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Scientific paper

Hydrothermal Scenario for Amino Acids and Sulfur-Containing Amino Acids Formation

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Abstract

The chemical evolution of amino acids, especially sulfur-containing ones, requires appropriate conditions and natural sources to provide starting prebiotic compounds. In the present study hydrothermal vents, volcanoes and oceans were chosen as a plausible environment, where prebiotic reactions take place. The suggested reaction network starts only with three compounds – water, hydrogen cyanide/formamide and hydrogen sulfide. The present study suggests one-pot hydrothermal experiment in laboratory conditions to demonstrate some vital prebiotic precursors formation. The reaction pathways from starting molecules to amino acids were modelled at SCS-MP2/cc-pVDZ/SMD level of the theory. The calculated energetic characteristics facilitate the determination of the plausible reaction pathways for amino acids – glycine, serine and alanine, along with sulfur-containing ones – cysteine and homocysteine under hydrothermal scenario.

Keywords: Sulfur-containing amino acids; chemical evolution; prebiotic chemistry; hydrothermal conditions

1. Introduction

Prebiotic chemistry is a fascinating scientific field, making scientists curious around the Globe. The discovery of hydrothermal springs in mid-ocean ridges in the 1970s led scientists to the assumption that these geological objects provide suitable conditions for the abiotic formation of biological molecules, especially amino acids. Although there are many plausible energy sources for generating organic compounds, the choice of an appropriate energy source for amino acid synthesis in the context of chemical evolution remains a challenging task. One of Earth's most notable energy sources is its internal heat, which can be discovered from anomalously high thermal gradients around volcanoes and hydrothermal vents (Figure 1).[1]

The formation of hydrothermal vents in volcanic regions with underwater chambers of rising magma involves three important components: fluid, heat and fluids circulation through the rocks. Cold seawater, interacting with heated magma, seeps into cracks in the sea floor and can heat up to 400 °C, stimulating chemical reactions. Depending on the type of hydrothermal vent, the temperature can vary from 90 °C to 400 °C.[2,3] For instance, the so-called black smokers are hydrothermal fumaroles with abundant sulfides in suspension and the water can reach temperatures above 400 °C. When these hot liquids come

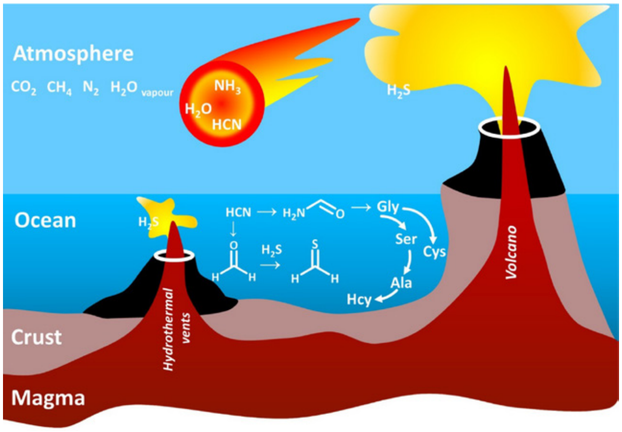


Figure 1. Prebiotic conditions on the Early Earth: simple precursors and some key amino acids formation in the Ocean under hydrothermal conditions. Reprinted and adapted with permission from Prof. Yannick Vallée (*Encycl. L'Environnement*).[1]

into contact with cold seawater, they generate rapid formation of sulfides and other minerals, resulting in a cloudy suspension resembling a cloud of black smoke. In contrast, white smokers are usually far from their heat source and contain sulfates. Their temperatures are lower than those of black smokers and can vary from 40 °C to 75 °C.

Therefore, primary volcanoes and hydrothermal vents are extreme environments for the abiotic production of amino acids from inorganic volcanic gases at high pressures and temperatures. The main components of volcanic gases are water (H_2O), carbon dioxide (CO_2), sulfur dioxide (SO_2) (high-temperature volcanic gases) or hydrogen sulfide (H_2S) (low-temperature volcanic gases), and ammonia (NH_3). For example, when chemical reactions took place deep in the Earth's crust, amino acids were subsequently ejected together with hydrothermal fluids into colder waters, where lower temperatures and the presence of clay minerals provide favorable conditions for the formation even of peptides.[4]

The presence of sulfur in volcanic hydrothermal systems is a unique natural source for sulfur-containing amino acids formation.[5] Youssef-Saliba and Vallée published a comprehensive review of the prebiotic synthesis of sulfur-containing amino acids and their impact on the origin of life.[6] Modern volcanoes represent a unique analog of conditions present during the Hadean Eon and be a suitable prototype for prebiotic synthesis in the laboratory.[7] It is well-known that the modern study of chemical evolution, aiming to obtain amino acids from simple molecules in early abiotic conditions, takes its origin from the pioneering work of Urey and Miller, published in 1953.[8]

However, the first and most famous experiment was not the only one established. The authors, and later their colleagues, performed a tremendous trilogy, making different variations of the experiments in their laboratory and methods of analyses applied. Anyway, all of Miller's experiments were aimed at discovering reaction pathways for non-biological amino acid synthesis, assuming various scenarios, including *UV* irradiation, lightning or hydrothermal vents. The idea to mimic hydrothermal vents led Miller to the creation of the so-called *volcanic apparatus* in 1955.[9] The principal difference from the classic version was only in the presence of a tapering glass *hood* attached to the water-containing flask, which injects a jet of steam and gas into the spark-discharge flask and thus increases the airflow through a hollow, hermetic glass device. The increasing of airflow creates a more dynamic reaction vessel in the apparatus and imitates a water-rich volcanic eruption accompanied by lightning, but Miller did not attach importance to *volcanic apparatus* modification and assumed that it gave results similar to the *classic apparatus.* In 2008, by modern analysis techniques, Johnson *et al.* detected 22 amino acids and five amines in Miller's volcanic experiment.[10]

In 2010, *Parker et al.* took archival samples of analysis from a 1958 *Miller* experiment that was never published.[11] These samples were obtained as a result of the *classic apparatus* experiment using a gaseous mixture of H_2S, CH_4, NH_3 and CO_2 that mimics possible primitive Earth conditions, including the source of H_2S – volcanic gases and hydrothermal vents. Hence, only methionine has been registered as a sulfur-containing amino acid. However, authors found some sulfur-containing amino acids derivatives – cysteamine, homocysteic acid and others.

Furthermore, it is important to take into account the volcanic hydrothermal experiment, provided by Botta *et al.* The authors report the syntheses, based on two initial prebiotic components – hydrothermal water and formamide.[12] A major role in this experiment is played by the employment of the mineral catalysts – meteorites. As a result, the reaction mixture contains a variety of prebiotic molecules, such as carboxylic acids, nucleobases, amino acids and sugars.

