

Enhanced Synthesis of Alkyl Amino Acids in Miller's 1958 H₂S Experiment

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Abstract Stanley Miller's 1958 H₂S-containing experiment, which included a simulated prebiotic atmosphere of methane (CH₄), ammonia (NH₃), carbon dioxide (CO₂), and hydrogen sulfide (H₂S) produced several alkyl amino acids, including the α -, β -, and γ -isomers of aminobutyric acid (ABA) in greater relative yields than had previously been reported from his spark discharge experiments. In the presence of H₂S, aspartic and glutamic acids could yield alkyl amino acids via the formation of thioimide intermediates. Radical chemistry initiated by passing H₂S through a spark discharge could have also enhanced alkyl amino acid synthesis by generating alkyl radicals that can help form the aldehyde and ketone precursors to these amino acids. We propose mechanisms that may have influenced the synthesis of certain amino acids in localized environments rich in H₂S and lightning discharges, similar to conditions near volcanic systems on the early Earth, thus contributing to the prebiotic chemical inventory of the primordial Earth.

Keywords Stanley Miller · Spark discharge · Hydrogen sulfide

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Introduction and Experimental Procedures

In the early 1950s Stanley Miller published results from his classic spark discharge experiment, which astonishingly produced several amino acids (Miller 1953). Archived samples prepared by Miller using his volcanic apparatus were recently reanalyzed and found to contain over 5 times as many organic compounds than Miller was able to identify in the 1950s (Johnson et al. 2008). More recently, Miller's previously unreported 1958 H₂S-containing experimental samples were found and analyzed and were composed of numerous organosulfur compounds (Parker et al. 2011a, b).

The H₂S samples were analyzed by high performance liquid chromatography with fluorescence detection (HPLC-FD) and ultraperformance liquid chromatography-fluorescence detection with time-of-flight mass spectrometry (UPLC-FD/ToF-MS). HPLC-FD was used primarily as a pre-screening method to assess the complexity of the sample mixtures. UPLC-FD/ToF-MS was then used to quantitate the products. Details of the preparation and analysis of these samples are stated in Parker et al. (2011a, b).

The possibility of biological and chemical contamination of Miller's 50 year old H₂S samples was a concern considered in this study. A preserved procedural blank that could be used for comparison to sample analysis results was not found with the discovered samples. Analytical reagent blanks were created and underwent identical preparation and analysis protocols as the samples themselves, to assess the amount of contamination introduced by the chemical reagents used in this study. It was found that contamination from chemical reagents used during sample preparation and analysis was negligible. Additionally, the original samples were sealed and stored as dried residues until analyzed, which minimized potential contamination pathways. However, the samples were not sealed under anaerobic conditions and thus the presence of oxygen in the sample vials could have induced oxidation of some organic compounds that were initially synthesized in Miller's H₂S experiment.

Results and Discussion

Miller's H₂S experiment synthesized 23 amino acids and 4 amines. Many non-protein amino acids, including several rare, or absent, in biology were detected. Amino acids with chiral centers were generally racemic within experimental error, suggesting that most of the compounds found in these samples were synthesized in the experiment. Details regarding the abundances and variety of compounds produced in Miller's H₂S experiment can be found in Parker et al. (2011b).

Alanine and β -alanine, α -aminoisobutyric acid (α -AIB), and α -, β -, and γ -ABA were more abundant in the H₂S experiment than amino acids such as aspartic and glutamic acids (Parker et al. 2011b). One potential explanation for this could be the production of alkyl amino acids from the degradation of aspartic and glutamic acids. In the presence of H₂S, aspartic and glutamic acids may form their respective cyclic thioimides (Fig. 1), which can be hydrolytically opened to produce α - and ω -thioacids. Dethiocarboxylation of these thioacids may then form the aforementioned alkyl amino acids. It is important to note that the dethiocarboxylation of thioacids would result in the production of COS, which has been implicated in the formation of dipeptides (Leman et al. 2004). This study focused explicitly on the detection and quantitation of primary amino-containing organic compounds and as a result COS was not a targeted product.

