SILICA, ALUMINA AND CLAY CATALYZED PEPTIDE BOND FORMATION: ENHANCED EFFICIENCY OF ALUMINA CATALYST

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Abstract. Catalytic efficiencies of clay (hectorite), silica and alumina were tested in peptide bond formation reactions of glycine (Gly), alanine (Ala), proline (Pro), valine (Val) and leucine (Leu). The reactions were performed as drying/wetting (hectorite) and temperature fluctuation (silica and alumina) experiments at 85 °C. The reactivity of amino acids decreased in order Gly > Ala > Pro ≈ Val ≈ Leu. The highest catalytic efficiency was observed for alumina, the only catalyst producing oligopeptides in all investigated reaction systems. The peptide bond formation on alumina is probably catalyzed by the same sites and via similar reaction mechanisms as some alumina-catalyzed dehydration reactions used in industrial chemistry.

1. Introduction

Silica, alumina and aluminosilicates catalyze a variety of the reactions and promote processes in organo-chemical systems. Their possible role in pre-biological chemical evolution has been proposed (Bernal, 1951; Rao *et al*., 1980; Ponnamperuma *et al*., 1982; Cairns-Smith and Hartman, 1988; Bujdák and Rode, 1995). Besides the prebiotic formation of bio-monomers, sugars and nucleic acids, the role of clays and related minerals in peptide bond formation catalysis has been assumed. Condensation reaction between amino acids proceeds readily after the activation of amino acid molecule with various kinds of condensation agents (Hulshoff and Ponnamperuma, 1976). Clay minerals promote the polymerization of such kind of activated species (amino acid adenylates) (Paecht-Horowitz, 1977; Paecht-Horowitz, 1978; Paecht-Horowitz and Eirich, 1988; Paecht-Horowitz and Lahav, 1977; Paecht-Horowitz *et al*., 1970). However, any possible pathways leading to the formation of amino acid adenylates under the assumed prebiotic conditions have not been found (Warden *et al*., 1974). The existence of other condensation agents on the primitive earth in sufficient amounts is also questionable (Hulshoff and Ponnamperuma, 1976; Keefe and Miller, 1995). Silica, alumina and related minerals were probably among the major components of primitive earth crust. At higher temperatures, oligomerization of various types of amino acids on silica or alumina was observed also without activation with condensation agents (Rohlfing and McAlhaney, 1976; Basiuk *et al*., 1990; Basiuk *et al*., 1991; Gromovoy *et al*.,

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1991; Basiuk, 1992; Basiuk *et al*., 1992; Basiuk and Gromovoy, 1994). However, there are some questions remaining, whether such conditions could have been realized during the prebiotic era to the necessary extent needed for playing an important role in peptide formation. Moreover, oligomerization at higher temperatures would be likely accompanied by the decomposition of reactants, as well as of reaction product (Basiuk *et al*., 1991; Basiuk, 1992). At temperatures below 100 °C, without previous amino acid activation and in the absence of any condensation agents, amino acid and dipeptide oligomerization proceeds to a certain extent when applying drying/wetting cycles on clays (Lahav *et al*., 1978; Lawless and Levi, 1979; Bujdák, *et al*., 1994; Bujdák, *et al*., 1995; Bujdák, *et al*., 1996a–c; Bujdák and Rode, 1996), silica and alumina (Bujdák and Rode, 1997a,b). Clay catalyzed amino acid oligomerization has been studied mostly with Gly, the simplest amino acid, and in many aspects the most reactive one. Some earlier, as well as recent papers showed that already Ala oligomers are produced to a much lower extent on clays (Lawless and Levi, 1979; Bujdák and Rode, 1997b; Bujdák *et al*., 1994; 1995; 1996a; 1997b). While the highest efficiency of clays was achieved in reaction systems involving drying/wetting cycles, silica and alumina gave the highest yields in temperature fluctuation experiments. Alumina was found to be the most efficient, producing more than 3% of dialanine (Ala_2) at 80 °C after two weeks (Bujdák and Rode, 1997b). Oligomerization of aspartic acid on kaolinite was reported, together with the preference for the reaction of L-optical isomers (Degens *et al*., 1970). However, Flores and Bonner (1974) did not observe any oligomerization of aspartic acid under the same conditions. Peptide bond formation was claimed for cysteine, proline and lysine on the basis of infrared spectra (Siffert and Kessaisia, 1978). However, there is no record supported by reliable data of any products formed by clay catalyzed oligomerization of any amino acid except Gly and Ala at temperatures below 100 °C.

