GLYCINE AND DIGLYCINE AS POSSIBLE CATALYTIC FACTORS IN THE PREBIOTIC EVOLUTION OF PEPTIDES

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Abstract. Mutual catalytic effects within the Salt-Induced Peptide Formation (SIPF) Reaction might be one little puzzle piece in the complicated process of the formation of complex peptidic systems and their chemical evolution on the prebiotic earth. The catalytic effects of glycine and diglycine on the formation of dipeptides from mixed amino acid systems in the SIPF Reaction was investigated for systems with leucine, proline, valine and aspartic acid and showed to result in a significant increase of the yield of the majority of the produced dipeptides. The results of the experiments strongly confirm previous theories on the catalytic mechanism and show the ability of the SIPF Reaction to produce a very diverse set of peptide products with relevance to the formation of a biosphere.

Keywords: amino acids, chemical evolution, copper complexes, mutual catalysis, origin of life, prebiotic peptide formation, Salt-Induced Peptide Formation Reaction

1. Introduction

Under the proposed conditions of the primordial earth the Salt-Induced Peptide Formation (SIPF) Reaction (Schwendinger and Rode, 1989b) is one of the most facile reaction pathways to explain the formation of the first peptides eventually leading to the emergence of the first life on earth. It has been discovered in the late 80s and since then has been investigated in many aspects. It works with every amino acid investigated so far and produces substantial peptide yields under a wide range of conditions. The major reactants needed are Cu(II) and NaCl which, according to geochemical data (Cloud, 1973; Levine *et al.*, 1982; Carver, 1981; Ochiai, 1978), have been present in abundance in the prebiotic scenario. Sodium chloride at concentrations above 3 M has an unsaturated first hydration shell (Limtrakul and Rode, 1985; Limtrakul *et al.*, 1985; Schwendinger and Rode, 1989a) and works as a very mild condensation agent to enable peptide bond formation in aqueous solution, and Cu(II) complexes amino acids and/or peptides, activates them and lowers the kinetic barrier for the reaction (Oie *et al.*, 1982, 1983, 1984; Schwendinger and Rode, 1989b, 1992). There are two modes of carrying out the SIPF Reaction. One type is the constant volume experiment that starts from high concentrations of Cu(II) ($\sim$0.5 M) and NaCl ($\sim$5 M) and simulates conditions in primitive hot salt lakes and lagunas with larger volumes, where the essential chemicals were

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accumulated. The other type is the evaporation cycle experiment, starting from 10 times lower concentrations, that mimicks a coastal region scenario with tidal pools, small lagunas and puddles that undergo wetting and drying cycles through the influence of sun, rain and tidal changes, constantly increasing and decreasing the concentration of the reactants. The latter form of experiment leads to peptide yields of up to 7% in the case of glycine and to lower values for other amino acids. As shown recently (Suwannachot and Rode, 1998, 1999) glycine, histidine, phenylalanine and diketopiperazine (cyc-Gly_2) have a catalytic effect on the formation of dialanine, divaline, dileucine and dilysine and raise the yields by a factor of up to 50 and enable dipeptide formation at much lower amino acid concentrations. The scope of the work presented in this article was to extend these experiments to mixed systems of two different amino acids out of valine, leucine, proline and aspartic acid under the catalytic influence of glycine or diglycine, the presumably most abundant of the aforementioned catalytically active amino acids and peptides in the prebiotic scenario, investigating the yield increase of not only the homodipeptides but also the heterodipeptides.

