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The origin of methanethiol in midocean ridge hydrothermal fluids

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Simple alkyl thiols such as methanethiol (CH_3SH) are widely speculated to form in seafloor hot spring fluids. Putative CH_3SH synthesis by abiotic (nonbiological) reduction of inorganic carbon (CO_2 or CO) has been invoked as an initiation reaction for the emergence of protometabolism and microbial life in primordial hydrothermal settings. Thiols are also presumptive ligands for hydrothermal trace metals and potential fuels for associated microbial communities. In an effort to constrain sources and sinks of CH_3SH in seafloor hydrothermal systems, we determined for the first time its abundance in diverse hydrothermal fluids emanating from ultramafic, mafic, and sediment-covered midocean ridge settings. Our data demonstrate that the distribution of CH_3SH is inconsistent with metastable equilibrium with inorganic carbon, indicating that production by abiotic carbon reduction is more limited than previously proposed. CH_3SH concentrations are uniformly low ($\sim 10^{-8}$ M) in high-temperature fluids (>200 °C) from all unsedimented systems and, in many cases, suggestive of metastable equilibrium with CH_4 instead. Associated low-temperature fluids (<200 °C) formed by admixing of seawater, however, are invariably enriched in CH_3SH (up to $\sim 10^{-6}$ M) along with NH_4^+ and low-molecular-weight hydrocarbons relative to high-temperature source fluids, resembling our observations from a sediment-hosted system. This strongly implicates thermogenic interactions between upwelling fluids and microbial biomass or associated dissolved organic matter during subsurface mixing in crustal aquifers. Widespread thermal degradation of subsurface organic matter may be an important source of organic production in unsedimented hydrothermal systems and may influence microbial metabolic strategies in cooler near-seafloor and plume habitats.

methyl mercaptan | biogeochemistry | origin of life | prebiotic chemistry

Since their discovery in 1977, seafloor hot spring fluids have been widely proposed as a potential source of organic molecules necessary for early life to emerge and thrive on a Hadean–Archaean Earth (1–5), and for metabolic energy and fixed carbon in modern hydrothermal systems (6, 7). Abiotic (nonbiological) reduction of inorganic carbon (CO_2 or CO) to methanethiol (methyl mercaptan, CH_3SH) is considered a crucial first step in the putative transition from prebiotic to primitive metabolic chemistry, leading to the emergence of hyperthermophilic microbial life (8–13). Specifically, methanethiol is the presumptive abiotic precursor of acetyl thioester (8, 12, 13)—the functional moiety of the Acetyl-CoA coenzyme central to many ancient metabolic pathways—and a sustainable abiotic source of acetyl thioesters is a key feature of models proposing the emergence of primordial metabolism in hydrothermal settings (5). Alkyl thiols are additionally implicated in the synthesis of the key metabolite pyruvate (10), which is speculated to have led to a primordial protometabolic network in a hydrothermal setting (14). In modern hot spring environments, hydrothermally produced thiols could constitute metabolic energy and carbon sources (15) for mesophilic and thermophilic microorganisms in subsurface, near-vent, and plume settings given that such compounds are intensively cycled in sedimentary microbial habitats (16). Moreover, due to their strong metal-binding abilities, thiol

functional groups are also increasingly implicated in the complexation and delivery of hydrothermally derived metals such as Fe and Cu to the deep ocean (17–19).

Abiotic reduction of inorganic carbon to CH_3SH has been shown to occur under experimental hydrothermal conditions (8, 20, 21), and thermodynamic considerations indicate that the abundance of CH_3SH in metastable equilibrium with inorganic carbon sources should increase strongly with dissolved hydrogen (H_2) abundance (22). This has led to the assumption that CH_3SH should be enriched in H_2 -rich fluids emanating from serpentine-hosted hydrothermal settings, such as the Lost City hydrothermal field (13). Without evidence from analogous modern hydrothermal fluids, however, the assumption of widespread thiol production by inorganic carbon reduction in prebiotic seafloor hot springs lacks support.

