Activated alumina as an energy source for peptide bond formation: Consequences for mineral-mediated prebiotic processes

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Summary. The catalytic properties of various forms of alumina were tested for alanine dimerization reaction. The catalytic efficiency of alumina depends on the structure, as well as on acid/base properties of the catalyst. The highest yields of Ala_2 were achieved on activated alumina with surface of neutral pH (about 3% conversion after 2 weeks). Thermal analysis of Ala + alumina reaction systems shows that the thermal behavior of amino acid changes substantially in contact with the activated surface of the alumina catalyst. The reaction of Ala is detected as being strongly endothermic by differential thermal analysis of pure amino acid (above 250°C). The alanine endothermic reaction is shifted substantially to lower values (below 200°C) and hardly detectable if activated alumina is present. The reaction mechanism of amino acid activation on alumina surface and its significance for mineral-catalyzed prebiotic peptide bond formation are discussed.

Keywords: Amino acids – Alumina – Alanine – Catalysis – Clay – Peptide bond formation – Prebiotic

Introduction

Possible ways of prebiotic formation of basic bioorganic compounds, such as amino acids, were demonstrated by the Miller-Urey experiments several decades ago (Miller, 1953). After the formation of these simple compounds, chemical evolution should have continued through condensation reactions of the monomers, in the case of amino acid leading to the formation of short peptides. The thermodynamic barrier of peptide bond formation reaction is a crucial point of this step. This barrier could have been theoretically overcome by energy rich compounds, acting as condensation agents (e.g. energy rich polyphosphates, heterocyclic compounds, dicyanamide, etc) (Hulshof and Ponnamperuma, 1976). However, such compounds could hardly have been formed under the prebiotic conditions; and if, they would fast have

decomposed immidiately, due to their high sensitivity to hydrolysis (Keefe and Miller, 1995; Rode, 1999 and references therein). Alternatively, Bernal (1951) pointed out a possible catalytic role of chemically active minerals. Several reviews on this topic have been published (Rao et al., 1980; Ponnamperuma et al., 1982; Cairns-Smith and Hartman, 1988; Bujdák and Rode, 1995), and catalytic effects of clays and related compounds in amino acid oligomerization have been observed. There are a few hypotheses used to explain the mechanisms of catalysis by these compounds:

1. The catalytic activity of silicate minerals is usually interpreted on the basis of amino acid activation by a condensation reaction with Si—OH groups leading to —Si—O—CO— bonds. The formation of such activated species was observed by infrared spectroscopy (Jewett and Lawless, 1981). Clay mineral structure and composition affect the catalytic efficiency via re-distribution and orientation of the reactant zwitterions at the clay's active sites (Bujdák and Rode, 1996, 1999a).

2. Another series of the reactants' activation is based solely on the mineral effects on amino acid orientation and molecular state (acid/zwitterion/base). For example, amino acid zwitterion cannot react via substitution reaction at carboxylic carbon atom, because of the absence of the nucleophilic amino group. Basic catalysis, induced by hydrolysis of carbonates or some silicates, efficiently converts inactive zwitterions of amino acids to anions with nucleophilic amino groups (Zamaraev et al., 1997). Another aspect of low reactivity of amino acid zwitterions includes the intramolecular H—bond interaction between NH_3^+ and COO^- groups. Alumina's positively charged Lewis acid sites interact with carboxyl anion groups and break this association (Basiuk et al., 1990).

3. The plausibility of the role of alumina and kaolinite to store and to trans-duce energy has been suggested. These solid materials exposed to wetting/drying treatment exhibit luminescent properties. Energy transfer takes place at the conditions relevant for prebiotic processes (Lahav et al., 1981; Coyne et al., 1984; Lahav et al., 1985), and may have been significant therefore for the catalysis of peptide bond formation reaction (Coyne et al., 1981, 1983). Kaolinite and related minerals are the only clay minerals, whose main part of the surface is similar to that of alumina. Kaolinite layers are composed of one sheet of tetrahedrons with Si central atoms (SiO_4) linked to one sheet of $AlO_2(OH)_4$ octahedrons. The kaolinite surface, namely the one of formed by aluminum polyhedrons, should exhibit similar properties as the surface of alumina. Adsorption and catalytic properties of the alumina surface are strongly dependent on the conditions of the thermal activation of material precursors (Walker et al., 1999). Thermally activated alumina shows a very high catalytic efficiency for peptide bond formation, much higher than any other tested mineral catalyst (Bujdák and Rode, 1997, 1999b).

