**Electrochemical CO₂ Reduction by Ni-containing Iron Sulfides: How Is CO₂
Electrochemically Reduced at Bisulfide-Bearing Deep-sea Hydrothermal Precipitates?**

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Abstract

The discovery of deep-sea hydrothermal vents on the late 1970's has led to many hypotheses concerning chemical evolution in the prebiotic ocean and the early evolution of energy metabolism in ancient Earth. Such studies stand on the quest for the bioenergetic evolution to utilize reducing chemicals such as H_2 for CO_2 reduction and carbon assimilation. In addition to the direct reaction of H_2 and CO_2 , the electrical current passing across a bisulfide-bearing chimney structure has pointed to the possible electrocatalytic CO_2 reduction at the cold ocean-vent interface (R. Nakamura, et al. *Angew. Chem. Int. Ed.* 2010, **49**, 7692–7694). To confirm the validity of this hypothesis, here, we examined the energetics of electrocatalytic CO_2 reduction by iron sulfide (FeS) deposits at slightly acidic pH. Although FeS deposits inefficiently reduced CO_2 , the efficiency of the reaction was substantially improved by the substitution of Fe with Ni to form $FeNi_2S_4$ (violarite), of which surface was further modified with amine compounds. The potential-dependent activity of CO_2 reduction demonstrated that CO_2 reduction by H_2 in hydrothermal fluids was involved in a strong endergonic electron transfer reaction, suggesting that a naturally occurring proton-motive force (PMF) as high as 200 mV would be established across the hydrothermal vent chimney wall. However, in the chimney structures, H_2 generation competes with CO_2 reduction for electrical current, resulting in rapid consumption of the PMF. Therefore, to maintain the PMF and the electrosynthesis of organic compounds in hydrothermal vent mineral deposits, we propose a homeostatic pH regulation mechanism of FeS deposits, in which elemental hydrogen stored in the hydrothermal mineral deposits is used to balance the consumption of the electrochemical gradient by H_2 generation.

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46 *Keywords:* Origin of life, Metal sulfide, hydrothermal vent, CO₂ reduction, proton-motive
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1. Introduction

In the last two decades, there has been considerable interest in understanding biogeochemical processes associated with deep-sea hydrothermal environments [1-8]. Such studies are motivated by the unique microbial and chemical processes occurring in the present and ancient deep oceans without energy input from sun light and using reductive chemicals such as H_2S and H_2 emitted from hydrothermal vents as energy sources [1,3,6,8]. In such environments, iron sulfides (FeSs), which are able to catalyze CO_2 reduction and carbon assimilation, are continuously precipitated [9]. Based on these phenomena, it has been proposed that the surface of FeS minerals, which often contains trace metals such as Ni, Cu, W and Mo [10], has played a critical role in prebiotic organic synthesis and early evolution of energy metabolisms in ancient Earth [10-27].

As a model of prebiotic organic synthesis, Russell et al. [28-31] have proposed that Ni-containing greigite (Fe_5NiS_8) embedded in a colloidal membrane of FeS acts as a catalyst for CO_2 reduction. This proposed model is based on the structural similarity of Fe_5NiS_8 to the NiFe_3S_4 cluster that functions as the reaction center of carbon monoxide dehydrogenase (CODH), a Ni-containing enzyme found in certain chemolithoautotrophic microorganisms including acetogens and methanogens that reversibly catalyzes the reduction of CO_2 to CO [32-34]. Russell et al. [18,35,36] have pointed that H_2 supplied from alkaline hydrothermal fluids serves as an electron and proton source for the conversion of CO_2 to formate by Fe_5NiS_8 . In contrast, Wächtershäuser et al. [37] suggested that FeS, rather than H_2 , act as a reductant and demonstrated that Ni-containing FeS particles catalyzed the synthesis of organic compounds using the reducing power of the

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7 71 FeS/FeS₂ couple. As a potential prebiotic pathway of such H₂-independent acetyl-CoA
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9 72 synthesis, the hydrothermal synthesis (100 °C) of acetate from CO and CH₃SH in a mixed
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12 73 solution of FeS and NiS has also been demonstrated [12].
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14 74 The two proposed pathways for prebiotic organic synthesis are based on the
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16 75 heterogeneous reactions of CO₂ with reductive chemicals such as H₂, H₂S, and FeS at the
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18 76 surface of Ni-containing FeS deposits. In any of the operative CO₂ reduction processes,
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21 77 however, the formation of organic molecules is ceased when the reducing chemicals are
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24 78 consumed. Therefore, a continuous influx of reductants to the catalytic centers via diffusion
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26 79 and/or convection is essential to drive the prebiotic organic synthesis system. Alternatively,
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29 80 it is also possible to promote a continuous energy influx by the transfer of electrons from
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31 81 the densely interconnected structure of micron- and submicron-crystalline particles of FeS
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33 82 deposits (Fig. 1). Recently, we examined the electrochemical functionalities of
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36 83 biogeochemical processes associated with FeS and found that a portion of mineral
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38 84 assemblage obtained from a black smoker chimney in the Mariner hydrothermal field of the
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41 85 Lau Basin displayed excellent metallic electrical conduction over a 10-cm distance [38,39].
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43 86 In addition, the chimney minerals functioned as efficient electrocatalysts for the oxidation
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45 87 of H₂S and H₂, and the reduction of O₂ and ferric ions. Based on these findings, we
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48 88 hypothesized that in addition to the reductive chemicals emitted from the hydrothermal
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50 89 fluids, high-energy electrons are also transported from the high-temperature hydrothermal
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52 90 fluid to the outer mineral interface with seawater via the hydrothermal sulfide deposits in
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55 91 the form of electrical current (Fig. 1). The FeS precipitates can also store, transport and
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57 92 discharge elemental hydrogen in the form of protons, as demonstrated from the use of FeS
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as an anode material in nickel-metal hydride batteries [40,41]. Therefore, it is possible that the natural setting of electrochemical reactors in the hydrothermal vents could sustain the energy flow for organic synthesis at the mineral interfaces between seawater and hydrothermal fluid.

