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Salt induced peptide formation: on the selectivity of the copper induced peptide formation under possible prebiotic conditions

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Abstract

The reactivity of α, β and γ-amino acids in the copper(II)/NaCl-induced peptide formation reaction has been investigated. The stability constants and species distribution of amino acid/copper complexes were determined by the potentiometric method, and they indicate that copper complexes of the type Cu(aa)Cl, being held responsible for the peptide formation, form preferentially with α-amino acids. The peptide formation itself has been investigated by experiments with constant volume and temperature and by evaporation cycle experiments with subsequent peptide analysis by means of HPLC. All possible dipeptide combinations of the investigated amino acids with glycine are observed, and α-amino acids are preferably linked to glycine when competing with their β analogues. This preference of biologically relevant amino acids supplies another strong argument for the possible significance of the salt-induced peptide formation reaction for the chemical evolution of first peptides on the primordial earth.

Keywords: Copper complexes; Amino acid complexes; Peptide complexes

1. Introduction

Many proposals have been made until now how peptides, a class of molecules indispensable for the evolution of life, could have been formed from amino acids in an aqueous environment on the prebiotic earth. The major barriers for the peptide formation are the ionic nature of the amino acids in water, which prevents the nucleophilic attack of the amino N at the carboxylic C at virtually any pH, and the large excess of water which forces the thermodynamic equilibrium to the side of the educts [1,2]. Most of the effort of hitherto investigations had the goal to overcome these obstacles. Peptides have been synthesized from amino acids in aqueous solutions by the aid of a variety of condensation reagents [3], e.g. cyanamides [4], cyanates [5], linear [6] and cyclic [7,8] inorganic polyphosphates or ATP [9], and in the dry state after complete evaporation of water and subsequent reaction of the amino acids in melt [10] or on clays [11,12]. Recently a novel peptide formation reaction was discovered [13–15], which utilizes the water binding abilities of high concentrations of inorganic salts and the catalytic effect of copper(II) ions on the condensation of amino acids and therefore represents the probably simplest known mechanism for this purpose.

The demands of this reaction on the environment are only small and in full agreement with the present knowledge about the actual conditions on the prebiotic earth (summarized in Refs. [13–15]). Sodium chloride should have been ubiquitously available, and the presence of copper is indicated by the typical 'green zones' of archaic (precambrian) rock formations, which consist mainly of azurite and malachite. Because copper(II) is reduced by the amino acids in a side reaction, traces of oxygen to regenerate the catalyst are profitable for the yield of the reaction. This requirement – an O_2 concentration of 10^{-35} atm has been supposed to be sufficient to oxidize Cu(I) [16] – is well within the proposals for the prebiotic atmosphere's oxygen content, which range from 10^{-15} [17] to 10^{-1} [18] pal.

Application to systems simultaneously containing Gly/Ala [15] and Gly/Val [19] has shown that the reaction is also applicable to α-amino acids other than the simplest one, glycine, and that preferential formation of certain sequence isomers occurs.

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In a typical prebiotic peptide formation experiment rather ideal conditions are used with respect to the quality of the educts. However, the results of most postulated prebiotic amino acid syntheses [20–22] immediately show that biologically relevant amino acids are not the only products obtained, and in most cases not even the major products. Therefore it seems to be an inevitable necessity that a reaction model which is claimed to have possible prebiotic significance displays a certain selectivity for the reaction of α-amino acids over 'useless' competitors. We have extended our investigations therefore to systems containing also non-biological amino acids. In particular the reactivity of α-alanine (aala) and β-alanine (bala), and α-aminobutyric acid (aaba), β-aminobutyric acid (baba) and γ-aminobutyric acid (gaba) were compared. In this context aaba is incorrectly given the status of an 'advantageous' amino acid, but it can be assumed that the selection of the modern set of the biological amino acids was made at a later stage of evolution [23]. The chosen α- and β-amino acids are the simplest representatives of this class of compounds, and they are formed in simulated prebiotic amino acid synthesis reactions in yields comparable to those of the simple α-amino acids. Therefore the evaluation of the relative reactivity of α- and β-amino acids should provide important information about the applicability of the model reaction to systems that do not contain only advantageous amino acids as sole educts, and therefore should resemble the composition of primordial amino acid solutions in a more realistic way.

