Peptide bond formation on the surface of activated alumina: peptide chain elongation

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Reactions of the dipeptides (Gly_2, Ala_2) themselves and in combination with amino acids (AA) (glycine, alanine, valine, leucine, proline) and glycine oligopeptides (Gly_3, Gly_4, Gly_5) on activated alumina surface were investigated. Reactions of glycine oligopeptides led to the formation of longer-chain oligomers up to Gly_{11}. Combinations of Gly_2 with other amino acids led to various reactions proceeding by different reaction mechanisms. In the reactions of Gly_2 with AA, cyclic anhydride ($cyc(Gly_2)$) formation was followed by AA addition and molecular rearrangement leading to the formation of Gly-Gly-AA tripeptides. In the reaction of Ala_2 with glycine, this type of reaction apparently does not proceed readily, although very high yields of $cyc(Ala_2)$ are formed. The reactivities of the individual components are not always reflected in reactions of their mixtures.

KEY WORDS: peptide bond formation; activated alumina; amino acids; chemical evolution.

1. Introduction

Silica, aluminum compounds and related minerals were probably among the major components of the primitive earth lithosphere. Some aluminosilicates and activated aluminas catalyze a variety of the reactions of organic compounds [1]. The possible role of such compounds as catalysts in chemical evolution of first bioorganic compounds on the primitive earth has been proposed [2,3]. Catalytic efficiency of silica, alumina and clays for peptide bond formation has been proven experimentally. At elevated temperatures and under vacuum, oligomerization of various types of amino acids on silica or alumina was observed [4–6]. However, there are some questions remaining: whether such conditions could have been realized during the prebiotic era, and whether oligomerization at higher temperatures would not lead to the decomposition of reactants and reaction products [5,6]. On the other hand, our previous studies have indicated that the condensation reaction of amino acids takes place on the surface of activated alumina also under much milder temperatures, i.e., below 100 °C. The reaction led to peptide bond formation without previous amino acid activation and in the absence of any condensation agents [7,8]. Activated alumina is a highly efficient catalyst for amino acid dimerization [9,10]. Under mild conditions, it catalyzes the reactions of amino acids, which are generally nonreactive on surfaces of silica and clay minerals [11,12]. A reaction mechanism of alumina-catalyzed peptide bond formation was proposed, according to which the high catalytic efficiency of activated alumina most probably related to the presence of active Lewis acid and base pairs on the surface of alumina, which interact with and activate both the ammonium and carboxyl groups of amino acid zwitterions [11]. Moreover, activated alumina is, in principle, also acting similar to condensation agents: it absorbs water in a reactive way, which proceeds as a multiple-step reaction leading to distinct structural changes of alumina bulk [13]. This reaction of activated alumina with water is exothermal. The role of alumina as an energy source in amino acid dimerization has already been evaluated by a thermal analysis study [10].

The efficiency of clay surfaces for the elongation of oligopeptide chains has already been reported [14]. However, for alumina, only the reactions of amino acids have been studied in detail [9,10]. The objective of this work was to investigate the oligomerization of dipeptides and some glycine oligopeptides separately, and in combination with some other amino acids. These reactions of amino acids, dipeptides or short oligopeptides are a rather complex combination of various processes (amino acid dimerization, cyclic anhydride formation, peptide chain elongation, sequence inversion, hydrolysis, etc.). Some of these partial reactions proceed via various mechanisms [15]. This work should help to decide which of them occur preferentially, and how the reactivity of the reactants influences the formation of peptide bonds.

2. Experimental

2.1. Materials

Neutral pH activated alumina (type Brockmann I, 150 mesh) was purchased from Aldrich and used as a

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catalyst. Amino acids, their respective oligopeptides and cyclic anhydrides, and polyglycine were purchased from Sigma Co., Bachem or Senn Chemicals Co. in analytical grade purity. For experiments, only L-optical isomers of amino acids were used.

