COPPER-CATALYZED AMINO ACID CONDENSATION IN WATER – A SIMPLE POSSIBLE WAY OF PREBIOTIC PEPTIDE FORMATION

BERND M. RODE and MICHAEL G. SCHWENDINGER

Institute of Inorganic and Analytical Chemistry, University of Innsbruck, Innrain 62a, A-6020 Innsbruck, Austria

Abstract. The recently reported condensation reaction of glycine to di- and triglycine in aqueous solution in the presence of higher concentrations of sodium chloride and copper ions has been investigated systematically and quantitatively using HPLC analytical methods. The influence of 'environmental' factors (temperature, concentration, atmosphere) are discussed. Numerous other metal ions have been investigated with respect to similar catalytic effects, and molybdenum results as the only one inducing peptide condensation, although to a much lesser extent. Experiments based on evaporation of water and redissolution lead to peptide condensation up to $(gly)_6$ in concentrated solutions and produces peptides even starting from initially low concentrations.

1. Introduction

The prebiotic formation of peptides/proteins, being very basic molecules for the evolution of living organisms, has been subject of numerous experiments and discussions, mostly based on the assumption of large amounts of condensation reagents on the primitive earth, even as cyanamides, ATP, inorganic polyphosphates and heterocyclic compounds (Hulshoff and Ponnamperuma, 1976; Hawker and Oro, 1981; Rishpon *et al.* 1982; Rabinovitz and Hampai, 1985; Yamanaka *et al.*, 1988). Other authors have shown that melting of amino acids within 120 and 200 °C also leads to condensation (Fox and Dose, 1977). All of these hypothetical prebiotic peptide formation reactions are associated, however, with problems concerning the availability of condensation reagents and the particular reaction conditions on primitive earth (Sawai *et al.*, 1975; Schwendinger and Rode, 1989). Search for other, more simple condensation reactions has lead recently to a new mechanism for possible prebiotic peptide formation in aqueous solvation, in which sodium chloride acts as condensation reagent in the presence of Cu(II) ions as a catalyst (Schwendinger and Rode, 1989). Since the ubiquitous presence of both inorganic components can be assumed with high probability (Cloud, 1973; Hay, 1984), this reaction merited further systematic investigation. Determination of Cu (II) complex formation constants in 5 M NaCl solution has shown recently, that chlorocuprates are formed with glycine and diglycine ligands, and that the complexes of the condensated ligands are weaker than those with the amino acid (Tauler and Rode, 1989). The catalytic role of Cu(II) in the peptide condensation can probably be attributed, therefore, to initial binding of two ligands which, after condensation, are released into solution.

In this work we have investigated the quantitative yield of this reaction, depending

on initial concentrations, temperature and atmosphere. Further, we have investigated the possibility of other metal ions likely to have existed in a primordial sea to catalyze peptide formation under otherwise constant conditions. Finally, the probability of this process to occur under assumed conditions of repeated evaporation and redissolution (as can be expected to happen in coastal-shelf processes) and with very low initial reactant concentrations has been studied.

Experimental Methods

1. Reaction system

Solutions of glycine, sodium and copper chloride (analytical grade compounds) were prepared in distilled water and kept at elevated temperatures (thermostated sand bath) in glass flasks with reflux cooler (except evaporation experiments) under argon atmosphere or air. The mixture was analyzed at regular intervals.

2. Analytical method

Twenty μL of the reaction solution were diluted with 1 mL of water and analyzed by a Hewlett-Packard HP-1090M HPLC apparatus by two methods: a) directly and b) after derivatisation with o-phtalaldehyde (OPA)/3-mercaptopropionic acid (MPA) (Schuster R., 1988). For routine separation, two columns were used, a Shannon Hypersil (ODS 5 μm / 200 × 2.1 mm) column. The mobile phase consisted of 50 mM KH_2PO_4/7.2 mM $C_6H_{13}SO_3Na$, pH = 2.5 adjusted by H_3PO_4, with a flow rate of 0.35 mL min^{-1}. For separation of the derivatized peptides, a HP Amino-Acid-Column (2.1 × 200 mm) was used, with mobile phase composition and gradient according to Schuster (1988).

Detection was performed with a diode array detector at 195 nm. All peptides were identified by retention time of analytical grade reference substances (Sigma) and of their respective derivatives. Full agreement of both (independent) HPLC methods prove the identity of the substances. In addition, after chromatographic separation diglycine was isolated and identified by the IR spectrum of its dinitrophenyl derivative.