It is generally assumed that low-molecular-weight organic compounds in hydrothermal fluids emanating from settings lacking significant sedimentary organic matter (unsedimented systems) are primarily derived from inorganic carbon (e.g., mantle-derived CO_2) via abiotic reduction reactions, either by homogeneous reduction or involving heterogeneous mineral catalysts (23). CH_4 , C_{2+} hydrocarbons, and formate, for example, are postulated to be the products of abiotic carbon reduction in Lost City vent fluids (24, 25). However, potential contributions of thermogenic (i.e., derived from abiotic thermal decomposition of either preexisting biomass or biologically derived compounds) or biogenic (i.e., derived from metabolic activities of viable

Significance

Simple alkyl thiols such as methanethiol are widely speculated to spontaneously form in seafloor hot spring fluids and are implicated in facilitating the emergence of protometabolism and microbial life in early Earth hydrothermal systems, the complexation of hydrothermally derived metals, and as fuels for microbial ecosystems. Existing models suggest that methanethiol forms by nonbiological reduction of hydrothermal inorganic carbon (CO_2 or CO). We demonstrate that methanethiol is actively produced in low-temperature mixing zones of hydrothermal systems, but our data suggest it is the thermal destruction of preexisting organic matter (likely subsurface microbial biomass) that is responsible. Formation of organosulfur compounds and other degradation products during subsurface mixing may influence the biogeochemistry of low-temperature hydrothermal fluids inhabited by microbial life.

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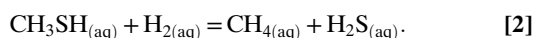
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reduction is not responsible for the production of CH_3SH in high-temperature endmember fluids. Aside from CO_2 , CH_4 is invariably the next largest stable pool of dissolved carbon in hydrothermal solutions, with all other aqueous single carbon species representing metastable states (7, 26). Indeed, models that propose reaction 1 as the source of CH_3SH in hydrothermal fluids (5, 13, 22) inherently assume that metastable CH_3SH is kinetically inhibited from destruction by further reduction to CH_4 . For many high-temperature endmember fluids presented here, however, calculated chemical affinities (see *Assessment of Metastable Equilibrium Using Chemical Affinities* and Eq. S1) show CH_3SH is indeed at or close to metastable equilibrium with CH_4 according to the reaction



For example, affinities for reaction 2 for all Rainbow endmember fluids (-3.8 to $+0.5$ kJ/mol) are within typical uncertainties of equilibrium at conditions of venting (see *Assessment of Metastable Equilibrium Using Chemical Affinities*). At Piccard ($+6.4$ to $+14.6$ kJ/mol) and Lucky Strike (-4.7 to -12.8 kJ/mol), endmembers are also close to equilibrium in several cases, as are some vents at $9^\circ 50' \text{N}$ (e.g., Tica, $+5.5$ kJ/mol). Thus, in contrast to reaction 1, measured CH_3SH concentrations in endmember fluids are more consistent with predicted values according to reaction 2. Although we cannot exclude that the reverse of reaction 2 is occurring, there is to date no evidence to suggest that aqueous CH_4 can react with H_2S under hydrothermal conditions. On the contrary, CH_3SH was observed to react to form small quantities of CH_4 at 100°C in the thioester synthesis experiments of Huber and Wächterhäuser (8). Thermodynamic data (22) indicate that reaction 2 would maintain CH_3SH at low levels with respect to CH_4 for most hydrothermal fluid compositions, with an inverse dependence on H_2 abundance. This is incompatible with the notion of greater abiotic CH_3SH production with increasing H_2 abundance, as invoked in scenarios for prebiotic hydrothermal thioester production (8, 12, 13). Regardless of whether or not CH_3SH forms by inorganic carbon reduction (reaction 1) or other biogenic or thermogenic processes during hydrothermal fluid circulation, the data presented here strongly suggest that metastable equilibrium with CH_4 at high temperatures is sufficiently fast that it regulates CH_3SH abundances in endmember fluids according to reaction 2.

Thermogenic CH_3SH Production. The highest CH_3SH concentrations were observed in endmember vent fluids from the sediment-covered Guaymas Basin rift zone, where the influence of hydrothermal alteration of immature organic matter and biomass is readily apparent (Fig. 2). At Guaymas Basin, basaltic dikes and sills intrude into 0.5-km-thick organic-rich diatomaceous ooze overlaying the ridge axis, resulting in rapid and widespread hydrothermal alteration of immature sedimentary organic matter and expulsion of hydrothermal petroleum at the seafloor (36, 38). In addition to abundant NH_4^+ and dissolved CO_2 , multiple classes of thermogenic organic compounds are added to circulating fluids during this process (35, 36, 39). Alkyl thiols are considered to form predominantly at low thermal maturities during petroleum generation in slowly subsiding sedimentary basins (40), and their production in this setting is therefore not surprising. Cyclic polysulfide organosulfur compounds (thiolanes, thianes, and thiepanes) have previously been reported in fragments of an active smoker chimney from Guaymas Basin, indicating organosulfur production during hydrothermal petroleum generation (38). Production of CH_3SH during hydrothermal alteration of sedimentary organic matter could reflect the removal of organosulfur moieties from macromolecular organic structures or the secondary reaction of thermogenic products such as CO. Indeed, fluids with abundant CH_3SH at Guaymas also have excess CO relative to equilibrium

