

# Prebiotic Synthesis of Methionine and Other Sulfur-Containing Organic Compounds on the Primitive Earth: A Contemporary Reassessment Based on an Unpublished 1958 Stanley Miller Experiment

Eric T. Parker · H. James Cleaves ·  
Michael P. Callahan · Jason P. Dworkin ·  
Daniel P. Glavin · Antonio Lazcano · Jeffrey L. Bada

Received: 5 October 2010 / Accepted: 19 October 2010

© The Author(s) 2010. This article is published with open access at Springerlink.com

**Abstract** Original extracts from an unpublished 1958 experiment conducted by the late Stanley L. Miller were recently found and analyzed using modern state-of-the-art analytical methods. The extracts were produced by the action of an electric discharge on a mixture of methane (CH<sub>4</sub>), hydrogen sulfide (H<sub>2</sub>S), ammonia (NH<sub>3</sub>), and carbon dioxide (CO<sub>2</sub>). Racemic methionine was formed in significant yields, together with other sulfur-bearing organic compounds. The formation of methionine and other compounds from a model prebiotic atmosphere that contained H<sub>2</sub>S suggests that this type of synthesis is robust under reducing conditions, which may have existed either in the global primitive atmosphere or in localized volcanic environments on the early Earth. The presence of a wide array of sulfur-containing organic compounds produced by the decomposition of methionine and cysteine

---

E. T. Parker · J. L. Bada

Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA 92093, USA

H. J. Cleaves

Geophysical Laboratory, Carnegie Institution of Washington, 5251 Broad Branch Rd. NW, Washington, DC 20015, USA

M. P. Callahan · J. P. Dworkin · D. P. Glavin

NASA Goddard Space Flight Center, Solar System Exploration Division, Greenbelt, MD 20771, USA

A. Lazcano

Facultad de Ciencias, UNAM, Apdo. Postal 70-407 Cd. Universitaria, 04510 Mexico D. F., Mexico

J. L. Bada (✉)

Scripps Institution of Oceanography, University of California at San Diego, 8615 Kennel Way, La Jolla, CA 92093-0212, USA

e-mail: [jbada@ucsd.edu](mailto:jbada@ucsd.edu)

*Present Address:*

E. T. Parker

School of Earth & Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA 30332, USA

indicates that in addition to abiotic synthetic processes, degradation of organic compounds on the primordial Earth could have been important in diversifying the inventory of molecules of biochemical significance not readily formed from other abiotic reactions, or derived from extraterrestrial delivery.

**Keywords** Prebiotic chemistry · Methionine · Amino acids · Sulfur

## Introduction

Even though the presence of sulfur-containing compounds in proteins had been known since the mid-19th century, it was only with the laborious work of John Mueller in the early 1920s that one of the components was identified as an amino acid other than cysteine. Using 45–68 kg of casein, Mueller successfully isolated 100–200 g of an amino acid that he assigned the empirical formula  $C_5H_{11}SNO_2$  (Mueller 1923a; Mueller 1923b). Using Mueller's procedure, Barger and Coyne (1928) also isolated the new amino acid from casein and showed that it was identical to the amino acid synthesized by the Strecker reaction ( $HCN + NH_3 + \text{aldehyde}$ ) when 3-methylthiopropional ( $HSCH_2CH_2CHO$ ) was used as the starting aldehyde. They determined its structure as the  $\gamma$ -methylthiol of  $\alpha$ -amino-n-butyric acid (2-amino-4-methylthio-butyric acid,  $CH_3SCH_2CH_2CH(NH_2)COOH$ ) and after conferring with Mueller, named the amino acid methionine.

Following methionine's discovery and chemical characterization, the study of its biochemical role together with that of cysteine and cystine (Lewis et al. 1936) soon led to the recognition of the important structural role of these sulfur amino acids in proteins. The metabolic importance of the sulfur amino acids was also elucidated, as well as that of other sulfur-bearing organic compounds like coenzyme A and iron-sulfur clusters. Cysteine and homocysteine were found to play a key role in transsulfuration and methyl transfer reactions in degradative and biosynthetic pathways. The recognition of the significance of sulfur in various aspects of contemporary biochemistry soon raised the issue of the presence of sulfur-containing organic molecules, including such sulfur amino acids as methionine and cysteine on the primitive Earth prior to the emergence of life (Heinen and Lauwers 1996).

There have been several attempts to synthesize sulfur amino acids from a variety of model reducing prebiotic atmospheres and different energy sources including spark discharges (Heyns et al. 1957), electron beams (Choughuley and Lemmon 1966) and UV light (Khare and Sagan 1971; Sagan and Khare 1971; Steinman et al. 1968). In all of these experiments methionine was either not reported as a product or was only tentatively identified (Van Trump and Miller 1972). A detailed investigation of the prebiotic synthesis of methionine was carried out by Van Trump and Miller (1972) who used an electric discharge acting on a simulated primitive Earth atmosphere containing methane ( $CH_4$ ), molecular nitrogen ( $N_2$ ), ammonia ( $NH_3$ ), water ( $H_2O$ ), and hydrogen sulfide ( $H_2S$ ) or methane thiol ( $CH_3SH$ ). The finding of acrolein (propenal,  $CH_2=CH-CHO$ ) as a product of the discharge and the demonstration of its likely involvement in the abiotic formation of methionine led to the suggestion that acrolein had played a central role as a precursor in the prebiotic synthesis of a number of amino acids that included methionine, glutamic acid, homocysteine ( $HSCH_2CH_2CHNH_2COOH$ ), homoserine ( $HOCH_2CH_2CHNH_2COOH$ ) and  $\alpha,\gamma$ -diaminobutyric acid (Van Trump and Miller 1972).