In the SIPF Reaction without catalyst two amino acid molecules coordinate to the Cu(II) center, one of them chelates via its amino nitrogen and one of the carboxylate oxygens, the other one only coordinates end-on via one of its carboxylate oxygens because of a chloride that can always be found coordinated to the Cu(II) at the described concentrations and at the pH of the solution as previously shown in theoretical calculations (Liedl and Rode, 1992) and experimentally (Tauler and Rode, 1990) with spectrophotometric and potentiometric titrations. Through this spatially close arrangement of the reaction partners the more nucleophilic amino acid attacks the carbonyl carbon of the other one in the next step and forms the peptide bond. Now the peptide, which is bound to the central atom weaker than either of the two amino acids before, can either decoordinate and the reaction can start again or it can stay end-on coordinated to the Cu(II) ion to form a longer peptide upon addition of another amino acid (see Figure 1).

The results of the work described in this article strongly support the proposed catalytic mechanism (Suwannachot and Rode, 1998, 1999) which suggests the initial formation of a peptide containing the catalyst and then, with another amino acid, the formation of a longer peptide which would hydrolyze either while still being coordinated to the copper or shortly after decoordination to yield the product (in the investigated experiments a homo- or heterodipeptide) and return the catalyst to the reaction system (a small portion of the catalyst can be incorporated in peptides).

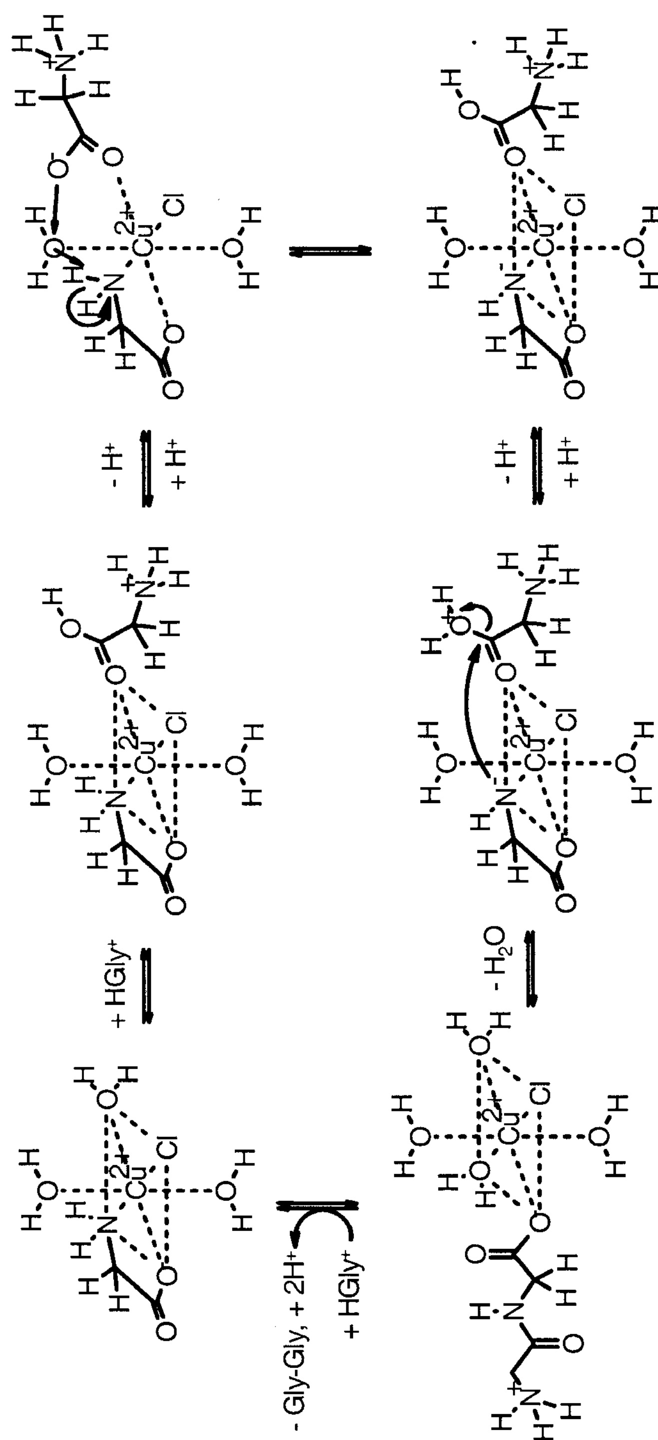


Figure 1. Postulated reaction mechanism scheme for the Salt-induced Peptide Formation Reaction (G + G → GG) (Eder and Rode, 1994).