2.2. Reaction system

Gly_2, Gly_3, Gly_4, Gly_5, Gly_2 + Ala (Leu, Val, Pro) and Ala_2 + Gly were used as reaction systems. Activated alumina was mixed with 100 mM aqueous amino acid (AA) solution in order to get a ratio of 1 mmol g^{-1}. For mixtures of two reactants, the overall concentration was adjusted to 10 mM, i.e., 5 mM for each compound. The mixtures were dried at 85 °C for 3 h, homogenized and directly used for the investigation of peptide bond formation by heating the samples to 85 °C for 1–14 days. The reaction of Gly_5 had to be conducted differently because of the low solubility of this compound in water. Solid Gly_5 was mixed with alumina at the ratio of 1 mmol g^{-1} and a small amount of water was added. The suspension was then treated in the same way as for the other reaction systems. After the reactions were completed, the products were extracted into distilled water and analyzed. In previous studies, we have used 0.1 M calcium chloride solution to release oligopeptides from clays [11]. However, we found in some preliminary experiments that distilled water is more efficient in the extraction for the mixtures with alumina, namely for the leaching of long-chain glycine oligopeptides. Probably, the presence of calcium chloride decreases the solubility of longer-chain glycine oligopeptides. Unfortunately, owing to generally low solubilities of these compounds, we were not able to quantitatively determine all these reaction products from alumina. Therefore, the determined amounts, especially of long-chain oligoglycines, should be considered only as indicative and probably underestimated. The solubilities of glycine and its oligopeptides were determined from one aqueous solution saturated with all substances. The saturation was achieved by the equilibrium between the solution and the excess of solid substances of each component at room temperature. Saturated solution was filtered (0.2-μm pore size), appropriately diluted and the amounts of all components were determined by HPLC analysis.

2.3. Analytical methods

Solutions, supernatants above the solids, were filtrated (0.2 μm) and analyzed with an Agilent 1100 Series LC System using an Agilent Hypersil (ODS, 5 μm, 200 × 2.1 mm) column. The mobile phase was a solution of 10 mM sodium hexanesulphonate (HPLC grade), adjusted to pH 2.5 by H_3PO_4 and acetonitrile of HPLC grade. Injection volume was 2 μL. Mobile-phase flow rate was 0.55 mL min^{-1} and column temperature was 35 °C. The combinations of the solvents in isocratic and gradient methods for the analysis have been published elsewhere [9]. The conditions for the analysis of Gly_2-Gly_5 reaction systems were the same as those used for glycine reaction [9]. The gradient analysis methods were used for the reaction systems of Gly_2 + AA, with identical conditions as applied for Gly + AA in a recent study [9]. Mobile-phase composition for Ala_2 + Gly was the same as previously used for the Ala + Gly reaction system [9]. Detection was performed with a diode array detector at 195 and 200 nm. All peptides were identified by retention times of authentic reference substances and UV–vis spectra. Linear oligopeptides and cyclic anhydrides were characterized by the light absorption at 195 nm. Oligopeptides with a peptide bond formed by proline secondary amine group absorbed most at 200 nm. The concentrations of the reaction products were determined by calibration using the solutions of reference substances. Since the absorptivity of oligopeptides and cyclic anhydride of glycine increases linearly with the number of peptide bonds, the concentrations of higher glycine oligomers (Gly_7-Gly_{11}) also could be estimated, although we did not have the reference substances of these compounds. The identification of these higher glycine oligopeptides is described below. All reaction yields were measured as the percentage of reactant incorporated in the reaction product.

3. Results and discussion

3.1. Reactions of Gly_2 and glycine oligopeptides

Analysis of the supernatant extracted from the reaction system starting with Gly_2 led to the identification of several reaction products: Cyc(Gly_2), Gly, Gly_3, Gly_5 and Gly_6. Some additional peaks in the chromatography patterns could not be identified by reference substances. The peaks following that of Gly_6 should be those of higher glycine oligomers, some of these at lower retention times could be side products, e.g., cyclic compounds [16,17]. Identification of the reaction products with peaks detected at higher retention times was attempted by their comparison with the retention times of the reference substances of glycine and its short-chain oligopeptides. Figure 1 shows the dependence of the retention times of glycine and its oligopeptides on the number of amino acid units in the molecule (solid symbols). The trend of increasing retention time with the number of glycine units in molecules was used for the identification of unknown reaction products (open symbols). The identification of the unknown reaction products with longer retention times fits very well to an overall trend of the dependence shown in figure 1. Furthermore, we confirmed the identity of the longer-chain glycine oligopeptides by measuring the mixture of glycine oligomers prepared by hydrolysis of polyglycine in water at 90 °C.