The late Stanley L. Miller performed a number of electric discharge experiments in the 1950s and saved portions of many of these as dried residues (Johnson et al. 2008). One

particularly interesting experiment used a  $\text{CH}_4$ ,  $\text{H}_2\text{S}$ ,  $\text{NH}_3$ , and  $\text{CO}_2$  gas mixture and was performed while he was at Columbia University in 1958. For unknown reasons, the results of the experiment were never analyzed or published by Miller. The discovery of several boxes containing vials of dried residues from this experiment led us to reanalyze the products of this unpublished experiment using modern analytical methods. As discussed below, the formation of methionine from a model prebiotic atmosphere containing  $\text{H}_2\text{S}$  suggests that its synthesis is robust under the reducing conditions that may have existed in the Earth's early atmosphere, either globally, or in localized environments around volcanic eruptions that were accompanied by intense lightning (Johnson et al. 2008; Tian et al. 2005; Urey 1952; Walker and Brimblecombe 1985).

## Experimental Procedures

### Identification of Vials and Experimental Description

Miller's archived samples were found stored in labeled four-dram vials. They were catalogued and identified by consulting Miller's original laboratory notebooks, which are kept in the Mandeville Special Collections in the Geisel Library at the University of California, San Diego (Stanley L. Miller collection, Laboratory Notebook 2, page 114, Serial number 655, MSS642, Box 25, Mandeville Collections, Geisel Library). The samples chosen for analysis came from a collection consisting of several vials containing dried residues prepared by Miller from his aforementioned 1958 experiment. In this experiment he used the classic two-chambered apparatus configuration that he originally tested in 1953 (Miller 1953, 1955). The apparatus was filled with 300 mL  $\text{H}_2\text{O}$  and a mixture of  $\text{CH}_4$  (258 mm Hg),  $\text{CO}_2$  (87 mm Hg),  $\text{H}_2\text{S}$  (100 mm Hg) and  $\text{NH}_3$  (250 mm Hg). According to Miller's 1958 laboratory notebooks, a few minutes after the experiment was initiated on March 24, 1958, a yellowing of the solution was observed, possibly from the formation of sulfur-bearing organic compounds or the polymerization of hydrogen cyanide (HCN). A day after the start of the experiment, Miller reported "a large amount of [elemental] sulphur had deposited in the 5 L flask. Shook up the flask to get the sulphur away from the electrode". No major changes were subsequently observed the day after, and on March 27, 1958 the sparking and boiling were stopped, and the water solution extracts sampled directly from the apparatus were placed in a freezer. A few days later, on March 30, a pressure of 854 mm Hg was registered, with a pH of approximately 8, with "little  $\text{NH}_3$ ,  $\text{H}_2\text{S}$  (or  $\text{CO}_2$ ) present" (S. L. Miller, 1958, Laboratory Notebook 2, page 114, Serial number 655, MSS642, Box 25, Mandeville Collections, Geisel Library). The increase in pressure at the end of the experiment was not addressed by Miller but may have been due to the production of carbon monoxide (CO) and molecular hydrogen ( $\text{H}_2$ ). The experiment was terminated 3 days later, and the products were placed in a freezer. On June 17, 1958 he passed the solution through filter paper with suction. The solution had a yellow-red color, "somewhat like cytochrome C" (S. L. Miller, 1958, Laboratory Notebook 2, page 114, Serial number 655, MSS642, Box 25, Mandeville Collections, Geisel Library). The solution from the experiment was separated into various fractions by ion chromatography (Miller 1955), which were dried and stored. Only the soluble portion of the products formed during Miller's 1958 experiment were saved and stored in sealed, sterilized four-dram vials. The sample extracts were kept dry at room temperature for ~50 years. These were the sample extracts studied here.

One concern when analyzing a preserved sample set that is 50 years old is the possibility of contamination over time. We did not find any blanks that had been stored under identical conditions as the sample extracts upon discovery of the sample set. Therefore sample analysis results could not be compared to blank analytical results retrieved from an identical analytical protocol to assess the level of blank contamination. However, procedural blanks were generated and subjected to the same sample preparation and analysis scheme as the samples themselves. Analysis of procedural blanks for targeted organic species revealed that contamination from sample preparation and analysis was negligible. Furthermore, the samples remained sealed and unopened until their analysis, to prevent contamination from water vapor and oxygen. However, the samples were not initially sealed under anaerobic conditions so it is possible that there was some oxygen present in the sealed tubes, which may have oxidized some of the species present in the sample extracts over time.

When Miller moved from Columbia University to the University of California, San Diego in 1960, he took the vials described above with him, together with the products of many other experiments he had conducted earlier while at the University of Chicago (Johnson et al. 2008). These were stored in a cardboard box until we rediscovered them a few months before his death on May 20, 2007.

## Chemicals and Reagents

All glassware and sample handling tools were rinsed with Millipore water (18.2 M $\Omega$ , <10 ppb total organic carbon), wrapped in aluminum foil, and then heated in air at 500°C overnight. All of the chemicals used in this study were purchased from Sigma-Aldrich or Fisher Scientific. Stock amino acid solutions ( $\sim 10^{-3}$  M) were prepared by mixing individual amino acid crystals (97–99% purity) with doubly distilled (dd) H<sub>2</sub>O. The reagent *o*-phthalaldehyde/*N*-acetyl-L-cysteine (OPA/NAC) was used as a chemical tag for the fluorescence detection and enantiomeric separation of primary amines. The derivatization solution was prepared by dissolving 4 mg OPA in 300  $\mu$ L methanol (Fisher Optima), and then adding 250  $\mu$ L 0.4 M sodium borate buffer (pH 9.4), 435  $\mu$ L H<sub>2</sub>O, and 15  $\mu$ L of 1 M NAC. The ammonium formate buffer used in the time of flight-mass spectrometry (ToF-MS) analyses described below was prepared by NH<sub>4</sub>OH titration of a 50 mM formic acid solution to pH 8. A 1  $\mu$ M phenolphthalein solution in acetonitrile with 0.1% formic acid was used for mass calibration of the ToF-MS via an independent electrospray emitter (Glavin and Dworkin 2009).

