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Reactions depending on iron sulfide and linking geochemistry with biochemistry

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ABSTRACT **Iron sulfide gives rise to unusual reducing reactions: some dependent on FeS/H_2S synergism [$NO_3^- \rightarrow NH_3$; $HC{\equiv}CH \rightarrow H_2C{=}CH_2$, $H_3C{-}CH_3$; $\text{-}CH_2{-}CO\text{-} \rightarrow \text{-}CH{=}CH\text{-}$, $\text{-}CH_2{-}CH_2\text{-}$; $HS{-}CH_2{-}COOH \rightarrow CH_3{-}COOH$; others dependent on FeS alone [$HS{-}CH_2\text{-}CH_2{-}X \rightarrow CH_2{=}CH_2$ (where X = OH, SH, or NH_2)]. The experimental conditions are geochemically plausible: 100°C, aqueous, nearly neutral, and fastidiously anaerobic. These reactions establish additional facts of soil chemistry, organic geochemistry, and the global nitrogen cycle. Further, they point to the common evolutionary denominator of geochemistry and biochemistry.**

Pyrite is the most stable iron mineral under anaerobic conditions—a thermodynamic sink for the reducing power of aqueous FeS/H_2S ($E^{\circ\prime} = -620$ mV). This reducing power has been postulated as the driving force for a pyrite-pulled chemo-autotrophic origin of life (1–4). Pyrite has been shown to reduce protons to molecular hydrogen under geochemical conditions (5), and it has been surmised as the basis for a vast repertory of hitherto unexplored reducing reactions (2–4). Naturally occurring oxidants like nitrate and biochemically relevant organic oxidants are the prime candidates for testing this potential.

EXPERIMENTAL PROCEDURES

All solutions were prepared from doubly distilled water through which argon had been bubbled for 2 hr. The H_2S gas was prepared by adding 50% H_2SO_4 to $Na_2S{\cdot}9\ H_2O$ in an evacuated serum bottle. All reactions were done at 100°C in a rotary shaker (100 rpm) in serum bottles (120 ml), closed with viton diaphragms (Ochs, Bovenden, F.R.G.), and supplied with an argon atmosphere (200 kPa). All chemicals were of the highest available purity.

Formation of Ammonia from Nitrate. A suspension of 2 mmol of FeS (pyrrhotite, 99.99%, Johnson Matthey) in 10 ml of H_2O was charged with 120 μmol of $NaNO_3$, dissolved in 0.2 ml of H_2O and with 2 mmol of H_2S gas, and finally adjusted to pH 4. NH_3 was determined after 3 days by the glutamate reductase method.

Formation of Ethene and Ethane from Ethyne, Acetaldehyde, and Mercapto Compounds. Amorphous FeS was prepared in an anaerobic chamber by adding $Na_2S{\cdot}9\ H_2O$ to 0.6 M $FeSO_4$, filtering the precipitate, washing it with H_2O, and drying it under N_2/H_2, 95:5; 2 mmol were suspended in 10 ml of H_2O. Ethyne (C_2H_2), ethene (C_2H_4), and ethane (C_2H_6) were determined by gas chromatography (Hewlett–Packard 5890). A column packed with Porapak N (Supelco) was used (injection temperature, 70°C; oven temperature, 70°C; detection temperature, 150°C; flame ionization detector; carrier gas, N_2). At the time of each measurement the pH was readjusted to 6.

Formation of Acetic Acid from Thioglycolic Acid. Serum bottles were charged with 2 mmol of FeS (pyrrhotite, 99.99%, Johnson Matthey), 10 ml of H_2O, and 50 μmol of thioglycolic acid and closed with rubber diaphragms. The reactions were continued for 4 weeks. Acetic acid was determined by gas chromatography. A column packed with Chromosorb 101 was used (injection temperature, 210°C; oven temperature, 160°C; detection temperature, 250°C; flame ionization detector; carrier gas, N_2).

Formation of Cinnamate and Phenylpropionate from Phenylpyruvate. Amorphous FeS (prepared as described above) in 20 ml of water was treated with 400 μmol of phenylpyruvate. The serum bottles were closed with rubber diaphragms. All reactions were continued for 12 days. The products were separated by HPLC [phosphate buffer/methanol-gradient; column RP18 (Kontron, Zurich)]. The compounds were detected by an UV detector (wavelength, 254 nm). Phenylpyruvate and cinnamate were identified by mass spectrometry.

