FORMATION OF AMINO ACIDS ON HEATING GLYCINE WITH ALUMINA

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Abstract. The conversion of glycine into amino acids on heating at 240°C with basic manganous carbonate and alumina is investigated. Alanine, α-aminobutyric acid, norvaline, norleucine, sarcosine, N-ethylglycine, N-methylalanine, N-ethylalanine, aspartic acid and glutamic acid are identified among the products of the reaction. Paper chromatography, ion-exchange chromatography and nuclear magnetic resonance are used for the analysis. A scheme for the observed transformations is presented and it is suggested that it may have been a pathway for the synthesis of amino acids from glycine under primitive Earth conditions.

1. Introduction

In the course of our attempts to obtain porphyrins on heating glycine and succinic acid we found that pyrrolic products were also formed on heating glycine alone under mild conditions (240 °C) in the presence of manganous carbonate and alumina. The difficulties we met in establishing the structure of these quite unstable compounds led us to study the ninhydrin positive products, formed in these experiments. Their initial study showed the presence of some lower amines, as well as of amino acids with a greater number of the carbon atoms than glycine. On the other hand the mixtures obtained in numerous experiments for prebiotic syntheses consist of glycine, almost always predominant, and of amino acids with a longer carbon chain (Ponnamperuma, 1971). The mechanism of their formation under catalytic and thermal conditions has not yet been elucidated. The formation of higher amino acids on heating glycine under mild temperature conditions indicates not only a new possibility for the prebiotic synthesis of amino acids from the simplest one, but could prove also a suitable model system for tracing the path of prebiotic complication of amino acid molecules. For these reasons we undertook the study of amino acids formed on heating glycine at 240°C with alumina and basic manganous carbonate.

Studies on the thermal behavior of amino acids from a point of view of prebiotic synthesis have been carried out under a large variety of conditions (Harada and Fox, 1964; Samochocka *et al.*, 1968) and in most of them the products formed have been identified only by means of chromatographic methods (Vallentyne, 1964). The compounds obtained by Heyns and Pavel (1957) on heating glycine in an open container in the presence of quartz sand have been proved mainly by paper chromatography (PC). Glycyl-glycine, alanine, methylamine, aspartic acid and some carboxylic acids (oxalic, succinic and fumaric) have been identified among the reaction products. In a study of pyrolysis of amino acids at 500 °C by gas chromatography – mass spectrometry the only ninhydrin positive products reported were aliphatic amines (Ratcliff *et al.*, 1973).

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2. Materials and Methods

A. Reagents

All reagents used were p.a. grade or chromatographically pure. The reference compounds N-ethylglycine, N-methylalanine and N-ethylalanine were prepared according to Fischer *et al.* (1916) and were freed from the corresponding alkylammonium salts by means of ion-exchange chromatography.

B. Heating of glycine and identification of the amino acids

Four millimoles of glycine, 100 mg of basic manganous carbonate ($MnCO_3$. $mMn(OH)_2.nH_2O$, Reachim) and 2.5 g of alumina (aluminium oxydatum, aktiv, basisch, für Chromatographie, Merck), well mixed together, were heated in sealed glass tubes under nitrogen atmosphere at 240 °C for one hour. The content of the tubes was extracted repeatedly with distilled water and freeze dried. The residue was hydrolyzed in 6 N HCl for 24 h and the hydrolyzate was passed through a column packed with Wofatit KPS 200 (H^+ form). The column was washed to neutral and the amino acids were eluted with 2 N ammonia. After treatment with active carbon and concentration the eluate was subjected to preparative vertical electrophoresis at 1500 V (pH of the buffer solution 5.6) and the neutral, basic and acidic fractions were eluted. The neutral fraction was separated by descending PC on Whatman 3 in the system *n*-butanol-pyridine-acetic acid-water (30 : 20 : 6 : 24). A second preparative electrophoresis was used for separating the individual acidic amino acids. The basic fraction was studied by PC.

Experiments were carried also in which different fractions of the unhydrolyzed neutral products were studied.

Besides the usual ninhydrin reaction, for detecting the N-alkylamino acids we used successfully the colour reaction recommended by Feigl (1966) for secondary amines – after spraying with a mixture of dilute solution of Na_2CO_3 and a solution of sodium nitroprussiate in acetaldehyde and cautiously heating, a typical blue colouring is observed, differing from the grey-violet colouring for the common α-amino acids.