The objective of the present work was to shade more light on the alumina catalyzed peptide bond formation reaction and its dependence on the catalyst structure and properties. Possible relationships between the mechanisms of

alumina and kaolinite catalysis and the relevance of thermal activation for kaolinite catalysts were investigated as well.

Materials and methods

Activated alumina samples of neutral, acidic, weakly acidic and basic properties (type Brockmann I) of 150 mesh and α-corundum of 100 mesh were purchased from Aldrich. Kaolinite (Pugu Hills, Tanzania) was purified by sedimentation of its dispersion and isolated as a fraction with particle size $<2\,\mu$m. Kaolinite dispersion was saturated with calcium chloride and washed in dialysis tubes with distilled water until negative reaction with silver nitrate, then dried and ground. Aliquot portions of kaolinite were heated to 300, 400, 450, 500, 550 and 600°C for three hours and directly used for the experiments. Amino acids Gly and L-Ala and their oligopeptides of analytical grade were purchased from Sigma.

Alumina samples were mixed with an appropriate amount of 100 mM Ala water solution to obtain mixtures of ratio 1 mmol Ala/g. The suspensions were evaporated at 80°C for three hours and directly used for the investigation of peptide bond formation by heating the samples to 80°C for 1–14 days. The solids were washed with 100 mM calcium chloride to leach out adsorbed Ala and reaction products. The liquid phase was obtained by filtration and used for HPLC measurements. Kaolinite samples (0.01 g) were mixed with 0.1 ml 100 mM Gly solution in 2 ml glass vials and subjected to a heating process at 80°C for 1–10 days. The treatment of the solid samples and the preparation of the supernatant liquid for HPLC analysis was identical to that in the alumina catalyzed reaction.

All solutions obtained from the reaction systems were analyzed by a Hewlett-Packard HP-1090M HPLC apparatus using a Shannon Hypersil (ODS 5 μm/200. 2.1 mm) column. The mobile phase for the separation of Gly oligopeptides contained 10 mM sodium hexanesulphonate adjusted to pH 2.5 with H_3PO_4. The solvent for the separation of Ala reaction products had the same pH and contained 2.5 mM sodium hexanesulphonate and 10 mM sodium phosphate. Detection was performed with a diode array detector at 195 nm. Oligopeptides were identified by retention times of authentic reference substances, as described previously (Bujdák and Rode, 1997, 1999b). The reaction yields were determined as percentage of the reactant converted to the reaction product.

The solid samples of alumina-Ala mixtures, prepared for the Ala oligomerization experiments, were also used for the thermal analysis measurement. Thermal analysis, including differential thermal analysis (DTA) and thermogravimetry (TG), was carried out by a SDT 2960 apparatus, TA Instruments, with a sample amount of about 25 mg, under nitrogen atmosphere, with a heating rate of 5 K/min within the range 20–310°C. Fused α-Al_2O_3 was used as a reference compound.

