

15th Water-Rock Interaction International Symposium, WRI-15

Sulfur chemistry of Cinder Pool, YNP: Implications for a chemical origin of life

Lindsey Sydow^a, Philip C. Bennett^{a,1}, D. Kirk Nordstrom^b*The University of Texas, Austin, TX 78712 USA**U.S. Geological Survey, Boulder, CO 80303 USA*

Abstract

One chemical origin of life theory posits the abiotic formation of alkyl thiols as an initial step to forming biomolecules and eventually simple chemoautotrophic cells. Alkyl thiols have not previously been identified in terrestrial hot springs as unequivocally abiogenic, but they have been synthesized in the laboratory under hydrothermal conditions in the presence of a catalyst. We found methanethiol as well as other sulfur gases in Cinder Pool, Yellowstone National Park in gas samples collected using the bubble strip method. Laboratory microcosm experiments were performed using synthetic Cinder Pool water in serum bottles containing different iron-sulfur minerals, including cinders collected from the pool itself, as catalytic surfaces for the CH_3SH generating reaction. The bottles were charged with H_2 , and CO_2 , with CS_2 added to some and incubated from 60 to 100°C . In bottles without introduced CS_2 , only the cinder material produced detectable CH_3SH . While CH_3SH is central to the autotroph-first theory and has been synthesized in the laboratory¹, it has not previously been observed to form abiotically in natural systems. We have identified abiogenic CH_3SH in a terrestrial hydrothermal feature using natural catalytic surfaces consistent with the Autotroph-First origin theory.

© 2017 Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Peer-review under responsibility of the organizing committee of WRI-15

Keywords: Methanethiol; Yellowstone; Cinder Pool; Origin of life, Alkyl thiols

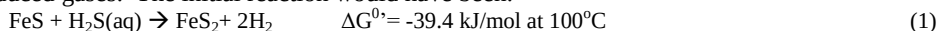
1. Introduction

The question of how life may have developed on Earth is as old as human existence. The two most commonly cited theories are the autotroph-first and heterotroph-first models. Heterotroph-first models are based on the idea that precursor biomolecules formed abiotically in a “primordial soup,” while autotroph-first models suggest that a primitive autotrophic metabolism is a necessary precursor to life.

* Corresponding author. Tel.: +1-512-471-3587; fax: +1-512-471-0959.

E-mail address: pbennett@jsg.utexas.edu

The autotroph-first hypothesis proposed that the first metabolism evolved from autocatalytic reactions on the surface of pyrite in hydrothermal environments². Pyrite (FeS₂) surfaces concentrate reactants, and hydrothermal fluids supply reactive reduced gases. The initial reaction would have been:



The energy and H₂ provides reducing power for other reactions on the pyrite surface³. The first primitive metabolic pathway is hypothesized to be the reductive acetyl-CoA pathway which depends on catalysis by acetyl-CoA synthase (which contains a Ni-Fe-S reaction center) to join carbon monoxide (CO) and coenzyme A (a thiol) into acetyl-CoA, a thioester which then enters the citric acid cycle:



A primitive version of this pathway, substituting simple bimodal Ni-Fe-S clusters for acetyl-CoA synthase and methanethiol (CH₃SH) for the more complex coenzyme A, was tested in laboratory experiments and produced thioacetic acid (CH₃COSH) via reaction (3)⁴.



This thioacid could then enter the reverse citric acid cycle to synthesize pyruvate, serving the purpose of the thioester acetyl-CoA in extant metabolisms⁴⁻⁶.

This hypothesis, however, requires the initial abiotic synthesis of CH₃SH. As proposed by¹:



Also observed was CS₂, and it was later found that (6) can be broken into (6a) and (6b, rate limiting), with (6b) requiring catalysis for the formation of CH₃SH⁷:



The H₂S produced in reaction (6b) could then react as in reaction (5) to produce more H₂, thus propagating the formation of more CS₂ and/or CH₃SH under the appropriate conditions. CS₂ has been observed in trace amounts in hydrothermal environments⁸.

Both CO and CO₂ dependent experiments yielded detectable CH₃SH, CS₂, and DMDS under simulated hydrothermal conditions^{1,9}. With transition metal sulfides and CO (both of which are required compounds for the theorized surface metabolism) often found in these environments, the confirmation of abiotic CH₃SH generation under similar conditions strengthens the idea that first life would have been a hyperthermophilic chemoautotroph. However, these findings still require identifying abiogenic CH₃SH synthesis in a natural hydrothermal system.