All NMR spectra were taken on a JEOL MS-100 NMR spectrometer in F_3CCOOH with TMS or HMDSO as internal standard. The chemical shifts are given in δ units.

The amino acid analyses were carried out on an automatic amino acid analyzer Bio-Cal BC 200 on Aminex A5, using the procedure of Spackman *et al.* (1958).

3. Results

By means of PC we could establish two types of amino acids in the fraction of the neutral amino acids:

(1) α-Amino monocarboxylic acids with a straight chain – alanine, α-aminobutyric acid, norvaline and norleucine.

(2) N-alkyl-substituted-α-amino monocarboxylic acids – sarcosine, N-ethylglycine, N-methylalanine and N-ethylalanine.

A third type of amino acids was found in the fraction of acidic amino acids:

(3) α-Amino dicarboxylic acids – aspartic acid and glutamic acid. The spot corresponding to aspartic acid gave the characteristic blue colouring when sprayed with a collidine solution after the usual spraying with ninhydrin.

The fraction of basic amino acids isolated by preparative electrophoresis was in a very small quantity, As the predominating amino acid in it had a little lower Rf value than that of ornithine we synthesized 2,3-diamino propanoic acid for reference (Caspary and Tollens, 1873; Cheronis, 1947), but it proved to have a different Rf value from the amino acid, formed in the experiment. The latter could possibly be 2,4-diamino butanoic acid, which we have not yet proved.

In spite of our efforts some neutral and acidic amino acids also remained unidentified. We assumed one of them, located on the chromatogram considerably lower than glycine, to be α,α′-diaminoglutaric acid, but this was not confirmed. The diaminoglutaric acid, synthesized via glutaric acid dichloride and α,α′-dibrom glutaric acid dichloride, had a different Rf value.

As in similar experiments where it is possible to obtain amino acids different from the reference compounds but of the same Rf value, we tried to support these results using another identification method. We choose NMR spectrometry because its data may be interpreted unambiguously, particularly when the spectrum of the reference compound is available, and because a high purity of the sample is not needed. In this way the structures of almost all amino acids identified by PC were confirmed: Ala, α-aminobutyric acid, norVal, norLeu, Sar, N-ethylglycine, N-methylalanine, Asp and Glu. Only N-ethylalanine was isolated in a quantity insufficient for taking its NMR spectrum. The spectrum of the fraction, whose Rf value corresponds to N-ethylglycine and N-methylalanine, showed the presence of both acids with the predominance of the former one. The chemical shifts and the general aspect of our NMR spectra corresponded to those described by Bovey and Tiers (1959), but with the higher resolution instrument used by us, the fine structure of the spectra could be observed. The chemical shifts of the protons in the NMR spectra of the amino acids are given in Table I. There are additional signals due to impurities in some of the spectra, but the coincidence of the chemical shifts, coupling constants and integration dated with those of the references prove their identity unambiguously.

We obtained additional data for the identification of the amino acids from the quantitative amino acid analyses of the mixtures from experiments heated for 1 and 24 h. Using corresponding reference compounds we confirmed the presence of Asp, Sar, Glu, Gly, Ala, α-aminobutyric acid, norVal and norLeu in these mixtures. Table II presents their quantities obtained from experiments heated 1 and 24 h. Thus the amino acids formed were identified by three different methods – PC, amino acid analysis and NMR spectrometry.

The unhydrolyzed products extracted from the reaction mixture after heating for 1 h at 240 °C were found to contain peptides besides amino acids. In the solvent system used for PC glycyl-glycine has a Rf value close to that of glycine, so that it could not be established because of the great quantity of glycine. For this reason the fraction containing glycine and the products with a little lower Rf value was isolated by preparative PC. Its amino acid analysis showed the presence of glycine, diglycine, triglycine and tetraglycine in decreasing quantities, as well as some

TABLE I

Chemical shifts of the protons of the isolated amino acids as well as of the reference compounds in trifluoroacetic acid