2. Experimental

2.1. Materials

Amino acids and peptides (purity >99%) were obtained from Bachem AG, Bubendorf, Switzerland (in all cases pure L-isomers were used). NaCl (p.a., >99.5%) and $CuCl_2 \cdot 2H_2O$ (p.a., >99%) were purchased from Merck, Darmstadt, Germany. Na-n-hexylsulfonate was acquired from Aldrich, acetonitrile (for HPLC, gradient grade, >99.9%), KH_2PO_4 (puriss. p.a. for HPLC, $\geqslant$99.5%), phosphoric acid (puriss. p.a. ACS, $\geqslant$85%) and hydrochloric acid (puriss. p.a., $\geqslant$32%) were bought from Fluka Chemie AG, Buchs, Switzerland. Ultrapure water was made in-house from deionized water. All chemicals were used without further purification.

2.2. Evaporation cycle experiments

To prepare the samples the amino acids and peptides were dissolved in ultrapure water containing 40 mM $CuCl_2$ and 0.5 M NaCl and when needed for dissolution one or more drops of HCl conc./50 mL. The samples were composed of two of four amino acids (aspartic acid, Asp, D; proline, Pro, P; leucine, Leu, L; valine, Val, V) each in 40 mM concentration; aspartic acid/proline, aspartic acid/leucine, leucine/proline, valine/aspartic acid, valine/proline and valine/leucine with either glycine or diglycine added as a catalyst in a concentration of 5, 10 or 20 mM and also without catalyst as control samples. For the experiment 1.0 mL of sample was filled into a 2 mL glass HPLC vial which was then put into a heating box at 85 °C for 24 hr leading to evaporation of all the water. For the next evaporation cycle the residue was filled up to the same volume again with ultrapure water and was subjected to the same conditions as in the first cycle. Samples were kept for analysis after the 1st, 4th and 7th day (= cycle) and also before the first cycle as a control.

2.3. Analysis of the products

The samples were analyzed by reversed phase ion pairing HPLC on an Agilent 1100 series HPLC system with diode array detection, identifying and quantifying the peptide products by comparison of retention times, UV-Vis spectra and response factors to pure reference substances in several concentrations. The analytical column used was an Agilent Hypersil ODS 5 μm/200 × 2.1 mm with a precolumn of the same type of dimensions 20 × 2.1 mm. The mobile phase consisted of two solvents. Solvent A contained 50 mM KH_2PO_4 and 7.2 mM $C_6H_{13}SO_3Na$ (as ion pairing agent) in ultrapure water, adjusted to pH 2.3 with phosphoric acid and filtered through a hydrophilized 0.22 μm polyvinylidene fluoride (PVDF) membrane obtained from Millipore. Solvent B was acetonitrile of gradient grade for HPLC. The gradient was developed to apply to all of the investigated systems (see Figure 2) and can be seen in Table I.

