine (ala), or a peptide such as a glycylglycine (gly-gly) in concentrations in the decimolar range, at different pH values, particularly at acidic pH values where the condensation of glycine seems to take place. Although the complexation of Cu(II) with glycine, alanine or glycylglycine has already been studied by several authors and reported in the literature [3–7], there are no investigations at high molar concentrations of sodium chloride, probably because in these previous studies, it was intended to avoid the known complex formation between Cu(II) and chloride, which – although apparently weak – becomes of major importance at the high concentrations of interest here. The following equilibria were studied in the present work:

(1) the complexation of Cu(II) with chloride ion at 5.0 M NaCl, which has not yet been sufficiently reported in previous works [8, 9];

(2) the complexation of Cu(II) with gly, ala and with gly-gly at 5.0 M $NaClO_4$;

(3) the complexation of Cu(II) with chloride and gly or ala or gly-gly at 5.0 M NaCl;

(4) the mixed ligand complexation of Cu(II), glycine, glycylglycine and chloride at 5.0 M NaCl;

(5) the protonation of all the ligands used in 5.0 M NaCl.

Experimental

Materials

5.0 M NaCl and $NaClO_4$ solutions were prepared from puriss reagents of Fluka and used without further purification. $CuCl_2$ 1.0 M stock solutions were prepared from Fluka puriss reagent and standardized iodometrically. $Cu(ClO4)_2$ 1.0 M stock solution was prepared by neutralization of CuO Carlo Erba 99% with the stoichiometric amount of $HClO_4$ acid (Merck), and standardized iodometrically. HCl and NaOH solutions 1.0 M were prepared from Merck Titrisol grade. Gly, gly-gly and ala were obtained from Sigma and used without further purification. Freshly boiled deionized H_2O was used in the preparation of all the solutions.

Apparatus

For the potentiometric determinations a Schott pH meter CG 803 and an INGOLD pH electrode with

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combined reference electrode were used. All pH measurements were carried out in a thermostatized cell at 25 °C. For the addition of the reactants in the titration procedure Metrohm Autoburettes Dosimat 665 and 645 were used. Spectrophotometric determinations were carried out with an Hitachi U-2000 UV–Vis spectrophotometer. Cells of 1 cm pathlength were used in all the determinations. All the calculations were carried out with the CDC CYBER 840 computer of the University of Innsbruck. In potentiometric determinations Gran's method [10, 11] was used for the *in situ* calibration of the potentiometric cell and for the determination of the standard potential E_0, in the ionic medium of 5.0 M NaCl or $NaClO_4$ M at 25 °C. From E_0 and e.m.f. readings in the basic part of a titration of a strong acid (HCl) with a strong base (NaOH), the ionic product of water in 5.0 M NaCl was obtained from the equation $E = E_0 + g \log Kw[OH]^{-1}$ ($g = 59.157$ at 25 °C). The value obtained was 14.45. In the potentiometric titrations the ligand concentrations were varied between 0.02 and 0.2 M, and the concentration of metal ion was varied between 0.005 and 0.1 M, for ligand to metal ion ratios between 1 and 3. The pH changes covered the range of complexation, for glycine and alanine between 1.5 and 6.0 (from where the complexation remains practically unchanged at least until very high pH values pH > 12), and for glycylglycine between 1.5 and 12. Other details of the procedure were as described in previous works [12–16]. The experimental data for the protonation and complexation equilibria were analyzed with the SUPERQUAD [17] program. In all the cases a fit lower than 3 sigma units was achieved for instrumental errors of 0.2 mV in the e.m.f. readings and 0.01 ml for the burette additions (see ref. 17 for details about the meaning of sigma).

Spectrophotometric Determinations

For the study of Cu(II)–chloride complexation, independent solutions containing 0.035 M Cu(II) ion, and different amounts of NaCl and $NaClO_4$ covering a range extending from 0.0 to 5.0 M NaCl and reciprocally from 5.0 M to 0.0 M $NaClO_4$ (keeping always the total amount of salt at 5.0 M) were prepared. A total number of 25 independently prepared solutions was analyzed. For the study of Cu(II)–glycine complexation, two series of experiments were carried out. The first one containing 5.0 M NaCl in the solutions, and the second series containing 5.0 M $NaClO_4$. The concentration of Cu(II) was kept at 0.020 M and that of glycine at 0.040 M in all analyzed solutions (50 solutions). After calibration of the potentiometric cell in the same way as for potentiometric measurements, pH was changed adding negligible amounts of highly concentrated NaOH and HCl or $HClO_4$ to the solutions which already contained Cu(II) and glycine. At each pH change a new Vis spectrum was recorded. The absorption spectra of the prepared solutions were measured between 400 and 900 nm, in steps of 20 nm. All data were analyzed using the SQUAD program [18]. In all cases a fit of experimental data with a standard deviation lower than 0.005 absorbance units was achieved for the set of proposed species.