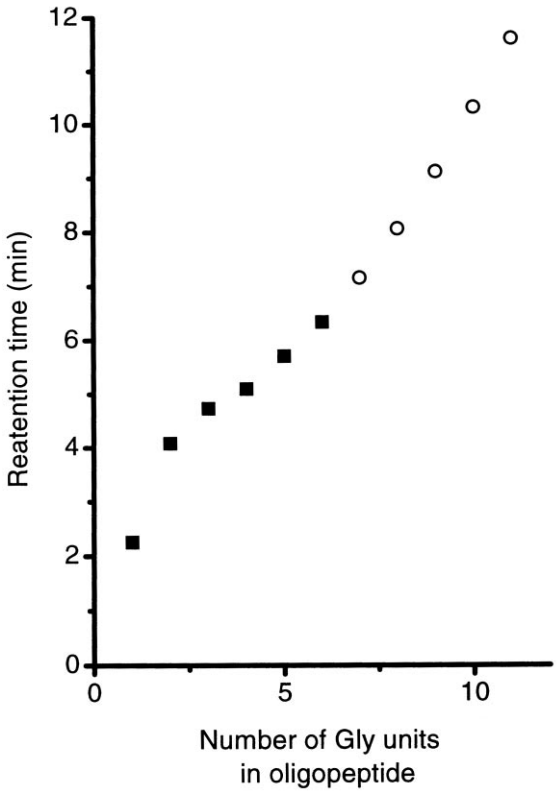

Figure 1. Relationship between retention times and the number of amino acid units in molecules of glycine and its dimer and oligomers. Peaks were assigned using the reference substances (solid symbols) or extrapolated (open symbols).

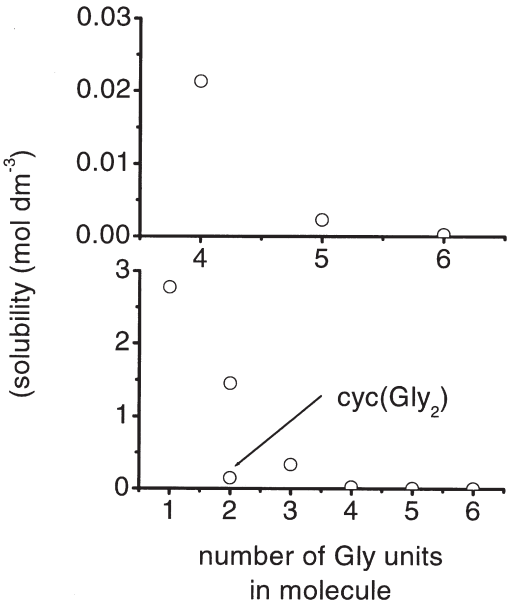

Figure 2. Solubilities of glycine, its dimer and oligopeptides in relation to the peptide size, expressed in number of amino acid units in the molecule.

For Gly_2 reaction system, we observed the formation of three reaction products eluted at longer retention times and identified according to figure 1 as Gly_7, Gly_8 and Gly_9 (table 1). Only low concentrations of these oligopeptides were found. Heating Gly_2 on alumina surface decreased its concentration to about 43% of the initial value after 14 days (table 1). The main reaction product of this reaction was the cyclic anhydride cyc(Gly_2) with the reaction yield exceeding 10%. Relatively high amounts of Gly_2 decomposed to Gly (7%). On the other hand, a series of longer oligopeptides was found. Gly_3 and Gly_4 were the main reaction products, each achieving a reaction yield of almost 4% after 14 days. Almost 1% and about 0.5% converted to Gly_5 and Gly_6 respectively. Only traces of the longer oligopeptides were found, not exceeding 0.1%. The sum of the reaction yields were significantly below 100% (table 1), which indicates that not all the reaction products and/or their amounts could be identified and determined. There could be some decomposition products of Gly_2, which were not identified. Another reason for the low total amount could be that due to the low solubilities of glycine oligomers with longer chains, not all of them could be dissolved. Consequently, the dissolved amounts of these compounds would be underestimated. One may also assume strong adsorption of the peptides on alumina surface. An almost irreversible adsorption of longer-chain peptides on clay surfaces has been reported and seems to support this assumption [18]. Figure 2 shows the dependence of glycine oligopeptide solubilities on the number of glycine units in the molecule. The upper part of the picture shows in detail the solubilities of Gly_4, Gly_5 and Gly_6, which decrease about 10 times with the increase of