In addition, a few theoretical studies were focused on modeling of the most famous experiment from Urey and Miller.[8] For instance, in 2014, Saitta and Saijac applied a new theoretical model to study the effect of electric field on mixtures of simple molecules, mimicking the famous spark discharge experiment .[13] When a mixture of H_2O, NH_3, CH_4, CO and N_2 is subjected to an electric field, the amino acid glycine is formed, which is one of the simplest building blocks for proteins and one of the most abundant products in Miller's original experiment.

In the same year, Wang *et al.* first reported results of *ab initio* nanoreactor simulations that discovered new reaction pathways and elementary steps for an ensemble of simple molecules.[14] To recreate the Miller-Urey experiment,[8] a system including H_2, H_2O, NH_3, CH_4, and CO is used. The authors show new routes for the synthesis of glycolaldehyde, cyanimide and glycine from the aforementioned gases.

In 2019, Das *et al.* again using the *ab initio* nanoreactor, showed that compounds important for chemical evolution can be generated from only two simple inorganic molecules, H_2O and HCN, which are prevalent on Earth during the Hadean eon.[15] Water and ammonia act as catalytic proton shuttles, which means that hydrogen cyanide and water can be sufficient to generate multiple building blocks, even when considering only reactions with energy barriers up to 40 kcal mol^{-1} occurring at 80-100 °C. The above-described experimental and theoretical studies on the abiotic production of amino acids, and in particular sulfur-containing amino acids, do not provide a sufficiently clear idea of their formation under hydrothermal conditions from simple molecules.

However, the formation of amino acids, including sulfur-containing ones during the chemical evolution does not always follow the Miller-Urey experiment. For instance, Surpateanu *et al.* demonstrate the formation of some amino acids, *e.g.* serine, cysteine and methionine via radical and photochemical-radical reaction mechanisms, starting from methylene, nitrene and carbon monoxide.

Interestingly, the central prebiotic precursor in their work is a compound with a three-membered cycle – aziridinone and its derivatives.[16] A possible reaction mechanism of glycine formation was shown by H. Lee and J. Choe, where the main reaction occurs after hydrolysis of malononitrile monoamide, which on turn was obtained after hydrolysis of amino malononitrile.[17] Shivani *et al.* described radical reactions to form serine and alanine from molecules, detected in the interstellar space.[18,19]

Herein, we report the investigation of some amino acids and sulfur-containing amino acid formation from simple molecules under hydrothermal conditions. To our best knowledge, this is the first report, which demonstrates non-trivial reaction pathways for the formation of sulfur-containing amino acids – cysteine and homocysteine, taking into consideration the geological context. Also, we suggest the non-trivial feasible reaction pathways and corresponding mechanisms for glycine, alanine and serine formation in the context of chemical evolution. Additionally, we present the results of an experimental study using a reactor of hydrothermal synthesis to explore the formation of important precursors such as urea, glycine and formylglycine.

2. Methods

2. 1. *Ab initio* Calculations

Quantum-chemical *ab initio* calculations were performed using *GAMESS* software package.[20,21] All transition states, reactants and products are localized at spin-component-scaled Möller-Plesset (SCS-MP2/*cc-pVDZ*) level of theory, taking into account water environment by the universal solvation model density (SMD).[22,23,24] SCS-MP2 was chosen to precise the estimation of the electron correlation energy during the geometry optimizations of reactants and products, along with transition states searching. The present computations consisted of two stages: the first one was geometry optimization of reactant and products or searching the transition states (TS) geometry; the second one – normal mode vibrational frequencies calculations. Numerically performed vibration frequency calculations were performed to define the nature of the stationary points - minima (absence of the imaginary frequency for the reactant and product), or maxima (one single imaginary frequency for the transition state) on the potential energy surface and to take vibrational zero-point energies and thermal corrections. The obtained enthalpy values referred to 0 K and free Gibbs energy – to standard conditions of 25 °C (298.15 K) and 1 atm of pressure. All reactants and products were validated so that they refer to the exact TS by intrinsic reaction coordinate (IRC) calculations. Launched from the TS, the IRC was generated as 250 steps at two directions, employing the Gonzalez-Schlegel algorithm with 0.05 Bohr small step size.[25]

2. 2. Hydrothermal Heating Experiment

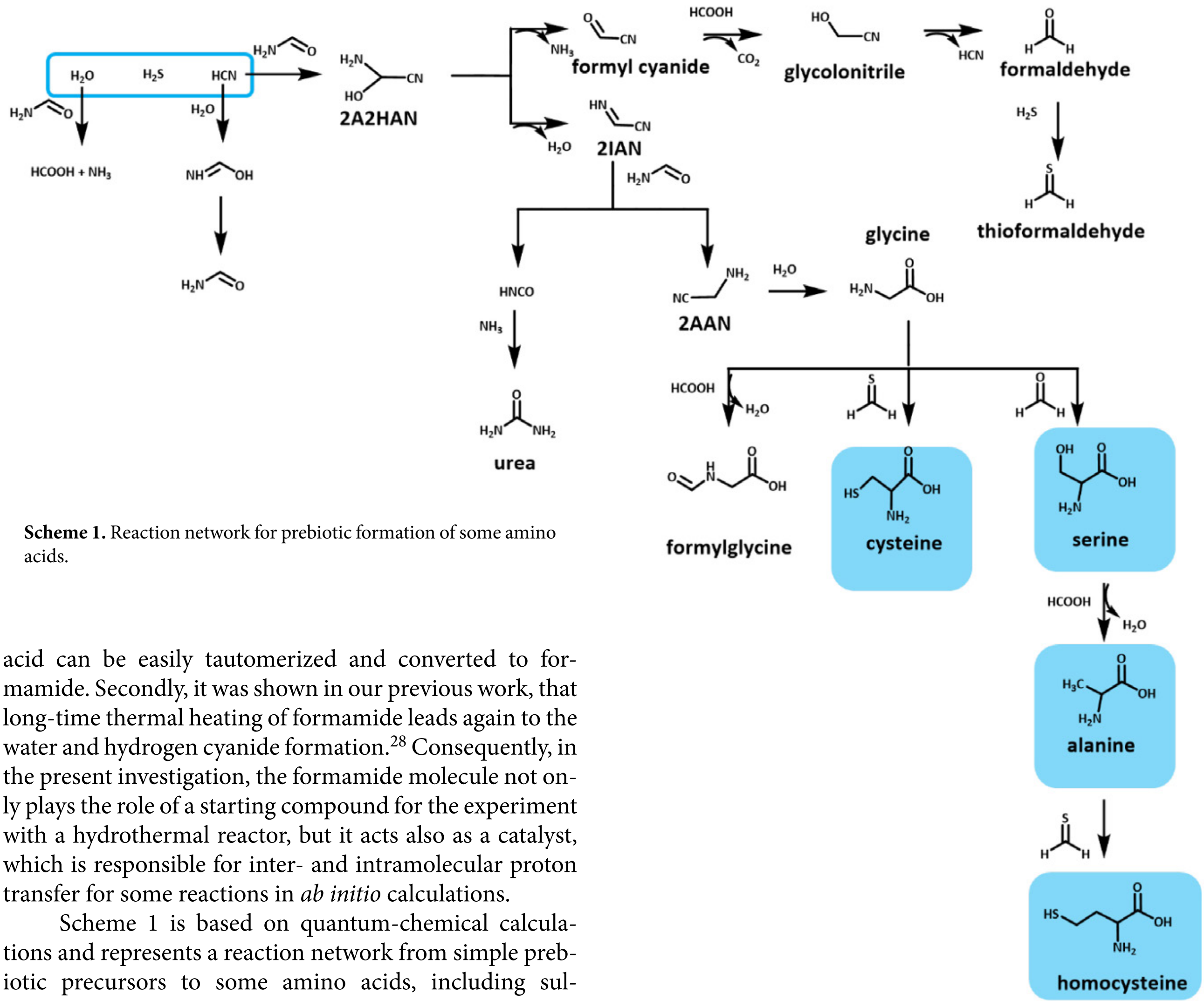
The hydrothermal experiment was carried out with a liquid sample, consisting of two components – water and formamide. Three types of water (V = 50.0 ml) were used in the experiment: ultrapure, distilled and hydrothermal water from Vlasa Spring (Velingrad, Bulgaria) (see Table S2, SI). Nonetheless, the amount of hydrogen sulfide was not determined in the provided data for hydrothermal water analysis. In the current experiment, 10 or 20 ml of liquid formamide (Fluka, 99.7%, Germany) were used (Table 1). Immediately after mixing, the obtained solution was transferred into a Teflon container from 100 ml and the autoclave was sealed in a hydrothermal high-pressure reactor Berghof *(BR-100*). Samples were heated at 140–160 °C with continuous stirring for different time intervals (61–164 hours). The reaction's autogenic pressure was estimated to be around 2.5 bar. The final products were cooled to room temperature.