2. Materials and Methods

Cinder Pool is an acid-SO₄-Cl spring in the Norris Geyser Basin, Yellowstone National Park, Wyoming (44°43'57.16" N 110°42'35.21" W). The name originates from the black sulfur spherules or "cinders" floating to the surface from a subaqueous layer of molten S⁰ at the bottom of the ~18 m deep pool. Gases bubbling up through this layer form 5-10mm spherules comprised primarily of sulfur with minor pyrite and FeS. The pH generally fluctuates between 4 and 4.5, surface temperature is approximately 90°C, and the sulfur pool is 118°C¹⁰.

Dissolved gases were collected using the bubble strip method modified for hydrothermal settings. 0.5 mL was injected immediately into a gas chromatograph (GC) for preliminary on-site analysis, while the remainder of the sample was injected to a positive pressure into a dry, sterilized, and pre-evacuated 60 mL serum bottle for later analysis in the laboratory. Samples were collected from 1, 2, 3, 10, 17, and ~18 m deep within Cinder Pool. Cinders were collected in sterile falcon tubes for structural and elemental analysis as well as for use in experiments and were stored in an anaerobic chamber with a constant 10% CO₂ and H₂ mix in N₂. Gas analysis was by gas-liquid chromatography using a SRI-310 GC with separation on a MXT-1 capillary column and detection by FPD and FID detectors. H₂S was also determined by colorimetry.

Laboratory microcosms were constructed using synthetic Cinder Pool water with the addition of reactive components potentially capable of forming methanethiol abiogenically. Microcosms contained either elemental sulfur, FeS, FeS₂, or cinder pool cinders, under a headspace of 66% H₂ and 33% CO₂ at a total pressure of 2.7 atm. Experiments were done at 121°C for 12 hours, and at 60, 80 and 100°C for one week under both semi-aerobic and anaerobic conditions. Additional experiments to test the method of¹ using 10 mL of synthetic Cinder Pool water or dilute HCl, 0.1-0.14 g of FeS, and a 1.4 atm headspace of 100% CO₂ were allowed to incubate overnight in the autoclave at 121°C.

3. Results

The $[H_2S]$ in Cinder Pool varied from 49.9 and 73.4 $\mu\text{mol/L}$ determined by colorimetry. CS_2 and COS were also detected in all of the field samples by GC, from both Cinder Pool and one nearby pool. Methanethiol was detected in several samples, and in general the highest concentrations of sulfur gases were recorded at the bottom of Cinder Pool. CH_3SH detection does not appear to correlate with the presence of any other gases.

The underlying molten sulfur had only small amounts of iron and aluminium. The composition of the floating cinders was significantly more heterogeneous. While it too was dominated by elemental sulfur, it also contained iron, aluminum, silicon, titanium, potassium, and significant oxygen.

Microcosms with S^0 as the sole sulfur source yielded only H_2S as a byproduct using either synthetic Cinder Pool water or dilute HCl, averaging 6.0 $\mu\text{mol/L}$ in the synthetic Cinder Pool water and 9.1 $\mu\text{mol/L}$ in dilute HCl. In anaerobic microcosms, concentrations of H_2S were over range.

Microcosms that used FeS and FeS_2 as the solid phase yielded only small amounts of H_2S unless there was added CS_2 . With added CS_2 at 60°C, H_2S , CH_3SH , CS_2 , and DMDS were detected with $CH_3SH < 0.5 \mu\text{mol/L}$. At 80°C, H_2S , CH_3SH , CS_2 , and DMDS were again present, with Eth-SH also measured. CH_3SH levels were much higher than those recorded at 60°C, while CS_2 and DMDS were substantially lower. At 100°C, 2 to 4 $\mu\text{mol/L}$ of CH_3SH was found and H_2S concentrations exceeded 30 $\mu\text{mol/L}$. No CS_2 or DMDS was detected in the 100°C experiments.