Results and Discussion

1. The Cu(II)/glycine/NaCl system

Previous semiquantitative investigations analyzing the formed peptides in this system by means of thin layer chromatography had indicated, that best yields of peptides are obtained at a concentration ratio Cu(II)/gly of 1:2, in 5.0 M NaCl, and at temperatures above 80 °C (Schwendinger and Rode, 1989). The peptide formation in such a system at 100 °C is illustrated by the HPLC results of Figure 1, showing the peaks for di- and triglycine evolving after 1, 4.5, 9, and 261 hr, respectively.
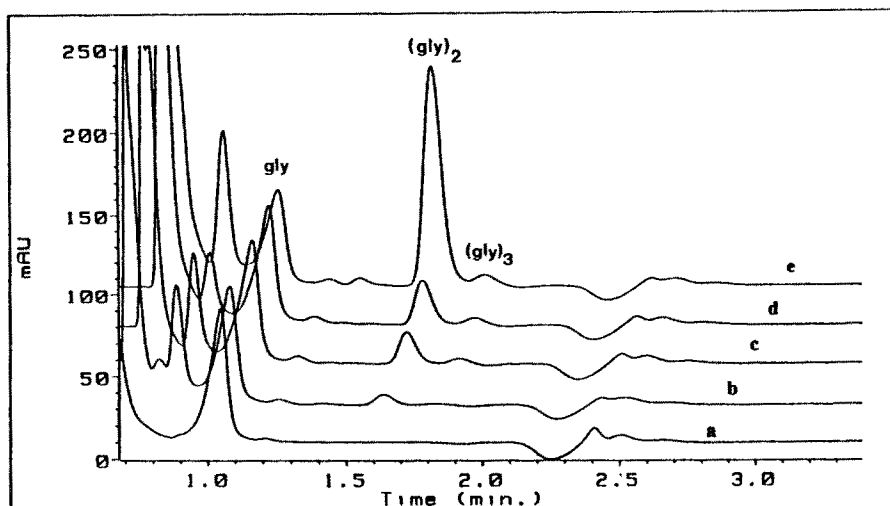
Fig. 1. Chromatogram showing peptide formation from glycine induced by 5M NaCl and Cu(II) ions at 100 °C after 0 (a), 1 (b), 5 (c), 9 (d) and 260 (e) hr.

Besides solvent peak (first on the left) and amino acid and peptide peaks, another peak appears, which could be identified to consist partly of diketopiperazine (DKP) by spiking with authentical DKP, the anhydride of glycine. The concentration of DKP is quite low, however, its strong appearance is mostly due to the excellent UV absorbance of this compound.

Table I summarizes the quantitative results for peptide formation after 10 days as a function of NaCl concentration. For these experiments, temperature was kept between 70 and 80 °C, and no inert gas atmosphere was employed. The data show that only minimal amounts of diglycine are formed without NaCl or at NaCl concentrations up to 1 M. Increasing NaCl concentration is directly related to the

TABLE I

Dependence of peptide formation on NaCl concentration[a]

^{c}NaCl (M)	^{c}Cu(II) (M)	$(gly)_2$	$(gly)_3$
0.0	0.4	0.25	–
0.5	0.5	0.52	–
1.0	0.5	0.47	–
1.8	0.45	2.50	+
2.5	0.45	3.20	+
3.5	0.45	3.30	++
5.0	0.40	4.10	++

