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ALUMINA CATALYZED REACTIONS OF AMINO ACIDS

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Abstract

Thermal reactions of glycine (Gly), alanine (Ala), leucine (Leu), valine (Val) and proline (Pro) adsorbed on activated alumina were studied by means of thermal analysis. In the absence of alumina, decomposition of amino acids was detected as a sharp endotherm above 200°C, whereas no thermal effects were detectable by differential thermal analysis (DTA) and differential scanning calorimetry (DSC) for amino acid/alumina mixtures. This could be explained by a continuous amino acid condensation to peptides and simultaneous absorption of formed water by alumina, the latter being gradually released at higher temperatures. Thermogravimetry (TG) and differential thermogravimetry (DTG) measurements revealed that the reactions of the amino acids adsorbed on alumina surface were spread over a wide range of temperatures. The catalysis of peptide bond formation on alumina surface at 85°C was proven directly by the identification of the reaction products, mainly dipeptides and cyclic anhydrides.

Keywords: activated alumina, amino acids, peptide bond formation, thermal analysis

Introduction

Inorganic compounds have certainly played a role in the molecular evolution of the first, simplest bioorganic compounds on primitive earth [1]. The most relevant scenarios have attributed an important role to the catalytic properties of inorganic compounds in the formation of first peptides [2]. Condensation reactions between amino acids do not proceed readily under the conditions considered to have been prevalent on the primordial earth [3]. Amino acids form short oligopeptides and cyclic anhydrides only if they are chemically activated and in the presence of catalysts or energy-rich compounds [4]. Only glycine (Gly), the simplest and the most reactive amino acid, forms traces of dimer and anhydride in reactions without catalyst and under relatively mild conditions [5]. A few reports in the last two decades presented the peptide bond formation from a few amino acids catalyzed by various inorganic compounds [1, 2, 5–10].

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Activated alumina is one of the most efficient catalysts for the peptide bond formation [6–8]. It produces dipeptides from all the amino acids tested recently [5]. Recent studies emphasized the unique properties of activated aluminas to catalyze reactions, where water had been one of reaction products [7]. Activated alumina has a large specific surface area and variable structure, including different chemically active sites, such as Lewis acid and base sites. Alumina does not adsorb water in the same way as most other similar solid materials do. Instead, it decomposes water molecules and partially changes its bulk composition and structure. This feature may play a key role in the alumina-catalysed peptide bond formation, which is in principle the condensation reaction, yielding water as one of reaction products.

Fast decomposition of amino acids at temperatures >200°C includes initial condensation reactions: cyclic anhydrides, linear oligopeptides, bicyclic and tricyclic amidines and further products are formed and subsequently decomposed [9]. Therefore, simple measurements of thermal analysis methods could be used as a tool for the estimation of catalytic efficiency of alumina for such reactions. Besides the condensation reactions other reactions also proceed but to a lower extent. For example, amino acid racemization of optically active amino acids may occur [10, 11] and, at higher temperatures, the formation of constituent gases (CO_2, H_2O, NH_3, CO) and a variety of simple, volatile organic compounds (amines, nitriles, amides, hydrocarbons, etc.) [12]. The objective of this work was to investigate the reactions of amino acids on alumina surface by the methods of thermal analysis. The reactions were conducted in the presence and absence of the catalyst.

Experimental

Activated alumina (γ-alumina, neutral pH, type Brockmann I, 150 mesh) was purchased from Aldrich. Amino acids of analytical grade were purchased from Sigma (Germany) and Bachem (Switzerland). For the reactions only *L*-optical isomers of amino acids were used. Alumina was mixed with aqueous amino acid solutions to get the ratios 1 mmol g^{-1}. The suspensions were dried at 85°C for 3 h, mixed and directly used for the thermal analysis measurements: differential thermal analysis (DTA), thermogravimetry (TG) and differential scanning calorimetry (DSC). DTA and TG were carried out by a SDT 2960 apparatus, TA instruments, with a sample amount of about 15 mg, under nitrogen atmosphere and with a heating rate of 2 K min^{-1} within the range 20–320°C. Fused α-Al_2O_3 was used as a reference compound. DSC measurements were carried out under similar conditions as DTA and TG on a 910 differential scanning calorimeter (DuPont). The products formed from the same samples, kept for 28 days at constant 85°C, were analyzed with an Agilent 1100 Series LC System using a Shannon Hypersil (ODS 5 μm/200×2.1 mm) column. The mobile phase contained 10 mM sodium hexanesulphonate and was adjusted to pH 2.5 with H_3PO_4. The solvent for the separation of hydrophobic oligopeptides (Ala_2, Val_2, Leu_2, Pro_2) was modified with appropriate amount of acetonitrile. Detection was performed with a diode array detector at 195 nm. The reaction products were identified according to retention times of reference substances. More details on the methods have been published elsewhere [13].