Table 1
Alumina-catalyzed oligopeptide formation from Gly_2

Time (days)	Yield (%)										
	cyc(Gly_2)	Gly	Gly_2	Gly_3	Gly_4	Gly_5	Gly_6	Gly_7	Gly_8	Gly_9	Sum
1	2.27	–	76.10	0.47	0.47	0.17	–	–	–	–	79.48
3	5.68	2.45	67.78	1.06	1.33	0.52	0.03	–	–	–	78.85
5	9.94	4.64	54.56	2.56	3.33	0.62	0.25	0.03	0.03	–	75.96
7	9.89	5.03	54.19	2.55	3.17	0.58	0.24	0.03	0.02	–	75.7
10	9.91	6.06	49.44	3.12	3.57	0.72	0.33	0.05	0.04	0.01	73.25
14	10.82	6.92	42.62	3.93	3.96	0.82	0.47	0.09	0.06	0.01	69.7

Table 2
Alumina-catalyzed oligopeptide formation from glycine oligopeptides after 14 days

	Yield (%)												
	cyc(Gly_2)	Gly	Gly_2	Gly_3	Gly_4	Gly_5	Gly_6	Gly_7	Gly_8	Gly_9	Gly_{10}	Gly_{11}	Sum
Gly_3	2.56	3.86	11.97	60.07	2.26	1.73	1.10	0.18	0.10	0.04	–	–	83.87
Gly_4	0.52	1.94	2.78	1.87	69.30	0.62	0.29	0.14	0.08	0.06	0.02	–	77.62
Gly_5	0.04	0.60	0.56	0.31	0.35	14.45	0.08	0.02	0.01	0.01	0.06	0.06	16.55

the oligopeptide chain by one glycine unit, changing from 21 mM for Gly_4, to about 2.5 mM for Gly_5 and 0.23 mM for Gly_6. Taking into account this trend, one may assume much lower solubilities for the oligopeptides of longer chains such as Gly_7-Gly_9.

Table 2 summarizes the yields of the products of Gly_3, Gly_4 and Gly_5 reactions after the reaction time of 14 days. Although there were Gly, Gly_2 and cyc(gly_2) produced from Gly_3, the formation of longer-chain oligopeptides was also proven. More unreacted Gly_3 (about 60%) was found in supernatant after 14 days (table 2) in comparison to 43% unreacted Gly_2 under the same conditions (table 1). The sum of all identified compounds in Gly_3 reaction system (84%) is also significantly higher. It indicates lower reactivity of Gly_3 in comparison to Gly_2 including side-reactions, which led to nonidentified products, even a larger amount of unreacted compound was found, corresponding to lower yields of hydrolysis products (Gly, Gly_2, Gly_3 and cyclic anhydride). The sum of the yields was lower compared to the case of Gly_3, which indicates the formation of some nonidentified side products and/or the formation of glycine oligopeptides with longer chains and characterized by low solubilities. Gly_{10} was also identified in the supernatants obtained from this reaction system. Overall, very low concentrations of all compounds were found for the supernatant prepared from Gly_5 reaction mixture. Owing to the low solubility of the reactant, the starting mixture could not be prepared from Gly_5 solution. Probably, only a small amount of Gly_5 dissolved and reacted with the alumina surface. Low amounts of hydrolysis products were identified, and the low yields of longer oligopeptides could be expected because of their low solubilities and consequently low concentrations. However, the formation of Gly_{10} and Gly_{11} from Gly_5 could be shown at least qualitatively.