## High Performance Liquid Chromatography with UV Fluorescent Detection (HPLC-UV)

This method was used to pre-screen the various samples to provide an indication of the relative abundances in order to optimize the more detailed analyses done with combined LC-FD/ToF-MS described below. The residues in the various vials were first re-suspended in 1.5 mL ddH<sub>2</sub>O and subjected to vortex stirring and sonication prior to being brought to dryness using a vacuum centrifuge set at 40°C. The samples were then resuspended into 1 mL aliquots of ddH<sub>2</sub>O and diluted from initial stock concentrations according to optimal fluorescent signal response. Amino acids and primary amines were separated and detected using a 5  $\mu$ m particle, 250 mm  $\times$  4.6 mm C-18 reverse phase HPLC column (*Phenomenex*) coupled with a *Shimadzu* RF-535 fluorescence detector ( $\lambda_{\text{ex}} = 340$  nm,  $\lambda_{\text{em}} = 450$  nm). Buffer flow rate was 1 mL/min with gradients optimized for separation of amino acid enantiomers (Zhao and Bada 1995). Buffers were Optima grade Methanol (A) and 0.05 M sodium acetate with 8% methanol (B). Samples were prepared for analysis by mixing 5  $\mu$ L sample aliquots with 10  $\mu$ L of 0.4 M, pH 9.4 sodium borate prior to 1 min derivatization

with 5  $\mu\text{L}$  OPA/NAC. Reactions were quenched with 0.05 M sodium acetate buffer (pH 5.5) to a final volume of 500  $\mu\text{L}$  and immediately analyzed. Concentrations of peaks were determined based on comparison with standard peak areas of known concentrations.

### HPLC-FD and Time of Flight-Mass Spectrometry (LC-FD/ToF-MS)

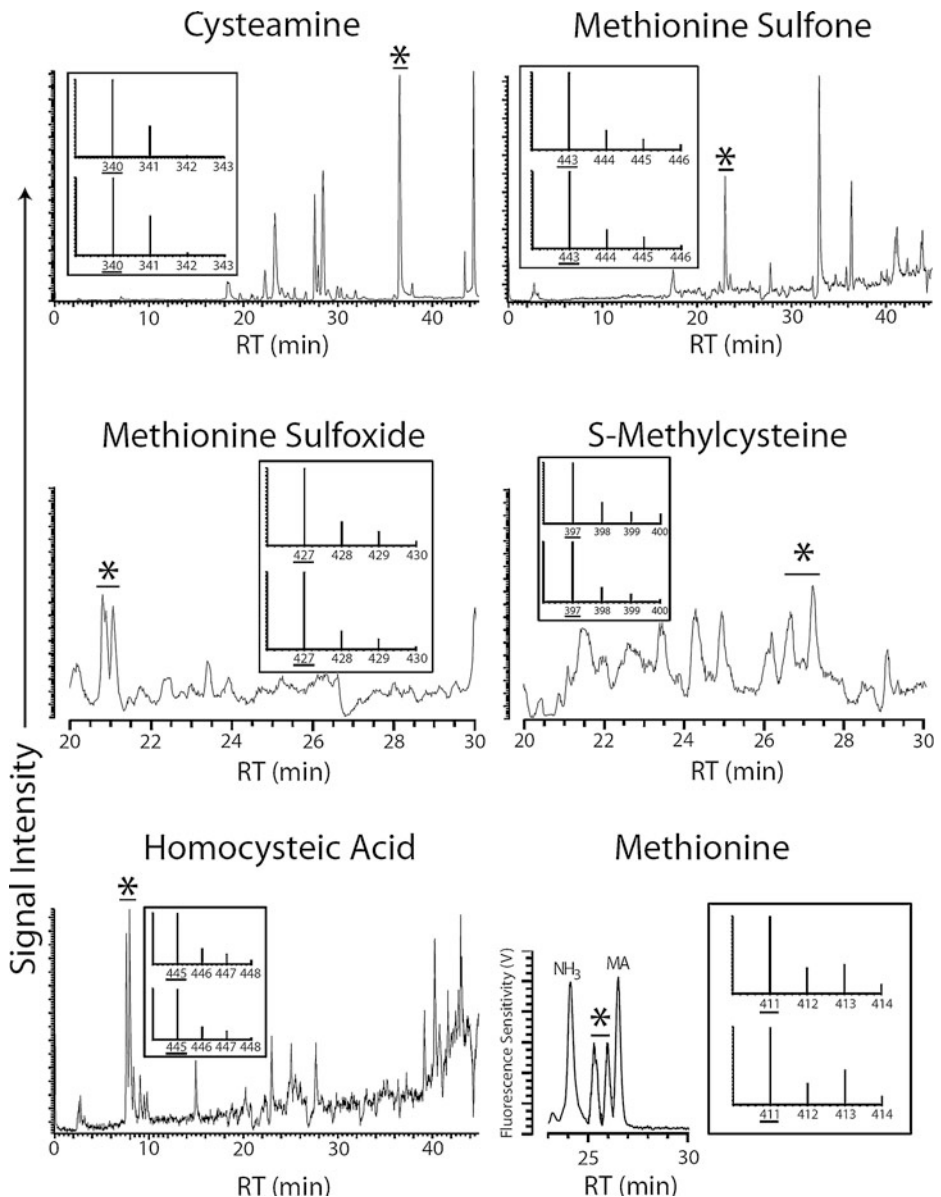
A fraction of each residue was prepared and similarly derivatized for analysis by LC-FD/ToF-MS as described elsewhere (Johnson et al. 2008). In addition to using retention times to identify fluorescent peaks in the LC-FD/ToF-MS chromatograms, we also determined compound identities by the presence of the appropriate monoisotopic mass at the correct retention time.