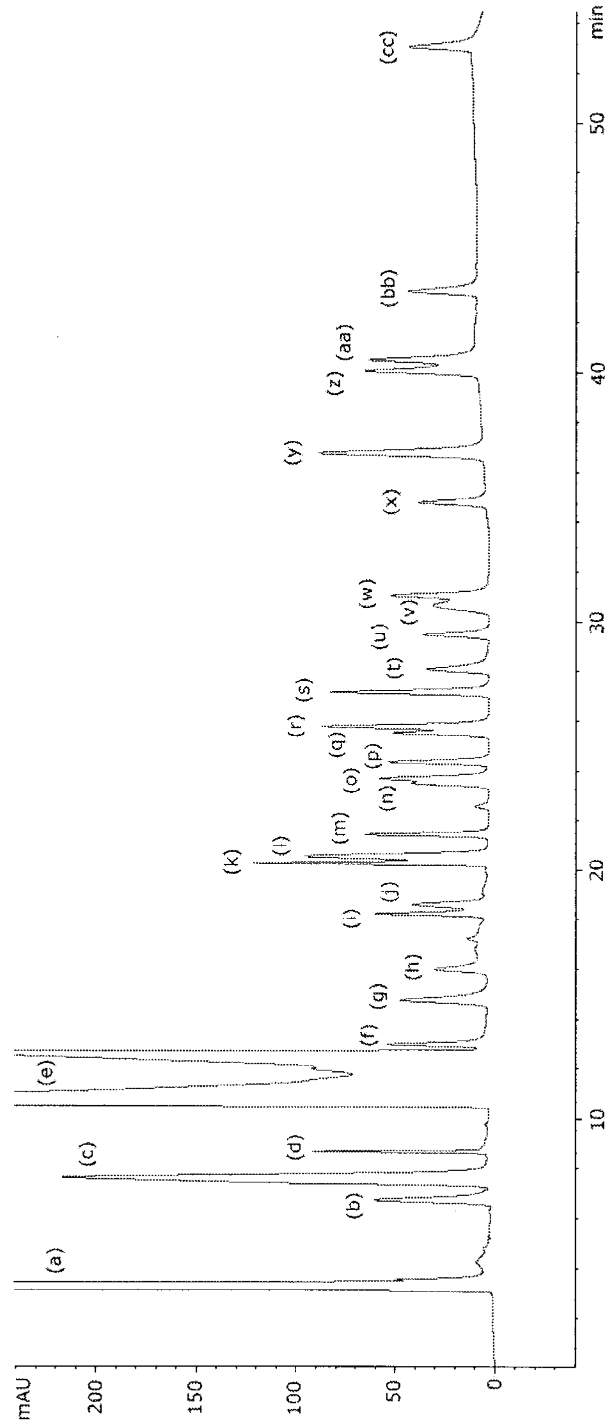


Figure 2. Elution profile of a mixture of standard substances of the observed peptides with the above described gradient method. (a) injection peak, (b) GG, (c) DD, (d) GGGG, (e) Cu-species, (f) PD, (g) PGG, (h) PG, (i) VD, (j) GP, (k) VGG, (l) GGP, (m) VG, (n) PP, (o) DV, (p) LD, (q) GV, (r) GGV, (s) LGG, (t) PV, (u) LG, (v) VP, (w) VV, (x) DL, (y) LV, (z) PL, (aa) LP, (bb) VL, (cc) LL.

TABLE I

Gradient profile (solvents and flow) for the HPLC analysis of the products

Time (min)	Solvent A (%)	Solvent B (%)	Flow (mL min^{-1})
0	100	0	0.15
6	100	0	0.15
10	100	0	
12	97	3	0.35
48	85	15	
55	100	0	0.35
60	100	0	0.15

TABLE II

Yield (%) of the peptide A–B without catalyst (w/o), in the presence of 10 mM glycine (G) and in the presence of 10 mM diglycine (GG) after 7 days. 0.0% yield refers to a value below the minimum detectable amount of on average 0.012% (0.008–0.015%)

	A ↓ B→											
	Asp			Val			Leu			Pro		
	w/o	G	GG	w/o	G	GG	w/o	G	GG	w/o	G	GG
Asp	0.1[a]	0.4[a]	0.5[a]	0.2	0.9	1.0	0.0	0.1	0.1	–[b]	–[b]	–[b]
Val	0.1	0.1	0.1	0.1[a]	0.5[a]	0.2[a]	0.0	0.1	0.2	0.0	0.2	0.2
Leu	0.0	0.1	0.1	0.0	0.0	0.1	0.0[a]	0.1[a]	0.1[a]	0.1	0.1	0.8
Pro	0.1	0.6	0.6	0.0	0.7	1.0	0.0	0.5	0.7	0.2[a]	1.0[a]	1.2[a]

[a] Mean value from three sample systems yielding the same dipeptide.
[b] No standard substance was available.