The superiority of copper to accelerate and enhance peptide formation in systems containing high concentrations of sodium chloride has been attributed to the special properties of a chlorocuprate complex containing one deprotonated amino acid ligand as a suitable nucleophilic reaction partner, and to the large difference between the stabilities of copper complexes containing amino acids or peptides as ligands. Therefore it could be expected that a comparison of the complex formation constants of the amino acid isomers would give a first idea about their relative reactivities.

The stability constants of copper complexes as well as the protonation constants of the amino acids under investigation have been for some time known, but they have been determined in very different media of much lower ionic strength. In the system Cu(II)/gly/NaCl it has been shown previously not only that constants themselves are substantially altered in solutions of high sodium chloride concentration, but also that formation of a chloroglycinato–cuprate complex species is observed due to the high educt concentration [24]. Therefore it seemed unavoidable to characterize the complexes formed in solutions containing 5 M of sodium chloride (corresponding to the peptide forming system) de novo by potentiometric titrations.

In a second step, these amino acids were reacted in two types of experiments leading to salt-induced peptide formation. In the first series, rather high concentrations of amino acid(s), copper ion and sodium chloride were kept at constant volume and temperature, and the progress of peptide formation was monitored by regular sampling. In the second series, evaporation experiments were performed, starting from relatively low concentrations. After each evaporation cycle of about 24 h, the solid residue was taken up in the same amount of water, and the formed peptides were analysed after each cycle. Whereas the first series allows a more exact monitoring of the reaction and corresponds to a possible archaic scenario with concentrated sea water solutions near to evaporation, the second series can claim some reality for reactions occurring off-shore and in flat lagunas, where rapid, complete evaporation is possible within a day, followed by new addition of water through tidal processes and/or rain.

2. Experimental

2.1. Materials

2.1.1. Potentiometric titrations

α-ala, β-ala, aaba, baba and gaba, NaCl and $CuCl_2$ were obtained from Fluka Co. and were used without further purification. The titrant solutions (0.4 M NaOH/5.0 M NaCl and 0.4 M HCl/4.6 M NaCl) were prepared from Merck Titrisol grade. 0.3 M $CuCl_2$/4.1 M NaCl stock solutions were standardized iodometrically. Concentrations and ratios have been chosen to keep the ionic strength at a constant value of 5 for all titrations. Freshly boiled deionized water was used for the preparation of all the solutions.

2.1.2. Peptide formation experiments

The same amino acids as listed above were used. The needed reference peptides of analytical grade were purchased from Fluka, Sigma and Senn Chemical Co., as far as they were commercially available. The other peptides had to be synthesized by us for this purpose, and the following procedure was followed.

The corresponding amino acids (if not commercially available in the appropriately protected forms) were protected according to standard methods [25]. Amino functions were furnished with the BOC (tert-butyloxycarbonyl) protecting group. Free acids were converted into the methyl esters. The peptide bond was formed by means of ethyl chloroformate and subsequent chromatographic work-up (MPLC, silica-gel, solvent: ethylacetate/methanol or ethylacetate/cyclohexane in various mixtures) of the peptides. After formation of the peptide bond, H_2/Pd/C in MeOH was used to remove *Z*(benzyloxycarbonyl) protecting groups and to convert

benzyl esters into the free acids. Methyl esters were saponified with aqueous NaOH in THF or MeOH. BOC protecting groups and the t-butyl group of the corresponding esters were removed by passing HCl gas through a solution or suspension of the peptides in ethyl acetate. The peptide hydrochlorides were converted into their free forms by means of ion-exchange chromatography (Dowex 50 W) in water and subsequent lyophilization.