[a] Reaction time is 10 days; the concentration of glycine is twice that of Cu(II).
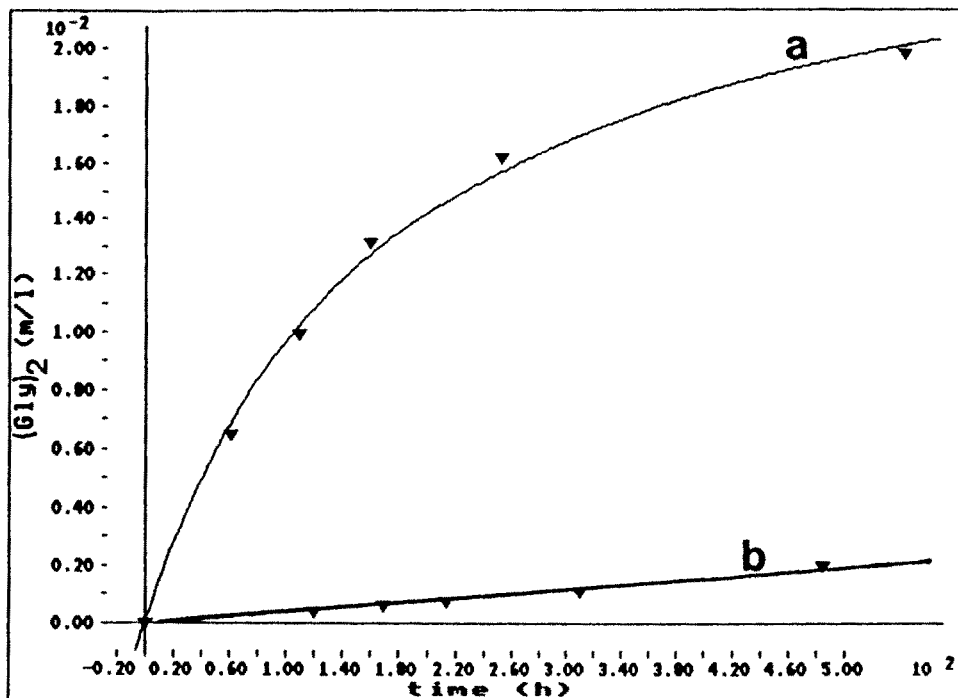
Fig. 2. Peptide formation vs. reaction time for 0.4 M Cu(II)/0.8 M glycine and 5 M NaCl, at pH=2 (a) and pH=3 (b).

TABLE II

Variation of atmosphere, glycine and Cu(II) concentration[a]

^{c}gly	^{c}Cu(II)	T °C	% (gly)$_2$[b]	% (gly)$_2$[c]	Atmosphere
0.09	0.09	80	0.3	not measured	Ar
0.18	0.09	80	0.7	not measured	Ar
0.35	0.09	80	0.5	not measured	Ar
0.4	0.2	80	1.2	2.3	Ar
0.4	0.4	83	3.0	3.3	Ar
0.5	0.25	80	1.9	3.3	air
0.8	0.2	75	0.4	0.8	air
0.8	0.4	86	4.1	5.2	Ar
0.8	0.4	86	4.1	5.0	air
1.0	0.5	75	3.5	3.9	Ar
1.0	0.5	82	4.6	4.6	Ar
1.2	0.4	80	1.8	2.7	Ar

[a] The concentration of NaCl was 5 M,
[b] Yield is after 11 days.
[c] Yield is after 23 days.

amount of dipeptide formed, and tripeptide can be observed only when such elevated concentrations are present.

In a further experiment NaCl 5M was present but no copper ions. The resulting dipeptide amounts after 11 days were below 0.1% in all samples, when the pH was varied from 2 to 6.

In the next series, pH was varied in the system Cu(II) 0.4 M / gly 0.8 M / NaCl 5 M by addition of NaOH, from 3 to 8. The temperature was kept at 70–80 °C, without inert gas atmosphere. Increasing pH to 3 strongly lowers the yield to 0.3% (cf. Figure 2), and then not much difference is observed until pH 6. In the further experiments no artificial adjustment of pH was performed.

Table II gives some representative results of experiments of variation of gly and Cu(II) concentrations, at a temperature of 80 °C, 5.0 M NaCl and an argon atmosphere. Identical experiments under air did not reveal a significant influence of oxygen on the results, similar to previous studies (Schwendinger and Rode, 1989).

Evaluation of the time dependence of peptide formation under most suitable conditions by means of a least-square fit to possible reaction kinetics showed (Figure 3), that the reaction is most likely of second order with reference to glycine concentration. The resulting velocity constant for the reaction is $1.0 \ \text{L mol}^{-1} \ \text{h}^{-1}$.

These results, together with the complex species forming in such solutions (Tauler and Rode, 1990) indicate that a relation of Cu(II) to gly of 1:2 and high concentrations of chloride form the most favourable condition for complex formation. If chloride or H^+ concentration becomes too low or a too large excess of glycine is present, mostly Cu(II)-glycine chelate complexes seem to form, and not the chlorocuprate-glycine complexes with carboxylate-coordinated glycine(s), which seem to be essential for the peptide condensation reaction. Visually this behaviour can be observed from the colour of the solution: solutions dominated by Cu(II)-glycine chelate complexes which form peptides to a very low extent are blue, whereas the chlorocuprate-glycine complexes which induce peptide synthesis are green.