## Results

Typical LC-FD/ToF-MS chromatograms and mass spectra detailing the detection of the various sulfur-bearing organic compounds in Miller's original 1958 sample fractions are shown in Fig. 1. A summary of the recoveries of these sulfur-containing compounds relative to glycine is shown in Fig. 2 (a more extensive manuscript describing the entire suite of amino acids and amines detected in this experiment is in preparation). The observation that chiral amino acids were racemic within the precision of the measurements, combined with the fact that racemization is far too slow of a process to produce racemic mixtures of chiral amino acids over the time span that the sample extracts were stored (Bada 1991), provide evidence that the species detected here are a product of the experiment and not contamination. Additionally, other amino acids detected in the mixture, namely the butyric acid isomers (detected here, but described in detail in another manuscript in preparation) are not common biological compounds. We were not able to calculate absolute yields for the various amino acids because there was no record of how much of the solution from the experiment was saved. However, Van Trump and Miller (1972) gave the yield of glycine from a similar experiment (based on carbon added as methane) as 0.068%.

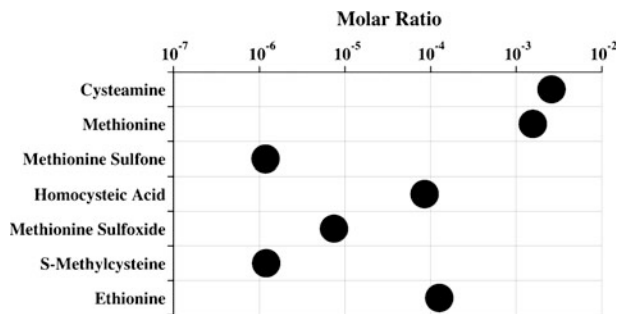
In addition to methionine and glutamic acid (detected here, data to be presented in a separate manuscript that is in preparation), which were reported by Van Trump and Miller (1972), we have also identified the non-proteinogenic sulfur-containing amino acid S-methylcysteine ( $\text{CH}_3\text{SCH}_2\text{CH}(\text{NH}_2)\text{COOH}$ ) and have tentatively identified the non-proteinogenic sulfur-containing amino acid ethionine (2-amino-4-ethylthiobutyric acid ( $\text{CH}_3\text{CH}_2\text{SCH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$ )), the lower and higher homologues of methionine, respectively. Several of the molecules listed in Fig. 2 are likely decomposition products of cysteine, homocysteine, and methionine, including cysteamine ( $\text{HSCH}_2\text{CH}_2\text{NH}_2$ ), homocysteic acid ( $\text{HO}_3\text{SCH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{CO}_2\text{H}$ ), methionine sulfone ( $\text{CH}_3\text{SO}_2\text{CH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$ ) and methionine sulfoxide ( $\text{CH}_3\text{SOCH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$ ), among others.

It is possible that cysteine and homocysteine may have been present in the original samples, but their presence could not be established with certainty because the OPA/NAC derivatization method does not provide high sensitivity for these species. This may be due to cyclization of compounds containing highly nucleophilic functional groups (such as amine or sulfhydryl groups) in 1, 2 or 1, 3 positions, either eliminating the fluorescent tag (OPA/NAC also does not effectively tag 2, 3-diamino propionic acid, 2,4-diamino butyric acid or 2, 3-diamino succinic acid, but does tag ornithine and lysine), but could also be due to internal fluorescence quenching of doubly-labeled compounds.



**Fig. 1** Sample chromatogram and mass spectra traces for 6 sulfur compounds detected in Miller's original sample extracts. All chromatogram traces displayed resulted from selective ion monitoring analysis except the methionine chromatogram trace, which was produced by HPLC-UV analysis. The chromatograms obtained by selective ion monitoring are plotted as signal intensity versus time, whereas the methionine chromatogram obtained by HPLC-UV is plotted as fluorescence sensitivity versus time. In each chromatogram, the asterisk demarcates the detection of the species in question. The mass spectra traces that accompany each chromatogram were obtained using ToF-MS analysis and are plotted as spectral intensity versus mass. Mass spectra traces were used to verify the sulfur distribution of the organosulfur species identified during selective ion monitoring. In all cases, the bottom mass spectra trace is the standard trace and the top mass spectra trace is the experimental trace. In each mass spectra trace, the underlined mass is the parent mass in question. Note: RT is retention time and MA is methylamine

**Fig. 2** Moles (relative to glycine = 1) of the various sulfur compounds detected in vials of dried residues obtained from the sparking of a  $\text{CH}_4$ ,  $\text{H}_2\text{S}$ ,  $\text{NH}_3$  and  $\text{CO}_2$  gas mixture



We also attempted to detect cysteine by GC-MS and DART (Direct Analysis in Real Time)-ToF-MS analysis. Cysteine was undetectable by GC-MS, and only traces of a compound with cysteine's mass were detectable by DART-ToF-MS analysis, suggesting that any cysteine produced in this experiment decomposed during storage, possibly due to oxidative dimerization to produce cystine. However, we were also unable to detect cysteine by LC-MS analysis, and cystine would presumably not suffer from the cyclization issues mentioned above, suggesting that any cysteine produced was rapidly degraded during storage into products other than cysteine and cystine.