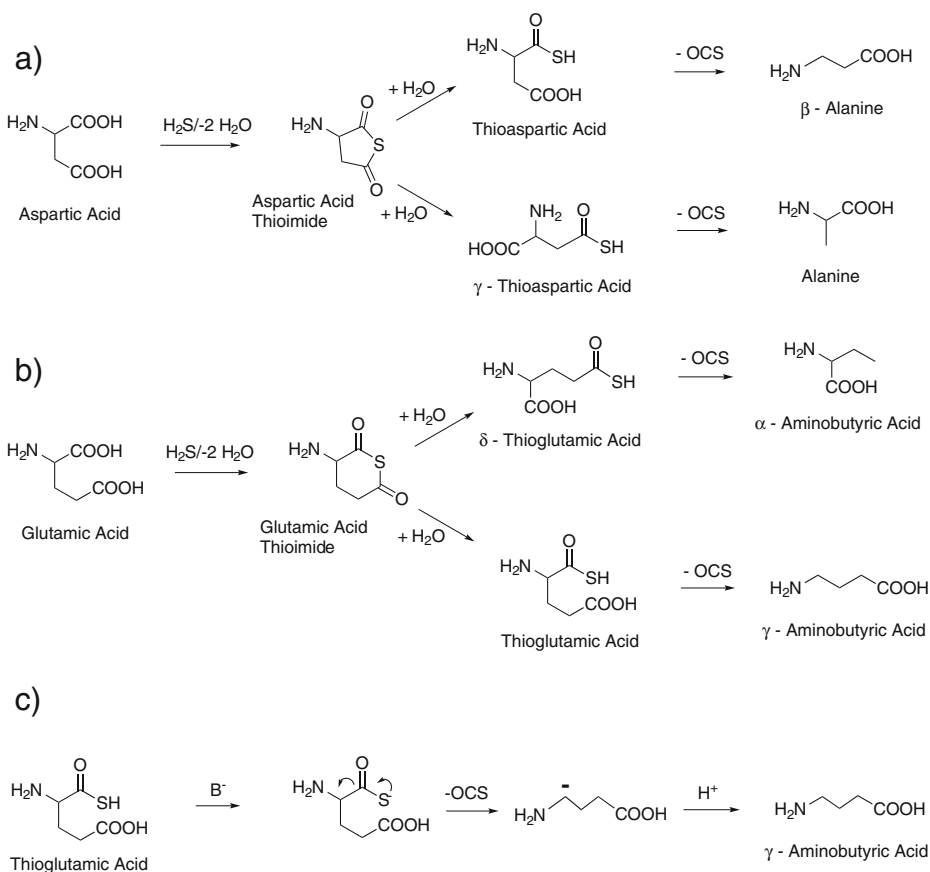


Fig. 1 Proposed mechanisms for the aqueous phase production of alkyl amino acids from **a** aspartic acid and **b** glutamic acid in the presence of H_2S . A more detailed mechanism starting from thioglutamic acid is shown in **c**. This mechanism may operate more easily starting with the Strecker nitrile or amide intermediates

The solubility of a gas in water depends primarily on the Henry's law constant of the gas, which takes into consideration factors such as the partial pressure of the gas. Based on their solubilities, the gases used in the H_2S experiment would have partitioned into the aqueous phase in the following order: $\text{NH}_3 \gg \text{H}_2\text{S} > \text{CO}_2 > \text{CH}_4$ (Wilhelm et al. 1977). Therefore, based on solubility alone, much more NH_3 than H_2S would have dissolved into the aqueous phase, but over the course of the experiment significant amounts of H_2S could have dissolved, possibly driving the aqueous mechanisms in Fig. 1. However, solubility is also influenced by the pH of the solution, especially for the species CO_2 , H_2S , and NH_3 . The effective Henry's law coefficient dictates the solubility of a gas in solution based largely on the partial pressure of the gas, and the pH of the solution. Calculations of the effective Henry's Law coefficients for H_2S , CO_2 , and NH_3 indicate that basic solutions are more suitable for the dissolution of H_2S and CO_2 , while acidic solutions are more conducive for dissolving gaseous NH_3 . In a solution of neutral pH, CO_2 and H_2S are more soluble than NH_3 , assuming all three species are present at identical partial pressures. The oxidation state of volcanic outgassing is largely dependent upon the oxidation state of the upper mantle. To produce an atmosphere abundant in CH_4 , NH_3 , and H_2S , similar to the one Miller used in

