

NOVEL FORMATION OF  $\alpha$ -AMINO ACIDS FROM  $\alpha$ -OXO ACIDS AND AMMONIA IN AN AQUEOUS MEDIUM

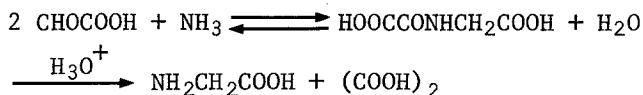
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**Abstract:** In the course of a study of possible mechanisms for chemical evolution in the primeval sea, we found the novel formation of  $\alpha$ -amino acids and N-acylamino acids from  $\alpha$ -oxo acids and ammonia in an aqueous medium. Glyoxylic acid reacted with ammonia to form N-oxalylglycine, which gave glycine in a 5-39% yield after hydrolysis with 6N HCl. Pyruvic acid and ammonia reacted to give N-acetylalanine, which formed alanine in a 3-7% overall yield upon hydrolysis. The pH optima in these reactions were between pH 3 and 4. These reactions were further extended to the formation of other amino acids. Glutamic acid, phenylalanine and alanine were formed from  $\alpha$ -ketoglutaric acid, phenylpyruvic acid and oxaloacetic acid, respectively, under similar conditions. N-Succinylglutamic acid was obtained as an intermediate in glutamic acid synthesis. Phenylacetylphenylalanineamide was also isolated as an intermediate in phenylalanine synthesis. Alanine, rather than aspartic acid, was produced from oxaloacetic acid. These reactions provide a novel route for the prebiotic synthesis of amino acids. A mechanism for the reactions will be proposed.

Egami pointed out that a close correlation exists between the concentration of transition elements in contemporary sea water and their biological behavior, and he postulated that transition elements that are relatively abundant in sea water such as molybdenum, iron and zinc must have played important roles in the chemical evolution in the primeval sea (1). With this idea as a basis, we have been studying the formation of biomolecules in a modified sea medium (2,3). In the course of this research, we recently found that glycine and alanine are

formed from various sugars such as glycolaldehyde, glyceraldehyde, erythrose, ribose and glucose with ammonia in a modified sea medium (4). In the course of further studies, it has been found that a certain compound accumulates in a reaction mixture containing glyoxylic acid and ammonium sulfate; this compound gives glycine and oxalic acid after 6N HCl hydrolysis. The structure of the compound has now been identified as N-oxalylglycine (5).



It is well known that ammonia combines with oxo compounds to give imines in the presence of acid (6,7), however, there has been as yet no example of the isolation of an amide compound such as N-oxalylglycine in the reaction.

We wish to report a novel reaction of  $\alpha$ -oxo acids with ammonia in an aqueous medium. The reaction provides not only a new pathway for the prebiotic synthesis of amino acids (8,9) but also a new and facile way for the formation of  $\alpha$ -amino acids and N-acylamino acids (10,11).

Glycine was obtained in 39% yield from the reaction of 0.5 M glyoxylic acid and 0.25 M ammonium sulfate at 27°C and pH 4, followed by lyophilization and hydrolysis in 6N HCl. A repeat of the same experiment without lyophilization provided a much lower yield (3%) of glycine. However, the yield of glycine obtained from the reaction at high temperature (105°C) was independent of lyophilization. Acidic pH was more favorable than alkaline pH for the formation of glycine. The yield after hydrolysis was considerably higher than that before hydrolysis. These facts suggest that there is a glycine derivative, produced by lyophilization, which yields glycine after 6N HCl hydrolysis.

A large scale reaction (50 ml) of glyoxylic acid (0.5 M) with ammonium sulfate (0.25 M) was carried out in a similar manner with the aim of isolating the intermediate compounds. The resulting solution was lyophilized to a pale yellow powder. To the powder were added 200 ml of ethanol, and the mixture was stirred at room temperature for 5 h. and then filtered. Removal of ethanol *in vacuo* yielded 385 mg of a reddish yellow oil. This oil was then dissolved in a mixture of equal amounts of methanol and 1N NaOH to give white crystals. The crystals were recrystallized from a mixed solvent of methanol and water. Selected physical data of these crystals were as follows: mp > 300°C, IR(KBr) 3470-3300 (s), 1680 (s), 1660 (s), 1603 (s), 1530 (s), 1403 (s), 1300 (m), 1240 (m)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$ 4.29 (s, 2H); elemental analysis, C, 23.83; H, 1.69; N, 6.71%. The

IR spectrum indicates the presence of a secondary amide group. Elemental analysis indicates the composition of  $C_4H_3O_5NNa_2 \cdot 0.5 H_2O$ . These data identify the crystals as probably the disodium salt of N-oxalylglycine. The free acid was obtained from the disodium salt through a column of Dowex 50 :  $^1H$  NMR ( $D_2O$ )  $\delta$ 4.14 (s, 2H);  $^{13}C$  NMR ( $D_2O$ )  $\delta$ 41.5 (methylene), 161.5 (carbonyl of amide), 162.8 (COOH of oxalic acid moiety), 173.1 (COOH of glycine moiety). To confirm the identity of the free acid it was methylated with diazomethane to give the corresponding methyl ester, which showed a molecular peak at 175 with significant peaks at 145, 88, 59, 56 and 44. These spectral data suggested that the free acid is N-oxalylglycine. This was confirmed by comparison with an independently synthesized sample of N-oxalylglycine.