## Discussion

It is likely that  $\text{H}_2\text{S}$  liberated from volcanic gases, hydrothermal vents, and other sites of fumarole activity, was present in the atmosphere of the primitive Earth (Urey 1952; Walker and Brimblecombe 1985; Kasting et al. 1989; Domagal-Goldman et al. 2008). This possibility is supported by models of thermal outgassing of volatiles based on ordinary chondritic material (Schaefer and Fegley 2007). As has been pointed out (Sagan and Khare (1971); Miller and Orgel (1974); Raulin and Toupance (1977)),  $\text{H}_2\text{S}$  can act as a long wavelength UV photon acceptor for the energetic activation of other molecules such as methane. Thus, it could have played a central role as a sulfur donor in the abiotic synthesis of thio-amino acids and other sulfur-bearing compounds.

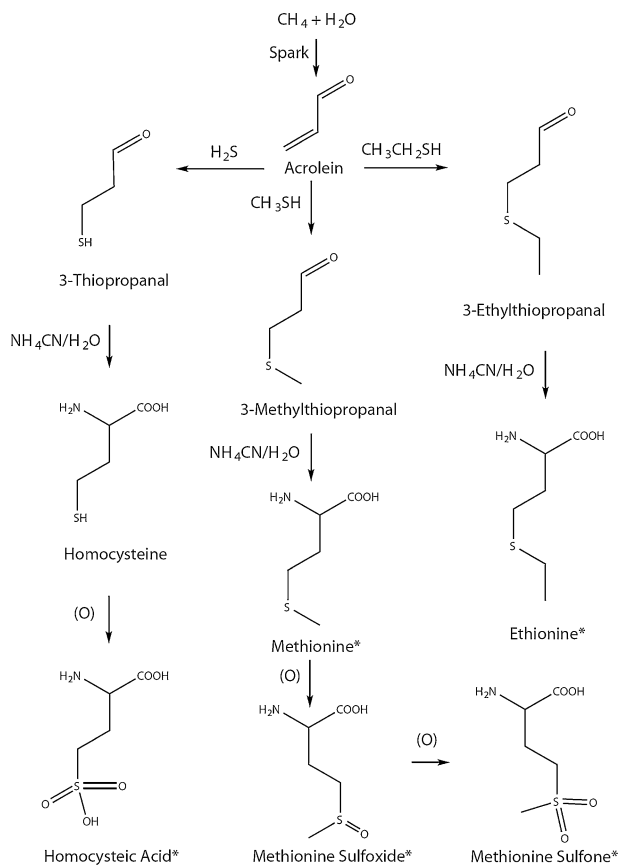
Van Trump and Miller (1972) demonstrated that methionine is synthesized by the action of an electric discharge on a simulated primitive Earth atmosphere containing  $\text{CH}_4$ ,  $\text{N}_2$ ,  $\text{NH}_3$ ,  $\text{H}_2\text{O}$ , and  $\text{H}_2\text{S}$  or  $\text{CH}_3\text{SH}$  at yields of  $\sim 3 \times 10^{-3}$  relative to glycine. This is very similar to the ratio we determined (Fig. 2). As shown here, analysis of the samples from experiments performed by Miller in 1958, 14 years before those he conducted in collaboration with Van Trump, demonstrate that methionine and other sulfur-bearing compounds, including S-methylcysteine, ethionine, homocysteic acid, methionine sulfone, methionine sulfoxide, and cysteamine, can be synthesized in good yields from a spark discharge acting on a  $\text{CH}_4$ ,  $\text{NH}_3$ ,  $\text{CO}_2$ , and  $\text{H}_2\text{S}$  gas mixture, in addition to the water vapor that was present in the 5 L flask due to the inclusion of 300 mL of  $\text{H}_2\text{O}$  in the system. The results presented here also expand the list of sulfur amino compounds that may have been formed prebiotically and are the first report of the synthesis of the non-proteinogenic amino acid S-methylcysteine. Additionally, a peak consistent with ethionine, but coeluting with a contaminant leads us to the tentative reporting of the synthesis of ethionine in a prebiotic simulation experiment.

The abiotic formation of methionine by a Strecker synthesis involving 3-methylthiopropional, KCN and  $\text{NH}_4\text{Cl}$  has been reported (Barger and Coyne 1928). Van

Trump and Miller (1972) suggested that 3-methylthiopropenal could be produced from a reducing atmosphere containing hydrogen sulfide by the addition of methane thiol to acrolein even under dilute conditions (Fig. 3). Acrolein is a byproduct of the decomposition of methionine (Lieberman et al. 1965) and can also be produced in significant amounts from very dilute solutions of formaldehyde and acetaldehyde under neutral to basic conditions (Cleaves 2003). It is significant to note that the two predominant amino acids produced in electric discharge experiments are glycine and alanine, the Strecker synthesis products of formaldehyde and acetaldehyde, respectively (Miller 1955). As suggested by Van Trump and Miller (1972), acrolein may have also played a key role as a precursor in the formation of glutamic acid, homocysteine, homoserine and  $\alpha,\gamma$ -diaminobutyric acid.