2.2. Apparatus

2.2.1. Potentiometric titrations

For the potentiometric determinations an Orion 8104 Ross combination pH electrode with combined reference electrode and an Orion pH meter SA 720 were used. The acid or base solutions were added with a Metrohm 665 Dosimat. The titrations were automatized and controlled through the RS232 interfaces of both the burette and the potentiometer using a small PC program. The stability criterium between two successive e.m.f. readings was 0.1 mV during 90 s. All measurements were carried out in a thermostatized cell at 25 °C under N_2 atmosphere.

To determine the protonation constants of the amino acids in 5 M NaCl, solutions with an amino acid concentration between 0.01 and 0.05 M were titrated from pH 2.0 to 12. For the investigation of the complexes with copper, the metal concentrations were varied between 0.005 and 0.02 M, and concentrations of the ligand were varied between 0.01 and 0.04 M, with a ligand to metal ratio between 4 and 1. As far as possible these titrations were carried out in the pH range 2.0–6.5. Due to the weak copper complexes of the β- and especially the γ-amino acids hydrolysis was observed already at a lower pH, therefore in these cases the pH range had to be reduced to a maximum of 4.5–5.5.

The method of Gran [26,27] was used for in situ calibration of the potentiometric cell and the determination of the standard potential $E°$ prior to every titration. Calibration was performed in the same ionic medium (5.0 M NaCl) in order to avoid differences in ionic activities and junction potentials, and all calibrations were performed immediately before each analysis. Diluted HCl in 5.0 M NaCl was titrated by NaOH, and the initial acid concentration of this solution was estimated according to Gran [26,27]. Assuming Nernstian behaviour, E_0 was evaluated. Under the conditions of this work (constant temperature of 298 K and constant ionic strength of 5.0 M) the standard potential was constant between pH 2 and 12. Also the calculated ionic product for water evaluated in every titration, was obtained constant at 14.45 for all experiments. Other details of the procedure were as described in previous works [24]. The experimental data for the protonation and complexation equilibria were analyzed with the SUPERQUAD [28] program which was adapted for use on a PC. In all the cases a fit lower than 3σ units was obtained for instrumental errors of 0.2 mV in the e.m.f. readings and 5 μl for the burette additions (σ [28] is the ratio of the root mean square of the weighted residuals to the standard deviation).

2.2.2. Peptide formation experiments

For the reactions with constant volume, solutions containing 0.4–0.8 M amino acid, 0.2–0.4 M Cu(II) and 5 M NaCl were kept at constant temperature of 80 °C in flasks with a reflux cooler (closed with Al foil for protection from impurities). Peptide formation was monitored over 3 weeks by HPLC analysis.

For the evaporation experiments, solutions containing 0.08–0.16 M amino acid, 0.04–0.08 M Cu(II) and 0.5 M NaCl were kept in open systems at elevated temperature (80–90 °C), guaranteeing complete solvent evaporation within 24 ± 1 h. After each cycle distilled water was added and the process repeated. Samples were taken after each cycle for a total of 5–7 cycles and analyzed by HPLC.

All the experiments were performed 2–5 times in order to prove reproduceability. The data given in the Tables are the mean values of all experiments, the results of which vary within $\pm 5\%$ of these values.

2.3. HPLC analysis of peptides

The samples were analyzed using a Hewlett-Packard HP-1090M HPLC apparatus after online derivatization with *o*-phthalaldehyde (OPA)/3-mercaptopropionic acid (MPA) [29]. Separation was performed on a Shannon Hypersil column (ODS, 5, 200×2.1 mm) with mobile phase according to Ref. [29] (solvent A: 30 mM EDTA, 0.2% THF; solvent B: 100 mM $NaOAc \cdot 3H_2O$, 0.1 mM EDTA, 80% MeCN). The gradient was chosen in the following way: $t = 0$ min: 100% A, $t = 2$ min: 100% A, $t = 17$ min: 80% A, $t = 20$ min: 80% A, $t = 23$ min: 0% A. During the analysis the flow was kept constant at 0.45 ml/min.