Finally, the possibility of formation of higher peptides was investigated by starting the reaction in 5.0 M NaCl/0.4 M Cu(II) solution from 0.4 M diglycine instead of glycine. After 10 days of reaction, 45% of the diglycine hydrolyzes to glycine, 0.8% has been converted to $(\text{gly})_3$, and 0.4% to $(\text{gly})_4$. No higher peptide could be detected under these conditions.

2. INFLUENCE OF ANIONS AND REACTION CONDITIONS

The influence of pH and atmosphere has been discussed already in Section 1. A certain difference can be expected in the presence of oxygen only with the respect of a parallel reaction of glycine oxidation by Cu(II), when oxygen of air can regenerate the amount of reduced copper (I)- chloro-complexes to Cu(II) complexes. This side reaction does not seem to have a significant influence under the reaction conditions studied here, but it is subject of ongoing electrochemical investigations.

Temperature influence has also been studied to a limited extent. According to these preliminary results, the reaction seems to become very slow below 60 °C, and to
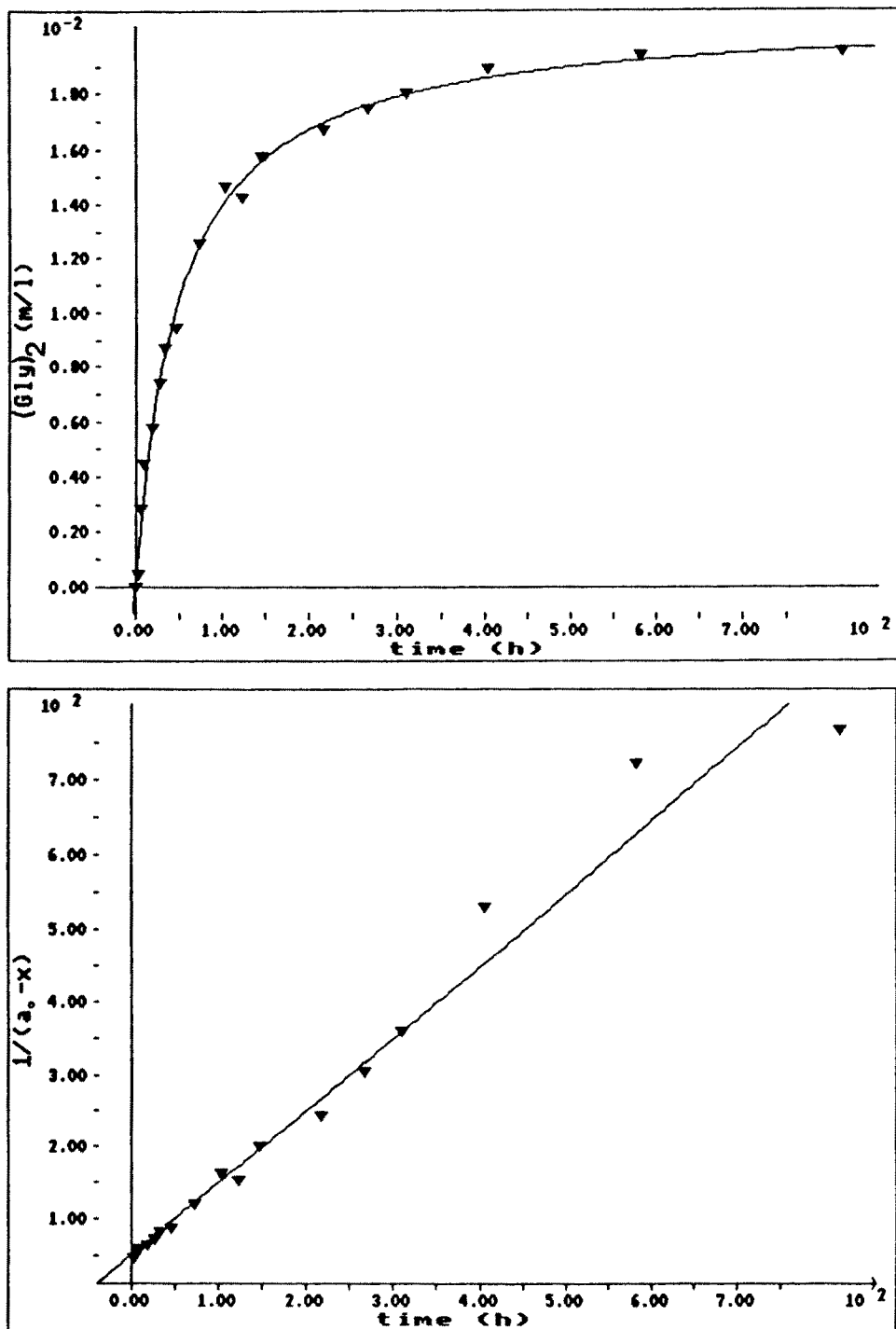
Fig. 3. Fitting of reaction data to 2nd-order reaction kinetics for Cu:gly:NaCl = 0.4M : 0.8M : 5.0 M: (a) concentration vs. time; (b) $1/(a_0) - x)$ vs time; a_0 = total amount of reacting glycine, x = amount of formed diglycine.