The objective of this work was to investigate the oligomerization of some amino acids as Pro, Val, Leu alone and combined with Gly and Ala on silica, alumina and hectorite at temperatures below the boiling point of water. The relative reactivities of these amino acids should be compared with those of Gly and Ala, comparing the yields of respective oligopeptides.

2. Experimental

2.1. MATERIALS

Silica (spectroscopic standard, B.D.H. LTD, England), alumina (neutral, for chromatography, Reanal, Hungary) and hectorite (SHCa-1) (the Source Clays Repository of The Clay Minerals Society) were used as catalysts. Silica and alumina were used as obtained. Hectorite was purified by the sedimentation of water dispersions and a Ca-saturated <2 μm fraction was prepared (Komadel *et al*., 1996). Amino

TABLE I

Mobile phase compositions used for the analysis of amino acids/peptides condensation products

Reaction system	Mobile phase composition
Gly, Gly + Ala	10 mM $C_6H_{13}SO_3Na$, pH = 2.5 by H_3PO_4
Ala	2.5 mM $C_6H_{13}SO_3Na$, 10 mM KH_2PO_4, pH = 2.5 by H_3PO_4
Pro, Pro + Gly, Pro + Gly_2, Pro + Ala	10 mM $C_6H_{13}SO_3Na$, 6% CH_3CN, pH = 2.5 by H_3PO_4
Val, Val + Gly, Val + Gly_2, Val + Ala	10 mM $C_6H_{13}SO_3Na$, 8% CH_3CN, pH = 2.5 by H_3PO_4
Leu	10 mM $C_6H_{13}SO_3Na$, 20% CH_3CN, pH = 2.5 by H_3PO_4
Leu + Gly, Leu + Gly_2	10 mM $C_6H_{13}SO_3Na$, 15% CH_3CN, pH = 2.5 by H_3PO_4

acids, their respective oligopeptides and cyclic anhydrides were purchased in analytical grade quality. For experiments only L-optical isomers of amino acids were used. For analysis, L-, D-dipeptides were used in some cases, but no racemization was observed during the reactions.

2.2. REACTION SYSTEM

The reactions investigated are summarized in Table I.

The most suitable reaction conditions for peptide bond formation were chosen for all catalysts according to the data of recent work (Bujdák and Rode, 1997b):

1. 0.01 g of hectorite powder was mixed with 1 mL of 10 mM reactant solution in 2 mL glass vials. For reactions of two reactants, the overall concentration was 10 mM, i.e., 5 mM for each compound. Suspensions were evaporated, dried and heated in a heating box at 85 °C for 24 hr (1 cycle). The next cycle started with addition of 1 mL of distilled water.
2. 0.01 g of silica or alumina was immersed in 0.1 mL of 100 mM reactant solution. The reaction mixture was heated in the same way as the hectorite-reactant suspension. However, no water was added to start the new cycle, but the samples were left at room temperature for one hour to adsorb water from air. Then the samples were heated in the heating box to complete the next 24 hr cycle.

All reactions finished after 7 cycles, and before the analysis 1 mL 0.1 M calcium chloride solution was added to the solids to release all formed oligopeptides. After 24 hr the liquid phase was analyzed.