In biological systems, CO₂ reduction by CODH is coupled with the oxidation of H₂ to protons [42-44]. Recently, it has been also shown that CODH directly exchanges electrons with conductive substrates and functions as an efficient electrocatalyst for CO₂ reduction to CO at potentials near the thermodynamic potential of the CO₂/CO couple (−0.54 V vs SHE at pH 7.3) [45]. This finding, together with the hypothesis by Russell et al. [28-31] that Ni/greigite could be the evolutionary origin for the NiFe₃S₄ cluster in CODH, led us further to hypothesize that high-energy electrons delivered from hydrothermal fluids directly activate Ni/FeS catalysts, as is observed for the immobilized catalytic center of CODH.

To obtain new insight into the electrocatalysis of FeS precipitates and to test our hypothesis that Ni/FeS catalysts can be activated by high-energy electrons, here, the electroreduction of CO₂ by hydrothermally synthesized Fe₃S₄ greigite was evaluated in an aqueous solution at pH 5.5 in order to mimic the pH of the CO₂-saturated early ocean [46]. Specifically, we investigated the role of Ni in the electroactivation of CO₂ by replacing an Fe atom in greigite with a hetero-metal atom, such as Ni, Cu, or Zn. Based on the observed electroreduction potential of CO₂ and hydrogen storage ability of FeS, we discuss how the natural electrochemical reactors in the hydrothermal vents would be able to trigger and maintain the electroreduction of CO₂ as the prebiotic organic carbon syntheses.

2. Experiments

2.1. Synthesis

Fe₃S₄ powder was prepared in aqueous solutions using standard hydrothermal techniques reported in the literature [41]. Briefly, 6 mmol iron (II) sulfate heptahydrate (Sigma-Aldrich) and 6 mmol L-cysteine (C₃H₇NO₂S, Wako) were separately dissolved in 120 mL deionized water. The two solutions were mixed under stirring for 20 min and were then combined in a Teflon-lined, 300-mL autoclave reaction vessel. After heating at 200 °C for 24 h, the reaction vessel was cooled to room temperature in air. Black precipitates were collected by filtration, and the obtained samples were washed with distilled water and ethanol, followed by evacuation for 2 h at 60 °C. The introduction of Ni²⁺, Cu²⁺, Zn²⁺ into iron sulfides was performed by the partial substitution of an appropriate ratio of iron (II) sulfate as a starting material with either nickel (II) sulfate hexahydrate (Wako), copper (II) sulfate pentahydrate (Wako) or zinc sulfate heptahydrate (Wako), respectively. All chemicals were of the highest grade possible and were used without further purification.

2.2. Electrochemical Reactions

A gas diffusion electrode system was adapted to the electrochemical CO₂ reduction experiments. The electrochemical cell of this system consists of two compartments, one for the liquid phase and one for the gas phase, and a gas diffusion electrode was positioned at the interface between these compartments. The gas diffusion electrode consisted of a hydrophobic gas diffusion layer facing the gas phase and a hydrophilic catalyst layer facing the liquid phase. To function as a working electrode, carbon paper (5.4 cm²) was coated

with the 25 mg of the prepared Fe_3S_4 powder and utilized for the catalytic layer. No binder materials were utilized in the coating of the electrode to avoid potential adverse effects on the catalytic ability of the metal sulfides. A Teflon membrane with 5- μm pore size (Millipore) was used as a gas diffusion layer. A Pt plate and Ag/AgCl (sat. KCl) electrode were used as counter and reference electrodes, respectively, and phosphate buffer ($\text{Na}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$; pH = 5.5) was used as an electrolyte. Electrochemical measurements were conducted using a HSV-100 potentiostat (Hokuto-Denko). After potentiostatic condition for 30 min with 760 Torr CO_2 in a gas chamber, the analysis of reduction products was performed by gas chromatography (Shimadzu) equipped with FID or TCD detectors. All measurements were conducted at room temperature.

2.3. Characterization

Characterization of obtained samples was performed by X-ray diffraction (XRD) on a Rigaku-Smart Lab X-ray diffractometer using Cu $K\alpha$ radiation ($\lambda = 1.5418 \text{ \AA}$) and an operation voltage and current maintained at 40 kV and 40 mA, respectively. FTIR spectra of samples and adsorbed species were recorded with a Vertex 70 FT-IR spectrometer (Bruker) in transmission mode at room temperature. The self-sustaining wafer of a sulfide sample (~10 mg) was mounted into an in-house constructed transmission infrared vacuum cell equipped with CaF_2 windows.