After heating, the obtained liquid probe was analyzed by gas-chromatography associated with mass-spectrometry (GC–MS). Firstly, 1 ml of the reaction mixture was evaporated using a CentriVap Vacuum Concentration System (Labconco) to remove formamide, then the remaining aqueous solution was lyophilized to dryness. After that, 5.0 g of the dry mass was dissolved in 100.0 µl of N,N-bis-trimethylsilyl trifluoroacetamide (BSTFA) and 100.0 µl of pyridine. The sample was heated on Thermo-Shaker TS-100 (40 min/70 °C/300 rpm). The GC–MS analysis was performed on a 7890A gas chromatograph (Agilent Technologies) connected to a 5975C mass selective detector (Agilent Technologies). Separations were conducted using a 30 m × 0.25 mm (i.d.) DB-5ms silica-fused capillary column with a 0.25 µm poly(dimethylsiloxane) film as the stationary phase. The carrier gas, helium (chromatography grade), was maintained at a flow rate of 1.0 ml/min. The injector and transfer line temperatures were set to 250 °C. The oven temperature program used was 100 °C for 2 min then 15 °C/min to 180 °C for 1 min then 5 °C/min to 300 °C for 10 min. 1.0 µl of the sample was injected in split mode at a ratio of 5:1. The mass spectrometer scanned from 50 to 550 m/z, with all mass spectra recorded in electron impact (EI) mode at 70 eV.

3. Results and Discussion

The results of the investigation include an experiment, which mimics hydrothermal conditions and *ab initio* quantum-chemical calculations. The experiment provides a background for *ab initio* simulations, demonstrating the formation of some prebiotic precursors under hydrothermal conditions.

It should be noted that for our experiment with a high-pressure reactor of hydrothermal synthesis, three starting compounds were selected: water is the environment (ocean), where the reactions take place; formamide, which is a universal prebiotic precursor and hydrogen sulfide, which originates from hydrothermal vents in volcanic regions.[26] The alternative that preferred formamide to be one of the starting compounds instead of hydrogen cyanide, which came to the ocean from asteroids and meteorites during the Early Earth period (Figure 1), was chosen not by chance.[27] First of all, formamide and its tautomer, formimidic acid, are products of the hydrogen cyanide hydrolysis reaction (Scheme 1). The obtained formimidic acid can be easily tautomerized and converted to formamide. Secondly, it was shown in our previous work, that long-time thermal heating of formamide leads again to the water and hydrogen cyanide formation.[28] Consequently, in the present investigation, the formamide molecule not only plays the role of a starting compound for the experiment with a hydrothermal reactor, but it acts also as a catalyst, which is responsible for inter- and intramolecular proton transfer for some reactions in *ab initio* calculations.



Scheme 1. Reaction network for prebiotic formation of some amino acids.

Scheme 1 is based on quantum-chemical calculations and represents a reaction network from simple prebiotic precursors to some amino acids, including sulfur-containing ones. The starting three molecules, water, hydrogen sulfide and hydrogen cyanide, are available under hydrothermal conditions, which facilitate their reactive capacity to generate complex prebiotic compounds. *Ab initio* calculations were performed with water as an environment, imitating reactions in the ocean. Hydrogen cyanide is a key source of carbon and nitrogen atoms in prebiotic chemistry and its relations with formamide molecules were explained above. Formamide is a compound with several functions since it is not only the starting precursor for the reaction network but effective catalyst for proton transfer. Of course, in the *prebiotic soup* not only formamide can be able to act as a *proton shuttle* for the successful proton transfer. For instance, formimidic acid, water molecules and formic acid are an alternative to the formamide and, depending on the structure of TS and other factors for some specific cases, when one molecule can act more efficiently than another, providing superior kinetic and energetic characteristics.

The main results of the one-pot hydrothermal experiment are presented in Table 1. As it can be seen from Table 1, both experiments, where 20 ml liquid formamide was mixed with 50 ml ultrapure water for 164 hours at 160 °C and 140 °C results in urea, glycerol and ethanolamine. The experiment, consisted of mixture of 50 ml distilled water and 10 ml liquid formamide, which were heated at 140 °C for 61 hours provide not only urea, glycerol and ethanolamine, but glycine and formylglycine. The same main products were obtained during the last experiment, where 10 ml liquid formamide was mixed with 50 ml hydrother-

Table 1. Condensation products (% from total ion current (TIC)) obtained from hydrothermal experiment. Formamide and water mixture (ml) are heated at different temperatures, T (°C), and time intervals, t (h).

Formamide	*Water*	*t*	*T*	*Result*	*% TIC*
20	50 (ultrapure water)	164	160	Urea Glycerol Ethanolamine	2.89 0.46 0.23
20	50 (ultrapure water)	164	140	Urea Glycerol Ethanolamine	3.65 0.39 0.12
10	50 (distilled water)	61	140	Urea Glycerol Ethanolamine Glycine Formylglycine	2.13 0.14 0.12 0.03 0.02
10	50 (hydrothermal water from Vlasa spring, Velingrad, Bulgaria)	61	140	Urea Glycerol Ethanolamine Glycine Formylglycine Hydrogen sulfide	2.08 0.56 0.41 0.14 0.09 0.02

mal water at 140 °C for 61 hours. Immediately after heating, all samples possessed a specific smell of ammonia and formic acid that is in agreement with theoretical simulations, described below. As a result, one-pot hydrothermal experiments provide valuable prebiotic precursors such as urea, glycerol, ethanolamine and the amino acid glycine with its derivative – formylglycine were detected.

