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AMINO-ACID SYNTHESIS IN AQUEOUS MEDIA UNDER ULTRASONIC IRRADIATION

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The now-famous "Miller-Urey experiment" used a reduced mixture of gases to form basic organic molecules, such as amino acids. Since then, several methods have tried to reproduce the synthesis of amino acids under unusual conditions. All authors agree that the formation mechanisms of amino acids under such conditions might be of radical origin. If so, sonochemistry, known to enhance or to promote radical pathways, should be a powerful tool to access the synthesis of these compounds. We explored the feasibility of amino acid synthesis under ultrasonic irradiation in aqueous medium under a reductive atmosphere. The effects of several experimental parameters such as incident ultrasound frequency, temperature, nature and concentration of initial reactants, reaction time, and acoustic energy were explored.

Keywords: amino acid, ultrasound, aqueous media, cavitation.

In 1953, Miller studied the formation of amino acids by the action of electric discharges on a mixture of methane, nitrogen, and water with traces of ammonia [1]. This synthetic reaction, known as the strecker reaction, provides one of the most efficient methods for the synthesis of α-amino nitriles, which are hydrolyzed to give amino acids [2–4]. These moieties are not only very versatile intermediates for the synthesis of α-amino acids [5] but also for 1,2-diamines [6], amides [7], and various nitrogen-containing heterocycles such as thiadiazoles and imidazoles [8].

Ultrasound is known to enhance some chemical processes through a physical phenomenon called cavitation, which is the formation, growth, and collapse of bubbles in an elastic medium. By imploding, these bubbles create local high pressures (up to 1000 bars) and temperatures (up to 5000 K), which leads to high-energy radical mechanisms with some physical effects such as micro-mixing, mass transport, or reduction of particles size [9–11]. It is well known that many organic reactions driven by a radical pathway can be accelerated or promoted when submitted to ultrasound [11–15]. Compared with traditional methods, this technique is more convenient taking green chemistry concepts into account.

Our hypothesis is based on the sonolysis of water, which leads to the formation of radical intermediates OH with a strong dependence of concentration on the working frequency [16]. If the production rate of OH is at a reasonable level, it should react with a very simple organic molecule to afford basic amino acids.

The water-formamide system is of prominent interest; it is one of the simplest molecules usually chosen as a model for studying biological systems exhibiting the peptide type of bonding and DNA structures [17–23]. The nature of the hydrogen bonding between formamide and water can explain the hydrogen-bonding mechanism expected in the hydration of proteins [24–26]. The formamide–water ($HCONH_2$–H_2O) system is an important model system for investigating hydrophilic and hydrophobic interactions. The endothermal effects observed in water–formamide mixtures indicate that water loses its structure as a result of specific hydrophilic and hydrophobic interactions. This complex may go into the cavitation bubbles and be exposed, when collapsing, to the extreme conditions of temperature and pressure to afford radicals. If not, it can react at the surrounding shell of the collapsing bubbles where the OH concentration is important.

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TABLE 1. Ultrasonic Irradiation of an Homogeneous Solution of Formamide–Water at 493 and 1600 kHz

Formamide, mole %	Time, h	T, °C	Amino acids, mmole				Formamide, mole %	Time, h	T, °C	Amino acids, mmole			
			Gly	Ala	Asp	unknown				Gly	Ala	Asp	unknown
at 493 kHz							at 1600 kHz						
10	24	RT	–	–	–	0.02	10	24	RT	–	–	–	–
10	120	RT	–	–	–	0.05	10	72	RT	–	–	–	0.02
10	120	ΔT[a]	0.11	0.18	0.11	0.01	10	120	RT	–	–	–	0.04
40	24	RT	–	–	–	0.03	10	120	ΔT[a]	0.03	0.08	0.04	0.03
40	120	RT	0.09	0.28	0.22	0.01	40	24	RT	–	–	–	0.04
40	120	ΔT[a]	0.04	0.27	0.14	0.03	40	120	ΔT[a]	0.03	0.11	0.05	0.03
25	24	ΔT[a]	–	–	–	0.02	2.5	24	RT	–	–	–	0.03
25	72	ΔT[a]	0.15	0.35	0.13	0.02	2.5	120	ΔT[a]	0.03	0.25	0.04	0.02

[a]ΔT means the temperature was not controlled with a cooling jacket but allowed to rise naturally from room temperature to an average 60°C.