3. Results

3.1. Characterization

XRD patterns for Fe_3S_4 powder obtained from a mixed solution of L-cysteine and Fe(II) sulfate heptahydrate, and those for Ni-doped iron sulfide (Ni-FeS) with a Fe to Ni ratio of 5 to 1 are shown in Fig. 2a-i and Fig. 2a-ii, respectively. The prepared Fe_3S_4 powder exhibited an XRD pattern assigned to that of greigite (indexed to JCPDS 16-0713), characterized by a reverse spinel structure composed of edge-sharing FeS_6 octahedra and corner-sharing FeS_4 tetrahedra (Fig. 2b left). Although Ni-FeS with an Fe/Ni ratio of 5 retained the greigite structure, the sample with an Fe/Ni ratio of 1 exhibited a new phase assigned to FeNi_2S_4 (violarite, indexed to JCPDS 42-1449) and NiS (indexed to JCPDS 02-1280) (Fig. 2a-iii). FeNi_2S_4 has a reverse spinel structure composed of edge-sharing FeS_6 octahedra, NiS_6 octahedra, and corner-sharing NiS_4 tetrahedra [47] (Fig. 2b center). The sample obtained from mixed solutions of Ni(II) sulfate hexahydrate and L-cysteine (Fe/Ni = 0) had an XRD pattern characteristic of pure NiS (Fig. 2a-iv). SEM images of the Fe_3S_4 powder showed that the particles were composed of abundant nanosheets that formed an aggregated structure with a diameter of approx. 5 μm (Fig. 3a). In the sample prepared with a Fe/Ni ratio of 5, numerous spherical particles of ~500 nm in diameter were observed (Fig. 3b), which became more abundant as the amount of Ni increased (Fig. 3c and d).

3.2. Electrochemical reduction of protons

To investigate the electrocatalytic functions of the FeS deposits, carbon paper coated with greigite or Ni-FeS powder was used as a gas diffusion electrode. In order to avoid potential adverse effects on the catalytic ability of metal sulfides, no binder materials was utilized for coating of metal sulfides particles on a carbon paper. Although the lack of binder materials

impedes the analysis of long-term electrocatalytic property due to a partial detachment of metal sulfides particles from a carbon paper, it is of importance to evaluate the intrinsic catalytic properties of metal sulfides. A control experiment for electrochemical CO₂ reduction was first performed by examining the electrocatalysis of greigite at pH 5.5 in the presence of 760 Torr N₂. The potential-dependent Faradaic efficiencies (FE) for H₂ production by greigite are shown in Fig. 4. H₂ was generated at an onset potential of –0.5 V (vs SHE), which is 0.2 V below the theoretical equilibrium potential. The FE increased when more negative potential was applied and approached saturation at –1.1 V. The total FE fell below 100 % at a potential of –1.1 V and was reduced to almost 0 % at –0.5 V. The reduced FE was attributed to hydrogen storage within the greigite particles. A recent report showed that hydrothermally synthesized greigite powder stores hydrogen at a density of 3.26×10^{–2} mol/cm³, which is comparable to that reported for Pd (6.83×10^{–2} mol/cm³) [41]. In the present work, we estimated the amount of stored hydrogen in the greigite particles by monitoring the time course of potential under galvanostatic conditions at 60 mA g^{–1} after electrolysis at –0.5, –0.7 and –0.9 V for 30 min (Fig. S1). Under these three conditions, the amount of stored hydrogen was approximately (3.86 ± 2.02)×10^{–3}, (3.95 ± 0.51)×10^{–3}, and (5.83 ± 1.32)×10^{–3} mol/cm³, respectively, which corresponded to FEs of 80 ± 30 %, 61 ± 23 % and 19 ± 8 % (mean ± standard deviation), respectively. These values were consistent with the observed reductions in FE observed in the range –0.5 to –1.1 V.

3.3. Electrochemical reduction of CO₂

The electrocatalytic potential of FeS for CO₂ reduction was next evaluated by performing

electrolysis with the greigite and Ni-FeS samples as catalysts in the presence of 760 Torr CO₂. After electrolysis at −1.3 V for 1 h, CO, CH₄, and H₂ were detected as gas phase products, and no products were detected by HPLC analysis in the liquid phase. As shown in Fig. 5a, the FEs of CO and CH₄ increased with increasing Ni content, with the sample prepared using a Fe/Ni ratio of 1 showing the highest activity. However, in the absence of FeS, CO and CH₄ were not produced. Thus, these results demonstrate that Ni-containing FeS has the ability to electrochemically reduce CO₂. As described in Fig. 2, the Ni-FeS (Fe/Ni = 1) sample is comprised of a mixture of FeNi₂S₄ and NiS. As pure NiS was less active than Ni-FeS (Fe/Ni = 1), FeNi₂S₄ appears to play a key role in CO₂ reduction processes.

The potential dependence of FEs for CO and CH₄ production for the greigite and Ni-FeS (Fe/Ni = 1) samples is presented in Fig. 6. Over the potential range examined, Ni doping led to an increase in FEs compared to those for greigite. For the Ni-FeS sample, CO and CH₄ were generated at onset potentials of −0.5 and −0.7 V, respectively, which are 0.07 and 0.54 V below the theoretical equilibrium potentials, respectively. Note that such improvement in FE was not observed for FeS that contained either Cu²⁺ or Zn²⁺ in place of Ni (Fig. 5b and c). From these results, it can be concluded that the tetrahedrally coordinated Ni ion center in FeNi₂S₄ plays an important role in the electrochemical reduction of CO₂ on the surface of FeS deposits.