It is reasonable to note, that in the current hydrothermal experiment, hydrogen sulfide, originating from hydrothermal water, did not react with formamide. However, such chemical behaviour was predictable, since the reaction mixture requires a remarkably longer period to *trigger* interactions in the *prebiotic soup*. For instance, in 1972, van Trump and Miller published the classic experiment, where the primitive Earth atmosphere consists of CH_4, N_2, NH_3, H_2O, H_2S, and indicated a trace of methionine.[29] However, after 52 years, the probe analysis of a never-published experiment by Miller (1958), indicates that the sample is rich in diverse compounds, including sulfur-containing amino acid methionine, which is a clear confirmation that not only modern analysis techniques, but *time* is a crucial factor for the effective prebiotic products formation.[11]

Our experiment could be compared with other studies, related to simulations of hydrothermal environment. For instance, Villafañe-Barajas *et al.* investigated the stability and reactivity of hydrogen cyanide under hydrothermal scenario – different pH values (acidic and basic), thermolysis of HCN at 100 °C and in the presence of inorganic surfaces.[30] As products of HCN thermolysis, the authors detected the formation of formaldehyde, urea, diaminomaleonitrile (DAMN) and some carboxylic acids – oxalic, maleic, glycolic and formic acid. In addition, GC-MS analysis identified glycine and alanine traces only in the acidic sample without clay; in basic samples – urea, oxalic and glycolic acid and some intermediate species such as carbamic acid, ethanolamine, glycerol, succinic and propanoic acid. Consequently, the obtained prebiotic precursors, such as urea, ethanolamine and glycerol from formamide and water in our work are in line with the hydrogen cyanide thermal heating experiment.

In addition, it is also quite curious to bring up some details in the above-mentioned hydrothermal experiment by Botta *et al.* and to compare them with our results. In their work, 1 ml of formamide and thermal water from the Pisciarelli solfataric area (30% in weight with respect to formamide) were heated in the hydrothermal reactor.[12] The synthesis was carried out for 5 days at a temperature range 85–87 °C in the presence of meteorites, which act as a mineral catalyst. The authors detected by GC-MS a panel of different compounds – nucleobases, some sugars, carbohydrates, carboxylic acids, *etc.*). The formation of urea, glycine and formylglycine during hydrothermal synthesis in our study is in agreement with results, obtained by Botta *et al.*[12] However, the authors provide the results of synthesis in the presence of mineral catalysts and great excess of formamide towards water, which is questionable ratio for real hydrothermal vents and volcanoes. Their experiment with formamide and water in the absence of a meteorite yielded only one compound – purine. It means, that our hydrothermal experiment without the presence of mineral catalyst without the exceed of formamide and shorter time-heating (61 hours), but at a higher temperature (160 °C), results in a variety of prebiotic compounds in comparison with the synthesis, mentioned above. Anyway, the obtained precursors after our hydrothermal volcanic experiment shed light on theoretically proposed reaction pathways and confirm that the *ab initio* study proceeds in the right way.

The general Scheme 1 represents a reaction network, which is based on the results of *ab initio* quantum-chemical calculations. The present scheme demonstrates how, starting from water, hydrogen sulfide and hydrogen cyanide, simple amino acids and sulfur-containing ones can be formed.

The cascade of reactions begins with the formation of formamide from hydrogen cyanide and water, which occurs in two steps (Figure 2). The first step is the formation of formimidic acid from hydrogen cyanide and a water molecule. The reaction begins when the water molecule acts as a nucleophile, attacking the carbon from the HCN, and forming a C-O bond. A simultaneous proton transfer from the latter to a second water molecule, acting as a catalyst, results in the protonation of nitrogen, thereby forming a formimidic acid. The reaction is slightly endothermic and the barrier is relatively high, 41.4 kcal mol^{-1}, though it is feasible under the experimental conditions. Once the formimidic acid is formed, it can easily tautomerize into formamide, due to the relatively low barrier.

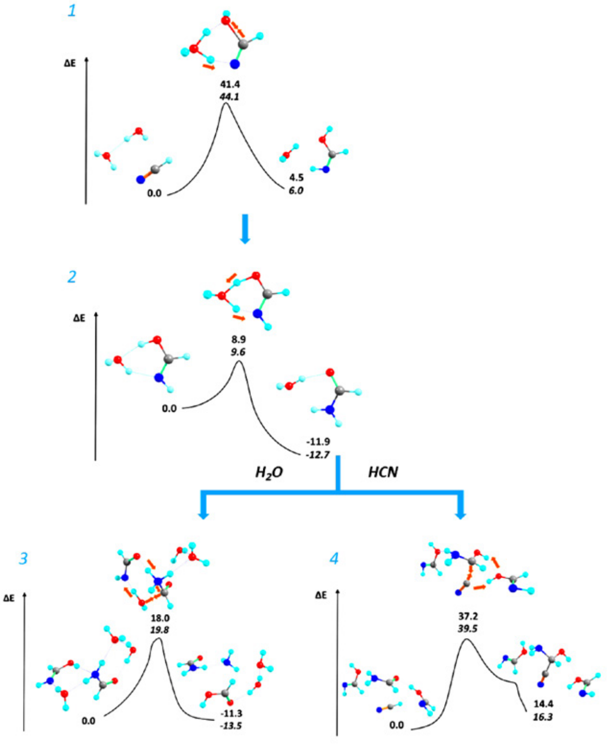


Figure 2. Schematic potential energy profile along the reaction pathways and optimized structures of reactants, products and TS for formation of: 1) formimidic acid from water and hydrogen cyanide; 2) formamide tautomerization; 3) formic acid and ammonia from formamide and water and 4) 2A2HAN from formamide and hydrogen cyanide. The values of ΔH_0 (top) and ΔG_{298} (bottom, italics) in kcal mol^{-1} are calculated at SMD/SCS-MP2/cc-pVDZ level of the theory. Color representations are: grey – carbon, red – oxygen, blue – nitrogen and cyan – hydrogen.

This process is catalyzed by a water molecule, where the hydrogen from the hydroxyl group of the acid is transferred through the water molecule to the nitrogen atom. This step is exothermic, with a relatively low energy barrier of 8.9 kcal mol^{-1}. The obtained formamide is crucial for the subsequent reactions. Formic acid and ammonia are the products of the hydrolysis of formamide. The reaction is exothermic with a barrier of 18 kcal mol^{-1} (Figure 2). From the interaction of hydrogen cyanide and formamide, the reaction pathway leads to the formation of an unstable key intermediate 2-amino-2-hydroxyacetonitrile (2A2HAN). The process begins with simultaneous proton transfer from formimidic acid (catalyst) to the adjacent formamide molecule and from HCN to the catalytic formimidic acid. Immediately afterward, the cyanide group attacks the carbon atom of the formamide, resulting in the formation of a hemiaminal. The barrier of this self-catalyzed endothermic reaction is 37.2 kcal mol^{-1} (Figure 2).