Results and discussion

The addition of water, present in the reactant solution of Ala, seems to have changed the surface properties of alumina. In general, the addition of water to activated alumina significantly decreases its catalytic activity. For some reactions, the alumina catalyst is even poisoned by very small amounts of water (Pines and Manassen, 1966). Nevertheless, the catalytic activities of the tested alumina samples are still sufficient for the Ala $\rightarrow$ Ala$_2$ condensation reaction also after contact with water. Figure 1 shows the formation of dialanine (Ala$_2$) catalyzed with various forms of alumina, namely corundum (α-alumina) and activated γ-aluminas. α-alumina is structurally not only
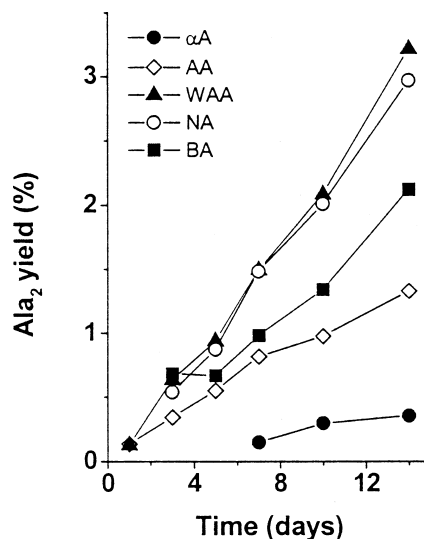
Fig. 1. Formation of Ala$_2$ from Ala on various forms of alumina: α-alumina (αA), acidic alumina (AA), weakly acidic alumina (WAA), neutral alumina (NA), basic alumina (BA)

different but also more uniform and has a lower surface area in comparison to the activated γ-aluminas. It is chemically rather inert compared to γ-aluminas, which are porous materials with an open structure and chemically active surface (Greenwood and Earnshaw, 1984). The structure and chemical properties of the different alumina samples significantly affect the catalytic efficiency of the material (Fig. 1): Using α-alumina as a catalyst, Ala$_2$ was detected after 7 days reaction and its yield did not exceed 0.5%. Activated γ-aluminas gave significantly higher yields and Ala$_2$ was detected after 3 days in all reaction systems containing this type of catalyst. Besides the structure, an influence of the acid-base properties of γ-aluminas was observed as well. Weakly acidic and neutral aluminas exhibit the highest catalytic efficiency. Lower yields were obtained on basic, and the lowest on acidic alumina catalyst (Fig. 1). The interpretation of this trend is based on the mechanism of amino acid activation on alumina surface, taking into account the form of catalytically active sites (Bujdák and Rode 1999b). In order to become active, hydrated aluminum oxide is pre-heated, leading to a condensation reaction between the pairs of hydroxyl groups of neighboring aluminum polyhedrons. This reaction forms pairs of acid and base sites (Fig. 2) (Pines and Manasses, 1966). Weakly acidic and neutral alumina, giving a pH of 6 and 7 $\pm$ 0.5 suspension, contain approximately an equal number of Lewis acidic (Al$^+$) and basic sites (Al—O$^-$) (Fig. 2). Lewis acid or base sites prevail on the surfaces of catalysts with stronger acidic or basic character, respectively. Basic alumina, forming suspensions of pH 9.5 $\pm$ 0.3, contains almost exclusively only Al—O$^-$ basic sites. On the other hand, acidic forms of alumina (with suspension pH of 4.5 $\pm$ 0.3) contain mainly Lewis acid sites (Al$^+$). As shown in Fig. 2, the optimal activation of amino acid zwitterions requires both acidic
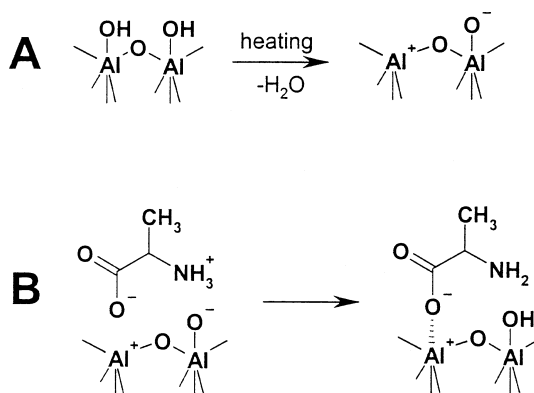
Fig. 2. Schemes showing formation of alumina active sites (**A**) and the mechanism of the amino acid activation on alumina surface (**B**)