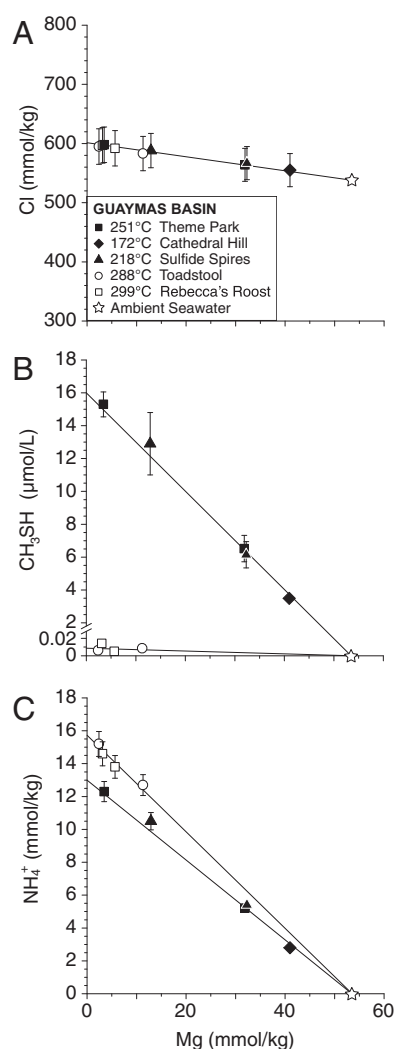


Fig. 2. Plots of measured concentrations of chloride (Cl; A), methanethiol (CH_3SH ; B), and ammonium (NH_4^+ ; C) versus Mg in Guaymas Basin vent fluid samples, showing CH_3SH and NH_4^+ production. Mg is used as an index of mixing between seawater and vent fluid (see *Endmember and Mixed Vent Fluid Compositions* for further details). Cooler endmember fluids (Theme Park, Cathedral Hill, and Sulfide Spires; solid symbols) from areas of hydrothermal petroleum expulsion have higher CH_3SH concentrations and lower $\text{C}_1/(\text{C}_2+\text{C}_3)$ ratios (<60) than hotter fluids from large flange structures (>124 ; Toadstool and Rebecca's Roost; open symbols), implying thermal maturity differences (see text). Cl (A) suggests a common source endmember. All fluids have interacted with sedimentary organic matter under hydrothermal conditions, yielding elevated NH_4^+ (C), but CH_3SH (B) and C_2+ hydrocarbons have likely decomposed to CH_4 at the higher thermal stress of the hotter fluids. Uncertainties (2s) not shown are smaller than data symbols.

with CO_2 and H_2 (see *Dataset S1* and *Widespread $\text{CO}_2\text{-H}_2\text{-CO}$ Equilibrium in Unsedimented Systems*).

The abundance of CH_3SH in fluids at Guaymas Basin is characterized by a bimodal distribution, with cooler endmember fluids being most enriched and hotter endmember fluids having similarly low abundances to fluids in unsedimented systems. CH_3SH -depleted Rebecca's Roost and Toadstool fluids ($288\text{--}299^\circ \text{C}$; Fig. 2) have substantially higher $\text{C}_1/(\text{C}_2+\text{C}_3)$ ratios ($124\text{--}132$ versus $55\text{--}60$) than the CH_3SH -rich cooler fluids ($172\text{--}251^\circ \text{C}$), suggesting higher thermal maturity in the hotter fluids and the conversion of longer-chain alkanes to shorter chains (39). These observations suggest that abundant CH_3SH is produced predominantly during early hydrothermal alteration of immature organic matter, consistent with observations from conventional