We studied the feasibility of synthesizing amino acids at two different frequencies with two laboratory-designed reactors at 493 and 1600 kHz. The 493 kHz frequency is in the range of the maximal production rate of OH, whereas at 1600 kHz interesting chemical activity is observed together with a very low electric consumption, adding a green touch to the concept. We avoided the use of low-frequency devices since, with such systems, effects are more of physical origin than chemical.

Table 1 presents the experimental conditions and results of the amino acid synthesis obtained at 493 kHz. Several parameters, such as temperature, time, and concentration, were varied.

Table 1 summarizes the experimental conditions and results of the amino acid synthesis obtained at 1600 kHz, and the effect of some parameters, such as temperature, time, and concentration, was studied.

The effect of temperature seems to be a key parameter, whereas the concentration of formamide has little or no influence on the reaction. Moreover, it seems that the longer the reaction time, the higher the probability of the presence of amino acids. A possible explanation is that sonolysis of water affords hydrogen peroxide through radical combination of two OH which increases in concentration with time [24]. By increasing the hydrogen peroxide radical concentration, [OH], in the solution, we increase the probability of it reacting with formamide. In addition, formamide vaporises at 60°C more than at room temperature and thus goes more easily into the cavitation bubbles through the rectified diffusion phenomenon and concentrates inside. When the bubbles are collapsing, formamide suffers extreme conditions together with water and reacts in the nearby collapsing area with the produced [OH]. This suggests that time and temperature might be synergic parameters to afford amino acids.

These results clearly confirmed the synthesis of amino acids in the experiments and show that this reaction is strongly thermally dependent. Indeed, with 10% of formamide, whatever the reaction time, no reaction occurs at room temperature whereas at 60°C, alanine is afforded. It is also confirmed that the initial concentration of formamide is not a limiting parameter since at as low as 2.5% of formamide, alanine is again detected. These results show also the same tendency as the previous ones, with a synergic effect of temperature and reaction time.

Formamide is itself hydrolyzed, meaning that it persists only in a relatively dry condition. In the proposed pathways, we have seen two similar intermediates, differing only in the conformation of *O*-protonated and *N*-protonated formamide [27, 28].

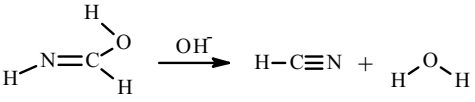
In this pathway, a NH proton may be transferred to water, forming an imidic acid-hydronium ion complex as the transition state, followed by back transfer of a different proton, leading back to *O*-protonated formamide and water. Back transfer of the proton was to the amide nitrogen, leading to *N*-protonated formamide and water.

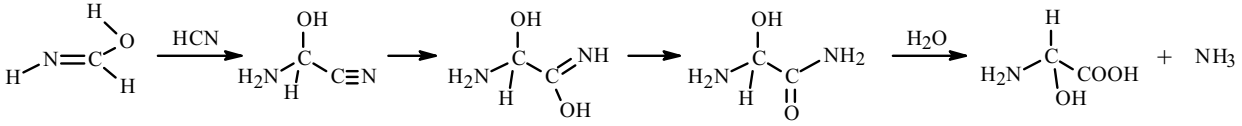
In the subsequent steps, the initiating step is the formation of HCN, or cyanic acid, by the attack of OH or hydrogen peroxide radical on formamide [29].

In homogeneous reaction mixtures, aldehydes, HCN, and ammonia are required for the Strecker type synthesis of α-amino nitriles, and over the experimental temperature range they were converted to amino acids.

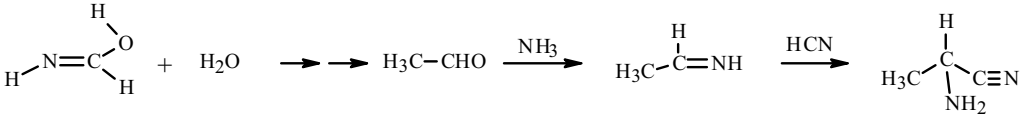
In the low-temperature region, amino acids are formed by formamide oxidation with water vapor, while in the high-temperature region HCN is formed in increasing yield.