and basic sites. The pairs of oppositely charged sites probably play a crucial role in adsorption of amino acid zwitterions, as the distribution of such sites controls the orientation of reactants on the alumina surface. Lewis acid sites activate the carboxyl carbons by electrostatic polarization of $—CO_2^-$ groups. Basic $Al—O^-$ sites react with inactive ammonium groups, producing nucleophilic amino groups (Fig. 2), which are necessary for the reaction of amino acids to peptides. Basic alumina without acidic sites does not activate carboxyl carbon of amino acids but non-activated anionic carboxyl groups of amino acids are still ready to exhibit although a lower degree of electrophilicity. Hence, the yields produced on basic alumina are lower than those formed on neutral or weakly acidic catalysts (Fig. 1). On the other hand, the Lewis acid sites in acidic alumina activate only carboxyl carbon of amino acid zwitterions. Due to the absence of basic sites, no sufficient amount of amino groups is formed and the catalytic activity of acidic alumina is even the lowest among all activated aluminas (Fig. 1). A part of Ala_2 formed at alumina surface is converted to cyclic anhydride ($Cyc(Ala_2)$) in an intramolecular condensation reaction. The reaction yields of cyclic anhydride qualitatively correlate with the amount of Ala_2. About 0.5% of $Cyc(Ala_2)$ forms on neutral and weakly acidic aluminas after 14 days. Lower yields, 0.35 and 0.15%, are produced in the presence of basic or acidic aluminum oxide, respectively, whereas $Cyc(Ala_2)$ was not detectable in the reaction system containing α-alumina.

The results for the catalytic activities of various forms of aluminum oxide observed for Ala_2 formation are supported by the results of thermal analysis measurements. DTA, TG and DTG of alumina-Ala mixtures were compared with the thermal behavior of neutral alumina (Figs. 3, 4). The typical sharp endothermic change, associated with Ala decomposition and present in the DTA pattern of pure Ala (not shown), appeared only in the DTA pattern of Ala α-alumina mixture. The only effect of an inert surface of α-alumina is the shift of the decomposition temperature to lower values (Fig. 3). The thermal decomposition of α-alumina Ala mixture is accompanied by a sharp loss of
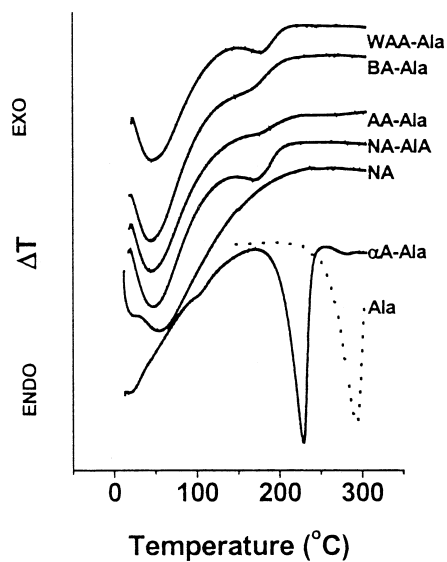
Fig. 3. Differential thermal analysis of alanine (*Ala*, dashed line), neutral alumina (*NA*) and of the mixtures of alanine with weakly acidic alumina (*WAA*), basic alumina (*BA*), acidic alumina (*AA*), neutral alumina (*NA*) and α-alumina (αA). The data are shifted on the y-axis. The measured values of ΔT for pure Ala are divided by ten
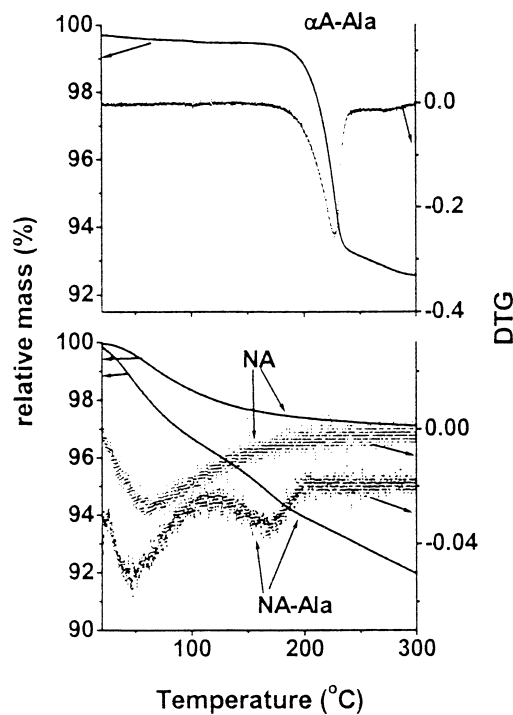
Fig. 4. Thermogravimetry and differential thermogravimetry (*DTG*) of α-alumina-Ala (αA-*Ala*) and neutral alumina (*NA*) and its mixture with alanine (*NA*-*Ala*)