(~150 °C) mixed fluids from other mafic-hosted hydrothermal systems based on N-isotope measurements (42). Thermal degradation of dissolved organic nitrogen (DON) from admixed ambient deep ocean seawater is also an unlikely source of NH_4^+ . Some mixed fluids are enriched in NH_4^+ above conservative endmember dilution by up to 4 μM (Fig. 3C), exceeding what might reasonably be expected for entrainment and complete N release from typical deep-ocean DON concentrations [$<3 \mu\text{M}$ (47)]. Although microbially mediated nitrogen transformations can increase the abundance of NH_4^+ in very low-temperature/diffuse fluids (42), most large NH_4^+ and CH_3SH enrichments observed during this study (e.g., Hot Chimlet #1) are in mixed fluids hotter than the 122 °C limit for microbial life (48), suggesting that microbial activity is not responsible for production of either NH_4^+ or CH_3SH .

It is widely proposed that the permeable and porous upper oceanic crust (Layer 2A)—where mixing predominantly occurs—harbors microbial communities that constitute a deep biosphere (41–43). Given that CH_3SH in low-temperature mixed fluids is consistently associated with thermogenic indicators (Fig. 3 and Fig. S1) in systems free of sedimentary influence, we propose that entrainment and thermal alteration of microbial biomass, and/or associated dissolved organic matter (DOM), are responsible for production of CH_3SH within subsurface zones of mixing between high-temperature fluids and seawater. Previous observations of organic compounds derived from microbial biomass pyrolysis in low-temperature fluids support this possibility. Brault et al. (49) first described the presence of nonvolatile hydrocarbons in a diffuse (~15 °C) and a high-temperature (>250 °C) vent from 13°N, East Pacific Rise, and found strong enrichments of microbial lipid residues in the cooler vent. More recent work also suggests that dissolved organic carbon (DOC) is enriched in low-temperature fluids relative to both endmember precursors and ambient seawater, for example, and correlates with microbial cell counts (50). Although these observations could reflect active microbial processes, our results suggest that higher-temperature portions of subsurface mixing zones generate dissolved organic compounds through abiotic thermal degradation. Fluids in the temperature range presented here (e.g., 126–191 °C) have rarely been reported but provide key insights into processes occurring within crustal hydrothermal aquifers beyond the known 122 °C (48) temperature limit of life.

The ubiquitous and uniformly low levels of CH_3SH (~ 10^{-8} M) in endmember fluids from unsedimented systems, despite the broad range of temperature, salinity, and H_2 concentrations, may be a remnant of pyrolysis of trace organic matter at high temperatures. Brault et al. (49) noted minor quantities of thermally mature hopanes in a hot fluid (>250 °C) from 13°N, suggesting that entrainment and rapid pyrolysis of biomass to a high extent of maturity occurs during fluid venting at the seafloor. Endmember fluids are also known to be depleted in DOC relative to ambient seawater (50), implying thermal decomposition. A thermogenic carbon source would provide an explanation for the presence of CH_3SH in fluids with no thermodynamic drive to create it from CO_2 . In a similar manner to the hotter endmember fluids at Guaymas Basin, consumption of CH_3SH by reaction 2 in high-temperature fluids could therefore represent the high maturity stage of much more limited organic matter pyrolysis during hydrothermal circulation or venting that produces insignificant changes in the abundance of major species like CH_4 . Lost City endmember fluids, despite quite low vent temperatures (94–96 °C; Dataset S1), are also consistent with this explanation. Several lines of evidence from the inorganic (51) and organic (52) compositions of endmember fluids there point to substantial conductive cooling and much higher temperatures (likely in excess of 250 °C) in the subsurface reaction zone. Collectively, our data therefore imply that the distribution of methanethiol in seafloor hydrothermal fluids is largely controlled by thermal maturation of preexisting biological organic matter, with endmember and mixed vent fluids representing higher and lower thermal maturities, respectively.