3. Results and discussion

3.1. Potentiometric titrations

The results of the potentiometric titrations and the stability constants of the species present in the system Cu^{2+}/amino acid/NaCl with ionic strength = 5 in the acidic range of the pH scale have been collected in Table 1, together with the literature values obtained at low ionic strength. As in the case of glycine, an additional chlorocuprate complex of the type $Cu(aa)Cl^+$ could be detected for all amino acids investigated due to the high metal and ligand concentrations. The values

Table 1
Comparison of logarithms of stability constants obtained in 5 M NaCl solution at 298 K to the corresponding values at low ionic strength [30] [a]. *pqr* refers to the species $(Cu)_p(aa)_q(H)_r$, aa = amino acid

Species		011	012	pK_a	111	110	120
α-ala	[30]	9.66	11.95	2.29		8.14	14.90
	Exp.	10.71[2] 3[b]	13.72[2]	3.01[2]	11.45[2] 3[b]	8.68[1]	15.62[1]
β-ala	[30]	10.06	13.53	3.47		7.04	12.54
	Exp.	11.14[2]	15.51[2] 4[b]	4.37[2]	12.38[1]	7.43[1] 5[b]	13.17[1]
aaba	[30]	9.63	11.93	2.30		8.07	14.85
	Exp.	10.61[1]	13.58[1] 4[b]	2.97[1]	11.39[2]	8.62[1] 5[b]	15.54[1]
baba	[30]	10.02	13.44	3.42		7.12	12.85
	Exp.	11.12[1]	15.35[1] 4[b]	4.23[1]	12.45[2]	7.51[1] 4[b]	13.29[2]
gaba	[30]	10.31	14.34	4.03			
	Exp.	11.33[1]	16.20[1] 3[b]	4.87[1]	12.77[2] 4[b]		

[a] [b]: No. of titrations with approx. 50 data points each. Exp.: this work. [1]: ±0.01 (σ). [2]: ±0.02 (σ).

Table 2
Reactions for pure aminobutyric acids with constant volume [a]

	Reaction time (days)							
	1	2	4	8	11	15	21	26
$(aaba)_2$ [b]	nf	nf	nf	0.02	0.05	0.15	0.29	0.34
$(gaba)_2$ [c]	0.51	0.82	1.03	1.21	1.22	1.25	1.10	1.06

[a] All results as percent of initial amino acid concentration; nf = not found.
[b] 0.8 M aaba and 0.4 M Cu^{2+} in 5.0 M NaCl solution.
[c] 0.8 M gaba and 0.4 M Cu^{2+} in 5.0 M NaCl solution.

of the corresponding stability constants are not defined very sharply, because they vary considerably with the slightest change in the protonation constants. However, the neglect of these species in the refinement algorithm leads to much worse fits.

In comparison to solutions of low ionic strength the stability constants of all complexes increase, but the relative trend remains the same: copper amino acid chelate complexes of α-amino acids are more stable than their β analogues, and they do not even form with γ-amino acids due to the instability of seven-membered rings. To illustrate the consequences of the difference in the stability constants on species distributions, the obtained β values have been used to calculate the concentrations of species in a hypothetical solution containing 0.3 M gly, each 0.1 M α-ala, β-ala, aaba, baba and gaba, and 0.4 M Cu^{2+} in 5 M NaCl. The results are shown in Fig. 1(a)–(e). They have to be regarded as only approximate representatives, since mixed complexes with two different amino acid ligands have not been considered in this simulation. The values for the stability constants of glycine have been taken from Ref. [24]. The plots of the percentage of amino acids existing as protonated (a) and neutral (b) species versus the pH reveal that at a pH below 4 β- and γ-amino acids are almost quantitatively free in solution, mainly as protonated species. The $Cu(aaH)^{2+}$ complexes (c) of all amino acids except glycine have similar concentrations, the maxima of the α-amino acid concentrations being at lower pH.

The most significant result is illustrated in Fig. 1(d): the copper monochelate complexes of the α-amino acids are the dominating copper species (at pH 4 more than 70% of copper is present in such complexes), whereas the concentration of their β analogues is insignificant.