In formamide, however, many species that are thermodynamically unstable in water with respect to hydrolysis are spontaneously synthesized.





The reactions of alanine production proceeds in essentially the same way as those of glycine:



The reaction is followed by hydrolysis to alanine, in solution.

We have shown that under ultrasonic conditions, a formamide–water mixture can afford alanine, glycine, and aspartic acid. In addition, several very simple organic molecules are used as starting materials to diversify the nature of the produced amino acids.

EXPERIMENTAL

Material and Methods. Glycine, alanine were obtained from Carl Ruth GmbH, 2-chlorobenzoxazole from Aldrich and formamide from Merck. The organic solution was analyzed by high-precision liquid chromatography (HPLC), infrared spectroscopy (Perkin–Elmer spectrometer) and mass spectra (JEOL JMS-01SG-2 spectrometer).

The reaction took place in a sonoreactor, which is a cylindrical vessel with an ultrasound equipped piezoelectric transducer at the bottom of the cylinder. A vessel with a ratio of liquid height to vessel diameter (R) of 2.5 was used.

Reaction were run under a reducing atmosphere of nitrogen.

General Procedure. A molar solution of 2.5, 10, or 40% formamide in 40 mL of ultrapure water (Millipore Milli-Q) is first prepared under a neutral atmosphere. Before pouring this solution into the ultrasonic device, the air atmosphere is evacuated by using N_2 gas to eliminate oxygen and to produce a reducing atmosphere. The aqueous phase is then poured into the reactor by syringe. The medium is then submitted to ultrasonic irradiation according to set experimental conditions (time, frequency, temperature, acoustic power, etc.). The temperature was either kept constant at 20°C by a cooling system or allowed to rise naturally to detect any thermal effect.

Detection Methods. a) Paper Chromatography. The classic procedure of detection of amino acids by paper chromatography was used with a BAW solvent [butanol–acetic acid–water 60:15:15 (4:1:1)]. After migration in an adapted chromatography tank, the paper is removed and sprayed with ninhydrin at 0.25% in ethanol in a fumed cupboard and then allowed to dry in the oven for a few minutes up to the development of colored spots. Several amino acid standards were qualified (color and R_f) by this method prior to experiments and then systematically compared with experiments for matching.

The standard amino acids, L-alanine (R_f 0.53), β-alanine (R_f 0.47), aspartic acid (R_f 0.25), glutamic acid (R_f 0.25), glycine (R_f 0.44), *iso*-leucine (R_f 0.73), leucine (R_f 0.74), lysine (R_f 0.48), serine (R_f 0.48), and valine (R_f 0.65) are used as reference detection points.

b) High-performance Liquid Chromatography (HPLC). We confirmed paper chromatography matching between standards and experiments by using an HPLC detection method. We used a derivatization-based UV-detection method of amino acids. The amino acids were derivatized by a UV-sensitive agent, 2-chlorobenzoxazole, and detected by UV-HPLC [30].

Firstly 50 µL of an aqueous solution of 2.5% sodium acetate trihydrate (w/v) mixed with 1.0 mL of methanol and then 25 µL of 2-chlorobenzoxazole (25 µL) were prepared, 50 µL of buffer (0.25 M sodium carbonate) was mixed with 1 nmol of a standard solution of amino acids in 0.1 N HCl and 100 µL of the above 2-chlorobenzoxazole solution for derivatization. The reaction was allowed to continue typically at 80°C for 30 min. The samples (BOX–AA) were diluted with sodium acetate solution and analyzed by HPLC at 245 nm. The 2-chlorobenzoxazole amino acid derivatives were separated on a C18 reversed-phase column (0.46 × 25 cm). Typical solvents consisted of A: 2% ammonium bicarbonate (1% w/v) in 20% methanol–water, and B: 10% methanol in acetonitrile.

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