It has been suggested that the reaction of ammonium thiocyanate, thiourea, and thiacetamide (all of which are produced from electric discharges acting on  $\text{NH}_3$ ,  $\text{CH}_4$ ,  $\text{H}_2\text{O}$ , and  $\text{H}_2\text{S}$  gas mixtures (Heyns et al. 1957)) with formaldehyde can lead to the production of glycine, cysteine, and cystine (Herrera 1942; Perezgasga et al. 2003). It has also been shown that  $\text{H}_2\text{S}$ , together with pyrite and other metal sulfides, can partake in surface-mediated reactions that provide electrons for the reduction of organic compounds under simulated volcanic conditions (Huber et al. 2010; and references therein). However, organic sulfur-containing amino acids and amines, such as homocysteic acid, cysteamine, taurine ( $\text{HO}_3\text{SCH}_2\text{CH}_2\text{NH}_2$ ) (Choughuley and Lemmon 1966), cysteine (Khare and Sagan 1971;

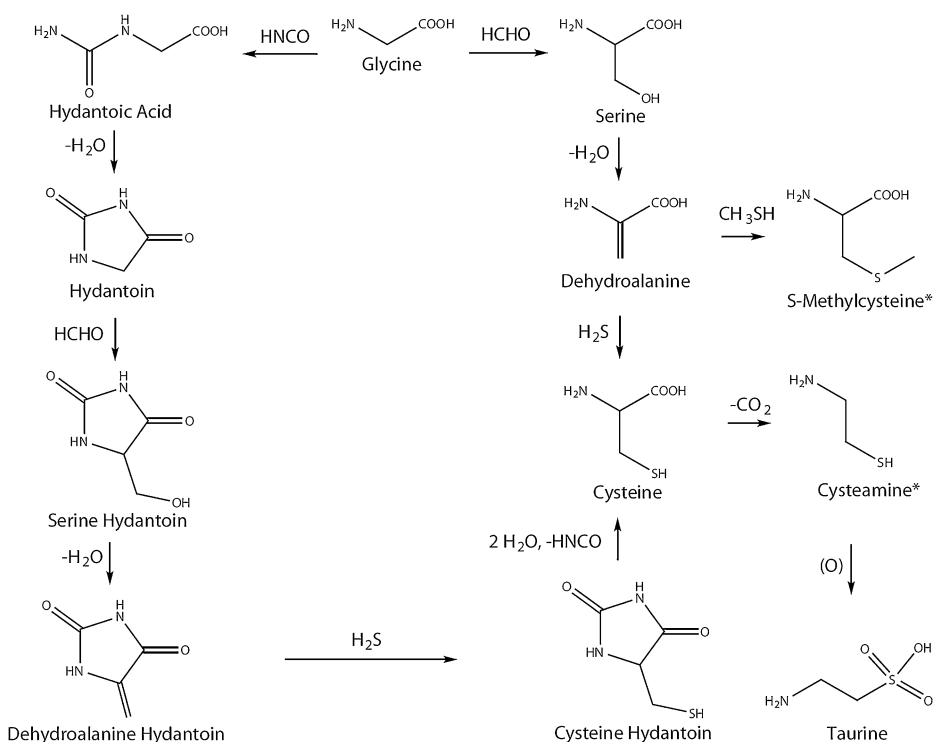
**Fig. 3** Prebiotic synthesis of methionine, methionine sulfoxide, methionine sulfone, ethionine, and homocysteic acid in the presence of acrolein, which is based in part on the scheme proposed by Van Trump and Miller (1972). Asterisks denote species that were detected in this study





Sagan and Khare 1971) and methionine, seem to be produced more readily from model  $\text{H}_2\text{S}$ -containing primitive atmospheres than from pyrite/metal sulfide reactions (Huber et al. 2010).

Two alternative pathways can be suggested for the production of cysteine from glycine under possible prebiotic conditions (Fig. 4). As suggested by Weber and Miller (1981), S-methylcysteine could have formed under primitive conditions by the Michael addition of  $\text{CH}_3\text{SH}$  to dehydroalanine (Fig. 4). We could not confirm the formation of dehydroalanine because it is very reactive and thus if present its levels could be below our detection limits, which are in the low femtomole range. The notion that methionine is a product of the addition of  $\text{CH}_3\text{SH}$  to acrolein (Van Trump and Miller 1972) is supported by the tentative detection of ethionine (Fig. 3), which could have been formed in part by the addition of ethane thiol ( $\text{CH}_3\text{CH}_2\text{SH}$ ) to acrolein. Cysteamine has also been produced in a model reducing atmosphere with electron beams, albeit in low yields (Choughuley and Lemmon 1966). Several of the compounds we have detected are known decomposition products of cysteine and methionine. Cysteamine, the simplest aminothiols, is produced by the decarboxylation of cysteine (Fig. 4), and methionine sulfone and methionine sulfoxide are produced by the oxidation of methionine (Lieberman et al. 1965). We did not detect taurine, which would be the product of the decarboxylation of cysteic acid or the oxidation of cysteamine (Fig. 4). Perhaps due to their relative instabilities, neither indigenous cysteine



**Fig. 4** Two possible mechanisms for the prebiotic synthesis of cysteine from glycine via serine or serine hydantoin, which would form dehydroalanine or its hydantoin. Reaction of the latter intermediates with  $\text{H}_2\text{S}$  would yield cysteine derivatives. Asterisks represent sulfur-containing compounds detected in this study

nor methionine has so far been conclusively detected in carbonaceous chondrites (Pizzarello and Shock 2010).

The presence of homocysteic acid in the samples we have analyzed could be explained by the Strecker degradation of methionine (Schönberg and Moubacher 1952). The Strecker degradation of methionine proceeds via the catalytic decarboxylation and deamination with a carbonyl compound or an inorganic catalyst to produce 3-methylmercaptopropanal (Schönberg and Moubacher 1952), which we did not attempt to detect. However, the Strecker degradation of methionine is also known to produce, among other compounds, homocysteine (Lieberman et al. 1965), which upon oxidation would yield homocysteic acid.