Results and discussion

Decomposition of free amino acids

First steps of the reactions of simplest amino acids induced by heating include the condensation reactions of carboxyl and amino groups leading to the formation of peptide bonds [14]. The main reaction products of such reactions are cyclic anhydrides and linear peptides. Only for amino acids with further reactive functional groups (e.g. $–NH_2$, –COOH, –SH, –OH) in side chains other reaction products are formed [15,16]. In this work we used only α-amino acids with aliphatic side chains on α-carbon atom without reactive functional groups: H– (Gly), CH_3– (Ala), $(CH_3)_2$–CH–CH_2– (Leu) and $(CH_3)_2$–CH– (Val). Pro is a cyclic aliphatic amino acid of more complicated structure with a secondary amino group, but also without any additional reactive groups. Therefore, we assume, that the role of the side chains in amino acid condensation played only a secondary role and that they were not much involved in the reactions. However, besides the condensation reactions the formation of other reaction products, which could be formed due to deamination or decarboxylation reactions, cannot be neglected [11, 17].

Thermal behaviour of free amino acids is illustrated in Figs 1, 2. Reactions of amino acids were detected in the temperature range between 200 and 300°C, but reactions at lower temperatures cannot be excluded, even they were not detectable by methods used. The endothermic reactions at high temperatures (200–300°C) recorded by DTA measurements (Fig. 1) were in principle the same as those reported elsewhere [14, 17]. For Gly, Ala, Val and Leu, the DTA patterns display a single endothermic peak. The positions of these peaks probably depend on amino acid reactivities under the given conditions and increased in the order: 234 (Gly), 265 (Val),

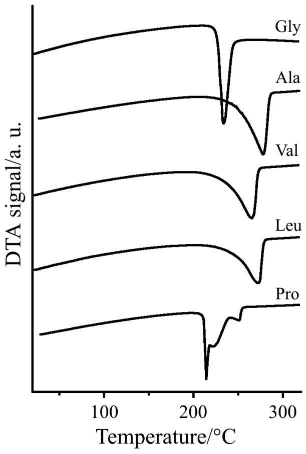

Fig. 1 Differential thermal analysis of amino acids

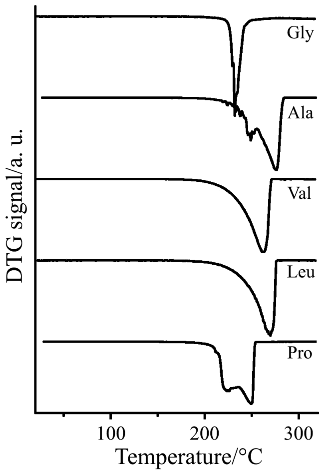

Fig. 2 Differential thermogravimetry of amino acids

271 (Leu), 277°C (Ala). On the other hand, more endothermic peaks appear in the DTA pattern of Pro. The decomposition of Pro began with the endothermic reaction characterized with a very sharp, negative peak (214°C) partially overlapped with a broad endothermic peak (220°C) and followed by another smaller one with a maximum at higher temperature (250°C). The existence of several bands in the DTA pattern of Pro may be related to the formation of a relatively stable cyclic anhydride as one of the main products of the heat-induced reaction [18], and this cyclic anhydride formation could be reflected by the sharp endothermic peak. For other amino acids the reaction products formed in the first steps of the decomposition were probably not more stable than the amino acid themselves and therefore, DTA did not recognize the distinct steps of the reaction processes. Instead, when the endothermal reactions of other amino acids had started, they proceeded very fast and were detected as single peaks. One should mention, that the endothermal reaction of Pro started at a temperature lower than those of the other amino acids. The above trends are basically in agreement with other work, studying the sensitivity of amino acids to pyrolytic decomposition [18].

DTG patterns (Fig. 2) are in principle very similar to the DTA ones (Fig. 1). The DTG curves reflect the release of volatile products formed during the heating. The points of the maximal release of the volatile compounds are indicated by the minima of the DTG negative peaks: 232 (Gly), 276 (Ala), 262 (Val), 269 (Leu), 224 and 250°C (Pro). There are only negligible differences between shapes and positions of the DTA and DTG peaks for the reactions of Gly, Ala, Val and Leu. The multi-step decomposition reaction of Pro was indicated by both DTA and TG measurements, although the sharp endotherm observed in the DTA pattern (Fig. 1, 214°C) was not reflected by a significant change of mass (Fig. 2). The maximal release of the volatile decomposition compounds proceeded in the last stage of the reaction, although the endothermic effect at these temperatures was rather weak (Fig. 1).