The efficiency of calcium chloride solution to release adsorbed oligopeptides was tested. No significant difference between the concentrations of added and released amino acid (oligopeptide) was detected by the analytical method. IR spec-

troscopy did not detect remaining organic compound adsorbed on the clay surface after the treatment of calcium chloride solution.

2.3. Analytical methods

All samples were analyzed by a Hewlett-Packard HP-1090M HPLC apparatus using a Shannon Hypersil (ODS 5 μm/200 $\times$ 2.1 mm) column. The mobile phase compositions for the analysis of respective reaction systems are summarized in Table I. Ion pairing and reverse phase chromatography enabled a good separation and analysis of compounds. Reactants as well as possibly formed unknown compounds from the reactant decomposition (deamination, decarboxylation, oxidation, etc.) appear at lower retention times, most oligomer products were eluted after relatively higher retention times. Before analysis each solution was diluted with mobile phase (1:1). The injection volume varied between 2 and 25 μL depending on the particular method and yields. Detection was performed with a diode array detector at 195 nm, peptides were identified by retention times of authentic reference substances and UV-VIS spectra.

Three parallel samples were used for each reaction systems and the reaction yields were determined as average percentage of the reactants converted to the reaction product. The relative difference between the concentrations of the reaction products in parallel samples was below 15%. In the cases of very low reaction yields ($<$0.20%) this difference was below 25%.

3. Results and Discussion

3.1. Reactions of glycine and alanine

The reactions of the simplest amino acids Gly and Ala catalyzed by silica, alumina and clays at temperatures $<$100 °C have been investigated in the works mentioned previously. The reactions of Gly, Ala and Gly + Ala were repeated in this work, in order to compare their reactivity with that of other investigated amino acids. The reaction yields proved the high reactivity of Gly (Table II). Gly produced diketopiperazine (cyc(Gly_2)) and Gly_2 also in blank experiments (0.25% of sum yield). About 15% of Gly oligomerized to cyc(Gly_2), Gly_2 and triglycine (Gly_3) on alumina, Gly_2 being a major product (13%). The sum yield achieved on hectorite was more than twice lower than that formed on alumina and cyc(Gly_2) was the major product. Surprisingly, only very low yields were produced on silica, the similar as observed in drying/wetting cycles at 80 °C (Bujdák and Rode, 1997b). Another series of experiment reconfirmed the low catalytic efficiency of silica. The reactivity of Ala on hectorite or alumina was lower than that of Gly. No oligomerization leading to oligopeptides containing Ala unit(s) was observed in blank experiments of Ala and Ala + Gly. Alumina produced relatively high yields of cyclic anhydride (cyc(Ala_2)) and Ala_2 (0.17 and 1.59%, respectively, Table II).

TABLE II

The yields of oligopeptides produced by the reaction systems

Experiment	Analyzed product	Silica	Alumina	Hectorite
Gly	cyc(Gly_2)	0.32	1.94	5.69
	Gly_2	0.12	13.06	1.54
	Gly_3	–	1.02	0.14
Ala	cyc(Ala_2)	0.80	0.17	+
	Ala_2	0.11	1.59	0.08
Ala + Gly	cyc(Ala-Gly)	0.16	0.12	0.05
	Ala-Gly	0.10	0.48	0.11
	Gly-Ala	–	0.44	+
Pro	Pro_2	–	0.60	–
Pro + Gly	Pro-Gly	–	0.96	0.14
	Gly-Pro	–	0.93	–
Pro + Gly_2	Pro-Gly-Gly	0.74	1.09	0.22
Pro + Ala	Pro-Ala	–	+	–
	Ala-Pro	–	0.42	–
Val	cyc(Val_2)	+	0.18	–
	Val_2	0.06	0.05	–
Val + Gly	Val-Gly	+	0.09	+
	Gly-Val	0.13	0.82	–
Val + Gly_2	Val-Gly-Gly	–	0.27	–
	Gly-Gly-Val	0.13	0.28	–
Val + Ala	Ala-Val	0.21	0.85	–
Leu	Leu_2	0.19	1.05	–
Leu + Gly	Leu-Gly	0.25	0.42	–
	Gly-Leu	0.12	0.53	–
Leu + Gly2	Gly-Gly-Leu	0.42	0.19	–
Leu + Ala	Ala-Leu	+	0.10	–
	Leu-Ala	+	0.07	–

– = Not detectable; + = traces.