As long as free oxygen was absent in the primitive atmosphere and oceans, methionine could have persisted for significant periods of geologic time (Van Trump and Miller 1972). However, as oxygen began to accumulate in the early atmosphere (Kump 2008), oxidation by metal ions, peroxides, etc. would have likely been important in limiting the concentration of methionine and cysteine present in the primitive oceans and other water bodies (Weber and Miller 1981). Methionine decomposes readily in the presence of oxygen and produces methionine sulfoxide, methionine sulfone, and various sulfides and thiols (Lieberman et al. 1965).

It is thus possible that the compounds detected here represent both products synthesized due to the action of electric discharges on an atmosphere of  $\text{CH}_4$ ,  $\text{H}_2\text{S}$ ,  $\text{NH}_3$  and  $\text{CO}_2$  and the various Strecker and oxidative decomposition products of methionine and cysteine formed during the storage of the extracts. Even though these samples were not preserved under anoxic conditions, the manner in which they were preserved (dry, room temperature, ~50 years) implies that prebiotic methionine may not have been stable once oxygen began to accumulate in the early atmosphere.

## Conclusions

Our findings confirm and extend previous work by Van Trump and Miller (1972) on the prebiotic synthesis of methionine and other sulfur-bearing organic compounds, which could have been formed under primitive Earth conditions. However, the results presented here indicate that in addition to abiotic synthetic processes, degradation of organic compounds of biochemical significance on the primordial Earth could have played a significant role in diversifying the inventory of molecules not readily formed from other endogenous abiotic reactions, or derived from extraterrestrial delivery.

Our results may have evolutionary implications in the understanding of the earliest metabolic pathways. It has been hypothesized that cysteamine, which is a chemical precursor of the pantetheine moiety of coenzyme A, was formed in the primitive oceans from ethylene sulfide and ammonia or from ethylene imine and hydrogen sulfide (Keefe et al. 1995). However, our results suggest that cysteamine could have also formed readily from electric discharges. The recently discovered enzymatic conversion of cysteate into sulfopyruvate in the biosynthesis of coenzyme M (2-mercaptoethanesulfonic acid,  $\text{HSCH}_2\text{CH}_2\text{SO}_3\text{H}$ ) in *Methanosarcina acetivorans* (Graham et al. 2009) supports the idea that products of cysteine degradation and other sulfur-bearing organic compounds of prebiotic origin may have been involved in early biological processes.

The selection of the two thio-amino acids present in proteins is likely the outcome of a combination of their availability coupled with their functional utility (Cleaves 2010; Weber and Miller 1981). It has been suggested that cysteine could be an evolutionary replacement of an ancestral sulfhydryl-containing coenzyme (White 1982). However, it is possible that

cysteine was first incorporated into proteins because of its ability to form RNA-recognizing zinc-fingers, to bind to Fe/S clusters and to dimerize and covalently link to form disulfide bonds that play a key role in maintaining functional three-dimensionally folded protein structures.

In addition to its role as a building block in proteins, methionine is the immediate precursor of S-adenosylmethionine (SAM), the major methyl-group donor in trans-methylation reactions in contemporary biochemistry. It has been proposed that methyl group transfer from SAM to amines may be vestigial of prebiotic methylation reactions involving formaldehyde (Waddell et al. 2000). However, the possibility that ribonucleotide-like coenzymes are remnants of an ancestral stage in which ribozymes played a more conspicuous role in metabolism (Orgel and Sulston 1971; White 1976) suggests that methionine may have been first incorporated into biological systems because of its involvement in methyltransferase activities that evolved in a primordial RNA-dependent world. In other words, it is possible that methionine was initially incorporated into the RNA world as a cofactor.

**Acknowledgements** We are grateful to the librarians of the Mandeville Special Collections in the Geisel Library at the University of California, San Diego campus. Support from a UC Mexus-CONACYT Fellowship to A.L. and the NASA Astrobiology Institute and Goddard Center for Astrobiology for J.P.D. and D.P.G. are gratefully acknowledged. H.J.C. and M.P.C. were supported by the NASA Post-Doctoral Program (NPP). We also thank Dr. Jamie Elsila for GC-MS analyses of these extracts and Professor Facundo Fernandez for DART-ToF analyses. JLB, HJC, and Facundo Fernandez are also affiliated with the Center for Chemical Evolution at the Georgia Institute of Technology, which is jointly supported by NSF and the NASA Astrobiology Program, under the NSF CHE-1004570.

**Open Access** This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

## References

- Bada JL (1991) Amino acid cosmogeochemistry. *Philos Trans: Biol Sci* 333:349–358
- Barger G, Coyne FP (1928) The amino-acid methionine; constitution and synthesis. *Biochem J* 22:1417–1425
- Choughuley ASU, Lemmon RM (1966) Production of cysteic acid, taurine and cystamine under primitive earth conditions. *Nature* 210:628–629
- Cleaves HJ (2003) The prebiotic synthesis of acrolein. *Monatsh Chem* 134:585–593
- Cleaves HJ (2010) The origin of the biologically coded amino acids. *J Theor Biol* 263:490–498
- Domagal-Goldman SD, Kasting JF, Johnston DT, Farquhar J (2008) Organic haze, glaciations and multiple sulfur isotopes in the Mid-Archean Era. *Earth Planet Sci Lett* 269:29–40
- Glavin DP, Dworkin JP (2009) Enrichment of the amino acid L-isovaline by aqueous alteration on CI and CM meteorite parent bodies. *Proc Natl Acad Sci USA* 106:5487–5492
- Graham DE, Taylor SM, Wolf RZ, Namboori SC (2009) Convergent evolution of coenzyme M biosynthesis in the Methanosarcinales: cysteate synthase evolved from an ancestral threonine synthase. *Biochem J* 424:467–478
- Heinen W, Lauwers AM (1996) Organic sulfur compounds resulting from the interaction of iron sulfide, hydrogen sulfide and carbon dioxide in an anaerobic aqueous environment. *Orig Life Evol Biosph* 26:131–150
- Herrera AL (1942) A new theory of the origin and nature of life. *Science* 96:14
- Heyns K, Walter W, Meyer E (1957) Modelluntersuchungen zur Bildung organischer Verbindungen in Atmosphären einfacher Gase durch elektrische Entladungen. *Naturwissenschaften* 44:385–389
- Huber C, Eisenreich W, Wächsterhäuser G (2010) Synthesis of  $\alpha$ -amino and  $\alpha$ -hydroxy acids under volcanic conditions: implications for the origin of life. *Tetrahedron Lett* 51:1069–1071