proceed the faster, the higher the temperature becomes. However, near the boiling point of the solution, reduction of Cu(II) and hydrolysis of the peptides formed seems to occur more readily so that a temperature of 70° to 80° seems to be most suitable for maximizing the peptide yield.

Another important question was, whether change of NaCl to other salts would have a significant influence on the reaction. Replacement by NH_4Cl (5M) leads to equal amounts of peptide, proving that Na^+ is not involved in the reaction, except for the role of taking water into its hydration shell, which can be performed by other comparable ions as well. The change to $NaNO_3$ however decreases drastically the peptide yield, showing that coordination of Cl^- to Cu(II) is an essential feature of the reaction.

3. OTHER METAL ION

Previous experiments had indicated that copper seems to be the only suitable ion for this condensation reaction, similar to its superiority in catalyzing amino acid ester condensation in nonaqueous solution (Yamada et al., 1971). We decided to reinvestigate the metal ions studied previously, and to include iron and molybdenum, as they can be also expected to have been ubiquitously present in primordial sea.

The following ions have been investigated: Cr(III), Al(III), Ca(II), Mg(II), Zn(II), Cd(II), Fe(II), Ni(II), Co(II) and Mo(VI), 0.5 M each, with 0.9 M glycine and 5.0 M NaCl, T=75°. For all ions except Mo(VI), no enhanced peptide formation takes place after 11 days of reaction (Figure 4) With Co(II), Ni(II) and Fe(II), traces of diglycine are found (0.03–0.06%), but in the presence of Mo(VI) the yield is better: 0.3% of glycine is converted to diglycine. Still, this amount is much smaller than in the case of Cu(II), but it indicates that molybdenum might be a second
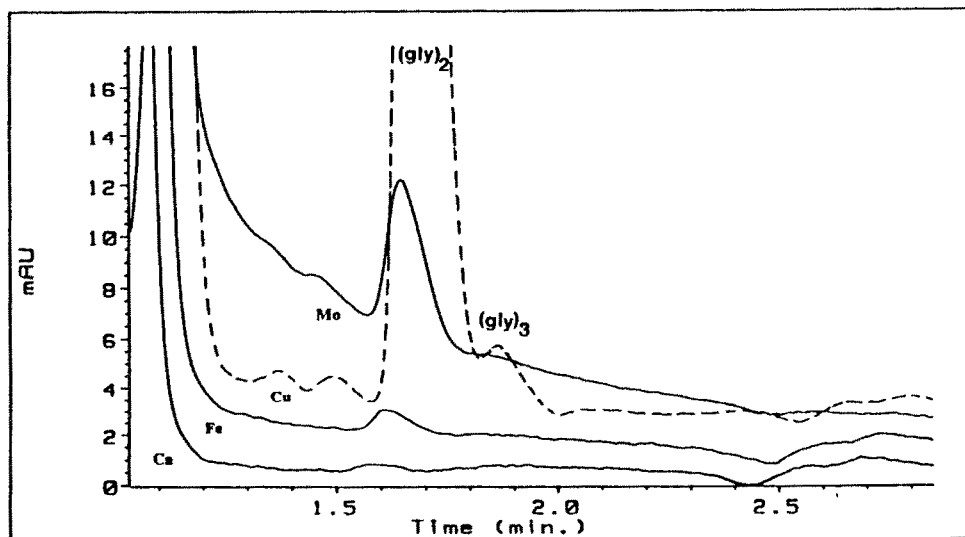

Fig. 4. Chromatogram of reaction with metal ions such as Ca(II), Fe(II), Cu(II) and Mo(VI).