Results and Discussion

Potentiometric Results

Cu(II)–glycine and Cu(II)–alanine systems

The study was carried out in both media, 5.0 M $NaClO_4$ and 5.0 M NaCl, in order to see whether there is some specific effect in the presence of high concentrations of chloride. However, pH measurements cannot distinguish whether the species contains chloride atoms or not, since the chloride ligand has no acid–base properties in the pH range under study. For this reason there is no assignment for the chloride stoichiometries in Table 1 which gives the potentiometric results. The differences observed between the values of the constants for protonation and complex formation in the two media are very minor and hence not listed separately. As far as they are significant, they should be related nevertheless, to the presence of chloride in the detected species at 5.0 M NaCl. In both media, 5.0 M NaCl and 5.0 M $NaClO_4$, the formation of a protonated complex species for the two ligands at very low pH values was detected, which should correspond to the complex formed between Cu(II) and the carboxylate group of glycine or alanine. This species could be detected potentiometrically in our conditions because of the relatively high amounts of the reagents used (see 'Experimental') compared to previous studies [5–7]. In fact the complexing abilities of carboxylate groups, although many times neglected, make a noticeable contribution at low pH values. The other two species with one and two molecules of ligand and their stability constants in dilute solution are already well known from the literature [3–7].

Cu(II)–glycylglycine and Cu(II)–glycine–glycylglycine systems

These two systems were only studied at 5.0 M NaCl, to see whether there is some difference to previous studies due to the high concentration of NaCl. In Table 1 the detected species and their stability constants are summarized. As already described by other authors, Cu(II) forms different complexes with glycylglycine than with glycine because of the interaction of the peptide N atom with the metal ion, which occurs already at low pH values (around pH 4 at the conditions of our experiments). As with

TABLE 1. Summary of potentiometric results[a]

Protonation			
Species[b] pqr	gly	gly-gly	ala
011	10.60(1)	9.11(1)	10.75(1)
012	13.58(1)	12.85(1)	13.76(1)

Binary complexation					
Species[b] pqr	gly	gly-gly	ala	gly[c]	ala[c]
111	11.40(1)	10.17(1)	11.55(1)	11.62(3)	11.79(1)
110	8.72(1)	6.08(1)	8.71(1)	9.11(1)	9.05(1)
120	15.75(1)		15.66(1)	17.09(1)	17.00(1)
11-1	0.91(1)				
11-2	−9.64(1)				
12-1	3.82(1)				
12-2	−8.28(2)				
22-3	−6.94(2)				

Mixed ligand complexation between Cu(II), glycine and glycylglycine

Species[b] pqrs	
1110	13.29(3)
111-1	4.75(1)

[a]Values of the log of the formation constants obtained using SUPERQUAD program [17]. Within brackets the standard deviations in the last figure are given; when in the refinement this value was lower than 0.01 log units a default value of 1 is given. [b]Species definition: pqr refers to stoichiometries (Cu)p (lig)q (H)r where lig is gly, gly-gly or ala. For the mixed ligand system pqrs refers to (Cu)p (gly)q (gly-gly)r (H)s. [c]Values obtained at 5.0 M $NaClO_4$; other values have been obtained at 5.0 M NaCl.

glycine and alanine, glycylglycine also forms a protonated complex at very low pH, through the carboxylate group bonded to the Cu(II) ion. The second complex is, as with glycine and alanine, a chelate complex involving the carboxylate and the amino group. However, it is interesting to note here that this complex for glycylglycine is of much lower stability than the same complex with glycine under the same conditions: 2.64 log units lower for glycylglycine compared to glycine. This effect cannot be attributed only to the lower donor ability of the amino group of glycylglycine (pK_a 9.11) compared to that of glycine (10.60), but also to the fact that this chelated species is not so stable for glycylglycine as for glycine. The analysis of the potentiometric titrations of solutions which contain Cu(II), glycine and glycylglycine, at approximately equal total concentrations and 5.0 M NaCl, reveals the formation of two new mixed ligand species, in addition to the binary complex species described above (see Table 1). From the stability of these mixed species it is concluded that mixed ligand complexes in this case are not really favoured over binary species, probably due to the already strong binary complex formation with the two ligands and to the limited number of appropriate coordination sites in the coordination sphere of the metal ion. Moreover, the species distribution of the mixed ligand system under the conditions where the condensation reaction of glycine takes place (glycine concentration is considerably higher than that of glycylglycine), reveals that under these circumstances, Cu(II) is practically complexed only by glycine and – due to the colour of solution – obviously also by chloride, and not by glygylglycine which remains freely in solution (less than 1 per cent at a relative excess of 2 to 1 for glycine over glycylglycine).