3.2. Reactions of Gly_2 with amino acids

Reactions of Gly_2 with other amino acids are basically more complicated and therefore produce numerous reaction products. The first group of the reaction products are those produced from Gly_2 itself, such as glycine, its cyclic anhydride and glycine oligomers. The reaction yields of these compounds were not analyzed. The next group of the products were dipeptides or cyclic anhydrides of the second reactant, i.e., the amino acid. The reactivity of amino acids decreased in the order Ala, Leu, Val, Pro, as indicated by the reaction yields of respective dipeptides (table 3). This confirms the trend of amino acid reactivity, which has been reported recently [9]. The list of the reaction yields of the cyclic anhydrides formed from these amino acids is not complete because we could not obtain all reference substances. Our interest was focused to the products formed by the reaction between Gly_2 and the amino acids as the second reactant. There are two main reaction products potentially formed, mainly the tripeptides Gly-Gly-AA and AA-Gly-Gly. It has been found that a peptide chain elongation of Gly_2 may start as the formation of cyclic anhydride as an intermediate reaction product [8]. The next step of the reaction is then a molecular rearrangement of the cyclic anhydride by a ring-opening mechanism and consecutive formation of an amide bond with a second reactant molecule (figure 3) [15,19,20]. If this reaction mechanism took place in the $Gly_2 + AA$ reaction, it would lead to the preferential formation of the tripeptide Gly-Gly-AA. The preference for this tripeptide was indeed found, regardless of the amino acid used (table 3). For example, almost three times more Gly-Gly-Ala (1.87%) than Ala-Gly-Gly (0.69%) was formed from Gly_2 and alanine. On the other hand, Ala-Gly has formed preferentially, compared with Gly-Ala, from Gly + Ala, as observed recently [9]. The preferential formation of Ala-Gly was also observed in Cu(II)-catalyzed reaction and explained by a sequence inversion reaction of Gly-Ala (produced the beginning) to Ala-Gly via cyclic anhydride [21]. For the case of Gly-Gly-Ala, the sequence inversion reaction cannot take place and, therefore, it remains the main product. However, Gly-Gly-Ala may hydrolyze either backward to the reactants Gly_2 and alanine, or to Gly + Gly-Ala. In a similar way, Ala-Gly-Gly, as the second main product of the reaction, hydrolyzes to the reactants or to Gly + Ala-Gly. The preferential formation of Gly-Ala (0.82%) compared with Ala-Gly (0.44%) is in accordance with higher yield of Gly-Gly-Ala compared to Ala-Gly-Gly (table 3), although Ala-Gly is preferentially formed from amino acids mixtures [9,21]. Besides, mixed di- and tripeptides, the mixed cyclic anhydride of glycine and alanine (cyc(Gly-Ala)) was also formed with considerable yield (0.78%). Its

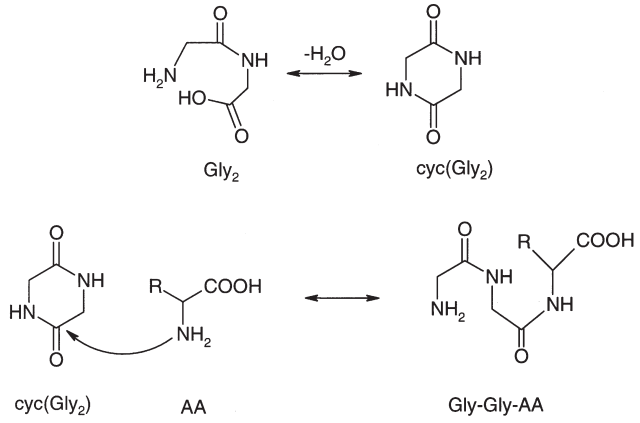

Figure 3. Two-step reaction of peptide chain elongation. The first step is an amide bond formation yielding a cyclic anhydride. The second reaction step is the reaction with an amino acid molecule via ring-opening molecular rearrangement.

formation can proceed by two mechanisms: (1) Direct cyclization reaction from dipeptides Ala-Gly and Gly-Ala, which had been formed by hydrolysis of tripeptides. (2) Reaction from tripeptides without inclusion of water, with a mechanism of a molecular rearrangement shown in figure 3, but proceeding in the opposite direction.

A markedly preferential formation of Gly-Gly-Val (1.18%) compared to Val-Gly-Gly (0.09%) was observed. It can be explained on the basis of a strong positive inductive effect of the side group in valine on the nucleophilicity of the amino group. This nucleophilicity of the amino group is essential for the condensation reaction with a second reactant molecule. However, the strong inductive effect reduces electrophilicity and consequently also the reactivity of the carboxyl group, which also contributes to the preferred linkage of valine via its amino group. This trend is fully in agreement with the reactions of valine with glycine on alumina or in Cu(II)-catalyzed reactions [9,21]. The same type of preference was observed for the reactions of Gly_2 with leucine and proline. Approximately similar yields of the preferred tripeptide (Gly-Gly-AA) were formed in the reactions with valine, leucine or proline. Slightly higher yields of Leu-Gly-Gly and Pro-Gly-Gly were formed compared to Val-Gly-Gly. It can be explained in terms of a relatively higher electrophilicity and reactivity of the carboxyl groups in leucine and proline. The preferential formation of Gly-Gly-Pro is surprising, since a low reactivity of the secondary amino group of Pro was observed in Gly + Pro reaction, where Pro-Gly was preferentially formed [9]. It indicates that the reaction with Gly_2 includes other aspects, perhaps proceeds by a different reaction mechanism. The reaction through cyclic anhydride is one of the possibilities, explaining preferential formation of Gly-Gly-Pro versus Pro-Gly-Gly. Another possible explanation is an extensive hydrolysis of the second tripeptide, which would be in agreement with the huge yield of Pro-Gly formed after 14 days (2.41%). The difference between the yields of mixed dipeptides is remarkable and the preferentially formed dipeptide Pro-Gly cannot be produced by hydrolysis of Gly-Gly-Pro. The formation of such a high yield of Pro-Gly by a reaction of glycine as a hydrolysis product of Gly_2 is not probable because of the low concentration of formed glycine. Moreover, no such high yields were observed for Pro + Gly reaction on alumina, where the starting concentration of glycine was much higher [9]. Consequently, the hydrolysis of Pro-Gly-Gly is the most plausible explanation of the formation of Pro-Gly with such high yields. However, we do not know yet, why the hydrolysis rate of Pro-Gly-Gly should be so high.