3.4. Effects of FeS surface modification with amine compounds

The NiFe₃S₄ catalytic center in CODH is held in place by cysteine and histidine side chains

of the protein [32]. It has been postulated in the iron sulfur world concept that the surface modification of metal sulfide minerals by peptides was an important evolutionary step from abiotic chemical reactions towards biotic metabolisms [48-50]. Specifically, the formation of Fe-N bonds through the interaction of peptide amine groups and FeS may have formed the primitive core of CODH. Alternatively, the peptide amine groups can also serve as co-catalysts for CO₂ reduction by donating electrons to the anti-bonding π^* orbital of CO₂, which is essential for the efficient electrochemical activation of CO₂, as it induces an anti-bonding character that facilitates disruption of the C=O bond, thereby decreasing the overpotential for CO₂ reduction. Recently, density functional theory (DFT) calculations indicated that the overpotential for the electroreduction of CO₂ by the NiFe₃S₄ center in CODH is largely due to the requirement for energetic stabilization of the adsorbed COOH formed by the one-electron reduction of CO₂ [51,52]. N-containing ionic liquids have been demonstrated to efficiently stabilize adsorbed COO⁻ and thus lower the overpotential and increase the FE for CO production [53]. Therefore, we speculate that the surface modification of FeS with amine compounds was critical for the evolutionary transition of inactive FeS minerals to active inorganic analogs of the NiFe₃S₄ center.

To examine the role of amine groups in the electrocatalytic reduction of CO₂, Ni-FeS powder (Fe/Ni = 1) was reacted with triethylamine (TEA) and poly-allylamine hydrochloride (PAH). Ni-FeS powder dispersed in an aqueous solution was reacted with different TEA concentrations and then examined by FT-IR following filtration, revealing that the reacted powder showed a symmetric vibration mode of $\nu(\text{C-H})$ at 2853 and 2923 cm⁻¹ (Fig. S2). These two infrared peaks remained at a similar intensity after vigorous

washing of the powder sample with water, indicating that chemical bonds had formed between TEA and the Ni-doped FeS particles.

Using the TEA-treated Ni-FeS (Fe/Ni = 1) as an electrocatalyst, the effect of amine bases on electrochemical CO₂ reduction was examined at pH 5.5. Analysis of gas phase products after electrolysis for 30 min at -1.3 V revealed a large increase in the FEs of both CO and CH₄ production relative to those for untreated Ni-FeS (Fig. 7). The FEs for CO and CH₄ production increased with increasing TEA content. As neither CO nor CH₄ was detected when electrolysis was conducted in the absence of CO₂, CO₂, as opposed to TEA, was the carbon source for the production of CO and CH₄.

The enhancement of the FE was also observed during the reaction of PAH with the Ni-FeS catalysts. A PAH-modified Ni-containing FeS sample was prepared by the dispersion of Ni-FeS powder in an aqueous solution containing different concentrations of PAH. Surface modification of Ni-FeS by PAH increased the FEs for CO and CH₄ by approx. 85 fold compared with unmodified greigite (Fig. 7). Taken together, these findings indicate that the co-existence of amine compounds with Ni-containing FeS minerals was critical for the formation of naturally occurring electrocatalysts capable of CO₂ reduction.

4. Discussion

The primary aim of the present research was to examine the electrocatalytic potential of FeS deposits for CO₂ reduction and obtain new insights into the metabolic evolution related to the utilization of reducing chemicals such as H₂ emitted from deep-sea hydrothermal vents for CO₂ reduction and carbon assimilation. Our results demonstrate that

hydrothermally synthesized iron-sulfide greigite is inefficient for the electrochemical reduction of CO₂ while the efficiency of this reaction is substantially improved (~85 fold) by Ni substitution of an Fe atom in greigite to form FeNi₂S₄, followed by the surface modification of FeNi₂S₄ with amine compounds such TEA and PAH. These findings highlight the possibility that abundant high-energy electrons delivered from hydrothermal fluids directly activate Ni/FeS deposits for CO₂ reduction.

Based on results of the electrocatalysis experiments performed in aqueous solution at pH 5.5, FeS precipitates containing FeNi₂S₄ as a catalytic site may function as an electrocatalyst for CO₂ reduction in hydrothermal environments (Fig. 8). In a previous study, we proposed that the electrical current across a sulfide-rich chimney wall represented a new form of energy transport that sustains CO₂ reduction and carbon assimilation (Fig. 1) [38,39]. The essence of this model is that an interconnected percolation network of micron and submicron crystalline FeS particles facilitates the highly efficient conversion of a spatially discrete redox potential to electrical current. Accordingly, high-energy electrons present in the hot hydrothermal fluid are directly transported to the mineral outer interface with the cold ambient seawater. Notably, this model differs from those previously proposed that assume CO₂ and reductive chemicals directly react at a narrow mixing interface between the hydrothermal fluids and seawater. At temperatures above approximately 250°C, organic carbons in contact with water become CO₂. As the electrosynthesis of organic compounds in the mineral outer interface may occur at much lower temperatures, the system can escape from rapid thermal degradation of synthesized organic compounds.

As shown in Figs. 6 and 8a, the electrochemical reduction of CO₂ to CO by FeS

deposits containing FeNi_2S_4 was initiated at approximately -500 mV at pH 5.5. This potential is 180 mV more negative than the redox couple of H^+/H_2 , which provides the most reducing power in a hydrothermal fluid, resulting in a strong endergonic electron transfer reaction (Fig. 8a). This energy imbalance suggests that a naturally occurring proton-motive force (PMF) as high as 200 mV, which is equivalent to a proton gradient of 3-4 pH units, must be established across the hydrothermal mineral structure such as chimney walls. The early ocean was saturated with CO_2 and had an estimated pH of 5.5 [45]. Thus, hydrothermal fluids with a pH above 8.5-9.5, such as alkaline bisulfide-bearing submarine vents described by Russell et al. [54], may utilize the spatially separated reductive energy of H_2 for electrosynthesis (Fig. 8b). Highly alkaline hydrothermal vents in the Hadean ocean are the most plausible system for prebiotic organic synthesis [55], as the hydrothermal vent chimney interior has a pH of 9-10 and the outer walls are bathed in ocean water with a pH of 5-6, generating a PMF of 180-300 mV.