Only experiments containing cinder material from the pool produced CH_3SH without the addition of external CS_2 . At 60°C, CS_2 was synthesized in-situ at higher concentrations than either the FeS or the FeS_2 experiments that were spiked with CS_2 : up to ~0.6 $\mu\text{mol/L}$. At 80°C CH_3SH was estimated to be ~3.4 $\mu\text{mol/L}$. H_2S and CS_2 exceeded detection limits in all three of the bottles, and were at least an order of magnitude greater in abundance in the cinder bottles than in the FeS or FeS_2 bottles at 80°C. Eth-SH ranged from 0.28 to 6.27 $\mu\text{mol/L}$. At 100°C, H_2S , CH_3SH , and CS_2 were detected in all cinder microcosms. CS_2 averaged 3.5 $\mu\text{mol/L}$ CH_3SH 1.2 $\mu\text{mol/L}$.

4. Discussion

The environment in Cinder Pool is highly dynamic, and a clear trend in dissolved gas concentrations with depth was not apparent. The highest concentrations of most gases were found at 18 m depth—approximately at the sulfur/water interface, but concentrations within the water column varied greatly. The greater abundance of all gases at the deepest sampling depth is likely a result of the higher temperature (at least 115.2°C) and proximity to the source of dissolved gases, and concentrations are likely higher within the sulfur layer due to its higher viscosity which can trap gases more easily.

Because the production of CH_3SH is probably H_2 limited in natural environments⁹, the higher concentrations of H_2 at the sulfur/water interface support the hypothesis that the cinder material is a reactive site for the key reactions for forming CH_3SH and beyond. Furthermore, because the pores of the cinders that float to the surface trap the gases found in this subaqueous material, they represent another possible refuge for the procession of surface metabolism reactions.

All three iron sulfide solids tested in this study catalyzed reaction (6b) when CS_2 was introduced to the microcosms, but only the cinders produced methanethiol (and CS_2) without introduced CS_2 . FeS and FeS_2 were only capable of catalyzing the hydrogenation of CS_2 into CH_3SH if CS_2 is added; they do not generate sufficient H_2S to complete reaction (6a) to produce CS_2 . Experiments with only S^0 generated H_2S , but there was no catalysis. In contrast, bottles containing pulverized cinders consistently generated the highest concentrations of H_2S , and produced measurable CS_2 and CH_3SH even when no CS_2 was initially added to the bottles. These findings were consistent with the gases detected in field samples. The large quantities of H_2S is sourced from the S^0 by the reduction of S^0 by H_2 . Under conditions found in Cinder Pool the formation of CS_2 (reaction 6a) is spontaneous whereas the formation of CH_3SH (reaction 6b) requires a catalyst.

It is clear from the experiments that FeS and FeS_2 can serve as catalysts only if there is another source of CS_2 . This is supported by recent research^{7,8}. Because cinders contain so much S^0 , they produce enough H_2S and subsequently CS_2 to fuel the formation of CH_3SH . The small amounts of iron sulfide minerals contained in the cinders then catalyze the CH_3SH generating reaction. Because the cinder material contains both S^0 and iron sulfide compounds, it can virtually serve as a CH_3SH factory if the right dissolved gases are present, as in Cinder Pool. Furthermore, thousands of floating cinders in addition to the molten cinder material at the bottom of the pool provide a large surface area on which these reactions can proceed.

Methanethiol has now been identified in Cinder Pool, and the water and dissolved gas composition are all

consistent with an abiotic synthesis pathway. An important question however, is whether the CH_3SH in Cinder Pool is truly abiotic in origin, or if it is part of a microbial synthesis pathway.

In modern systems methanethiol is microbially generated, and is a substrate for methanogens and aerobic methylotrophs. Currently the only microbial population identified in Cinder Pool is *Aquificales Hydrogenobaculum*, an organism that uses the reductive citric acid or reverse Krebs cycle¹¹. This metabolism is the extant version of the surface metabolism proposed by⁶, and it oxidizes sulfide compounds such as CH_3SH , as well as H_2 . This makes it extremely unlikely that CH_3SH detected in the pool is of biogenic origin, and much more likely that the microbial populations could be utilizing it as an energy source.