RESULTS AND DISCUSSION

In our quest for phylogenetically deep-branching hyperthermophilic bacteria and archaea (6) we tried to enrich organisms from sites with abundant pyrite deposits by using FeS/H_2S as a hydrogen source. Surprisingly, in the presence of nitrate, large amounts of ammonia were formed abiotically within the noninoculated controls. Subsequent systematic experiments (Table 1) gave unequivocal evidence for a synergistic effect of H_2S and FeS for the reduction of NO_3^- to NH_3—FeS alone causing only minor conversion, and H_2S alone causing none. As these conditions are geochemically plausible, this kind of nitrate reduction may be assumed to proceed in nature. This result solves an old geochemical problem (7). The global nitrogen cycle can now be seen as operating partly by microbial nitrate reduction and partly by an FeS/H_2S-driven abiotic nitrate reduction. The latter must have operated before the advent of nitrate-reducing microorganisms.

Our result is also of ecological significance. Nitrate ions and anaerobic pyrite-forming conditions cannot coexist. This fact means that nitrate reduction, exhibited in the laboratory by anaerobic organisms from pyrite-forming habitats, may well be ecologically irrelevant.

Finally, our result has a biochemical significance. It suggests a mechanistic commonality between the iron–sulfur clusters of nitrite reductases and nitrate reduction by FeS/H_2S. The evolution of enzymatic nitrate reduction may now be traced back to abiotic nitrate reduction.

The reduction of nitrate to ammonia by FeS/H_2S has a remarkable feature. This reaction channel bypasses the energetically favorable N_2 molecule. We, therefore, tested, as a model for nitrogen fixation, the reduction of ethyne by FeS/H_2S. Ethyne was, indeed, converted to ethene and

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Table 1. Formation of ammonia from nitrate in the presence of FeS and H_2S at pH 4

Run	Starting materials: FeS, mmol	Starting materials: H_2S, mmol	Starting materials: $NaNO_3$, μmol	Product: NH_3, μmol
1	2	2	480	200
2	2	2	480	206
3	2		480	54
4	2		480	53
5		2	480	0
6			480	0

ethane (Table 2). The product ratio strikingly resembles that of Fe-nitrogenase (8) and vanadium (V)–Fe-nitrogenase (9), thus encouraging attempts to find conditions for the predicted (2) nitrogen fixation by FeS/H_2S. Catalytic hydrogenation might be seen as a mechanism of the ethyne reaction, FeS or FeS_2 surfaces serving as a hydrogenation catalyst. Molecular hydrogen is, indeed, known to be produced by FeS/H_2S under geochemical conditions (5) [and incidentally by electrocrystallization with a titanium surface under the conditions of a heavy-water plant (10–13)]. Yet, if H_2S was replaced by H_2 by more than the amount that accumulated during the test duration (5), the result was the same as with FeS alone. The situation did not change when FeS was previously aged with H_2S to produce FeS_2 (Table 2, runs 9 and 10). Thus, molecular hydrogen appears inert under the reaction conditions.

We propose a mechanism involving the addition of H_2S to ethyne and subsequent desulfuration by FeS:

$$H—C\equiv C—H + H_2S \rightarrow H_2C=CH—SH$$

$$H_2C=CH—SH + FeS \rightarrow H_2C=CH_2 + FeS_2$$

$$H_2C=CH—SH + H_2S \rightarrow H_3C—CH(SH)_2$$

$$H_3C—CH(SH)_2 + 2\ FeS \rightarrow H_3C—CH_3 + 2\ FeS_2.$$

From this mechanism we concluded that acetaldehyde should give similar products by way of the following reactions:

$$H_3C—CHO + H_2S \rightarrow H_3C—CH(SH)OH$$

$$H_3C—CH(SH)OH \rightarrow H_2C=CH—SH + H_2O.$$

Acetaldehyde was, indeed, found to be converted by FeS/H_2S to ethene and ethane (Table 2). Again, FeS and H_2S exhibit synergism. The direct, one-step, one-pot conversion of $-CH_2—CO-$ to $-CH=CH-$ is an unusual reaction type. It proceeds under neutral, aqueous conditions.