The effect of drying on the formation of N-oxalylglycine from glyoxylic acid and ammonia was examined. N-oxalylglycine was not formed from glyoxylic acid and ammonia without drying. It was obtained by lyophilization, drying with a blower, drying with a rotary evaporator, and drying at room temperature. The yield increased with repetition of the drying cycle. Lyophilization and drying with a blower gave a good yield of N-oxalylglycine. The pH optimum for the formation of N-oxalylglycine was pH 3-4.

N-Formylglycine was obtained in a reaction of glyoxylic acid and ammonium sulfate at 105°C and pH 4. This fact suggests that N-oxalylglycine is formed at low temperature and N-formylglycine is formed at high temperature from glyoxylic acid and ammonia. A plausible mechanism for the formation of N-oxalylglycine from glyoxylic acid and ammonia is shown in Fig. 1.

In order to ascertain whether this type of reaction occurs generally between an oxo acid and an amine, we carried out the reaction of glyoxylic acid (0.5 M) with methylamine (0.5 M) in a similar manner. Glyoxylic acid reacted with methylamine to produce sarcosine in a 11-25% yield after 6N HCl hydrolysis. High-performance LC of the reaction product obtained before hydrolysis showed a peak corresponding to N-oxalylsarcosine. Collection of the high-performance LC peak gave a product with chromatographic behavior identical to that of N-oxalylsarcosine. Thus N-oxalylsarcosine may be formed in a similar manner from glyoxylic acid and methylamine.

A reaction of pyruvic acid (0.5 M) and ammonium sulfate (0.25 M) provided alanine both before and after hydrolysis in 6N HCl; separation by high-performance LC of the product before hydrolysis gave a peak corresponding to N-acetylalanine, which was identified by direct comparison with an authentic sample.

$\alpha$ -Ketoglutaric acid (0.5 M) and ammonium sulfate (0.25 M) reacted to give the corresponding N-succinylglutamic acid, which formed glutamic acid in 5-22% yield upon hydrolysis. The yield of glutamic acid in acidic and alkaline solutions was higher than that in neutral solution. Heating of N-succinylglutamic acid at 105°C gave pyroglutamic acid and succinic acid. N-succinylglutamic acid may be formed in a similar manner from  $\alpha$ -ketoglutaric acid and ammonia.

Treatment of phenylpyruvic acid (0.5 M) with ammonium sulfate (0.25 M) led to immediate precipitation of the white needles of N-phenylacetylphenylalanineamide (mp 190-191°C), which was identified by IR (3170, 1660, 1640, 1530  $\text{cm}^{-1}$ ),  $^1\text{H-NMR}$  ( $\text{d}_6\text{-DMSO} + \text{D}_2\text{O}$ )  $\delta$ 7.18 (s, 10H), 4.45 (m, 1H), 3.38 (s, 2H), 3.20-2.60 (m, 2H), and Mass  $m/e$  282 ( $\text{M}^+$ ). This identification was also confirmed by an independent synthesis. A small amount of N-phenylacetylphenylalanine was also detected in the reaction mixture. Phenylalanine was obtained from phenylpyruvic acid and ammonium sulfate in a 14% yield after hydrolysis.

Oxaloacetic acid reacted with ammonium sulfate to give alanine in 9% yield after hydrolysis instead of the expected aspartic acid. Similarly, a reaction of hydroxypyruvic acid with ammonium sulfate led to the production of serine, glycine and alanine. The formation of the N-acylamino acids and amino acids from the corresponding  $\alpha$ -oxo acids and ammonia is summarized in Table I and a reasonable mechanism for their formations is shown in Fig. 2.

Finally we describe a possible scheme for the formation of amino acids from oxo acids and ammonia in the primeval sea. At the initial stage of chemical evolution, formaldehyde may have been formed from carbon monoxide (12), carbon dioxide (13) and water by UV irradiation, or from methane and water by electric discharge (14). It is well known that formaldehyde reacts by aldolcondensation to form formose (15). The resulting  $\text{C}_2$ -compounds such as glyoxylic acid in formose reacted with ammonia to form glycine *via* N-acylglycine. The resulting  $\text{C}_3$ -compounds such as pyruvic acid reacted with ammonia to form alanine *via* N-acylalanine. Similarly,  $\alpha$ -ketoglutaric acid and phenylpyruvic acid reacted with ammonia to give glutamic acid and phenylalanine *via* their corresponding N-acylamino acids. Other amino acids may have also been formed from the corresponding oxo acids and ammonia. The amino acids formed were then further converted to proteins and protocell-like structures, marigranules (3), in the primeval sea.