Two competitive reactions – deamination and dehydration of 2A2HAN, can lead to the formation of formyl cyanide and 2-iminoacetonitrile, respectively (Figure 3). The deamination reaction, which results in the formation of formyl cyanide is kinetically and thermodynamically preferred. It begins with a proton transfer from the hydroxyl group of formimidic acid to the amino group of 2A2HAN. This results in the formation of a protonated amino group in 2A2HAN. After that the proton of the hydroxyl group of 2A2HAN transfers to the nitrogen atom of the formimidic acid. This causes the formimidic acid to undergo tautomerization into formamide. The protonated amino group is released as ammonia giving formyl cyanide. This reaction has a relatively low barrier (5.6 kcal mol^{-1}) and is slightly endothermic, making it kinetically preferred. Additionally, formyl cyanide can directly react with formic acid in a highly exothermic reaction where the decarboxylation product will be glycolonitrile. This reaction is carried out through simultaneous double proton transfer from the formic acid to the formyl cyanide which has an activation energy of 37.3 kcal mol^{-1} (Figure 3). Formimidic acid could catalyze the degradation of glycolonitrile to formaldehyde and hydrogen cyanide. First, the glycolonitrile C-C bond is significantly lengthened, and at the same time, the proton from the hydroxyl group is transferred to the nitrogen of the formimidic acid. The glycolonitrile molecule then degrades into formaldehyde and cyanide ion (34.4 kcal mol^{-1}) which takes the proton from the hydroxyl group of the formimidic acid resulting in the formation of formamide. Although, the reaction to form 2-iminoacetonitrile is thermodynamically less favorable than that of formyl cyanide, it leads to the formation of some highly desirable compounds. The formation of 2-iminoacetonitrile from 2A2HAN begins with the cleavage of the carbon-oxygen bond of the OH group of 2A2HAN. Simultaneously, the proton from the amino group of a formamide molecule (catalyst) transfers to the

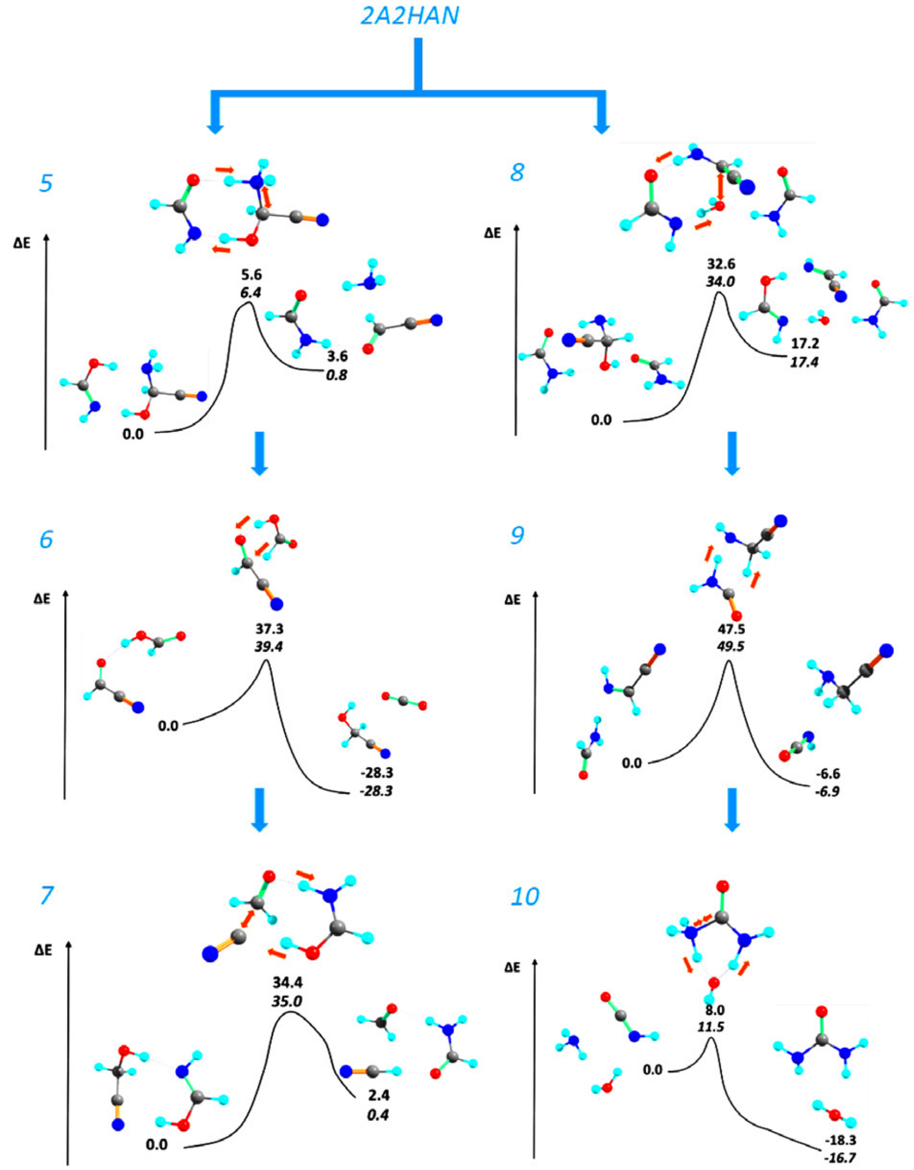


Figure 3. Schematic potential energy profile along the reaction pathways and optimized structures of reactants, products and TS for formation of: 5) formyl cyanide as a result of deamination of 2A2HAN; 6) glycolonitrile from formyl cyanide and formic acid; 7) formaldehyde from the reaction of degradation of glycolonitrile; 8) 2-iminoacetonitrile from 2A2HAN; 9) isocyanic acid and 2AAN from 2-iminoacetonitrile and formamide; 10) urea from isocyanic acid and ammonia. The values of ΔH_0 (top) and ΔG_{298} (bottom, italics) in kcal mol^{-1} are calculated at SMD/SCS-MP2/cc-pVDZ level of the theory. Color representations are: grey – carbon, red – oxygen, blue – nitrogen and cyan – hydrogen.

leaving hydroxyl group. Additionally, the proton from the amino group of 2A2HAN migrates to the carbonyl oxygen of the catalytic molecule. The estimated energy barrier of the reaction is 32.6 kcal mol^{-1}.

The formed 2-iminoacetonitrile can further react with formamide to produce isocyanic acid and 2-amino acetonitrile (2AAN). The reaction occurs through double proton transfer: from the amino group of the formamide to the imino group of 2-iminoacetonitrile and from the CH of formamide to the methylene carbon of 2-iminoacetonitrile. The cleavage of the C-H bond possibly contributes to the high activation energy of 47.5 kcal mol^{-1}. However, the reaction products, 2AAN and isocyanic acid, are thermodynamically favourable, with an energy change of –6.6 kcal mol^{-1}. Urea can be obtained as a product of isocyanic acid and ammonia in the presence of water as a catalyst. The reaction of nucleophilic addition has a relatively low barrier and is highly exothermic (–18.3 kcal mol^{-1}) (Figure 3).