- Johnson AP, Cleaves HJ, Dworkin JP, Glavin DP, Lazcano A, Bada JL (2008) The Miller volcanic spark discharge experiment. *Science* 322:404
- Kasting JF, Zahnle KJ, Pinto JP, Young AT (1989) Sulfur, ultraviolet radiation, and the early evolution of life. *Orig Life Evol Biosph* 19:95–108
- Keefe AD, Newton GL, Miller SL (1995) A possible prebiotic synthesis of pantetheine, a precursor to coenzyme A. *Nature* 373:683–685
- Khare BN, Sagan C (1971) Synthesis of cystine in simulated primitive conditions. *Nature* 232:577–579
- Kump LR (2008) The rise of atmospheric oxygen. *Nature* 451:277–278
- Lewis HB, Brown BH, White FR (1936) The metabolism of sulfur: XXIII. The influence of the ingestion of cystine, cysteine, and methionine on the excretion of cystine in cystinuria. *J Biol Chem* 114:171–184
- Lieberman M, Kunishi AT, Mapson LW, Wardale DA (1965) Ethylene production from methionine. *Biochem J* 97:449–459
- Miller SL (1953) A production of amino acids under possible primitive Earth conditions. *Science* 117:528–529
- Miller SL (1955) Production of some organic compounds under possible primitive Earth conditions. *J Am Chem Soc* 77:2351–2361
- Miller SL, Orgel LE (1974) The origins of life on Earth. Prentice Hall, Englewood Cliffs
- Mueller JH (1923a) A new sulfur-containing amino-acid isolated from the hydrolytic products of protein. *J Biol Chem* 56:157–169
- Mueller JH (1923b) A new sulfur-containing amino-acid isolated from the hydrolytic products of protein: II. Sulfur excretion after ingestion. *J Biol Chem* 58:373–375
- Orgel LE, Sulston J (1971) Polynucleotide replication and the origin of life. In: Kimball AP, Oró J (eds) *Prebiotic and biochemical evolution*. North Holland, Amsterdam, pp 89–94
- Perezgasga L, Silva E, Lazcano A, Negrón-Mendoza A (2003) The sulfocyanic theory on the origin of life: towards a critical reappraisal of an autotrophic theory. *Int J Astrobiol* 2:301–306
- Pizzarello S, Shock E (2010) The organic composition of carbonaceous meteorites: the evolutionary story ahead of biochemistry. *Cold Spring Harbor Perspect Biol* 2:a002105
- Raulin F, Toupan G (1977) The role of sulphur in chemical evolution. *J Mol Evol* 9:329–338
- Sagan C, Khare BN (1971) Long-wavelength ultraviolet photoproduction of amino acids on the primitive Earth. *Science* 173:417–420
- Schaefer L, Fegley B Jr (2007) Outgassing of ordinary chondritic material and some of its implications for the chemistry of asteroids, planets, and satellites. *Icarus* 186:462–483
- Schönberg A, Moubacher R (1952) The Strecker degradation of  $\alpha$ -amino acids. *Chem Rev* 50:261–277
- Steinman G, Smith AE, Silver JJ (1968) Synthesis of a sulfur-containing amino acid under simulated prebiotic conditions. *Science* 159:1108–1109
- Tian F, Toon OB, Pavlov AA, De Sterck H (2005) A hydrogen-rich early Earth atmosphere. *Science* 308:1014–1017
- Urey HC (1952) The planets: their origin and development. University of Chicago Press, Chicago
- Van Trump JE, Miller SL (1972) Prebiotic synthesis of methionine. *Science* 178:859–860
- Waddell TG, Eilders LL, Patel BP, Sims M (2000) Prebiotic methylation and the evolution of methyl transfer reactions in living cells. *Orig Life Evol Biospheres* 30:539–548
- Walker JCG, Brimblecombe P (1985) Iron and sulfur in the pre-biologic ocean. *Precambrian Res* 28:205–222
- Weber AL, Miller SL (1981) Reasons for the occurrence of the twenty coded protein amino acids. *J Mol Evol* 17:273–284
- White HB III (1976) Coenzymes as fossils of an earlier metabolic state. *J Mol Evol* 7:101–104
- White HB III (1982) Evolution of coenzymes and the origin of pyridine nucleotides. In: Everse J, Anderson B, You B-S (eds) *The Pyridine nucleotide coenzymes*. Academic, New York, pp 1–17
- Zhao M, Bada JL (1995) Determination of  $\alpha$ -dialkylamino acids and their enantiomers in geological samples by high-performance liquid chromatography after derivatization with a chiral adduct of *o*-phthaldialdehyde. *J Chromatogr A* 690:55–63