The catalytic effect of silica was higher for the reaction system with Ala than for the one with Gly. It converted almost 1% of Ala to dimer and cyclic anhydride. Hectorite was much less efficient for this reaction than silicon and aluminum oxides. The determined yield of Ala_2 on this clay (0.08%) is in fact the same as observed earlier at 80 °C (Bujdák and Rode, 1997b). The yields of Ala-Gly, Gly-Ala and cyc(Ala-Gly) obtained on each catalyst were in general very low which is certainly also due to the additional concurrent reactions proceeding in this system, such as Gly_2 and cyc(gly_2) formation.

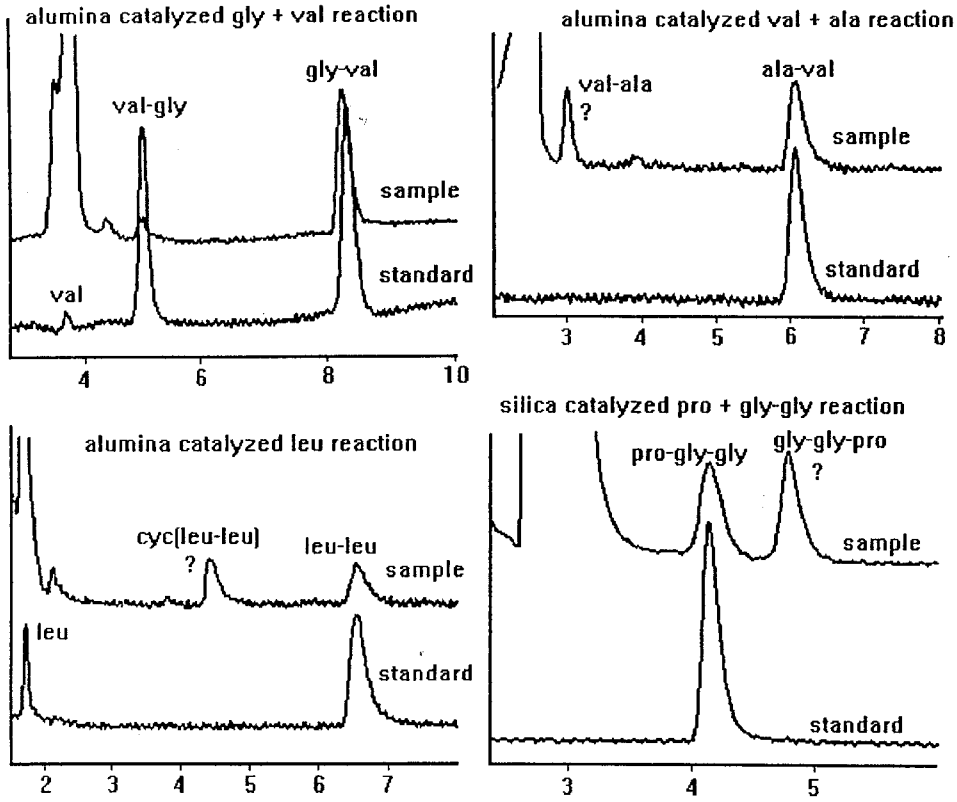

Figure 1. Chromatograms showing oligopeptide formation in some reaction systems. '?' means that the identity of this oligopeptide is assumed only and could not be confirmed by the chromatogram due to the lack of reference substance.