3.3. Reactions of Ala_2

Ala_2 and Ala_2 + Gly were the last reaction systems studied and the yields produced after 14 days are summarized in table 4. Surprisingly, large yields of cyc(Ala_2) are formed from Ala_2 (around 50%), but the amounts of Ala_4 were also relatively high. The readiness of Ala_2 to form cyclic anhydride was also encountered in the reaction system with glycine. In spite of the high reactivity of glycine [22], Ala_2 preferentially formed the tetramer (1.96%), or converted to cyclic anhydride with an exceptionally large reaction yield of 48.33%. Small yields of Ala_3 (0.33%) were also obtained. The yield of

Table 3
Alumina-catalyzed oligopeptide formation from mixtures of alanine, valine, leucine or proline with diglycine after 14 days

AA	Yield (%)						
	cyc(AA_2)	AA_2	Gly_2AA	$AAGly_2$	GlyAA	AAGly	cyc(GlyAA)
Ala	9.46	4.23	1.87	0.69	0.82	0.44	0.78
Val	R[a]	0.47	1.18	0.09	0.39	0.12	0.12
Leu	R[a]	3.90	1.20[a]	0.40[a]	0.20[a]	0.10[c]	R
Pro	R[a]	–[b]	1.21	0.59	0.16	2.41	R

"-" means below detection limit.
R – reference substances unavailable.
[c]The peaks of Gly-Gly-Leu and Gly-Leu; Leu-Gly-Gly and Leu-Gly in chromatogram were not well separated by the analytical method. The concentrations of these compounds were calculated using peak areas obtained by the deconvolution of peak doublets.

Table 4
Alumina-catalyzed oligopeptide formation from Ala_2 and Ala_2 + Gly reactions

	Yield (%)							
	cyc(Ala-Gly)	cyc(Ala_2)	Gly_2	cyc(Gly_2)	Ala_3	Ala_4	$GlyAla_2$	Ala_2Gly
Ala_2		54.00			0.20	2.59		
Ala_2 + Gly	0.21	48.33	6.84	5.25	0.08	1.96	0.68	0.42

cyc(Ala_2) was remarkably high, so were the yields of cyc(Gly_2) and Gly_2. However, the reactivity of cyc(Ala_2) is apparently lower than that of cyc(Gly_2) was. Taking into account the high yields of cyc(Ala_2), one would expect its reaction with glycine by the same mechanism as shown in figure 3 for the reaction of cyc(Gly_2) with other amino acids. However, only 0.43% Ala-Ala-Gly was formed, even less than Gly-Ala-Ala (0.68%). The reaction of Ala_2 + Gly indicates that the type of the reaction products and reaction yields in peptide bond formation on mineral surfaces do not always reflect the reactivities of the reactants themselves (Gly and Ala_2), but may be determined by other factors as well. The concentrations of Gly-Ala, Ala-Gly dimers (0.21, 0.28%) and cyc(Ala-Gly) (0.21%) were considerably lower than those of the products containing either only alanine or only glycine units in their molecules (table 4). Specific adsorption on the surface of catalyst and its influence on interaction and association between reactant molecules could be possible reasons for this interesting phenomenon. All these aspects may play a key role for understanding reactivity and chemical behavior as well as chemical evolution of short peptides on primitive earth.

Acknowledgments

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