The proposed first intermediate in the reaction of acetaldehyde, $H_3C—CH(SH)OH$, is an instable isomer of 2-mercaptoethanol, $HS—CH_2—CH_2—OH$. 2-Mercaptoethanol is quite stable in aqueous solution. We, therefore, tested whether it could react with FeS/H_2S. Surprisingly, it produced ethene with a much higher rate than acetaldehyde (Table 3). Again, this reaction type is odd from the view point of conventional organic chemistry. This reaction seems to comprise an elimination of water with the possible involvement of an anionic intermediate. Ethylene glycol, $HO—CH_2—CH_2—OH$, was stable under the same conditions (Table 3, run 5). This result shows that the interaction of the sulfhydryl group with FeS is essential. The conclusion is corroborated by the reaction of 1,2-dimercaptoethane, $HS—CH_2—CH_2—SH$ (Table 3, runs 6 and 7) and of 2-mercaptoethylamine, $HS—CH_2—CH_2—NH_2$ (Table 3, run 11). The reactions of these sulfur compounds are not promoted by H_2S.

The facile eliminative desulfuration of 1,2-dimercaptoethane by FeS means that the former cannot exist in the presence of FeS. However, coenzyme M ($HS—CH_2—CH_2—SO_3H$), a derivative of 1,2-dimercaptoethane, does exist in the anaerobic metabolism of archaeal organisms. We, therefore, tested its stability in the presence of FeS/H_2S. Remarkably, coenzyme M turned out to be relatively stable (Table 3, run 10). Similarly, coenzyme A, a derivative of 2-mercaptoethylamine is moderately stable (Table 3, run 12). These results are compatible with an early biochemical evolution under pyrite-

Table 2. Formation of ethene and ethane from ethyne and acetaldehyde in the presence of FeS and H_2S

Run	Starting materials: FeS, mmol	Starting materials: H_2S, mmol	Starting materials: X, μmol	Starting materials: H_2, μmol	Products, μmol: 7 days C_2H_4	7 days C_2H_6	7 days C_2H_2*	14 days C_2H_4	14 days C_2H_6	14 days C_2H_2*	21 days C_2H_4	21 days C_2H_6	21 days C_2H_2*
X = ethyne													
1	2	2	50		4.9	0.11	9.0	6.7	0.15	1.1	7.6	0.16	0.13
2	2	2	50		7.0	0.15	2.5	8.5	0.17	0.05	9.0	0.21	0
3	2		50		0.36	0	12	0.47	0	0.40	0.82	0	0
4		2	50		0.03	0	35	0.08	0	19	0.11	0	10
5		2	50		0.005	0	24	0.01	0	5	0.02	0	1
6			50		0	0	50	0	0	50	0	0	48
7	2		50	40	0.37	0	10	0.53	0	1.7	1.1	0	0.04
8	2		50	40	0.32	0	11	0.43	0	2.0	0.75	0	0.08
9†	2		50	40	0.18	0	37	0.31	0	35	0.50	0	35
10†	2		50	40	0.35	0	21	0.42	0	21	1.1	0	16
X = acetaldehyde													
11	2	2	50		0.94	0.03		1.5	0.05		1.6	0.05	
12	2	2	50		1.10	0.03		1.74	0.03		1.9	0.06	
13	2		50		0.01	0		0.01	0		0.01	0	
14	2		50		0.005	0		0.01	0		0.01	0	
15		2	50		0	0		0.01	0		0.02	0	
16			50		0	0		0	0		0	0	

*Residual ethyne.

†Runs 9 and 10 were done with batches of FeS that had been treated at 100°C with 10 ml of H_2O and 2 mmol of H_2S under an atmosphere of argon for 14 days to produce FeS_2.

Table 3. Formation of ethene from mercapto compounds in the presence of FeS

Run	Starting materials: FeS, mmol	H_2S, mmol	X, μmol	C_2H_4 product, μmol: 3 days	7 days
	X = HS—CH_2—CH_2—OH				
1*	2	2	50	10.6	23.2
2*	2		50	11.0	22.4
3		2	50	0.04	0.05
4			50	0.2	0.3
	X = HO—CH_2—CH_2—OH				
5*	2	2	50		0.04
	X = HS—CH_2—CH_2—SH				
6	2	2	50	22.8	33.5
7	2		50	27.4	29.9
8		2	50	0.006	0.008
9			50	0.22	0.22
	X = HS—CH_2—CH_2—SO_3H (CoM)				
10	2		50	0.02	0.04
	X = HS—CH_2—CH_2—NH_2				
11	2		50	7.6	12.9
	X = HS—CH_2—CH_2—NH—R† (CoA)				
12	2		50	0.19	0.44

CoM, coenzyme M.
*In runs 1, 2, and 5 amorphous FeS was precipitated directly in the serum bottle from 2 mmol of $FeSO_4$ and 2 mmol of H_2S gas in the presence of 10 ml of H_2O.
†R = rest of CoA.

forming conditions and an emergence of coenzyme A and coenzyme M under those conditions.