Amino acids	NH_3^+	α-CH	β-CH_2	γ-CH_2	CH_3	NCH_3	NCH_2	NCH_2CH_3
Alanine	7.36	4.01			1.49			
	(b)	(m)			(d)			
α-Aminobutyric acid	7.30	4.33	2.21		1.18			
	(b)	(m)	(m)		(t)			
Norvaline	7.27	4.34	2.10	1.58	1.04			
	(b)	(m)	(m)	(m)	(t)			
Norleucine[a]	4.81	3.60	1.92	1.41	0.90			
	(s)	(t)	(m)	(m)	(t)			
Aspartic acid	7.28	4.32	3.07					
	(b)	(m)	(d)					
Glutamic acid	7.26	4.12	2.09	2.53				
	(b)	(m)	(q)	(t)				
Sarcosine	7.30	3.80				2.68		
	(b)	(t)				(t)		
N-ethylglycine	7.10	3.77					3.03	1.07
	(b)	(t)					(m)	(t)
N-methylalanine	7.12	3.83			1.37	2.62		
	(b)	(m)			(d)	(t)		

In all the cases (b) means broad peak.
[a] The spectra are taken in D_2O. The peaks of the δ protons coincide with those of the γ protons.

unidentified ninhydrin positive products. After hydrolysis in the usual manner the peaks of di-, tri- and tetraglycine disappeared. It is very probable some of the other ninhydrin positive products to be also peptides of glycine and of amino acids formed in predominant amounts. The Rf value of one of the components of the unhydrolyzed mixture coincides very well with that of glycylalanine.

The formation of ammonia under the experimental conditions was established by the NMR spectrum of the gas, evolved at opening the tubes and caught in dilute HCl. The spectrum consists of a strong triplet at 7.06 ppm with J = 52 Hz and very weak signals for the aliphatic protons.

TABLE II

Amino acid analyses of the reaction mixtures obtained on heating 4 mmoles of glycine with alumina and basic manganous carbonate at 240 °C for 1 h and 24 h (in μmoles)

Amino acids	1 hour	24 hours
Aspartic acid	8.0	6.2
Glutamic acid	13.8	19.0
Glycine	587	46.2
Alanine	108	26.6
α-Aminobutyric acid	54.6	19.0
Norvaline	12.5	9.2
Norleucine	1.66	2.0

4. Discussion

It is well known that decarboxylation is one of the main ways of degradation of amino acids on heating. The formation of a considerable quantity of ammonia on the other hand (besides its presence in the gas evolved, it is undoubtedly partially bound with CO_2 as ammonium carbamate) shows that the elimination of ammonia is the other essential process leading to formation of higher amino acids. Therefore they are formed by lengthening, probably step by step, of the glycine molecules, and every single step includes elimination of NH_3 and CO_2. In a most general way these processes can be expressed by the following equation:

$$n\mathrm{NH_2.CH_2.COOH} \longrightarrow \mathrm{NH_2.CH(CH_2)_{n-2}CH_3.COOH} + (n-1)\mathrm{NH_3} + (n-1)\mathrm{CO_2}$$

As to the sequence of these reactions, it is logical to assume that the elimination of ammonia necessary for the binding, e.g., of two molecules glycine, precedes the decarboxylation. Thus the amino dicarboxylic acids are an early stage in the formation of the amino monocarboxylic ones. For instance the first stage in the formation of alanine can be presented schematically in the following way:

$$\begin{matrix}\mathrm{NH_2.CH_2.COOH} \\ \mathrm{NH_2.CH_2.COOH}\end{matrix} \xrightarrow{-\mathrm{NH_3}} \mathrm{NH_2.CH(CH_2.COOH).COOH}$$

The formation of N-alkylamino acids shows clearly that the elimination of ammonia from two glycine molecules may follow the other possibility:

$$\begin{matrix}\mathrm{NH_2.CH_2.COOH} \\ \mathrm{NH_2.CH_2.COOH}\end{matrix} \xrightarrow{-\mathrm{NH_3}} \mathrm{NH(CH_2.COOH).CH_2.COOH}$$

It is clear that a consequent decarboxylation will transform the first product in alanine and the second in sarcosine. It must be borne in mind that there is another possibility for the formation of the N-alkylamino acids – alkylation of amino acids with aliphatic amines, for the formation of which we have preliminary indications. For instance sarcosine can be obtained in this way:

$$\begin{matrix}\mathrm{NH_2.CH_2.COOH} \\ \mathrm{NH_2.CH_3}\end{matrix} \xrightarrow{-\mathrm{NH_3}} \mathrm{NH(CH_3).CH_2.COOH}$$

The other amino acids we established can be obtained in a similar way from the corresponding amino acids: N-ethylglycine from glycine and alanine or from glycine and ethylamine; N-methylalanine from alanine and glycine or from alanine and methylamine; N-ethylalanine from two molecules alanine or from alanine and ethylamine.