the H₂S experiment, the upper mantle must have been relatively reducing itself, containing metallic iron (Catling and Claire 2005) as may have been the case during the Hadean Eon. Ocean pH during the Hadean is thought to have been slightly acidic or neutral (Morse and Mackenzie 1998), which could have allowed for the dissolution of sufficient quantities of gaseous H₂S over geologic timescales to help facilitate the sulfur chemistry observed here.

Gas phase reactions may also be responsible for the higher relative yields of alkyl amino acids in the H₂S samples. Homolysis of water vapor by spark discharge would form hydroxyl radicals ($\cdot\text{OH}$), which can abstract hydrogen from CH₄ (Gierczak et al. 1997) and H₂S (Mousavipour et al. 2003), forming methyl ($\cdot\text{CH}_3$) and mercapto ($\text{HS}\cdot$) radicals, respectively (Fig. 2a). Hydrogen radicals ($\text{H}\cdot$) are also generated from the spark discharge dissociation of water and are known to help decompose H₂S to elemental sulfur (Helfritsch 1993), which was a byproduct Miller observed in this experiment (S.L. Miller, 1958, Laboratory Notebook 2, page 114, Serial number 655, MSS642, Box 25, Mandeville Collections, Geisel Library). Hydrogen atoms can be directly abstracted from CH₄ by $\text{H}\cdot$ to form $\cdot\text{CH}_3$ (Jursic 1996) (Fig. 2a, b). Alkyl amino acid precursor formation via an $\cdot\text{OH}$ -mediated mechanism alone may not explain the higher relative yields of alkyl amino acids