Thermal behaviour of amino acids adsorbed on alumina

Catalysts decrease the activation energy of chemical reactions, increasing the reaction rate and decreasing temperatures of reactions. One could, therefore, expect shifts of the DTA peaks to lower temperatures for the experiments with amino acid/alumina mixtures, taking into account that the catalyst may also increase the rate of side-reactions and decomposition. On the other hand, one of the roles of the solid surfaces in the heterogeneous catalysis can be the stabilisation of one of the reaction products. Indeed, no band was detected in the temperature range above 100°C as shown in the DTA patterns measured for the mixtures with alumina (Fig. 3). The absence of any discontinuous reaction detectable by DTA is in contrast to the decrease of mass proceeding in the broad range of temperatures (Fig. 4). The mass decrease at higher tem-

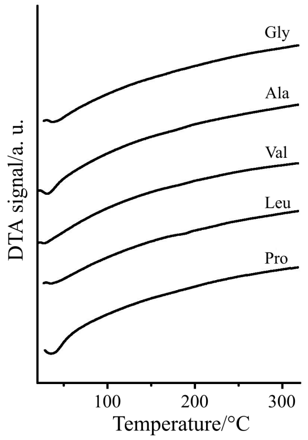

Fig. 3 Differential thermal analysis of amino acid/alumina mixtures

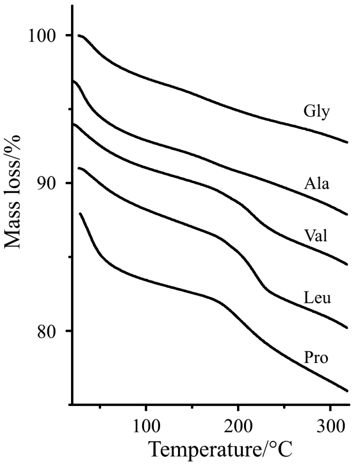

Fig. 4 Thermogravimetry of amino acid/alumina mixtures

peratures (>100°C) might be related to the release of volatile compounds formed by the reactions of amino acids, in particular of water during condensation. A hardly detectable discontinuous process of mass decrease seems to occur (only for Val, Leu and Pro) near 200°C. It becomes visible even more clearly in Fig. 5 presenting the DTG patterns calculated from the TG ones. No such peaks appeared in the DTG patterns of Gly and Ala. Reactions including alumina are thermodynamically and probably also chemically very different from the decomposition reactions of pure amino acids. The absence of any observable thermal effect in these reactions does not necessarily mean, that the amino acids did not react at all on the alumina surface. Instead, the reactions were rather spread evenly over a broad range of temperature and accompanied by a slow and constant decrease of mass as shown in the TG measurements (Fig. 4). On the basis of previous studies [5, 7] we can assume that the reactions included mainly condensation reactions leading to the formation of peptide bonds. By adsorption to alumina, the reaction products seem to be protected against further decomposition in contrast to the reactions in absence of the catalyst.

The slight endothermic reactions below 100°C could be assigned rather to the release of adsorbed water from the alumina surface or from the condensation reaction. It led to the decrease of mass shown in TG and DTG patterns (Figs 4, 5). This mass change was lower for the dehydration reactions of alumina mixtures with Leu and Val, confirmed by relatively lower endothermic signals detected in the DTA patterns of these samples (Fig. 3). This is consistent with the less easy formation of the dipeptides divaline and dileucine observed in the salt-induced peptide formation [2].

As mentioned above, no decomposition reaction was detected by the DTA measurements for amino acid/alumina mixtures. In order to track the thermal decomposition of these reactions more sensitively, we conducted another series of measurements applying a DSC method (Fig. 6). The decomposition reactions of pure amino acids as shown in the DSC and DTA patterns were very similar (Figs 1, 6). The DSC