Implications. Despite the diversity of geologic settings and potential catalytic minerals present in hydrothermal reaction zones, our results show no evidence for abiotic methanethiol synthesis from the inorganic precursors CO_2 , H_2 , and H_2S in modern hydrothermal fluids. This suggests that analogous hydrothermal systems on early Earth may not represent an abundant source of abiotic CH_3SH necessary for thioester production (4, 8, 12). The production of thermogenic organic compounds in crustal mixing zones, however, has numerous interesting biogeochemical implications for modern seafloor hydrothermal systems. Not only does widespread pyrolysis of subsurface organic matter provide further indirect support for a putative deep biosphere in unsedimented hydrothermal aquifers, it implies that such carbon may be recycled and returned to cooler near-surface environments by subsurface mixing processes. Production of methylated organic compounds by subsurface pyrolysis raises diverse possibilities for microbial organotrophic metabolisms (e.g., methylotrophy) in hydrothermal systems traditionally considered to have limited available organic compounds. A predominance of thermogenic products in low-temperature fluids could support larger populations of organotrophic microbes in these mixing zones relative to those immediately surrounding high-temperature vent structures, for example. Given that thiol functional groups are hypothesized to play a significant role in the complexation and delivery of hydrothermal trace metals (e.g., Fe and Cu) to the deep ocean (17–19), thermogenic production of organosulfur compounds with a high affinity for metals may constitute a key mechanism for this process in unsedimented hydrothermal systems.

Materials and Methods

All fluid samples were collected using isobaric gas-tight (IGT) samplers (27) during cruises to the Mid-Atlantic Ridge, Guaymas Basin, and East Pacific Rise in 2008 and Mid-Cayman Rise in 2012, using either ROV *Jason* or HOV *Alvin*. In most cases a minimum of two IGT samples were taken from each vent. Reported vent temperatures are the maximum measured in real time during fluid collection (27).

Dissolved CH_3SH concentrations were determined at sea upon sampler recovery by purge-and-trap gas chromatography (GC) with flame ionization detection (FID). FID, unlike sulfur-specific detection, is insensitive to the extremely high H_2S concentrations in vent fluids. Gas-tight fluid aliquots (<4 mL) were acidified with ~1 mL of 25 wt % phosphoric acid, and CH_3SH was sparged with He gas (30 mL/min for 10 min) and cryofocused on an *n*-octane-coated silica trap (–78 °C), then thermally desorbed (145 °C) directly onto a Carbograph 15C packed GC column (30 mL/min He, 40 °C isothermal). To limit potential losses of gaseous CH_3SH during sparging (53, 54), deactivated glass and polytetrafluoroethylene tubing were used wherever possible in the purge-and-trap system. Sparging was assumed to be quantitative given the long sparge time and volatility of CH_3SH , and resparging tests on samples revealed no significant evidence of incomplete removal. Before calibration and between samples, the trap was heated at >145 °C with He flowing (30 mL/min) for a minimum of 10 min to completely eliminate carryover of any residual CH_3SH remaining in the trap (typically <1%) from previous analyses. In almost all cases, resulting CH_3SH concentrations from separate discrete samples of the same vent yield mixing lines between bottom seawater (no detectable CH_3SH) and a hydrothermal endmember or mixed fluid composition when plotted against Mg (see *Endmember and Mixed Vent Fluid Compositions* for further details). This indicates not only that methanethiol is conservative with respect to accidental seawater entrainment during sample collection but that any losses or additions of CH_3SH due to the analytical method are not significant. Reported uncertainties (2s) for CH_3SH (Dataset S1) are the larger of either the error of reproducibility or the uncertainty of the commercial gas standard used ($\pm 5\%$).

H_2 and CO were analyzed at sea by a headspace extraction GC technique, using thermal conductivity and helium ionization detection, respectively (37, 39). H_2S (total dissolved, $\Sigma\text{H}_2\text{S}$) was determined either gravimetrically by precipitation as Ag_2S (55) or at sea by electrochemical (28, 34) or iodometric titration. pH(25 °C) was determined at sea by electrode (28, 34, 55). Aliquots for dissolved inorganic carbon (ΣCO_2 , abbreviated as CO_2), CH_4 , and C_{2+} hydrocarbons were stored in evacuated glass serum vials (poisoned with HgCl_2) for headspace gas GC analysis (28, 55). Cl was determined by ion chromatography [IC, $\pm 5\%$ (55)] or electrochemical titration [$\pm 0.5\%$ (28, 34)], and Mg was determined by either IC or inductively coupled plasma methods (28, 34, 55, 56). NH_4^+ was determined by either flow injection analysis (57) for unsedimented systems or IC (Guaymas Basin), with reported errors representing the larger of either error of reproducibility (2s) or the

typical $\pm 5\%$ reproducibility of prepared standards. Analytical uncertainties (2 σ) are ± 0.05 for pH(25 °C); $\pm 10\%$ for H₂S, CO, H₂, and C₂₊ hydrocarbon concentrations; and $\pm 5\%$ for Mg, ΣCO_2 , and CH₄ concentrations (28, 39, 55).

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