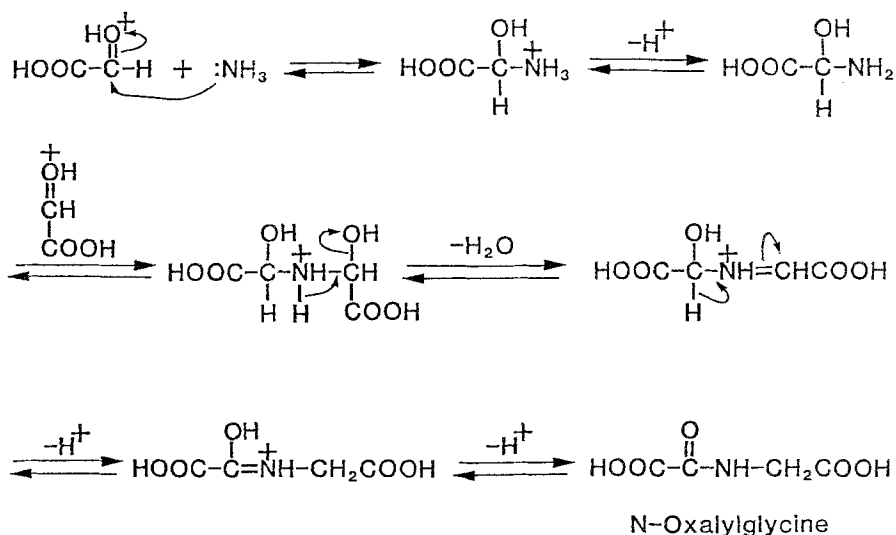


Fig. 1 A plausible mechanism for the formation of N-oxalylglycine from glyoxylic acid and ammonia in an aqueous medium.

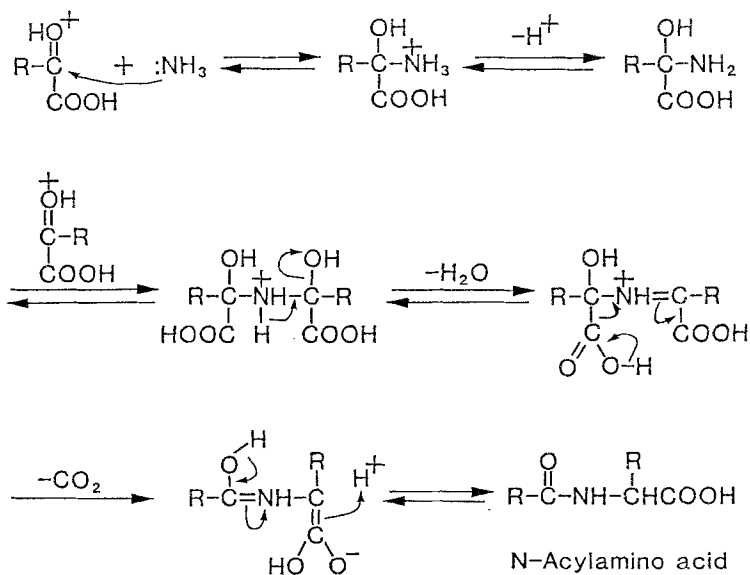
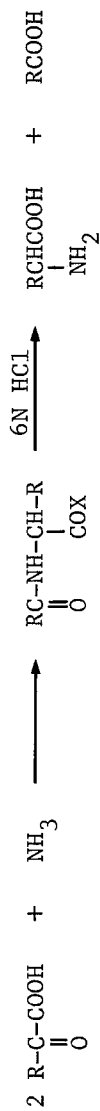


Fig. 2 A plausible mechanism for the formation of N-acylamino acids from  $\alpha$ -oxo acids and ammonia in an aqueous medium.

TABLE 1

Formation of N-Acylamino Acids and  $\alpha$ -Amino Acids from  $\alpha$ -Oxo Acids and Ammonia in an Aqueous Medium



$\alpha$ -Oxo acid                      N-acylamino acid (X = OH)  
N-acylamino acid amide (X = NH<sub>2</sub>)                      Carboxylic acid

R	$\alpha$ -Oxo acid	N-acylamino acid	$\alpha$ -Amino acid	Carboxylic acid
H-	Glyoxylic acid	N-Oxalylglycine & N-Formylglycine	Glycine	Oxalic acid & Formic acid
CH <sub>3</sub> -	Pyruvic acid	N-Acetylalanine & its amide	Alanine	Acetic acid
C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> -	Phenylpyruvic acid	N-Phenylacetylphenylalanine & its amide	Phenylalanine	Phenylacetic acid
HOOCCH <sub>2</sub> CH <sub>2</sub> -	$\alpha$ -Ketoglutaric acid	N-Succinylglutamic acid	Glutamic acid	Succinic acid
HOOCCH <sub>2</sub> -	Oxaloacetic acid	N-acetylalanine & its amide	Alanine	Acetic acid

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