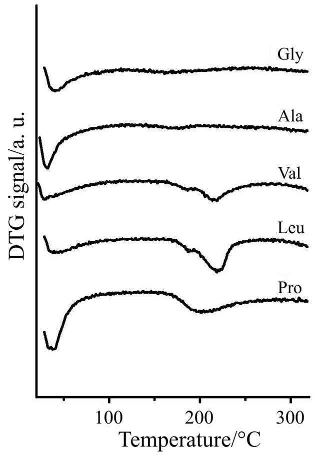

Fig. 5 Differential thermogravimetry of amino acid/alumina mixtures

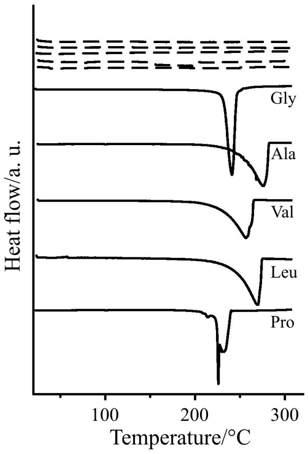

Fig. 6 Differential scanning calorimetry of amino acids (solid) and their mixtures with alumina (dashed). The curves for the mixtures are arranged in the same order as those for pure amino acids

measurements could be used for the estimation of the overall heats of the reactions, which increased in order Pro, Gly, Ala, Val, Leu, i.e. the heats increased with the size of the aliphatic side-chains in amino acid molecules, with the cyclic Pro being an expected exception. The measurements of pure amino acids were then compared with those of the amino acid/alumina mixtures. Despite of the higher sensitivity of the DSC, the results were in principle the same as those observed by the DTA: No discontinuous endothermic reaction was detected during the heating of amino acids adsorbed on the alumina surface.

Peptide bond formation

Amino acids in molten state can produce compounds with peptide bonds [2]. Enhanced reactivity of amino acids was also observed in the adsorbed state of reactants. Especially activated alumina exhibits high catalytic efficiencies at relatively low temperatures [13]. Ala formed traces of dipeptide even at 55°C on activated alumina [6]. Peptide bond formation from amino acids on the alumina surface was also investigated in this work, and the reaction products were detected by liquid chromatography. The most reactive amino acid was Gly. Separation and identification of the reference substances of Gly and its oligopeptides is demonstrated by the chromatogram in Fig. 7. Gly converted to oligopeptides (5.6% Gly_2, 1.9% Gly_3, 0.8% Gly_4) and cyclic anhydride cyc(Gly_2) (16.1%) when heated at 85°C for 28 days. Ala formed approximately equal amounts of Ala_2 and cyclic anhydride cyc(Ala_2) (7.5%) and traces of Ala_3 (0.1%). Lower yields were achieved for Leu and Val: 4.7% Leu_2 and 2.0% Val_2, respectively. No dipeptide was detected with Pro under the same conditions. Hence the reactivity of amino acids decreased in order Gly, Ala, Leu, Val and Pro under these specified conditions. The reactivity of amino acids at 85°C was low and

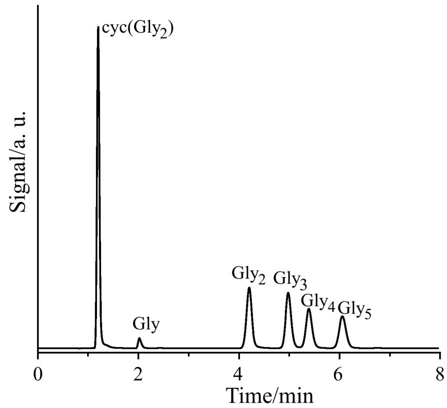

Fig. 7 Chromatogram showing separation of glycine, its cyclic anhydride and oligopeptides

only yields of several % were achieved after heating for 3 weeks. Hence, the reactions at temperatures <100ºC could not have been detected by means of thermal analysis. On the other hand, the finding that alumina catalyses peptide bond formation at temperatures below the boiling point of water is significant from the viewpoint of molecular evolution. The amino acids alone did not produce any oligopeptides under the same conditions, with the exception of Gly, which, however, formed only traces of Gly$_2$ and cyc(Gly$_2$).

Conclusions

Decomposition of amino acids, which includes also the formation of peptide bond, is detectable for all tested amino acids by means of DTA, DTG and DSC at temperatures above 200°C.

The mixtures of amino acids with activated alumina do not exhibit the same features of the thermal discontinuous decomposition as amino acids alone did. Instead, the absence of any endothermic reaction detectable in the DTA and DSC patterns indicates that reactions of amino acids occur smoothly over a broad range of temperature. Moreover, the activated alumina may have acted as a catalyst for amino acid condensation. The activated alumina reacts reversibly with water molecules, changing its structure and releasing energy [19]. Hence, the endothermal reactions of amino acids would be partially balanced by the exothermal reaction of alumina with water, which had been formed by the condensation reaction of amino acid molecules.

The catalytic effect of alumina can be proven by the reaction products, formed by steady heating at 85°C. Dipeptides and cyclic anhydrides are found as the main products. Further, the alumina surface apparently stabilizes these reaction products against decomposition, which could be seen as another relevant factor in prebiotic peptide evolution, in addition to the catalysis of peptide bond formation from amino acids.

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