A pivotal moment in the reaction scheme is the formation of the experimentally found amino acid glycine through the interaction of 2AAN and water (Figure 4). The first step, glycinimide formation, is similar to the reaction mechanism of the formimidic acid formation from hydrogen cyanide and water. The barrier is estimated to be as high as 43.7 kcal mol^{-1} and according to the reaction curve, it should be endothermic by 9.8 kcal mol^{-1}. It further transforms to glycinamide through water-assisted intermolecular proton transfer, which is a thermodynamically favorable process (−11.5 kcal mol^{-1}). The hydrolytic deamination in the third step is initiated by a water-assisted proton transfer from the water molecule to the amide amino group of glycinamide. The remaining hydroxyl group attacks the carbonyl carbon forming a single carbon-oxygen bond, while the protonated amino group leaves as ammonia. The activation barrier of the reaction is calculated to be 41.2 kcal mol^{-1}. The product of this reaction is glycine (Figure 4).

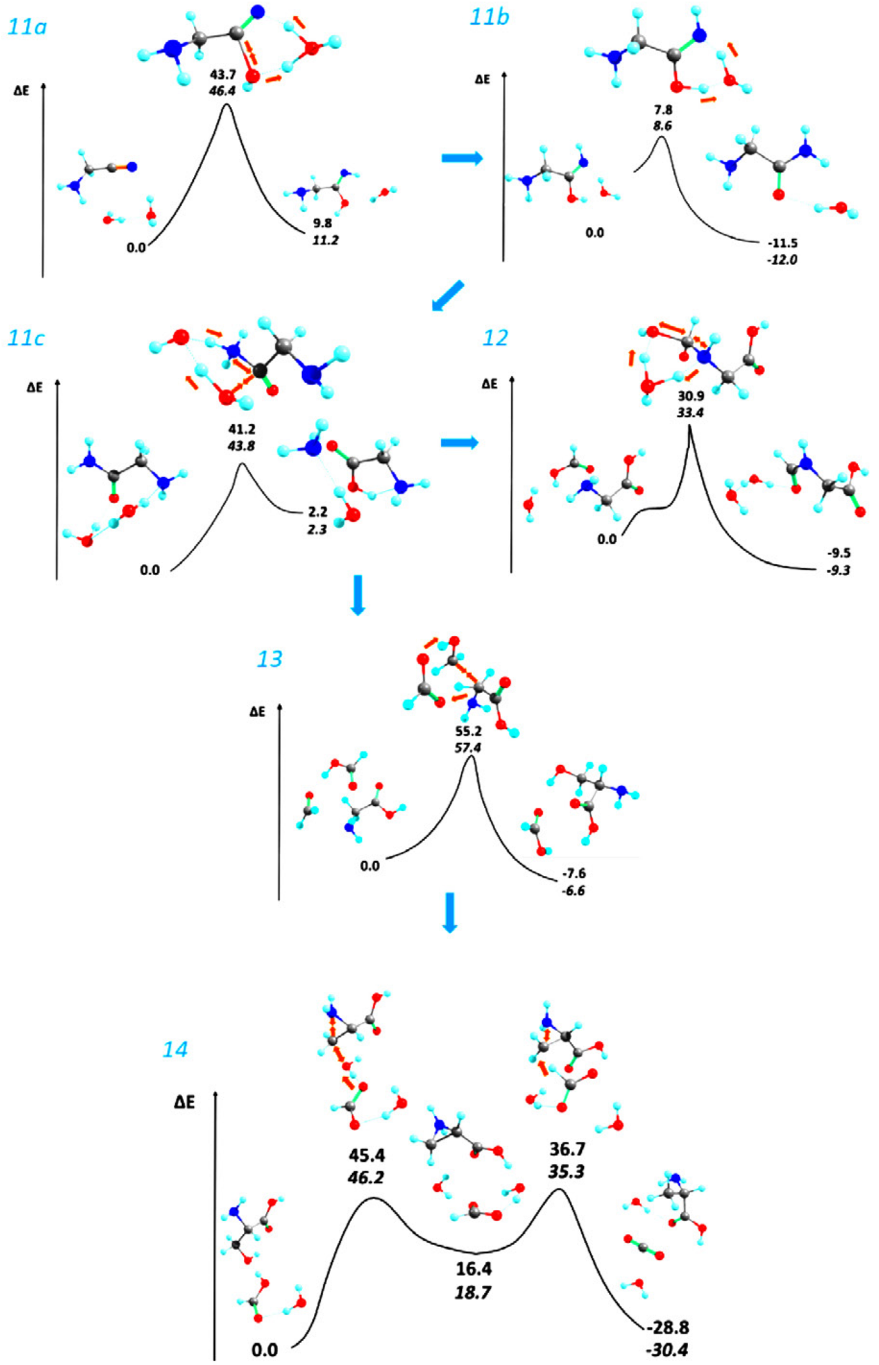


Figure 4. Schematic potential energy profile along the reaction pathways and optimized structures of reactants, products and TS for formation of: 11a) glycinimide from 2AAN and water; 11b) glycinamide; 11c) glycine as a result of glycinamide deamination; 12) formylglycine from glycine and formic acid; 13) serine from glycine and formaldehyde 14) alanine formation. The values of ΔH_0 (top) and ΔG_{298} (bottom, italics) in kcal mol^{-1} are calculated at SMD/SCS-MP2/cc-pVDZ level of the theory. Color representations are: grey – carbon, red – oxygen, blue – nitrogen and cyan – hydrogen.

Three reaction pathways are considered starting from glycine (Scheme 1). Kinetically and thermodynamically, the most favored process is the formation of formylglycine from glycine and formic acid (Figure 4). The amino nitrogen atom of glycine attacks the carbonyl carbon of formic acid. Once the carbon and nitrogen atoms are close enough (1.55 Å), a proton form the amino group transfers to the adjacent water molecule which transfers another proton to the hydroxyl group of the formic acid. The hydroxyl group leaves as water, while the formyl group is attached to the nitrogen atom of the glycine molecule. The mechanism described above leads to the formation of experimentally detected formylglycine. Another possible reaction is the serine formation that could occur by the interaction of glycine and formaldehyde, catalyzed by formic acid. The process begins with