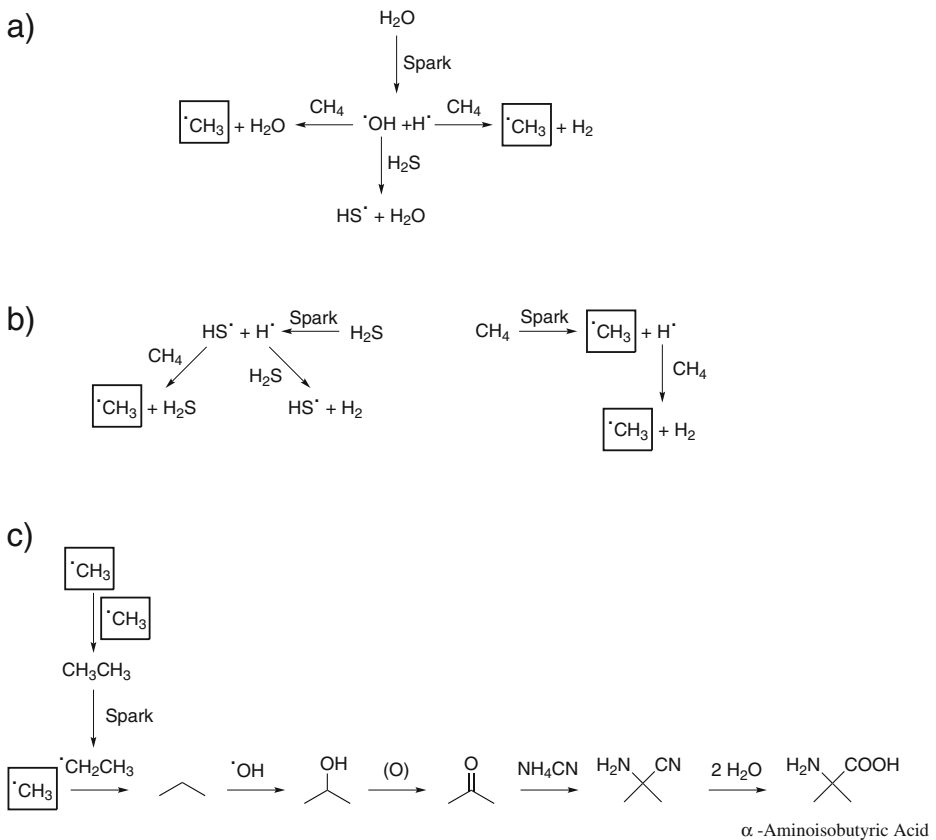


Fig. 2 Proposed mechanisms for the gas phase production of alkyl amino acid precursor methyl radicals by **a** $\cdot\text{OH}$ -mediated pathways and **b** pathways involving H₂S and CH₄ in the presence of a spark discharge. A proposed mechanism for the formation of alkyl amino acids from $\cdot\text{CH}_3$ is shown in **c**. The production of α -AIB is shown as an example in scheme **c**). Note: boxed species indicate alkyl amino acid precursor methyl radicals

in the H₂S experiment compared to other spark discharge studies, since the classic and volcanic experiments also incorporated a spark, CH₄, and water.

Dissociation of CH₄ and H₂S by spark discharge can form $\cdot\text{CH}_3$ and HS \cdot , respectively. Labile hydrogen atoms, such as those in CH₄, can be abstracted by HS \cdot to form $\cdot\text{CH}_3$ (McElroy et al. 1980) (Fig. 2b). Furthermore, H \cdot may be formed by the homolysis of H₂S and CH₄. Radicals produced by these spark discharge interactions may undergo similar pathways to produce the alkyl amino acid precursor $\cdot\text{CH}_3$, as those described for $\cdot\text{OH}$ -mediated mechanisms. The additional production of H \cdot and HS \cdot from the homolysis of H₂S, compared to those radicals produced by the dissociation of water alone, may partially cause enhanced yields of gaseous $\cdot\text{CH}_3$ when H₂S is present.

In a prebiotic atmosphere containing CH₄, which Miller used in his H₂S experiment, low mass hydrocarbons like ethane could have been formed by the joining of two $\cdot\text{CH}_3$, and larger hydrocarbons could have formed via similar polymerization reactions (Lasaga et al. 1971). Alkyl radicals could then recombine to make larger alkyl chains prior to conversion to aldehydes and ketones in the presence of $\cdot\text{OH}$, which may then take part in the Strecker synthesis of amino acids (Pascal et al. 2005) (Fig. 2c).

In addition to radical chemistry that could have facilitated the synthesis of alkyl amino acids in Miller's H₂S experiment, ion chemistry may have also played a role. Spark discharges produce large quantities of electrons and ions. The principle mechanisms by which ionization occurs via spark discharge in the gas phase are by electron and positive ion impact (Loeb 1936). Although the influence of ion chemistry enhancing alkyl amino acid synthesis was not a focus of this study, its potential involvement in the overall reactions taking place to form organic compounds in plausible primordial Earth conditions warrants further investigation.

Conclusions

We propose mechanisms that may help explain the high relative yields of alkyl amino acids found in Miller's 1958 H₂S experiment. In the presence of H₂S aspartic and glutamic acids could have yielded alanine, β -alanine, and α - and γ -ABA through the formation of aspartic and glutamic acid thioimides. The generation of $\cdot\text{CH}_3$ on the early Earth via HS \cdot - and $\cdot\text{OH}$ -mediated mechanisms could have increased the synthesis of alkyl radicals that may have eventually reacted to form amino acid precursor aldehydes and ketones, which could have undergone the Strecker synthesis to form alkyl amino acids. Dissociation of CH₄ by lightning may have also formed $\cdot\text{CH}_3$. These types of chemical reactions may have occurred in localized regions of the early Earth, such as near island-arc type volcanic systems, where there was an abundance of reduced gases including CH₄ and H₂S, volcano-associated lightning, and water vapor. These mechanisms could have helped produce a diverse array of prebiotic compounds on the primitive Earth.

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