Spectrophotometric Results

Cu(II)–chloride system

From the results obtained in the numerical analysis of the spectra of the solutions containing Cu(II) and different amounts of chloride (see 'Experimental'), it is concluded that up to two complex species between Cu(II) and one and two chloride ions, respectively, are formed at 5.0 M NaCl concentration. Under these conditions, the relative amounts of free Cu(II), $CuCl^+$ and $CuCl_2$ are 21%, 54% and 24%, respectively. The species distribution in Cu(II) solutions containing different amounts of chloride is shown in Fig. 1 and the spectra of the two complex species are shown in Fig. 2.

Cu(II)–chloride–glycine system

Analysis of the solutions at 5.0 M $NaClO_4$ provides the species spectra and stability constants of the three complexes which do not contain any chloride atoms (Table 2 and Fig. 2). Further analysis of the spectra of solutions at 5.0 M NaCl leads to the resolution of the species present in this medium. An important aspect to discuss here is the deduction of whether chloride ion is involved in the detected complex species between Cu(II) and glycine at 5.0 M NaCl. This was an initial hypothesis, since Cu(II) is already complexed by chloride from the beginning of the titrations, and also because of the green colour of the acidic solutions. From the comparison of the results obtained using NaCl and $NaClO_4$ it can be concluded that one chloride ion should be involved in the complex species formed between Cu(II) and glycine at 5.0 M NaCl. This is the reason for the apparent lower stability of Cu(II)–glycine complexes in this solution, compared with the same species in $NaClO_4$ solution. Assumption of a higher number of chloride ligands would give constants inconsistent with the ones found in the potentiometric analysis and unreasonable species spectra. As the species

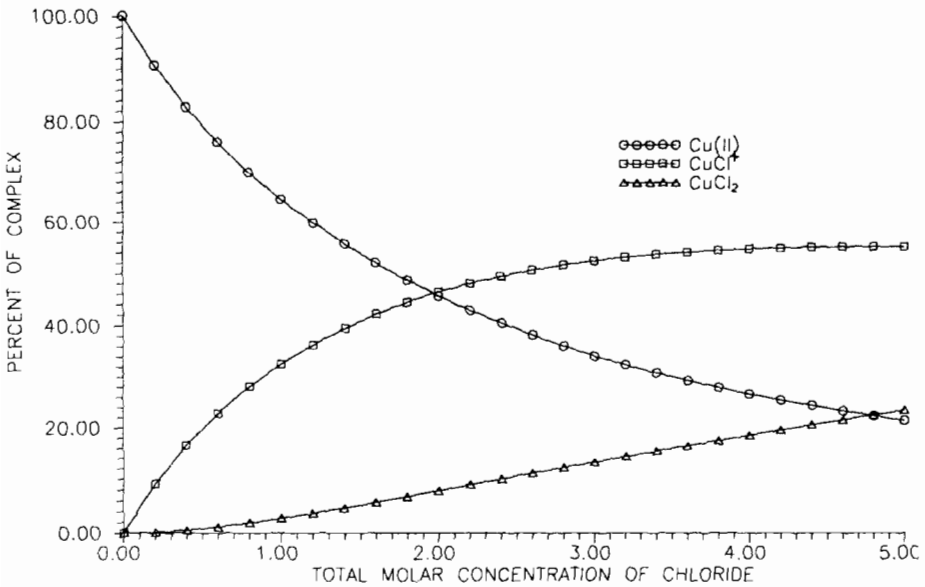

Fig. 1. Distribution plot of Cu(II)-containing species in the system Cu(II)–Cl.