3.2. REACTIONS INCLUDING OTHER AMINO ACIDS

Reactants, such as Pro, Val and Leu are more hydrophobic than Gly and Ala. In order to separate the reactants and the reaction products within a suitable range of retention time, the appropriate hydrophobicity of the mobile phase was adjusted by acetonitrile (Table I). These modified analytical methods achieved good separation of the reaction products from reactants, i.e. di- and tripeptides were characterized with higher hydrophobicity and longer retention times than the corresponding reactants (amino acids, Gly_2) and possibly formed decomposition products. Only in the case of the reactions with Pro, an unidentifiable compound was found, characterized by much higher retention time (15 min) than the analyzed reaction products. However, an identification of this compound has not been performed.

Figure 1 presents chromatograms of some analyzed samples as well as respective standards of the reference substances. Not all possible products could be analyzed since some dipeptides and mainly cyclic anhydrides were not available. Some of the reaction products, not confirmed by the reference substances, were

only assumed to have been formed as shown in Figure 1. Incomplete reaction yields could not be used for the quantitative comparison of the reactivity of tested amino acids on the catalysts. On the other hand, the obtained results clearly show qualitative aspects of the catalytic efficiency of hectorite, silica and alumina for peptide bond formation of Pro, Leu and Val, compared to that of the simplest amino acids – Gly and Ala.

The reaction systems including other amino acids, such as Pro, Val and Leu, produced much lower yields of oligopeptides (Table II) than Gly and Ala. The blank reaction systems produced none of the analyzed oligopeptides. Similar to the reactions of Ala (Table II), the catalytic efficiency of hectorite for the reactions of Pro, Val and Leu was very low. Very small amounts of Pro-Gly and Pro-Gly-Gly ($<0.25\%$) were detected by the reactions of Pro with Gly and Gly_2, respectively. Val-Gly formed from Gly and Val on hectorite was only qualitatively detectable. Major parts of the clay surface belongs to basals, which are inefficient for peptide bond formation catalysis. Only clay particle edges, a very small fraction of the total surface, are efficient in amino acid activation, as assumed in earlier works (White *et al*., 1984; Collins *et al*., 1988). Low yields were produced in some reaction systems with silica, but the catalytic efficiency was in average higher than that of hectorite. Silica surface contains a higher fraction of active sites (Si-OH) available for the reactant molecules. The relatively highest yields formed on silica were achieved in the reactions with Gly_2, which can be ascribed to the higher reactivity of Gly_2 (Bujdák *et al*., 1995; Takaoka *et al*., 1991). Silica catalyzed the oligomerization in most reactions of Val and Leu, but was not efficient in the catalysis of Pro reactions (with the exception of Pro + Gly_2 reaction).

The highest catalytic efficiency was observed in the case of alumina. Each amino acid alone, or in the reactions with Gly, Gly_2 and Ala produced at least detectable amounts of analyzed di- or tripeptides. Despite that, the yields were mostly below 1%, much lower as obtained in the reaction with some organic condensation agents or energy-rich phosphates (Rabinowitz *et al*., 1997; Hulshoff and Ponnamperuma, 1976), whose prebiotic availability is, however, highly questionable.

3.3. THE MECHANISM OF ALUMINA CATALYZED PEPTIDE BOND FORMATION

Alumina is known to catalyze many reactions of organic compounds, which is often used in chemical industry (Boehm, 1966). The most important catalytic applications concern dehydration, mainly of alcohols (giving alkenes and/or ethers), discovered already in the end of 18th century. Huge amount of works studying alumina catalyzed dehydration reactions have been published. However, for many years there was no agreement in the literature with regard to the aspects of the reaction mechanism of alumina catalysis and assignment of the structural sites (Pines and Manassen, 1966). Nowadays, intrinsic acidic sites on alumina surface are considered to be the catalytic sites for various dehydration reactions (Olah *et al*., 1984; Shiffino *et al*., 1993; Garvesini *et al*., 1995; Lahousse *et al*., 1995). These