It should be noted, however, that the PMF in alkaline vents would be rapidly dissipated by the H_2 generation reaction. As shown in Fig. 6, CO_2 reduction mediated by Ni/FeS competes for electrons with the proton reduction reaction that forms H_2 . Thus, the low FE for CO_2 reduction limits electrosynthesis, as the H_2 generation inside the cavity of the densely-connected percolation structure of FeS rapidly decreases the proton concentration, dissipating the PMF (Fig. 8c, left). Therefore, the presence of amine bases, which substantially improve the FE for CO_2 reduction (Fig. 7), is critical for maintaining the proton gradient. Inorganic bases, such as Mg^{2+} and Ca^{2+} , are able to serve the same function as amine bases in the cavity of FeS .

The dissipation of the electrochemical gradient across the vent wall by H₂ generation may be minimized by chemical energy stored in the hydrothermal mineral precipitates. In the hydrothermal vents, in addition to electrons generated by H₂ oxidation, elemental hydrogen is constantly generated at the interface between the hydrothermal fluid and the inner mineral precipitates via the dissociative insertion of H₂ and is subsequently stored within the mineral precipitates [40]. The storage of surplus energy is critical to maintain energy flow within the system, as described by Hengeveld et al [56]. FeS minerals can store, transport and discharge elemental hydrogen in the form of protons, as has been demonstrated from the use of FeS as anode materials in nickel-metal hydride batteries [40,41]. Therefore, we hypothesize that elemental hydrogen stored in the hydrothermal mineral precipitates is used to lower the pH in the inner cavity by the reductive dissolution of HS⁻ from FeS₂ ($2\text{H} + \text{FeS}_2 \rightarrow \text{FeS} + \text{HS}^- + \text{H}^+$), when the electrochemical gradient within the reactors is dissipated as a result of H₂ (Fig. 8c, right). Such a homeostatic pH regulation mechanism is expected to balance the consumption of the electrochemical gradient by H₂ generation, and thus maintain the electrosynthesis of organic compounds at sulfide-rich hydrothermal deposits for long.

5. Conclusions

The present electrochemical experiments for CO₂ reduction by FeS attempted to elucidate the energetic basis for the electrosynthesis of organic compounds at bisulfide-bearing deep-sea hydrothermal vent deposits. Our analyses demonstrated that FeS precipitates function as efficient electrocatalysts for CO₂ reduction upon the substitution of Fe with Ni

to form FeNi₂S₄, and that the FE is further increased by modification of the catalytic surface with amine compounds. These findings are consistent with the concept that Ni/greigite serves as a prebiotic catalyst for CO₂ reduction due to its structural similarity with the catalytic centers of CODH enzyme [28-31]. However, the present results indicate that surface modification of the active FeNi₂S₄ (violarite) center embedded in FeS precipitates with amine compounds was more important for the formation of naturally occurring electrocatalysts capable of the electroreduction of CO₂. The present study also demonstrated that H₂ generation competes with CO₂ reduction for electrical current, resulting in the consumption of the PMF. As amine compounds substantially improve the FE for CO₂ reduction, we conclude that the presence of amine compounds or inorganic bases such as Mg⁺ and Ca²⁺ is indispensable to limit dissipation of the electrochemical gradient by H₂ generation. Alternatively, homeostatic pH regulation mechanisms of FeS, as demonstrated from the use of sulfide-based anode materials in nickel-metal hydride batteries [40,41], may also limit the consumption of PMF by H₂ generation. Thus, the findings in the present study are expected to provide great insights into not only the electron conduction and electrocatalytic functions of bisulfide-bearing hydrothermal vent deposits, but also the energy storage ability of the hydrothermal vent deposits, for triggering and maintaining the electrosynthesis of organic compounds prior to the emergence of the primordial living forms.

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References

- [1] A. –L. Reysenbach, E. Shock, Merging Genomes with Geochemistry in Hydrothermal Ecosystems, *Science* 296 (2002) 1077.
- [2] K. Takai, K. Horikoshi, Genetic Diversity of Archaea in Deep-Sea Hydrothermal Vent Environments, *Genetics* 152 (1999) 1285.
- [3] W. Martin, J. Baross, D. Kelley, M. J. Russell, Hydrothermal vents and the origin of life, *Nat. Rev. Microbiol.* 6 (2008) 805.
- [4] S. M. Sievert, C. Vetriani, Chemoautotrophy at Deep-Sea Vents, *Oceanography* 25 (2012) 218.
- [5] T. Mogi, T. Ishii, K. Hashimoto, R. Nakamura, Low-voltage electrochemical CO₂ reduction by bacterial voltage-multiplier circuits, *Chem. Commun.* 49 (2013) 3967.
- [6] H. W. Jannasch, M. J. Mottl, Geomicrobiology of Deep-Sea Hydrothermal Vents, *Science* 229 (1985) 717.
- [7] S. Nakagawa, K. Takai, Deep-sea vent chemoautotrophs: diversity, biochemistry and ecological significance, *FEMS Microbiol. Ecol.* 65 (2008) 1.
- [8] J. A. Baross, S. E. Hoffman, SUBMARINE HYDROTHERMAL VENTS AND ASSOCIATED GRADIENT ENVIRONMENT AS SITES FOR THE ORIGIN AND