As to the mechanism of formation of alanine and the other higher amino acids with a straight chain, in view of the mobility of the α-hydrogen atoms of glycine an attractive pathway is the formation of aspartic acid by the direct binding of two glycine molecules with elimination of ammonia. Bearing in mind, however, the presence of a metal of variable valency and the milder experimental conditions we

consider as more likely a pathway involving the preliminary dehydrogenation of part of the glycine molecules. The iminoacetic acid formed reacts with glycine in a way analogous to the condensation of glycine with carbonyl compounds (Erlenmeyer, 1894). The so formed aminoethylenedicarboxylic acid tautomerizes to iminosuccinic acid and after β-decarboxylation (as β-imino acid) is converted to iminopropanoic acid, the latter being hydrogenated to alanine. In the presence of a metal of variable valency the dehydrogenation of the amino acids and the hydrogenation of the imino acids formed can take place as a catalyzed process of reciprocal hydrogenation – dehydrogenation. Thus the formation of the amino acids established by us can be presented in Figure 1. In this scheme the binding of every imino acid with glycine under elimination of ammonia leads to unsaturated di- or tricarboxylic acid which tautomerizes into the corresponding imino acid and then decarboxylizes.

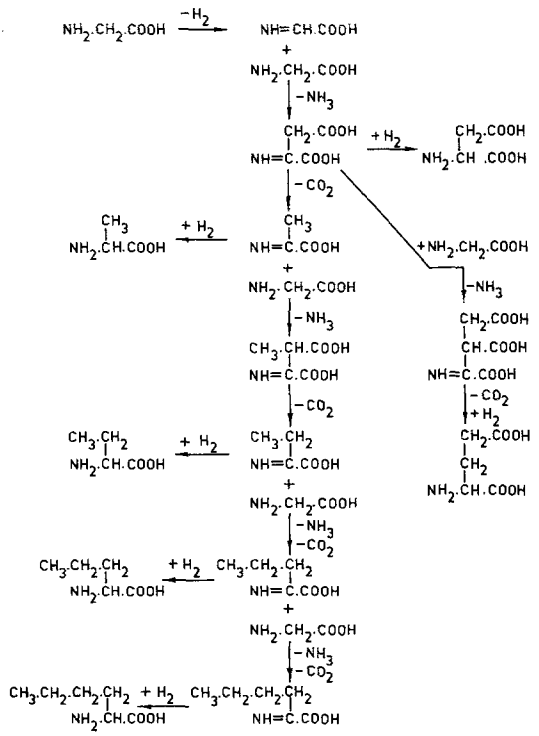

Fig. 1. Suggested scheme of the conversion of glycine into higher amino acids.

This scheme remains essentially the same if we assume that the imino acids are first hydrolyzed to the corresponding aldehydo- or keto-acids which then are condensed in the same manner with elimination of water instead of ammonia.

A similar conversion of the amino acids into their next higher homologs is supported by the observed ratios, increasing on prolonged heating: α-aminobutyric acid/Ala (0.51 at 1 h, 0.71 at 24 h), norVal/α-aminobutyric acid (0.23 and 0.48 respectively), norLeu/norVal (0.13 and 0.29 respectively) and Glu/Asp (1.73 and 3.07 respectively).

This scheme explains the formation of all the amino acids, established by us until now, by one and the same processes, with the exception of the alkylamino acids. As above described, these are formed by another pathway. In view of the different energy sources it is logical for the mechanism discussed to differ from the pathway for the formation of amino acids on γ-irradiation of N-acetylglycine and ammonia (Dose and Ponnamperuma, 1967).

We consider the established possibility for prebiotic conversion of glycine into other amino acids, some of which are components of contemporary proteins, quite important for determining the pathways for the chemical evolution of amino acids. The fact that glycine is present in 50 or more per cent in almost all mixtures of amino acids obtained in simulating experiments for prebiotic syntheses suggests that its formation from simple gas mixtures is comparatively easy. On the other hand the results of our experiments show that it can be transformed into more complicated amino acids under comparatively mild temperature conditions. The conditions necessary for a similar transformation of glycine (composition of the mineral template, temperature) could easily arise on the primitive Earth and thus the occurrence of analogous processes become quite probable. Therefore we can assume that the transformations of glycine studied by us are one of the possible pathways for prebiotic synthesis of more complicated amino acids in simulating experiments and on the primitive Earth.

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