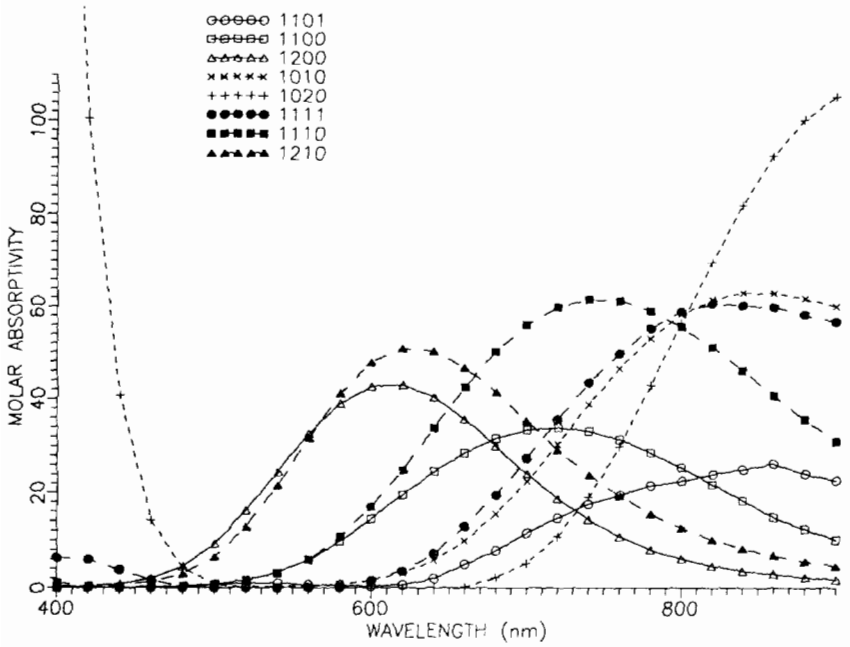

Fig. 2. Species spectra obtained using SQUAD program (species definition as in Table 2).

which contains only one chloride ligand is also the dominant species in solutions of Cu(II) and 5.0 M NaCl, the above conclusion seems reasonable. Therefore, the species detected in the systems studied at 5.0 M NaCl are in fact mixed ligand complex species, where both glycine and chloride ligands are coordinated to the metal ion, and these mixed ligand complexes are the only ones present at such high concentrations of NaCl. The simultaneous inclusion of the binary species detected in $NaClO_4$ solutions gives a much more worse fit or unreasonable constants and spectra. The complex involving two glycine molecules and one chloride atom is less stable than the related complex with only two glycine molecules, a fact which might be related to an effect similar to the 'pentammine effect' [19], i.e. that the axial coordination of a fifth molecule in the coordination sphere of the metal ion leads to a decrease in the stability of the complex in relation to the four-coordinated complex with two glycine molecules obtained at

5.0 M $NaClO_4$. In Figs. 3 and 4, the distribution plots of the species containing Cu(II) are given for 5.0 M $NaClO_4$ and 5.0 M NaCl, respectively. These plots reveal that under these conditions Cu(II) is already bonded to glycine and to chloride (in 5.0 M NaCl) at very low pH values. The presence of high concentrations of chloride decreases the concentration of free Cu(II) at acidic pH, and shifts the formation of the 2 ligand/1 metal complex towards higher pH values.

The agreement between constants of both spectrophotometric and potentiometric methods is very satisfactory. In general, data for species formed at very low or high pH values might be somewhat more accurately obtained by spectrophotometry, whereas in the other regions potentiometric values are expected to be superior.

TABLE 2. Summary of spectrophotometric results[a]

Medium[b]	Species[c] pqrs	log β
Na (Cl^-, ClO_4^-), 5.0 M	1010	−0.28(1)
	1020	−1.34(1)
NaCl, 5.0 M	1111	11.48(2)
	1110	8.68(1)
	1210	15.66(1)
$NaClO_4^-$, 5.0 M	1101	11.62(6)
	1100	9.23(1)
	1200	17.28(2)

[a]Values of the log of the formation constants obtained using SQUAD program [18]. Within brackets the standard deviation in the last figure is given; when in the refinement this value was lower than 0.01 log units a default value of 1 is given. [b]Medium at which the constants were obtained. [c]Species definition: pqrs refers to (Cu)p (gly)q (Cl)r (H)s.

Conclusions

With respect to the mechanism underlying the condensation reaction of amino acids to peptides in aqueous solution containing high concentrations of NaCl, our investigations allow us to conclude that the catalytic function of the Cu(II) ion apparently involves the formation of a complex with one chloride and two amino acid ligands. Upon an eventual condensation of the two amino acids, the dipeptide has to be supposed to be quickly released to the solution due to the lesser stability of this complex, at least as long as further amino acid ligands are available from the solution. According to the species distributions obtained in this work, such a process could occur within a fairly wide pH range starting from 2.5 and extending until alkaline conditions. These assumptions are in good agreement with all observations made so far in peptide condensation experiments [1, 2].