3.2. Peptide formation experiments

3.2.1. With constant volume (T = 80 °C)

Table 2 shows the formation of dimers of aaba and gaba. Dimerization of baba has not been observed under these conditions. Table 3 lists the reaction products formed from mixtures of glycine with aaba, baba or gaba, respectively. In Table 4 the results of 'competition experiments', where either α- and β-alanine or aaba and baba are simultaneously present with glycine, are given.

The best dimerization among the aminobutyric acids is found for gaba; only small amounts of the aaba dimer are formed after more than a week of reaction time. In the case of baba, dimerization is not observed even after 3 weeks. The better dimerization of gaba could be caused by the possibility of formation of a monomeric cyclic anhydride (lactame) under the dehydrating influence of the high salt concentrations, which could react with another gaba molecule.

In the systems, where glycine is present together with one of the aminobutyric acids, diglycine is found in all experiments, and also the dimer of gaba. In all three systems both types of mixed peptides are observed, gly–aba and aba–gly. Very similar peptide yields are found throughout the observation time for baba and aaba, less products are formed with gaba. After long reaction times, all dipeptides start to decompose again, but are still present in significant amounts after more than 3 weeks. Another interesting difference is observed in the preferred sequence of amino acids in the products: whereas aaba forms dominately aaba–gly, baba and gaba are mainly found as gly–aba peptides. In analogy to the gly/ala system [15], a sequence inversion process should be responsible for this preference.

A most interesting result, however, is found when α- and β-alanine compete for peptide formation with glycine: the α-alanine/gly peptides are formed in much higher quantities than gly/β-ala (see Table 4). The

Table 3
Reactions for mixed aba/gly systems with constant volume

Reaction time (days)	0.4 M gly, 0.4 M aaba and 0.4 M Cu^{2+} in 5 M NaCl solution			0.4 M gly, 0.4 M baba and 0.4 M Cu^{2+} in 5 M NaCl solution			0.4 M gly, 0.4 M gaba and 0.4 M Cu^{2+} in 5 M NaCl solution			
	$(gly)_2$	gly–aaba	aaba–gly	$(gly)_2$	gly–baba	baba–gly	$(gly)_2$	$(gaba)_2$	gly–gaba	gaba–gly
1	0.56	0.12	0.44	1.16	0.54	0.09	0.08	nf	nf	nf
2	1.47	0.11	0.80	1.37	0.81	0.11	0.63	nf	0.25	0.38
4	2.33	0.21	1.32	1.64	1.10	0.15	0.70	nf	0.23	0.35
8	3.08	0.44	2.07	2.02	2.27	0.27	0.64	0.08	0.58	0.41
11	5.04	0.86	2.21	1.26	2.47	0.31	0.53	0.22	0.78	0.39
15	4.26	0.74	2.15	1.17	2.62	0.43	0.07	0.21	1.23	0.52
21	2.28	0.35	1.66	nf	2.09	0.44	nf	0.24	1.35	0.31
26	0.22	0.07	1.02	nf	1.26	0.42	nf	0.21	1.09	0.29