379 EVOLUTION OF LIFE, *Origins Life Evol. Biosphere* 15 (1985) 327.
 380 [9] G. Wächtershäuser, Pyrite Formation, the First Energy Source for Life: a
 381 Hypothesis, *Syst. Appl. Microbiol.* 10 (1988) 207.
 382 [10] W. Nitschke, M. J. Russell, Hydrothermal Focusing of Chemical and Chemiosmotic
 383 Energy, Supported by Delivery of Catalytic Fe, Ni, Mo/W, Co, S and Se, Forced Life to
 384 Emerge, *J. Mol. Evol.* 69 (2009) 481.
 385 [11] E. V. Koonin, W. Martin, On the origin of genomes and cells within inorganic
 386 compartments, *Trends Genet.* 21 (2005) 647.
 387 [12] J. A. Huber, G. Wächtershäuser, Activated Acetic Acid by Carbon Fixation on (Fe,
 388 Ni)S Under Primordial Conditions, *Science* 276 (1997) 245.
 389 [13] D. Rickard, G. W. Luther III, Chemistry of Iron Sulfides, *Chem. Rev.* 107 (2007) 514.
 390 [14] M. Pósfai, R. E. Dunin-Borkowski, Sulfides in Biosystems, *Rev. Mineral. Geochem.*
 391 61 (2006) 679.
 392 [15] P. Baaske, F. M. Weinert, S. Duhr, K. H. Lemke, M. J. Russell, D. Braun, Extreme
 393 accumulation of nucleotides in simulated hydrothermal pore systems, *Proc. Natl. Acad. Sci.*
 394 USA 104 (2007) 9346.
 395 [16] M. A. A. Schoonen, Y. Xu, J. Bebie, **ENERGETICS AND KINETICS OF THE**
 396 **PREBIOTIC SYNTHESIS OF SIMPLE ORGANIC ACIDS AND AMINO ACIDS WITH**
 397 **THE FES-H₂S/FES₂ REDOX COUPLE AS REDUCTANT**, *Origins Life Evol. Biosphere*
 398 29 (1999) 5.
 399 [17] T. M. McCollom, J. S. Seewald, Abiotic Synthesis of Organic Compounds in
 400 Deep-Sea Hydrothermal Environments, *Chem. Rev.* 107 (2007) 382.

- 1
2
3
4
5
6
7 401 [18] M. J. Russell, The Alkaline Solution to the Emergence of Life: Energy, Entropy and
8
9 402 Early Evolution, *Acta. Biotheor.* 55 (2007) 133.
10
11
12 403 [19] G. D. Cody, N. Z. Boctor, R. M. Hazen, R. M. J. A. Brandes, H. J. Morowitz, H. S.
13
14 404 Yoder, Geochemical roots of autotrophic carbon fixation: Hydrothermal experiments in the
15
16 405 system citric acid, H_2O -($\pm\text{FeS}$)-($\pm\text{NiS}$), *Geochim. Cosmochim. Acta.* 65 (2001) 3557.
17
18
19 406 [20] N. Lane, W. F. Martin, The Origin of Membrane Bioenergetics, *Cell* 151 (2012) 1406.
20
21
22 407 [21] O. Dannenmuller, K. Arakawa, T. Eguchi, K. Kakinuma, S. Blanc, A. M. Albrecht, M.
23
24 408 Schmutz, Y. Nakatani, G. Ourisson, Membrane Properties of Archaeal Macrocyclic Diether
25
26 409 Phospholipids, *Chem. Eur. J.* 6 (2000) 645.
27
28
29 410 [22] M. G. Vladimirov, Y. F. Ryzhkov, V. A. Alekseev, V. A. Bogdanovskaya, V. A.
30
31 411 Otroshchenko, M. S. Kritsky, ELECTROCHEMICAL REDUCTION OF CARBON
32
33 412 DIOXIDE ON PYRITE AS A PATHWAY FOR ABIOGENIC FORMATION OF
34
35 413 ORGANIC MOLECULES, *Origins Life Evol. Biosphere* 34 (2004) 347.
36
37
38 414 [23] U. J. Meierhenrich, J. J. Filippi, C. Meinert, P. Vierling, J. P. Dworkin, On the Origin
39
40 415 of Primitive Cells: From Nutrient Intake to Elongation of Encapsulated Nucleotides,
41
42 416 *Angew. Chem. Int. Ed.* 49 (2010) 3738.
43
44
45 417 [24] A. G. Cairns-Smith, A. J. Hall, M. J. Russell, MINERAL THEORIES OF THE
46
47 418 ORIGIN OF LIFE AND AN IRON SULFIDE EXAMPLE, *Origins Life Evol. Biosphere*
48
49 419 22 (1992) 161.
50
51
52 420 [25] G. D. Cody, TRANSITION METAL SULFIDES AND THE ORIGINS OF
53
54 421 METABOLISM, *Annu. Rev. Earth Planet Sci.* 32 (2004) 569.
55
56
57
58
59
60
61
62
63
64
65