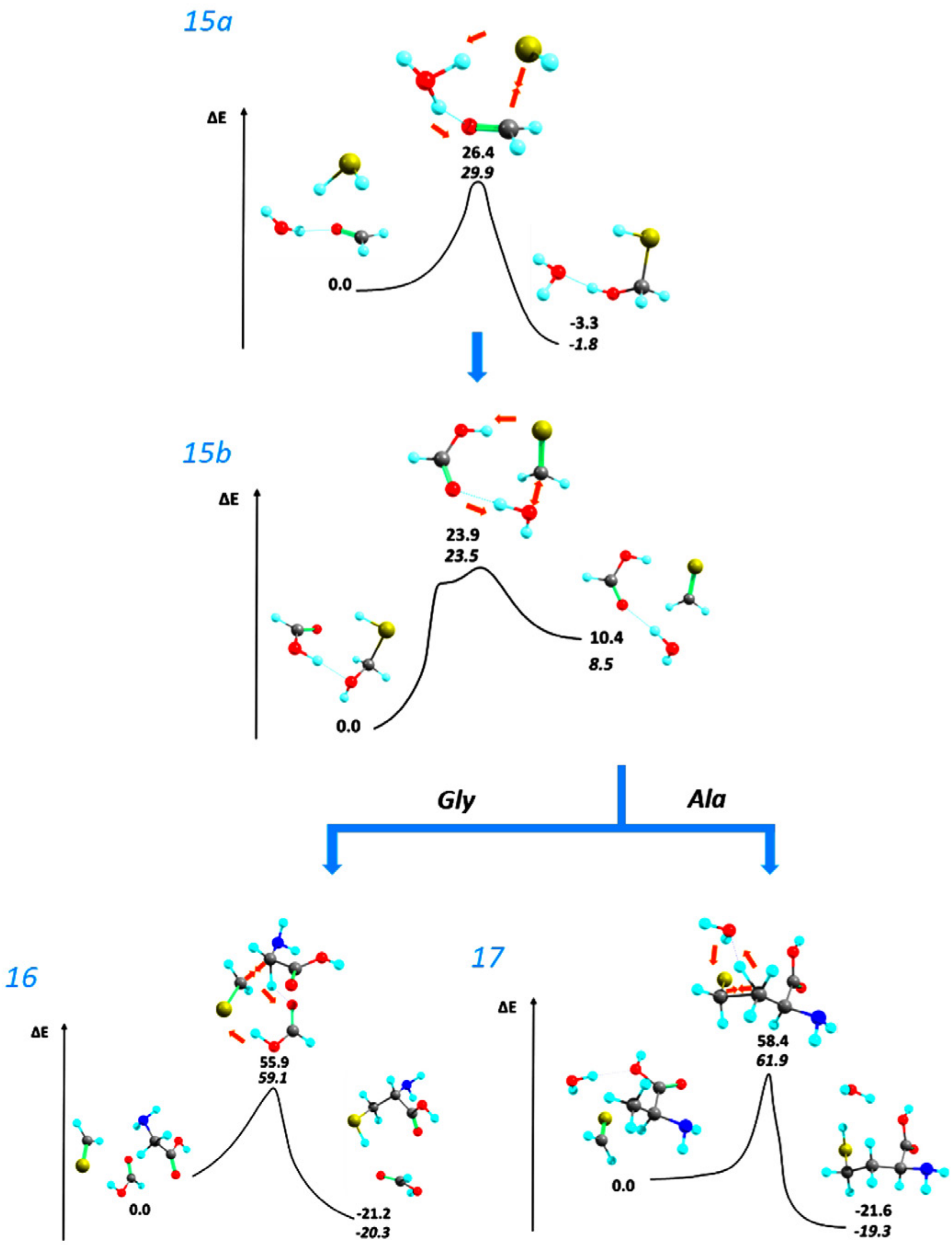


Figure 5. Schematic potential energy profile along the reaction pathways and optimized structures of reactants, products and TS for the formation of: 15a) thiohemiacetal from formaldehyde and hydrogen sulfide; 15b) thioformaldehyde as a reaction of thiohemiacetal dehydration; 16) cysteine from glycine and thioformaldehyde; 17) homocysteine from alanine and thioformaldehyde. The values of ΔH_0 (top) and ΔG_{298} (bottom, italics) in kcal mol^{-1} are calculated at SMD/SCS-MP2/cc-pVDZ level of the theory. Color representations are: grey – carbon, red – oxygen, blue – nitrogen and cyan – hydrogen.

the protonation of formaldehyde by the formic acid. Subsequently, one of the methylene protons of glycine transfers a proton to the carbonyl oxygen which restores the formic acid. Then the deprotonated methylene carbon of glycine attacks the carbon of the protonated formaldehyde. Although, this reaction has relatively high activation energy (55.2 kcal mol^{-1}) it is exothermic by 7.6 kcal mol^{-1} (Figure 4).

The obtained in the previous step serine is a starting compound for alanine formation. The alanine formation is a fascinating example of a complex reaction, since is takes place through s stable ionic intermediate structure and occurs in two stages. In the first stage, direct proton transfer from the hydroxyl group of formic acid to the same group of serine molecules is observed. Meanwhile, the C–O bond from hydroxymethyl group of serine is lengthening, resulting in the dehydration of serine. After that the methylene carbon creates a bond with amino-nitrogen, provoking three-membered ring closure. The obtained intermediate includes 2-carboxyaziridin-1-ium and a formate ion. The calculated energy barrier of this endothermic stage is 45.4 kcal mol^{-1}. Since three-membered rings are strained and unstable, the second stage starts with ring-opening and the 2-carboxyaziridin-1-ium transforms to 2-amino-2-carboxyethan-1-ide. Hard on the heels of the three-membered ring opening, the proton from the formate ion directly migrates to the methylene carbon, resulting in alanine and carbon oxide formation. The second stage of the reaction is a strongly exothermic (–28.8 kcal mol^{-1}) with an evaluated energy barrier of 36.7 kcal mol^{-1} (Figure 4). Nevertheless, the successful alanine formation through the three-membered structure (aziridinonil radical) was shown earlier by Surpateanu *et al.*, which is in agreement with the proposed in the current investigation non-trivial reaction mechanism.[31]

Formaldehyde can react with hydrogen sulfide, which is one of the starting compounds, to form thioformaldehyde (Figure 5). The first step is catalyzed through a water molecule, which transfers a proton from H_2S to the oxygen atom of the formaldehyde molecule. This leads to the formation of a thiohemiacetal. The barrier is calculated to be 26.4 kcal mol^{-1}, and the reaction is slightly exothermic. The second step is a dehydration reaction where the thiohemiacetal converts to the decisive thioformaldehyde. This formic acid-catalyzed, endothermic reaction has a barrier in 2.5 kcal mol^{-1} lower than the previous step. Glycine can react with thioformaldehyde to produce the sulfur-containing amino acid cysteine. Formic acid acts as a *proton shuttle*, removing the methylene proton, making the alpha carbon of glycine nucleophilic, and transferring another proton to the sulfur. This nucleophilic alpha carbon then attacks the thiocarbonyl carbon. Although the reaction has a high energy barrier (55.9 kcal mol^{-1}), it is highly exothermic. Once alanine is obtained, it can react