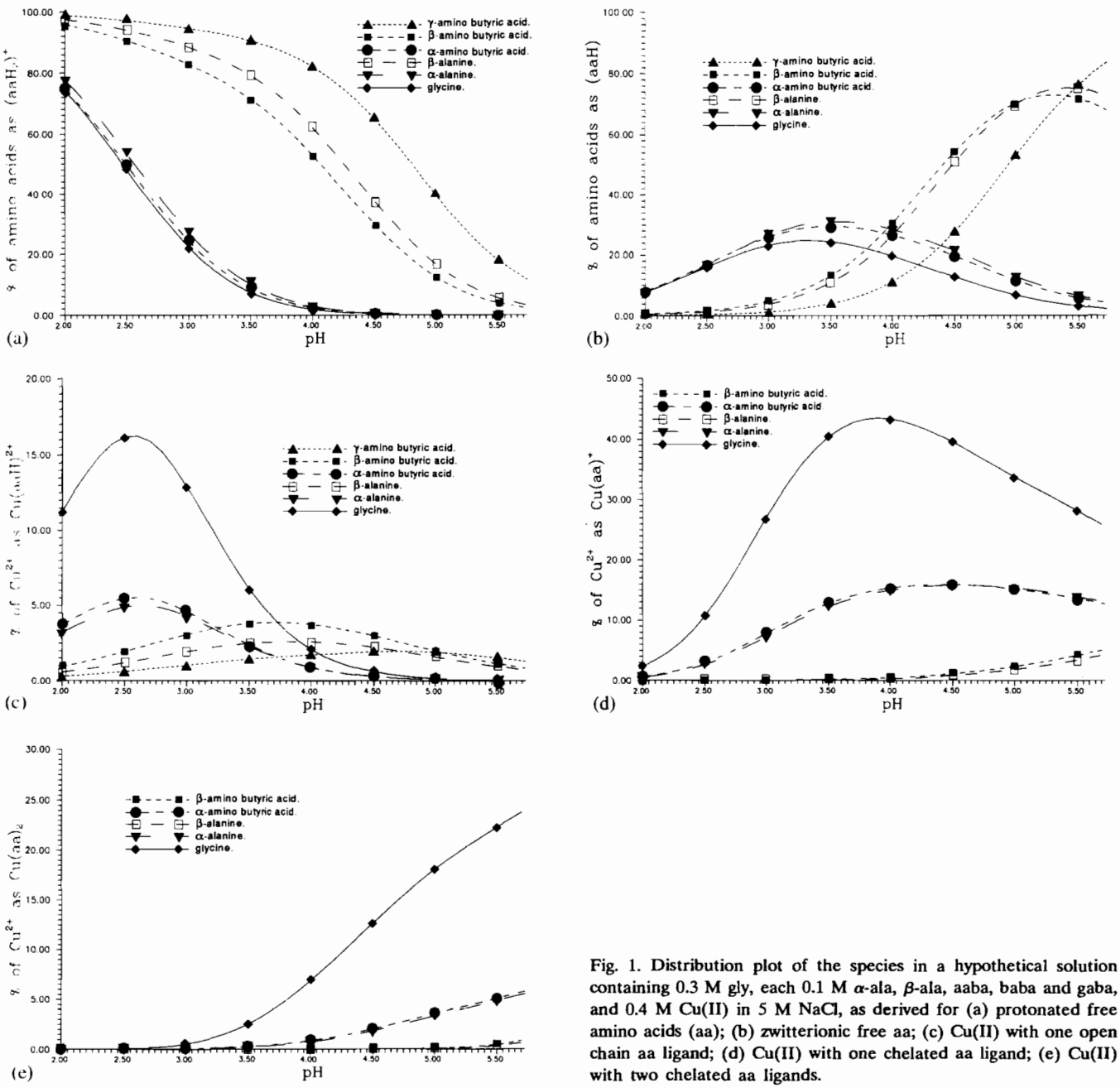

Fig. 1. Distribution plot of the species in a hypothetical solution containing 0.3 M gly, each 0.1 M α-ala, β-ala, aaba, baba and gaba, and 0.4 M Cu(II) in 5 M NaCl, as derived for (a) protonated free amino acids (aa); (b) zwitterionic free aa; (c) Cu(II) with one open chain aa ligand; (d) Cu(II) with one chelated aa ligand; (e) Cu(II) with two chelated aa ligands.

Table 4
Constant volume competition reaction for gly peptide formation with (a) aala and bala (0.49 M gly, 0.24 M aala, 0.24 M bala and 0.49 M Cu^{2+} in 4.9 M NaCl solution) and (b) aaba and baba (0.27 M gly, 0.27 M aaba, 0.27 M baba and 0.4 M Cu^{2+} in 5.0 M NaCl solution)