- 1
- 2
- 3
- 4
- 5
- 6
- 7 422 [26] G. Wächtershäuser, From volcanic origins of chemoautotrophic life to Bacteria,
- 8 423 Archaea and Eukarya, Phil. Trans. R. Soc. B 361 (2006) 1787.
- 9
- 10 424 [27] F. L. Sousa, T. Thiergart, G. Landan, S. Nelson-Sathi, I. A. C. Pereira, J. F. Allen, N,
- 11
- 12 425 Lane, W. F. Martin, Early bioenergetic evolution, Phil. Trans. R. Soc. B 368 (2013)
- 13
- 14 426 20130088.
- 15
- 16
- 17 427 [28] M. J. Russell, R. M. Daniel, A. J. Hall, J. A. Sherringham, A Hydrothermally
- 18
- 19 428 Precipitated Catalytic Iron Sulphide Membrane as a First Step Toward Life, J. Mol. Evol.
- 20
- 21 429 39 (1994) 231.
- 22
- 23
- 24 430 [29] M. J. Russell, W. Martin, The rocky roots of the acetyl-CoA pathway, Trends
- 25
- 26 431 Biochem. Sci. 29 (2004) 358.
- 27
- 28
- 29 432 [30] M. J. Russell, A. J. Hall, The emergence of life from iron monosulphide bubbles at a
- 30
- 31 433 submarine hydrothermal redox and pH front, J. Geol. Soc. Lond. 154 (1997) 377.
- 32
- 33
- 34 434 [31] W. Nitschke, S. E. McGlynn, E. J. Milner-White, M. J. Russell, On the antiquity of
- 35
- 36 435 metalloenzymes and their substrates in bioenergetics, Biochim. Biophys. Acta 1827 (2013)
- 37
- 38 436 871.
- 39
- 40
- 41 437 [32] H. Dobbek, V. Svetlitchnyi, L. Gremer, R. Huber, O. Meyer, Crystal Structure of a
- 42
- 43 438 Carbon Monoxide Dehydrogenase Reveals a [Ni-4Fe-5S] Cluster, Science 293 (2001)
- 44
- 45 439 1281.
- 46
- 47
- 48 440 [33] C. L. Drennan, J. Heo, M. D. Sintchak, E. Schreiter, P. W. Ludden, Life on carbon
- 49
- 50 441 monoxide: X-ray structure of *Rhodospirillum rubrum* Ni-Fe-S carbon monoxide
- 51
- 52 442 dehydrogenase, Proc. Natl. Acad. Sci. USA 98 (2001) 11973.
- 53
- 54
- 55 443 [34] P. Amara, J-M. Mouesca, A. Volbeda, J. C. Fontecilla-Camps, Carbon Monoxide
- 56
- 57
- 58

- Dehydrogenase Reaction Mechanism: A Likely Case of Abnormal CO₂ Insertion to a Ni-H⁻ Bond, *Inorg. Chem.* 50 (2011) 1868.
- [35] M. J. Russell, I. Kanik, Why Does Life Start, What Does It Do, Where Will It Be, And How Might We Find It?, *The journal of Cosmology* 5 (2010) 1008.
- [36] M. J. Russell, A. J. Hall, W. Martin, Serpentinization as a source of energy at the origin of life, *Geobiology* 8 (2010) 355.
- [37] G. Wächtershäuser, Evolution of the first metabolic cycles, *Proc. Natl. Acad. Sci. USA* 87 (1990) 200.
- [38] R. Nakamura, T. Takashima, S. Kato, K. Takai, M. Yamamoto, K. Hashimoto, Electrical Current Generation across a Black Smoker Chimney, *Angew. Chem. Int. Ed.* 49 (2010) 7692.
- [39] M. Yamamoto, R. Nakamura, K. Oguri, S. Kawagucci, K. Suzuki, K. Hashimoto, K. Takai, Generation of Electricity and Illumination by an Environmental Fuel Cell in Deep-Sea Hydrothermal Vents, *Angew. Chem. Int. Ed.* 52 (2013) 10758.
- [40] M. Bungs, H. Tributsch, Electrochemical and Photoelectrochemical Insertion and Transport of Hydrogen in Pyrite, *Ber. Bunsenges. Phys. Chem.* 101 (1997) 1844.
- [41] F. Cao, W. Hu, L. Zhou, W. Shi, S. Song, Y. Lei, S. Wang, H. Zhang, 3D Fe₃S₄ flower-like microspheres: high-yield synthesis *via* a biomolecule-assisted solution approach, their electrical, magnetic and electrochemical hydrogen storage properties, *Dalton Trans.* (2009) 9246.
- [42] J. G. Ferry, Co Dehydrogenase, *Annu. Rev. Microbiol.* 49 (1995) 305.
- [43] S. W. Ragsdale, M. Kumar, Nickel-Containing Carbon Monoxide

Dehydrogenase/Acetyl-CoA Synthase, Chem. Rev. 96 (1996) 2515.

[44] W. B. Jeon, S. W. Singer, P. W. Ludden, L. M. Rubio, New insights into the mechanism of nickel insertion into carbon monoxide dehydrogenase: analysis of *Rhodospirillum rubrum* carbon monoxide dehydrogenase variants with substituted ligands to the [Fe₃S₄] portion of the active-site C-cluster, J. Biol. Inorg. Chem. 10 (2005) 903.

[45] W. Shin, S. H. Lee, J. W. Shin, S. P. Lee, Y. Kim, Highly Selective Electrocatalytic Conversion of CO₂ to CO at -0.57 V (NHE) by Carbon Monoxide Dehydrogenase from *Moorella thermoacetica*, J. Am. Chem. Soc. 125 (2003) 14688.

[46] G. Macleod, C. Mckeown, A. J. Hall, M. J. Russell, Hydrothermal and oceanic pH conditions of possible relevance to the origin of life, Orig. Life Evol. Biosph. 24 (1994) 19.

[47] S. Haider, A. Roldan, N. H. de Leeuw, Catalytic Dissociation of Water on the (001), (011), and (111) Surfaces of Violarite, FeNi₂S₄: A DFT-D2 Study, J. Phys. Chem. C 118 (2014) 1958.

[48] E. J. Milner-White, M. J. Russell, SITES FOR PHOSPHATES AND IRON-SULFUR THIOLATES IN THE FIRST MEMBRANES: 3 TO 6 RESIDUE ANION-BINDING MOTIFS (NESTS), Origins Life Evol. Biosphere 35 (2005) 19.