The column temperature was set to 35 °C, the chromatograms were recorded at a wavelength of 200 nm with 4 nm bandwith, the reference wavelength was set to 550 nm with 100 nm bandwidth. Spectra from 190 to 950 nm in 2 nm steps were recorded continously during the runs. The injection volume was 2 μL of the filtered sample (0.2 μm Pall Gelman GHP Acrodisc hydrophilized polypropylene syringe filter) with a needle wash with ultrapure water before the injections to avoid carry-over and the samples were kept at 4 °C in the autosampler during the analysis.

TABLE III

Increase in yield of the peptide A–B in presence of 10 mM glycine (G) and in presence of 10 mM diglycine (GG) after 7 days

A ↓ B→	Asp		Val		Leu		Pro	
	G	GG	G	GG	G	GG	G	GG
Asp	4[a]	5[a]	5	5	8	8	–[b]	–[b]
Val	0	0	5[a]	2[a]	8	17	17	17
Leu	8	8	0	8	8[a]	8[a]	0	8
Pro	6	6	58	83	42	58	5[a]	6[a]

[a] Mean value from three sample systems yielding the same dipeptide.
[b] No standard substance was available.

3. Results and Discussion

All samples (without catalyst, with 5, 10, 20 mM catalyst; after the 0th, 1st, 4th and 7th day) were analyzed and evaluated but for a comparison of all the reaction systems (see Tables II and III) the 7th day samples with a concentration of 10 mM catalyst were chosen since at those conditions the catalytic effects are observed best for the majority of the systems.

3.1. Reaction systems

3.1.1. *Aspartic Acid and Proline*

The expected distribution of the yields (Eder and Rode, 1994) of the monitored products in this system, being Pro-Pro > Asp-Asp > Pro-Asp, deducted from their polarity and nucleophilicity and also as observed without catalyst, is changed to a preference of Asp-Asp > Pro-Asp > Pro-Pro. All yields are increased but in this system especially the one of Asp-Asp, which is on average increased more by diglycine (12 times with 5 mM Gly_2 as opposed to 5 times with 10 mM Gly after the 7th reaction day), in accordance with the proposed mechanism of catalysis in previous publications (Suwannachot and Rode, 1998, 1999).

3.1.2. *Aspartic Acid and Leucine*

In this system the distribution of the yields without catalyst (Asp-Asp > Asp-Leu > Leu-Asp > Leu-Leu) is determined again generally by the polarity and nucleophilicity of the amino acids. Since leucine is quite big and its sidechain is apolar its mobility is low and it does not react as well as expected from its nucleophilicity, and hence without catalyst after 7 reaction cycles the concentration of Leu-Leu was too small to be detected. In the presence of Gly or Gly_2 especially the yield of Leu-Asp, which is normally less favoured than Asp-Leu, was increased by a factor of up

to 14. The yield of Asp-Leu was increased more by Gly_2 which can be explained by the proposed catalytic mechanism (Suwannachot and Rode, 1998; Suwannachot and Rode, 1999). Leu-Leu which could not be detected without catalyst was formed in yields of around 0.2% upon use of either Gly or Gly_2 after the 7th reaction cycle.