mass, as shown by TG and DTG measurements (Fig. 4). The chemical aspects of Ala decomposition under these conditions was not studied or analyzed in this work. Ala reactions on alumina surface may include condensation reactions (e.g. oligomerization, cyclic anhydride formation), decarboxylation, deamination and/or evaporation of the reaction products (Lien and Nawar, 1974, Rodante et al., 1997, Calin et al., 1998).

The reaction systems containing mixtures of Ala with active γ-aluminas, exhibit completely different thermal behavior. The course of the DTA curves does not indicate any extensive decomposition of amino acid in the narrow range of the heating temperature. The very broad endotherm below 100°C rather corresponds to a dehydration of the γ-alumina surface (Mackenzie, 1970), which is not associated with any reaction of the adsorbed amino acid. A small conversion of Ala, observed by the experiments of reaction yield measurements under similar conditions, would not be detected by thermal analysis measurements. The low temperature endotherm in the DTA curve of neutral alumina is composed of a shoulder at about 30°C and the main endotherm near 60°C. DTA curves of Ala-γ-alumina samples have only one, slightly less broad endotherm at 50°C in this range. Adsorption of Ala on the alumina surface is probably accompanied by changes in the distribution of water, which is detected in changes of DTA curves in the low-temperature range. A very slight endothermic reaction is detected at higher temperatures (150–200°C) in the cases of the samples containing mixtures of γ-aluminas and Ala. Pure neutral alumina does not exhibit this reaction, which indicates the origin of this endotherm to be amino acid reactions. The shift of the endotherm to considerably lower temperatures with respect to that of pure Ala confirms the catalytic effect of γ-aluminas on Ala reactions. The lowering of the endothermic character may be explained in two ways:

1. Ala reactions proceed only to a limited degree, with respect to the thermal decomposition of Ala itself or to that on the surface of α-alumina.
2. γ-alumina surface, activated by thermal treatment, may act as a source of energy for Ala reactions (Fig. 2). Hence, the endothermal decomposition of amino acid would be partially balanced with the exothermal reaction of alumina with water, formed from amino acid reactions. The overall reaction heat would then average to nearly zero.

The trends observed by DTA measurements of γ-alumina Ala mixtures are also supported by the results of TG and DTG measurements. A big difference is observed compared to the TG and DTG measurements of the Ala α-alumina reaction system. The heating of neutral γ-alumina Ala mixture induces a larger mass loss than that of pure alumina itself. This difference can be attributed to mass losses due to both the reaction of Ala and release of the water, having been adsorbed to the alumina surface from the Ala solution. It is difficult to estimate the temperature ranges for distinct partial reactions for the sample of neutral γ-alumina-Ala from TG measurements. DTG detects mass loss below 100°C, being of the same origin as in the case of pure alumina. The mass loss in the range of 100–200°C is associated with the reactions of Ala. This process is overlapped with a continuous decrease of mass, probably

due to the condensation of structural OH groups and liberation of water from the surface or pores (Pines and Manasses, 1996) or partially due to reactions of the amino acid.