Reaction time (days)	$(gly)_2$	gly–bala	gly–aala	aala–gly	
(a)					
1	0.28	nf	0.12	0.04	
2	0.44	nf	0.13	0.15	
4	0.68	0.03	0.15	0.26	
7	0.90	0.06	0.17	0.36	
11	0.95	0.13	0.20	0.39	
22	1.05	0.28	0.18	0.43	
	$(gly)_2$	gly–baba	gly–aaba	baba–gly	aaba–gly
(b)					
1	0.44	0.34	0.24	0.18	0.12
2	0.78	0.40	0.31	0.18	0.29
3	0.92	0.45	0.41	0.20	0.44
4	1.09	0.55	0.44	0.20	0.59
6	1.22	0.75	0.49	0.25	0.82
8	1.04	0.94	0.54	0.27	0.96
10	0.93	1.01	0.49	0.31	0.97
14	0.59	0.97	0.40	0.39	0.88
18	nf	0.77	0.18	0.45	0.50
24	nf	0.39	0.04	0.50	0.20

afore-mentioned sequence inversion process is responsible for the domination of aala–gly after longer reaction times. Similar observations are made, when aaba and baba are present simultaneously with glycine (see Table 4); also in this system, a preference can be seen for aaba over baba as partner for peptide formation with glycine, and aaba–gly is the preferred sequence. In such competitions, the favourable complex formation constants of the α-amino acids seem to play a decisive role for the choice of the 'relevant' amino acid. Only after long reaction times, do baba peptides seem to prevail as they resist hydrolysis and oxidation processes better than their aaba analogues and also diglycine.

This preference for the biologically 'advantageous' amino acids seems to be a particular and most significant feature of the copper-induced peptide formation reaction and thus gives further credibility to its prebiotic relevance in chemical evolution of natural peptides.

3.2.2. *Evaporation experiments*

Tables 5, 6 and 7 display the results for the evaporation experiments, performed with the aminobutyric acids. The general features of their dimerization are similar to the systems with constant volume, but the reaction occurs faster and gives better yields. Under these conditions $(aaba)_2$ formation is also observed, the yield of which is still increasing after 1 week, whereas the gaba

Table 5
Evaporation experiments for pure aba systems

	Reaction cycle						
	1	2	3	4	5	6	7
$(aaba)_2$[a]	0.04	0.09	0.17	0.26	0.45	0.48	0.62
$(gaba)_2$[b]	0.71	1.86	1.23	1.23	1.27	1.92	1.41

[a] 0.08 M aaba and 0.04 M Cu^{2+} in 0.5 M NaCl solution.
[b] 0.08 M gaba and 0.04 M Cu^{2+} in0.5 M NaCl solution.

dimer concentration is already decreasing. When glycine is also present, the $(aaba)_2$ yield is even better than that of $(gaba)_2$ from the beginning. Again, baba does not form detectable amounts of a dimer.

When glycine is present (see Table 6), the total peptide yields are similar or better than those under constant volume conditions, if compared after 7 days. Also in this case a higher stability of the α-peptides after a longer reaction time can be recognized; the slight decrease of gly–aba observed in the final cycle of aaba and baba mixtures could be attributed to a sequence inversion process (probably via cyclic anhydrides) leading to aba–gly, similar to the gly–aala/aala–gly conversion [15].

The preferred formation of the aaba–gly peptide due to sequence inversion parallels the observations in the constant-volume experiments: the amount of gly–aaba seems to remain rather constant after 4 cycles, whereas the initially almost equivalent amount of aaba–gly increases continuously. However, while for both baba and gaba the gly–aba peptides had dominated at constant reaction volume, in the evaporation experiments gaba–gly is formed in higher quantities. This variable sequence preference, which could also play a role for further prolongations of the peptide chain, indicates a strong sensitivity of preferred peptide sequences to environmental conditions.

In the competition experiments (see Table 7), the aaba/gly peptides are produced in larger amounts from the second cycle on, and only at the seventh cycle does the amount of the baba/gly peptides become identical. This behaviour confirms the postulated preference for α-amino acids within the important initial phase of the reaction. An excess of glycine should improve this trend due to less $(aaba)_2$ formation, and also by preventing some of the hydrolysis/oxidation processes of the peptides occurring. The total yields of aaba and baba peptides are illustrated in Fig. 2, demonstrating the favoured use of the α-amino acid for peptide formation. An investigation of such 'competition situations' with varying amino acid concentrations seems to be worthwhile and will be the subject of future studies.