[49] R. P. Enriquez, T. N. Do, Interactions of iron-sulfur clusters with small peptides: Insights into early evolution, Comput. Biol. Chem. 41 (2012) 58.

[50] J. Meyer, Iron-sulfur protein folds, iron-sulfur chemistry, and evolution, J. Biol. Inorg. Chem. 13 (2008) 157.

[51] H. A. Hansen, J. B. Varley, A. A. Peterson, J. K. Nørskov, Understanding Trends in the Electrocatalytic Activity of Metals and Enzymes for CO₂ Reduction to CO, J. Phys.

488 Chem. Lett. 4 (2013) 388.
 489 [52] J. B. Varley, H. A. Hansen, N. L. Ammitzbøll, L. C. Grabow, A. A. Peterson, J.
 490 Rossimeisl, J. K. Nørskov, Ni-Fe-S Cubanes in CO₂ Reduction Electrocatalysis: A DFT
 491 Study, ACS Catal. 3 (2013) 2640.
 492 [53] B. A. Rosen, A. Salenhi-Khojin, M. R. Thorson, W. Zhu, D. T. Whipple, P. J. A. Kenis,
 493 R. I. Masel, Ionic Liquid-Mediated Selective Conversion of CO₂ to CO at Low
 494 Overpotentials, Science 334 (2011) 643.
 495 [54] R. E. Mielke, K. J. Robinson, L. M. White, S. E. McGlynn, K. McEachern, R. Bhartia,
 496 I. Kanik, M. J. Russell, Iron-Sulfide-Bearing Chimneys as Potential Catalytic Energy Traps
 497 at Life's Emergence, Astrobiology 11 (2011) 933.
 498 [55] T. Shibuya, T. Komiya, K. Nakamura, K. Takai, S. Maruyama, Highly alkaline,
 499 high-temperature hydrothermal fluids in the early Archean ocean, Precambrian Res. 182
 500 (2010) 230.
 501 [56] R. Hengeveld, M. A. Fedonkin, Bootstrapping the Energy Flow in the Beginning of
 502 Life, Acta. Biotheor. 55 (2007) 181.
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Fig. 1. Proposed model for electrical current generation in the deep-sea hydrothermal vent chimney [38,39]. In this model, electrons generated via the oxidation of H_2S and/or H_2 in the hydrothermal fluid are transported through the bisulfide-bearing chimney wall to seawater where they reduce Fe^{3+} , O_2 , CO_2 , and H^+ . The charge balance between an inner and outer surface of a hydrothermal vent is maintained by the diffusion and/or convection of ionic substances in sea water and/or a hydrothermal fluid.

Fig. 2. (a) XRD patterns of (i) pristine greigite (Fe_3S_4), Ni-doped iron sulfides (Ni-FeSs) with ratios of Fe to Ni of (ii) 5 to 1 and (iii) 1 to 1, and (iv) NiS . The patterns assigned to greigite (Fe_3S_4), FeNi_2S_4 and NiS are indicated with asterisks, pound signs and open circles, respectively. (b) Ball and stick models for the crystal structures of (left) Fe_3S_4 (greigite), (center) FeNi_2S_4 and (right) NiS . White: S, black: Fe, and gray: Ni.

Fig. 3. SEM images for (a) pristine greigite, (b) Ni-doped iron sulfides (Ni-FeSs) with Fe to Ni ratios of 5 to 1 (c) and 1 to 1, and (d) NiS.

Fig. 4. Potential dependence of the FE (Faradaic efficiency) for hydrogen production from greigite after potentiostatic conditions in N₂ atmosphere.

Fig. 5. Product distributions for (a) Ni-, (b) Cu- and (c) Zn-containing greigite with various Fe to metal ratios under a potentiostatic condition of -1.3 V vs SHE for 30 min.

Fig. 6. Potential dependence of FE (Faradaic efficiency) for reaction products from (a) greigite and (b) Ni-containing greigite (Fe/Ni = 1) in the presence of 760 Torr CO₂. Filled squares, CO; open triangles, CH₄; open squares, H₂ × 10⁻³.

Fig. 7. FEs (Faradaic efficiencies) for CO, CH₄ and H₂ production by greigite, Ni-containing greigite (Fe/Ni = 1) and Ni-containing greigite (Fe/Ni = 1) modified with TEA or PAH under a potentiostatic condition at – 1.3 V vs SHE for 30 min.

Fig. 8. Schematic illustration for the electrochemical synthesis of organic compounds in bisulfide-bearing deep-sea hydrothermal mineral deposits. (a) In the absence of the proton-motive force (PMF) across the mineral structure, electroreduction of CO_2 at the outer mineral interface by H_2 in hydrothermal fluid is the dominant endergonic reaction. (b) In the presence of the PMF, which can be as high as 200 mV, CO_2 reduction proceeds in the cavity of the bisulfide-bearing mineral deposits. (c-left) Due to the low FE for CO_2 , the

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7 679 electrical current is predominantly used for H₂ generation, which lowers the proton
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9 680 concentration in the cavity, dissipating the electrochemical gradient. (c-right) Dissipation of
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11 681 the PMF by H₂ evolution leads to the release of elemental hydrogens stored in the
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13 682 hydrothermal mineral deposits into the cavity in the form of protons by the reductive
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15 683 dissolution of HS⁻ from FeS₂ ($2\text{H} + \text{FeS}_2 \rightarrow \text{FeS} + \text{HS}^- + \text{H}^+$), thereby increasing the
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17 684 proton concentration and regenerating the PMF to initiate the electroreduction of CO₂.
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