3.1.3. *Leucine and Proline*

As the amino acids in this system are quite hydrophobic the dipeptide yields without catalyst are very low or even not detectable. The yield of Pro-Pro is higher than that of the other dipeptides (Pro-Pro > Leu-Pro > Pro-Leu, Leu-Leu) as could be predicted by its better mobility due to steric reasons. Leucine does not react well without catalyst and Pro-Leu and Leu-Leu could not be detected. Both investigated catalysts are very efficient in activating the amino acids in this system and lead to considerable yields (0.5%/0.7% of Pro-Leu with 10 mM Gly/Gly_2 after 7 days and 0.1% of Leu-Leu with 10 mM of either catalyst after 7 days), also for the products not detectable in the experiment without catalyst. The yield of Pro-Pro is increased by a factor of around 5 by glycine in all investigated concentrations and up to a factor of 11 by diglycine (5 mM Gly_2 after 7 reaction cycles). The yield of Leu-Pro is increased by a factor of 2 to 15 by either Gly or Gly_2 after 7 reaction cycles.

3.1.4. *Valine and Aspartic Acid*

The polarity and the order of nucleophilicity of the two amino acids leads to the following order of the yields of the analyzed products in the system without catalyst: Asp-Val > Val-Asp > Asp-Asp > Val-Val. Since Asp is the more reactive amino acid in this system the yield of Asp-Val is increased more (by a factor of 3 by 10 mM Gly after the 7th day and by a factor of 6 by 10 mM Gly_2 after the 7th day) than the yield of Val-Asp (which stays approximately the same with Gly and is increased by a factor of 3 by 20 mM Gly_2 after 7 days). The yield of Asp-Asp is increased by both Gly (10 mM lead to an increase by a factor of 3 after 7 days) and Gly_2 (5 mM result in an increase factor of 7 after 7 days). Most remarkable is the increase in yield of Val-Val, the product with the smallest yield in the system without catalyst, which amounts to an increase factor of 4 with 10 mM Gly and a factor of 8 with 10 mM Gly_2 after the 7th reaction cycle.

3.1.5. *Valine and Proline*

Without catalyst the yields of the homodipeptides are approximately the same (0.7% after 7 days) and the yields of the heterodipeptides are below the detection limit. With the use of either catalyst the yields of all monitored products are greatly increased, Pro-Val and Val-Pro can now be found with 0.7%/1.0% and 0.2%/0.2% (with 10 mM Gly/Gly_2 after 7 reaction days). The yield of Pro-Pro is increased by a major factor (28 with 20 mM Gly and 26 with 10 mM Gly_2 after 7 reaction cycles). Also the yield of Val-Val is increased considerably especially by glycine (by a factor of 16 with 10 mM Gly and by a factor of 4 with 5 mM Gly_2 after the 7th cycle).

3.1.6. *Valine and Leucine*

With this combination of hydrophobic amino acids, that are both quite unreactive, the size of leucine and hence its low mobility are determining factors for the distribution of yields without catalyst which is, as expected, Leu-Val > Val-Val > Val-Leu > Leu-Leu, Leu-Leu not being formed in detectable yields. With catalyst the yield of Leu-Leu is raised to a detectable amount (0.3%/0.7% with 10 mM Gly/Gly_2). In general Gly_2 turns out to be the better catalyst for this system as it (20 mM Gly_2 after 7 reaction cycles) raises the yield of Val-Val by a factor of 3, the one of Leu-Val by a factor of 5 and the yield of Val-Leu by a factor of 6, whereas Gly (after 7 days of reaction) raises them only by factors of 2 for Val-Val (with 10 mM Gly) and Leu-Val (with 5 mM Gly), and 4 for Val-Leu (with 10 mM Gly).

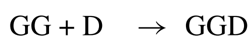
3.2. SUMMARY

In the investigated systems diglycine is generally the more effective catalyst than glycine. It leads to the same or a higher increase than glycine in the yields of homo- and heterodipeptides in most cases, in a few cases (Leu-Val, Leu-Pro) only diglycine leads to a yield increase. The formation of Val-Asp is not catalyzed by either glycine or diglycine and in the formation of Val-Val glycine shows a better catalytic efficiency than diglycine. The formation of homodipeptides is always promoted well but the formation of heterodipeptides, especially the ones containing leucine, that are hardly formed in detectable amounts without catalyst, is catalyzed considerably better. All peptides that are formed only in sub-detectable amounts without catalyst, which are in most cases heterodipeptides, can be synthesized with the use of one of the investigated catalysts. Of the peptides being more readily formed without catalyst, dipeptides containing proline or aspartic acid are the ones most remarkably promoted by catalysis.