Table 6
Evaporation experiments for mixed aba/gly systems

Reaction cycle	0.04 M gly, 0.04 M aaba and 0.04 M Cu^{2+} in 0.5 M NaCl solution				0.04 M gly, 0.04 M baba and 0.04 M Cu^{2+} in 0.5 M NaCl solution			0.04 M gly, 0.04 M gaba and 0.04 M Cu^{2+} in 0.5 M NaCl solution			
	$(gly)_2$	$(aaba)_2$	gly–aaba	aaba–gly	$(gly)_2$	gly–baba	baba–gly	$(gly)_2$	$(gaba)_2$	gly–gaba	gaba–gly
1	0.22	0.23	0.07	0.10	0.36	0.17	0.27	1.06	0.04	nf	0.40
2	1.25	0.36	0.28	0.32	1.89	1.14	0.54	2.49	0.01	0.16	0.40
3	1.80	0.45	0.36	0.60	2.50	1.77	0.64	3.06	0.03	0.18	0.68
4	2.41	0.62	0.55	0.82	3.21	2.06	0.70	3.56	0.18	0.19	0.76
5	2.48	0.64	0.58	1.04	2.08	1.90	0.91	4.11	0.13	0.38	0.79
6	2.66	0.73	0.71	1.24	1.59	1.88	1.08	3.51	0.27	0.48	0.84
7	2.90	0.93	0.60	1.43	0.69	1.42	1.21	3.13	0.18	0.55	0.88

Table 7
Competition reaction (evaporation experiment) for gly peptide formation with aaba and baba: 0.027 M gly, 0.027 M aaba, 0.027 M baba and 0.04 M Cu^{2+} in 0.5 M NaCl solution

Reaction cycle	$(gly)_2$	gly–baba	gly–aaba	baba–gly	aaba–gly	$(aaba)_2$
1	0.98	0.42	0.49	0.19	0.03	0.02
2	1.88	0.54	0.63	0.23	0.17	0.03
3	2.16	0.53	0.63	0.24	0.36	0.06
4	2.38	0.66	0.71	0.28	0.50	0.11
5	2.38	0.74	0.78	0.42	0.57	0.16
6	2.38	0.86	0.77	0.45	0.66	0.17
7	2.12	0.91	0.71	0.49	0.67	0.16

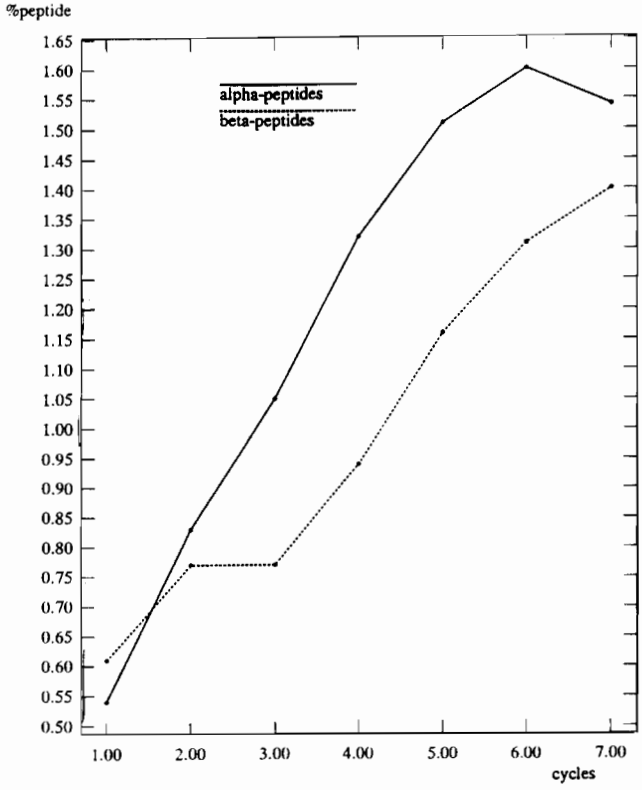

Fig. 2. Total concentrations of α- and β-aminobutyric acid containing peptides (in % of initial amino acid concentration) during the cycles of evaporation experiments.

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