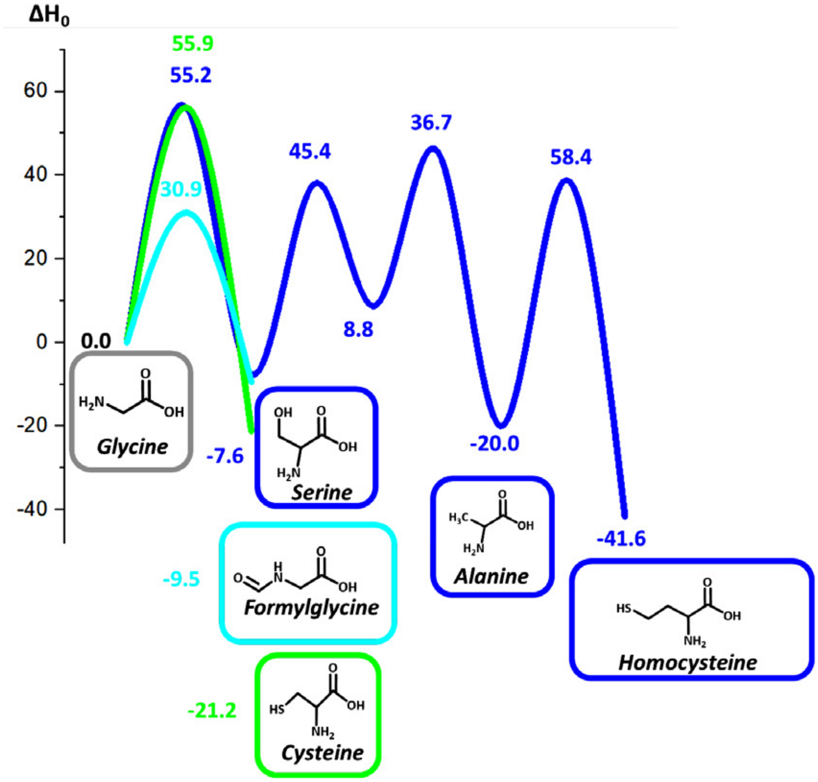


Figure 6. Schematic potential energy profiles along the reaction pathways of the amino acids (cysteine, serine, alanine and homocysteine) formation from glycine. The values of ΔH_0 in kcal mol^{-1} are calculated at SMD/SCS-MP2/cc-pVDZ level of the theory.

with thioformaldehyde to form homocysteine. When the methyl carbon of alanine and the carbon of thioformaldehyde are close enough (1.86 Å), a successive proton transfer occurs, mediated by the water molecule, from the methyl group of alanine to the sulfur atom, followed by the formation of a carbon-carbon bond. This reaction has a high energy barrier, estimated at 58.4 kcal mol^{-1}, but is highly exothermic (Figure 5).

Figure 6 presents an intrinsic reaction coordinate of the reaction pathways from glycine to cysteine, serine, alanine and homocysteine, which facilitates the opportunity to compare the reaction pathways and to determine the feasible processes. As it can be seen, the energy barrier (30.9 kcal mol^{-1}) of formylglycine formation is in about 15 kcal mol^{-1} lower in comparison with the cysteine (55.9 kcal mol^{-1}) or serine (55.2 kcal mol^{-1}) ones. It means that the formylglycine formation (cyan line) is kinetically favorable, which is in unison with our hydrothermal experiment, where formylglycine was indicated.

Paying attention to the energy of the product formation for reactions, mentioned above, the reaction for cysteine production (green line) is the most exothermic (–21.3 kcal mol^{-1}). That means that this reaction is also possible, but it will take a longer period of time than in the case of formylglycine formation. The longest reaction pathway of homocysteine formation (blue line), consists of four steps, where the last one is a rate-determining one (58.4 kcal mol^{-1}). As it can be seen, the alanine formation is a strongly exothermic process (–20.0 kcal mol^{-1}) along with the consequent even more exothermic homocysteine formation (–41.6 kcal mol^{-1}). Taking this into account, it can be assumed that high-energy reaction barriers for serine, alanine and homocysteine reaction pathways will be overcome through high temperatures or a long time of heating.

4. Concluding Comments

We suggest the plausible reaction pathways for the formation of the following amino acids – glycine, alanine and serine, as well as sulfur-containing ones – cysteine and homocysteine, taking into consideration the hydrothermal scenario of the chemical evolution.

Only three components are needed to provide a plausible environment for hydrothermal amino acid formation – water, hydrogen cyanide/formamide and hydrogen sulfide. The proposed reaction scheme reveals step by step the thorny reaction pathway for the mentioned above amino acids formation. The experiment with thermal water and liquid formamide heated at 140 °C for 60 hours in the reactor of hydrothermal synthesis demonstrates the appearance of urea – a key prebiotic precursor, along with simple amino acid glycine and its derivative – formylglycine. The provided hydrothermal experiment and simulations played the auxiliary role for the main part of the work – quantum-chemical calculations at SCS-MP2/cc-pVDZ/SMD level of theory.

The *ab initio* computations follow the suggested reaction network, providing insight into reaction mechanisms and their corresponding kinetic and energetic characteristics. Theoretical results demonstrate feasible reaction pathways for the main prebiotic compound formation, such as formamide, urea, formyl cyanide, formaldehyde, 2IAN, 2AA, thioformaldehyde *etc.* Moreover, the quantum-chemical calculations bring up the original reaction mechanisms for alanine, serine, cysteine and homocysteine formation from glycine. The results confirm kinetically preferable formylglycine formation in comparison with cysteine and serine, which explains its presence after the hydrothermal experiment. In addition, the present schematic potential energy profiles along the reaction pathways for alanine, serine, cysteine and homocysteine demonstrate that their formation also takes place, since the considered reactions are exothermic. However, the relatively high activation energy barriers of the abovementioned reactions of amino acids required more robust conditions and longer time.

We believe that the results of the current investigation increase the insight of new reaction pathways and mechanisms of hydrothermal amino acid formation, including sulfur-containing ones and demonstrate a solid foundation for future investigations in the field of prebiotic chemistry in terms of hydrothermal scenario.

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Data availibility

Data are provided within the manuscript or supplementary information files.

Declarations

Conflict of interest

The authors declare no conflict of interest.

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Povzetek

Kemijska evolucija aminokislin, zlasti tistih, ki vsebujejo žveplo, zahteva ustrezne pogoje in naravne vire za zagotavljanje začetnih prebiotskih spojin. V tej študiji so bili kot verjetno okolje za prebiotske reakcije izbrani hidrotermalni vrelci, vulkani in oceani. Predlagano reakcijsko omrežje se začne le s tremi spojinami – vodo, vodikovim cianidom/formamidom in vodikovim sulfidom. Študija predlaga izvedbno enostopenjskega hidrotermalnega eksperimenta v laboratorijskih pogojih za prikaz tvorbe nekaterih ključnih prebiotskih predhodnikov. Reakcijske poti od začetnih molekul do aminokislin so bile modelirane na ravni teorije SCS-MP2/cc-pVDZ/SMD. Izračunane energijske značilnosti omogočajo določitev verjetnih reakcijskih poti za aminokisline – glicin, serin in alanin ter za tiste, ki vsebujejo žveplo – cistein in homocistein v hidrotermalnem scenariju.