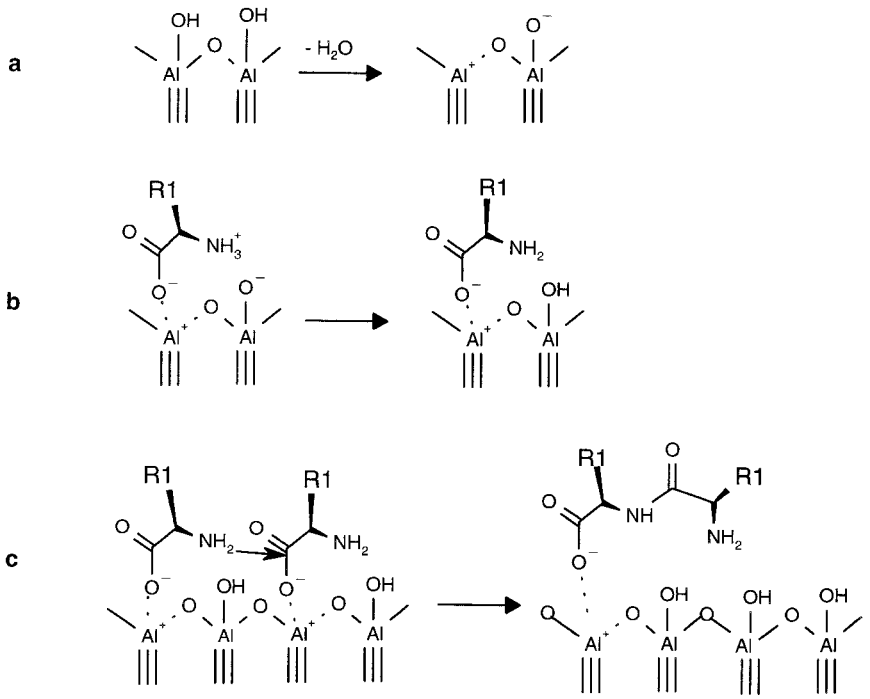

Figure 2. Interaction of alumina catalytic sites and amino acid zwitterions leading to peptide bond formation. a) The formation of catalytically active sites on alumina surface. b) The activation of functional groups of amino acid zwitterion at alumina surface. c) Assumed mechanism of peptide bond formation on alumina surface.

sites are formed after the condensation reactions of hydroxyl groups bound to the neighboring aluminum atoms (Figure 2a) and include a Lewis acid site on first aluminum atom and a base site on the second one (Pines and Manassen, 1966). The formation of salts between anions of carboxylic or amino acids and cations of alumina Lewis acidic sites were observed experimentally (Macklin and White, 1985; Basiuk *et al.*, 1990). Such salts are formed at lower temperatures and are more stable against hydrolysis than amino acid esters formed by condensation on silica surface. Whether the interaction between Lewis acidic sites and carboxyl group lead to any activation of carbonyl carbon was disputed, since this bond is rather ionic. The catalytic effect of alumina in peptide bond formation has been attributed only to the removal of intramolecular interaction between $–COO^-$ and $–NH_3^+$ groups within the amino acid molecule. However, the role of Al-OCO- bonds in amino acid activation cannot be completely neglected. Such ionic bonds have a partially covalent character, and the electrophilicity of carbonyl carbon should be higher as in the amino acid zwitterion. Consequently, carbonyl carbon electrophilicity would increase in case of an interaction of both carboxyl oxygen atoms with more Lewis acid centers on the alumina surface. The possibility of

such interactions is supported by the observation that dehydration of alcohols in fact does not proceed on the alumina external surface but occurs within either in submicroscopical pores, or crevices or channels. The reactant molecules are surrounded by alumina acidic and basic sites and alumina acts as 'pseudosolvent' activating dehydration (Pines and Manassen, 1966). The significance of basic sites Al-O^- was not considered in the literature concerning amino acid condensation on alumina surface (Basiuk *et al.*, 1990), but they play a role in other alumina catalyzed dehydration processes. Their role in peptide bond formation could be as following:

1. Catalytically active sites, including acidic and basic sites of opposite charge would control a suitable orientation of amino acid zwitterions while being adsorbed from aqueous solutions. Moreover, this orientation would remove intramolecular interaction of carboxyl and ammonium groups of amino acid molecules.
2. Deprotonation of ammonium group by basic Al-O^- group would result in the formation of nucleophilic amino groups necessary for the condensation reaction (Figure 2b). In summary, intrinsic acid and basic sites of alumina would activate both carboxyl and amino groups. The condensation reaction would proceed between two neighboring activated amino acid molecules. Intrinsic acidic sites, similar to the other dehydration reactions, would not act as real catalyst, since they would convert during the reaction back to an inactive state (Figure 2c), but could be recovered by re-dehydration of neighboring Al-OH groups, induced by heating.

One feature of alumina catalyzed peptide bond formation correlates well with other known dehydration processes (dehydration of alcohols) proceeding on this compound: The catalytic efficiency depends strongly on the amount of water in the reaction system and in some cases a small amount of water may poison the catalyst (Pines and Manassen, 1966). Higher amounts of water lead to lower amounts of acidic and basic sites favoring Al(OH)-O-Al(OH) sites. The latter ones (present in certain amount also under anhydrous conditions) are known to catalyze the reverse process – hydration. Indeed, the negative influence of water on oligopeptide yields was found to be much higher than in the case of silica (Bujdák and Rode, 1997b). To confirm this, Gly_2 oligomerization was performed on silica and alumina in temperature fluctuation experiments and the yields were compared also with those obtained in drying/wetting cycles reported recently (Bujdák and Rode, 1997a). In temperature fluctuation experiments, both silica and alumina produced large amounts of cyc(Gly_2), (43 and 19%, respectively). Both catalysts formed also tetraglycine (Gly_4) and hexaglycine (Gly_6) (4.86, 0.64% on silica and 6.58, 0.50% on alumina). However, only in the case of alumina, Gly_3 was formed (2.15%) and pentaglycine (Gly_5) was detectable under such 'anhydrous' conditions. When drying/wetting experiments were performed (more water was in the reaction system) the yield of Gly_3 produced on silica was much lower than that of Gly_4, i.e., 0.36

and 3.46%, respectively. On the other hand, they were more similar when produced on alumina under the same conditions (2.00, 3.15% of Gly_3 and Gly_4, respectively) (Bujdák and Rode, 1997b). Much higher yield ratios Gly_3/Gly_4 characteristic for alumina catalyzed reactions indicate a higher extent of reactant and/or reaction product hydrolysis, proceeding on alumina surface.

4. Conclusions

The reactivities of amino acids in the presence of clay, silica and alumina vary significantly. Only Gly yields traces of Gly_2 and cyc(gly_2) in experiments without catalyst. Ala reactivity on clay is significantly reduced in comparison to Gly. More hydrophobic amino acids with longer side chains (Leu, Val) and secondary amine bond (Pro) exhibit much lower reactivity than the simpler ones, Gly and Ala.

The efficiencies of tested catalysts for peptide bond formation increased in the order hectorite, silica, alumina. Only aluminum oxide was able to catalyze the formation of all analyzed oligopeptides within the tested reaction systems. Catalytically active sites, including coupled Lewis acid and basic sites, probably play a role in alumina catalyzed peptide bond formation.

The yields of Leu, Val and Pro oligopeptides produced on alumina at the temperature <100 °C were very low. On the other hand, alumina, aluminum hydroxides, hydrated oxides and related minerals occur all over the earth crust. Therefore, formation of peptides via matrix polycondensation of amino acids in prebiotic era catalyzed by aluminum minerals should not be neglected in the experimental modeling of prebiotic processes leading to peptides.

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