TABLE III

Evaporation experiments

Starting conditions (Gly/Cu/NaCl/Volume)[a]	Cycle No.	Duration (hr)	% $(gly)_2$	% $(gly)_3$	n_{max}[b]
0.8/0.4/4.6/100 mL	1	12	3.3	0.2	4
	2	+12	4.1	0.2	4
	3	+12	5.4	0.3	4
	4	+24	6.2	0.3	4
	5	+24	6.4	1.9	5
0.8/0.4/4.6/100 mL	1	24	5.2	n.e.	5
	2	+24	6.7	n.e.	5
	3	+24	7.9	n.e.	5
	4	+24	8.3	2.0	5
0.1/0.05/0.5/250 mL	1	24	4.4	0.7	4
	2	+24	6.2	2.5	4 (4=0.5%)
	3	+24	5.4	2.9	6 (4=1.0%)
0.003/0.01/0.05/500 mL	1	48	1.0	n.e.	2

n.e.: not quantitatively evaluated.

[a] Concentrations are given in molarity.

[b] n_{max} is the number of monomers in the highest molecular weight peptide identified.

possible catalyst for peptide synthesis in aqueous solution. Precipitation of MoO_3 aq. occurs under the conditions of this experiment, leaving the question of heterogeneous processes open.
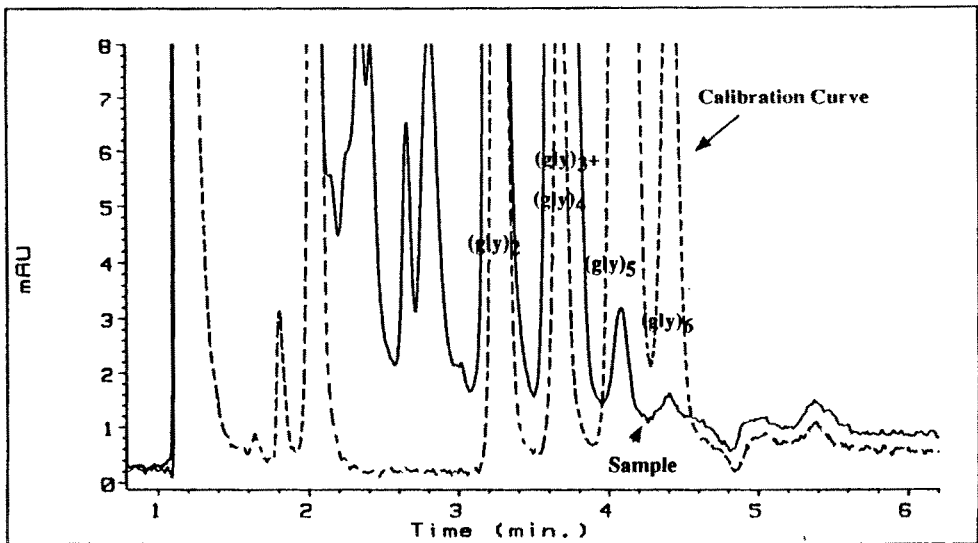
4. EVAPORATION EXPERIMENTS

All experiments performed so far have started from optimized laboratory conditions. It seemed to be of interest, therefore to make an approach to possibly more 'realistic' scenarios which could be imagined to happen on the primitive earth. Assuming primordial sea water with low concentrations of amino acid and Cu(II) ions and NaCl contents similar to nowaday's sea water, one could imagine on-shore formation of highly concentrated solutions until complete evaporation of the solvent and redissolution, due to tidal effects and day/night-related temperature changes.