4. Conclusions

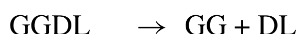
4.1. POSSIBLE CATALYTIC PATHWAY

For the example of aspartic acid and leucine with diglycine as a catalyst, the catalytic formation of Asp-Leu would possibly work as follows:



$$\mathrm{GG + D \rightarrow GGD}$$

$$\mathrm{GGD + L \rightarrow GGDL}$$



$$\mathrm{GGDL \rightarrow GG + DL}$$

Since the peptide always functions as the electrophile in the reaction due to its lower complex formation constant (Tauler and Rode, 1990) and thus its end-on

coordination and polarization of the carboxyl group in the copper complex (see Introduction), the amino acid (chelated to the copper and with an increased electron density at the nitrogen) connects to the C-terminus of the peptide (see Figure 1). Further sequence preferences are determined by the polarity, the size, the hydrophobicity and the nucleophilicity and electrophilicity of the amino acids. The hydrophobicities of the amino acids can be determined by their non-polar surface areas which are in the order of Leu (164 Å^2) > Val (135 Å^2) > Pro (124 Å^2) > Gly (47 Å^2) > Asp (45 Å^2) (Karplus, 1997) and the nucleophilicity/electrophilicity can be shown by the pK values of their amino and carboxyl group. In a combination A–B, B will preferably be the more nucleophilic partner (Suwannachot and Rode, 1998) if not other factors like in the case of leucine, which is quite hydrophobic and voluminous, play a bigger role. Polar, hydrophilic amino acids react better, and also smaller amino acids show better reactivity because of their increased mobility and because of steric reasons in the polar Cu–Cl–Na network (Schwendinger and Rode, 1992). The order of the yields in the investigated systems always conforms to the mechanistic ideas, the peptides that would be formed easier due to their aforementioned chemical characteristics show the highest yields and sequences that can only be formed via sequence inversion or chemically unfavourable reaction of the amino acids have lower yields.

In the system with valine and proline, for example, most standards were available in our investigations and the yields of the monitored sideproducts conform with the aforementioned mechanistic theories. After a rather continous increase of the product yields, after the 7th reaction cycle (= day) with 10 mM diglycine as catalyst the yield of Pro-Val is higher than the yield of Val-Pro (see Table II). The catalytic mechanism is supported by the yields of the observed intermediates (GGP more than double the amount of GGV) and moreover the sideproducts PGG and VGG are found in lower yields than the intermediate products. The products GP and GV that can be formed either by hydrolysis of the intermediate products between the two glycine or by hydrolysis of the diglycine and subsequent reaction with either proline or valine can also form the observed heterodipeptides PV and VP and as in the case of GGP and GGV the one leading to the higher yield of product (GP → PV) was found in higher yield.

4.2. Consequences for Chemical Evolution

Glycine can be assumed to have been one of the most abundant amino acids in the prebiotic scenario due to its simple structure and according to its comparatively high yield obtained in several Miller-type experiments (Miller *et al.*, 1976). Diglycine can be formed easily and in high yields (up to 10%) from glycine in the Salt-Induced Peptide Formation Reaction. Upon action of the two, the SIPF reaction yields an even more diverse set of products and increases the possibility for the formation of a big pool of peptides that could have undergone further chemical evolution, eventually leading to to the precursors of today's proteins.

Acknowledgement

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References

Carver, J. H.: 1981, Prebiotic Atmospheric Oxygen Levels, *Nature* **292**, 136–138.

Cloud, P.: 1973, Paleoecological Significance of the Banded Iron Formation, *Econ. Geol.* **68**, 1135–1143.