The cinders in the pool have an intriguing structure and composition that has implications for the theorized prebiotic synthesis of organic biomolecules. Previous research has suggested that permeable membranes may have formed along redox boundaries between alkaline submarine hot spring discharge and an acidic Hadean ocean^{12,13}. Cinders—large bubbles themselves—contain numerous enclosed spaces of the same size proposed by those previous studies, many on the order of 10 μm . Furthermore, cinders provide this structure in an environment that doesn't necessitate the alkaline solution proposed by others, allowing the reactions to be facilitated in more than one type of hydrothermal environment. While the membrane proposed by Russell and Hall is purely an “iron monosulfide bubble” or FeS , and the cinders are composed largely of elemental sulfur with minor iron sulfide components, the similarity between the two structures and their potential function to enclose and catalyze the first surface metabolic reactions is worthy of note. The composition of the cinders in an Archaean ‘Cinder Pool’ with a more reducing environment would have had a much higher concentration of ferrous iron and potentially a greater abundance of reactive catalytic surfaces.

One assertion of a hyperthermophilic chemoautotrophic origin of life is that it would not require a deeply reducing atmosphere or limit the location for reactions to a water/air interface⁴. The required processes could occur anywhere the chemical and mineralogical conditions are met. Abiotic CH_3SH production was originally hypothesized to occur only at mid-ocean ridge vents¹⁴, we have now documented a potential site in a terrestrial hydrothermal environment.

Acknowledgements

The authors gratefully acknowledge the Geology Foundation of the Jackson School of Geosciences and the National Research Program of the US Geological Survey.

References

1. Heinen W, Lauwers AM. Organic Sulfur Compounds Resulting from the Interaction of Iron Sulfide, Hydrogen Sulfide, and Carbon Dioxide in an Anaerobic Aqueous Environment. : *Origins Life Evol Biosphere* 1996; 26:131–150.
2. Wächtershäuser G. The Case for the Chemoautotrophic Origin of Life. *Origins Life Evol Biosphere* 1990; 20:173–176.
3. Huber C, Wächtershäuser G. Activated Acetic Acid by Carbon Fixation on (Fe,Ni)S Under Primordial Conditions. *Science* 1997; 276:245–247.
4. Wächtershäuser G. On the Chemistry and Evolution of the Pioneer Organism. *Chemistry and Biodiversity* 2007; 4:584–602.
5. Cody GD. Primordial Carbonylated Iron-Sulfur Compounds and the Synthesis of Pyruvate. *Science* 2000; 289:1337–1340.
6. Wächtershäuser G. Evolution of the first metabolic cycles. *Proc Natl Acad Sci* 1990; 87:200–4.
7. Gutiérrez OY, Kaufmann C, Hrabar A, Zhu Y, Lercher JA. Synthesis of methyl mercaptan from carbonyl sulfide over sulfide $\text{K}_2\text{MoO}_4/\text{SiO}_2$. *J. Catalysis* 2011; 280:264–273.
8. Rushdi AI, Simoneit BRT. Abiotic Synthesis of Organic Compounds from Carbon Disulfide Under Hydrothermal Conditions. *Astrobiology* 2005; 5:749–769.
9. Schulte MD, Rogers KL. Thiols in hydrothermal solution: standard partial molal properties and their role in the organic geochemistry of hydrothermal environments. *Geochim Cosmochim Acta* 2004; 68:1087–1097.
10. Xu Y, Schoonen MAA, Nordstrom DK, Cunningham KM, Ball JW. Sulfur geochemistry of hydrothermal waters in Yellowstone National Park, Wyoming, USA. II. Formation and decomposition of thiosulfate and polythionate in Cinder Pool. *J. Volca. Geotherm. Res.* 2000; 97:407–423.
11. Reysenbach A-L, Hamamura N, Podar M, Griffiths E, Ferreira S, Hochstein R, Heidelberg J, Johnson J, Mead D, Pohorille A, Sarmiento M, Schweighofer K, Seshadri R, Voytek MA. Complete and draft genome sequences of six members of the Aquificales. *J. bacteriology* 2009; 191:1992–3.
12. Martin W, Russell MJ. On the origin of biochemistry at an alkaline hydrothermal vent. *Phil trans Royal Soc London Series B, Biol sci* 2007; 362:1887–925.
13. Russell MJ, Hall AJ. The emergence of life from iron monosulphide bubbles at a submarine hydrothermal redox and pH front. *J Geol Soc* 1997; 154:377–402.
14. Dias D, do Nascimento PC, Jost CL, Bohrer ; de Carvalho LM; Koschinsky A. Voltammetric Determination of Low-Molecular-Weight Sulfur Compounds in Hydrothermal Vent Fluids - Studies with Hydrogen Sulfide, Methanethiol, Ethanethiol and Propanethiol. *Electroanalysis* 2010; 22:1066–1071.