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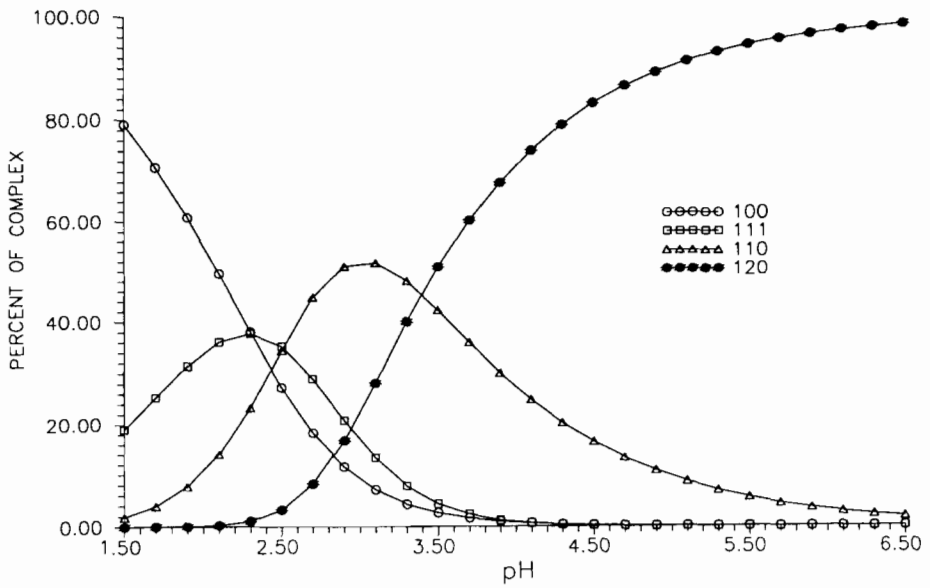

Fig. 3. Distribution plot of Cu(II)-containing species in the Cu(II)–gly system at 5.0 M $NaClO_4$; species definition as in Table 1 (Cu(II) = 0.4 M, gly = 0.8 M).

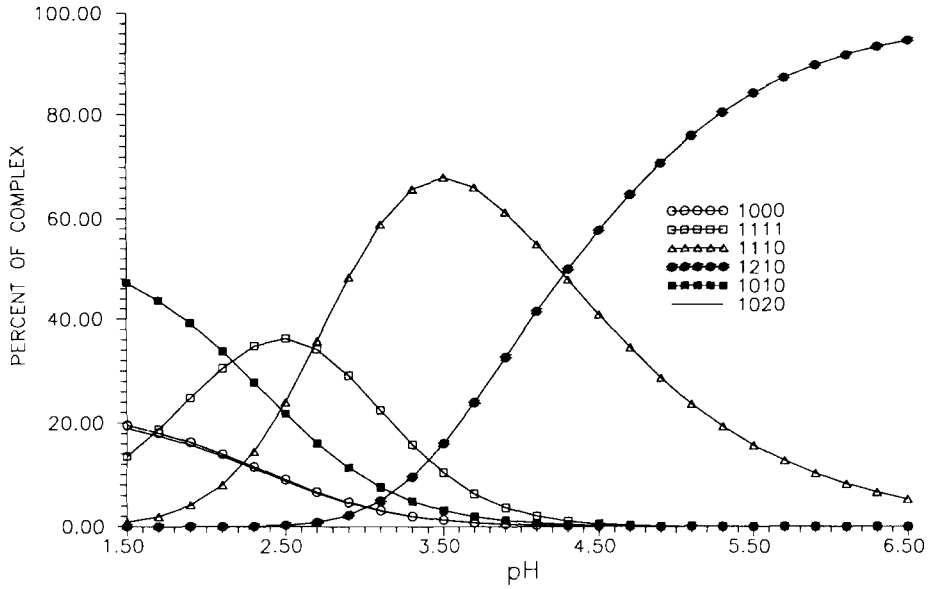

Fig. 4. Distribution plot of Cu(II)-containing species in the Cu(II)–gly–Cl system at 5.0 M NaCl; species definition as in Table 2 (Cu(II) = 0.4 M, gly = 0.8 M).

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