Eder, A. H. and Rode, B. M.: 1994, Influence of Alkali- and Alkaline-earth-metal Cations on the 'Salt-induced Peptide Formation' Reaction, *J. Chem. Soc. Dalton Trans.* **7**, 1125–1130.

Karplus, P. A.: 1997, Hydrophobicity Regained, *Protein Sci.* **6**, 1302–1307.

Levine, J. S., Augustsson, T. R. and Natarajan, M.: 1982, The Pre-biological Paleoatmosphere: Stability and Composition, *Origins Life* **12**, 245–259.

Liedl, K. R. and Rode, B. M.: 1992, *Ab Initio* Calculations Concerning the Reaction Mechanism of the Copper(II) Catalyzed Glycine Condensation in Aqueous Sodium Chloride Solution, *Chem. Phys. Lett.* **197**, 181–186.

Limtrakul, J. P., Fujiwara, S. and Rode, B. M.: 1985, A Quantum Chemical Analysis of the Structural Entities in Aqueous Sodium Chloride Solution and their Concentration Dependence, *Anal. Sci.* **1**, 29–32.

Limtrakul, J. P. and Rode, B. M.: 1985, Solvent Structures around Sodium and Chloride Ions in Water, *Mh. Chem.* **116**, 1377–1384.

Miller, S. L., Urey, H. C. and Oro, J.: 1976, Origin of Organic Compounds on the Primitive Earth and in Meteorites, *J. Mol. Evol.* **9**, 59–72.

Ochiai, E.: 1978, The Evolution of the Environment and its Influence on the Evolution of Life, *Origins Life* **9**, 81–91.

Oie, T., Loew, G. H., Burt, S. K., Binkley, J. S. and MacElroy, R. D.: 1982, Quantum Chemical Studies of a Model for Peptide Bond Formation: Formation of Formamide and Water from Ammonia and Formic Acid, *J. Am. Chem. Soc.* **104**, 6169–6174.

Oie, T., Loew, G. H., Burt, S. K. and MacElroy, R. D.: 1983, Quantum Chemical Studies of a Model for Peptide Bond Formation. 2. Role of Amine Catalyst in Formation of Formamide and Water from Ammonia and Formic Acid, *J. Am. Chem. Soc.* **105**, 2221–2227.

Oie, T., Loew, G. H., Burt, S. K. and MacElroy, R. D.: 1984, Quantum Chemical Studies of a Model for Peptide Bond Formation. 3. Role of Magnesium Cation in Formation of Amide and Water from Ammonia and Glycine, *J. Am. Chem. Soc.* **106**, 8007–8013.

Schwendinger, M. and Rode, B. M.: 1989a, Monte Carlo Simulation of a Supersaturated Sodium Chloride Solution, *Chem. Phys. Lett.* **155**, 527–532.

Schwendinger, M. G. and Rode, B. M.: 1989b, Possible Role of Copper and Sodium Chloride in Prebiotic Evolution of Peptides, *Anal. Sci.* **5**, 411–414.

Schwendinger, M. G. and Rode, B. M.: 1992, Investigations on the Mechanism of the Salt-Induced Peptide Formation Reaction, *Origins Life Evol. Biosphere* **22**, 349–359.

Suwannachot, Y. and Rode, B. M.: 1998, Catalysis of Dialanine Formation by Glycine in the Salt-Induced Peptide Formation Reaction, *Origins Life Evol. Biosphere* **28**, 79–90.

Suwannachot, Y. and Rode, B. M.: 1999, Mutual Amino Acid Catalysis in Salt Induced Peptide Formation Supports this Mechanism's Role in Prebiotic Peptide Evolution, *Origins Life Evol. Biosphere* **29**, 463–471.

Tauler, R. and Rode, B. M.: 1990, Reactions of Cu(II) with Glycine and Glycylglycine in Aqueous Solution at High Concentrations of Sodium Chloride, *Inorg. Chim. Acta* **173**, 93–98.