Consequences for mineral-mediated prebiotic processes

Alumina catalyzed amino acid dimerization is a good example for a reaction leading to the formation of peptide bonds, fulfiling the criteria of a pre-biotically possible reaction system. The yield of this model reaction for Ala dimerization reaches 3% after 14 days and, which is very important, continually increases within the whole time range. The reaction proceeds at temperatures below the boiling point of water and is most efficient on the surface of pH-neutral γ-aluminas. However, synthetic γ-alumina in activated forms as used in our experiments does not occur in the earth crust. On the other hand, α-alumina, very common in nature as mineral corundum, also exhibits catalytic activity under the same conditions although to a much lower extent. This indicates that catalytic sites identical to those on the surface of activated aluminas, may also occur on the surface of other natural aluminum oxide forms and related minerals. Therefore, the same activation mechanism may have been relevant also for other compounds of similar structure, e.g. for the clay minerals which have been considered frequently because of their chemical activity and occurrence in earth crust. The surface of the clay mineral kaolinite and other related minerals is structurally similar to that of alumina. Al—OH groups at the sheets of octahedrons $AlO_2(OH)_4$ form a chemically active fraction of the kaolinite surface. Moreover, there are some other similarities of alumina and kaolinite samples exposed to thermal treatment:

1. Both pre-heated kaolinite and alumina exhibit luminiscent properties (Coyne et al., 1981, 1983) as mentioned in the introduction.
2. Kaolinite changes its structure upon heating in a similar way as γ-aluminum hydroxide: It looses its hydroxyl groups bound to Al atoms by dehydroxylation reaction. Metakaolin is partially dehydroxylated kaolinite, which contains, depending on the heating conditions, variable fractions of "activated" sites, formed by the dehydroxylation of surface Al—OH groups. Tetrahedral coordination of Al atoms with Lewis acid properties in metakaolin has been reported (Stubican and Roy, 1961; Brindley and McKinstry, 1961) and phases of γ-alumina were detected in samples of heated kaolinite (Roy et al., 1958; Tsuzuki, 1961).

Assuming therefore, that thermal activation might positively affect the catalytic efficiency of kaolinite, thermally treated kaolinite samples were prepared and their catalytic efficiency for peptide bond formation was tested. The reaction systems, containing kaolinite heated above 450°C, produced detectable amounts of Ala_2 but only <0.05%. Kaolinite samples pre-heated at lower temperatures did not form any Ala_2. Figure 5 shows the reaction yields of Gly_2 formed from Gly as a function of pre-heating temperature.
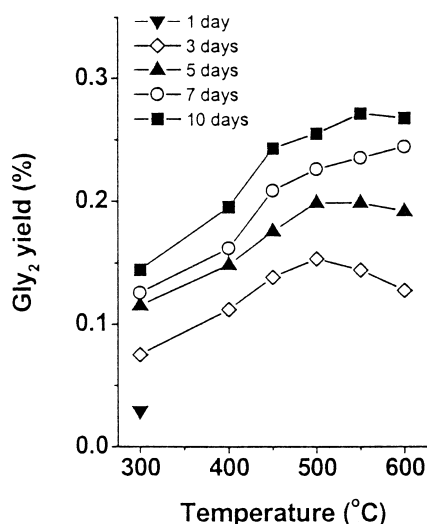
Fig. 5. The effect of kaolinite pre-heating temperature on the reaction yields of Gly$_2$ from Gly

The reaction yields of parent, unheated kaolinite was not significantly different from those of "blank" experiments (below 0.1%). Thermal activation, however, leads to an increase of the reaction yields up to 0.27%. Hence, the difference between the catalytic efficiencies of unheated and heated kaolinite samples is significant, but the yields obtained on thermally treated kaolinites are still very low. The observed yields' increase, however, indicates a possible relevance of thermal activation for kaolinite catalytic efficiency for peptide bond formation. A definitive answer would require further studies with detailed investigation of the changes of kaolinite structure upon heating.

Acknowledgements

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