A series of experiments were designed on the basis of such a scenario, where solutions of the aforementioned components with varying initial concentrations were kept at temperatures of 80 to 90 °C in open beakers to allow water evaporation. After drying, water was added again and the process repeated 3 to 4 times. After every process, concentration of peptides was analyzed by HPLC. The results are summarized in Table III. The most impressive result is the formation of higher peptides than in all previous experiments: Figure 4 shows the HPLC separation of the peptides found in the solution starting with the same concentration as in the previous experiments, together with the peaks of the reference peptides. Higher peptides up to $(gly)_6$ can be clearly identified, proving the capability of the reaction to synthesize oligopeptides in aqueous solution under the assumed conditions.
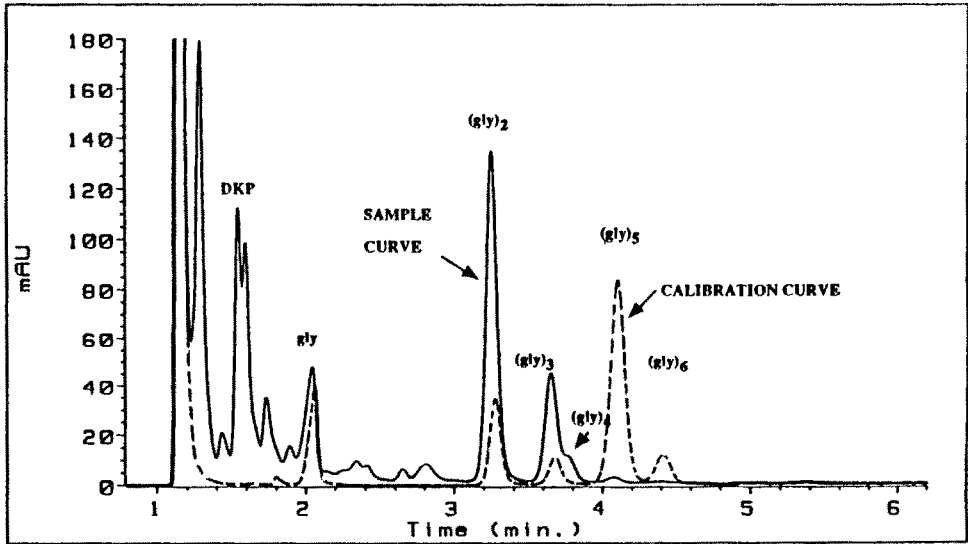
Fig. 5. Analysis of peptides formed in evaporation experiment (1) after 84 hr and 5 cycles ((a) original and (b) enlarged).

Conclusions

(1) The quantitative investigations of the copper induced peptide condensation in aqueous NaCl solutions has shown that this reaction leads within a relatively short time to yields of peptides, similar to that of other mechanisms suggested to possible prebiotic evolution steps, as that based on 1:10:1 ATP/imidazole/gly (Sawai and Orgel, 1975) or long-term cycles involving kaolinite/clay (Paecht-Horowitz, 1970). The reaction components, water, NaCl and Cu(II) were likely to have been available on the primitive earth, and the reaction is rather insensible to environmental factors. Higher oligopeptides can be formed under simple conditions.

(2) Although still subject to further detailed studies, the importance of chlorocuprate/amino acid complexes for peptide formation seems to be established, and the basic kinetics of the reaction indicate a second-order mechanism.

(3) The reaction occurs in the presence of NaCl and Cu(II) in water, is enhanced by the evaporation of water and hence represents one of the simplest known peptide evolution mechanism under possible prebiotic conditions so far.

Acknowledgment

References

Cloud, P. E.: 1973, *Econ. Geol.* **68**, 1135.
Fox, S. W. and Dose, K.: 1977, in *Molecular Evolution and the Origin of Life*, Rev. Ed., Marcel Decker Inc., N.Y.
Hawker, J. R. and Oro, J.: 1981, *J. Mol. Evol.* **17**, 285.
Hay, R. W.: 1984, in *Bioinorganic Chemistry*, E. Horwood Series of Chemical Science, Chichester / GB.
Hulshoff, J. and Ponnamperuma, C.: 1976, *Orig. Life* **7**, 197.
Paecht-Horowitz, Berger, J., and Katchalsky, A.: 1970, *Nature* **228**, 636.
Rabinovitz, J. and Hampai, A.: 1985, *J. Mol. Evol.* **21**, 199.
Rishpon, J., O'Hara, P., Lahav, N., and Lawless, J.: 1982, *J. Mol. Evol.* **18**, 179.
Sawai, H., Lohmann, R. and Orgel, L. E.: 1975, *J. Mol. Evol.* **6**, 165.
Schwendinger, M. G. and Rode, B. M.: 1989, *Analytical Sciences* **5**, 411.
Schuster, R.: 1988, *J. Chromatogr.* **431**, 271.
Tauler, R. and Rode, B. M.: 1990, *Inorg. Chim. Acta.* **173**, 93.