For gaining further insight into the mechanism of a pyrite-pulled desulfuration we tested the reaction of thioglycolic acid (HS—CH_2—COOH). A desulfuration was, indeed, found. Surprisingly, however, it depends on a synergism of FeS and H_2S (50 μmol of thioglycolic acid gave 26 μmol of acetic acid with FeS/H_2S; 1.8 μmol with FeS alone and 0.7 μmol with H_2S alone). H_2S is not required for the stoichiometry of this reaction, but must be catalytic. We propose the following mechanism:

$$HS—CH_2—COOH + H_2S \rightarrow HS—CH_2—COSH + H_2O$$

$$HS—CH_2—COSH + FeS \rightarrow CH_3—COSH + FeS_2$$

$$CH_3—COSH + H_2O \rightarrow CH_3—COOH + H_2S.$$

This mechanism is based on the assumption that the activation energy of the desulfuration reaction is higher for HS—CH_2—COOH than for HS—CH_2—COSH. It is remarkable that a similar set of reactions has been proposed for the mechanism of glycine reductase (14):

$$RSe—CH_2—COOH + R'SH \rightarrow RSe—CH_2—CO—SR' + H_2O$$

$$RSe—CH_2—CO—SR' + R''SH \rightarrow RSe—SR'' + CH_3—CO—SR'.$$

Our finding supports this mechanism. Moreover, it points to a possible archaic origin in a pyrite-pulled metabolism.

The discovered reactions of FeS corroborate one of the main postulates of the theory of a pyrite-pulled chemoautotrophic origin of life (postulate 5 in ref. 3). This postulate is of particular significance for the reduction of 2-ketoacids in the proposed archaic citrate cycle (3). We, therefore, treated phenylpyruvate with FeS/H_2S and found, indeed, high amounts of phenylpropionate together with traces of cinnamate (Table 4). H_2S alone gave only cinnamate. A reaction of FeS/H_2S with cinnamate gave only traces of phenylpropionate. Thus, the main reaction channel from phenylpyruvate to phenylpropionate does not proceed via cinnamate. The reaction requires pH ≤ 8. Similarly, the conversion of oxaloacetate to fumarate and succinate was detected by TLC. The results support the proposal that the extant reductive citrate cycle arose by detours from a simpler pyrite-pulled archaic cycle (3) and by turning FeS/H_2S into the iron–sulfur clusters of fumarate reductase (15), aconitase (16), and homoaconitase (17). Moreover, our results offer a solution to one of the open problems of enzymology. They suggest a mechanism for the enzymatic dehydration of 2-hydroxy acids (e.g., phenyllactyl-CoA → cinnamyl-CoA) (18) by three steps: (*i*) flavine-dependent oxidation of the 2-hydroxy group to a 2-keto group; (*ii*) reductive conversion of the 2-keto group to a double bond by an iron–sulfur cluster, akin to the reaction reported here; (*iii*) reestablishment of the redox status of the enzyme components.

All these redox reactions have a common component: the oxidation of sulfide units. The course of this oxidation is an open problem. It might be assumed that the sulfide units are first oxidized to elemental sulfur, which is known to react rapidly with FeS to form pyrite. As an alternative, these sulfide units may be assumed to react directly with FeS to form pyrite.

Our results suggest that anaerobic aqueous FeS/H_2S will tend to drive organic compounds toward exhaustive reduction (hydrocarbons). This geochemistry is promoted by the thermodynamic stability of the C—S bond compared with C—O or C—N, by the nucleophilicity of H_2S, and by the demonstrated facile desulfuration of organic sulfhydryl groups by FeS. This geochemistry bears on the chemistry of fossil pyritization and of the formation of peat, coal, oil, and natural gas. The FeS/H_2S-driven production of ethene from C_2 compounds may explain the widespread occurrence of ethene in (sedimentary) rocks (19) and hydrothermal vents (20). This geochemistry suggests that the hormone function of ethene in plants may have been started by external ethene. The biosynthesis of ethene from methionine would then be a latecomer, invented in areas devoid of an FeS/H_2S-driven production of ethene.

The spectre of an exhaustive reduction of organic compounds by FeS/H_2S, as exemplified herein, may be decisive for the fate of the widely held theory of a prebiotic broth. The rates of the reducing reactions discussed here and similar ones not yet investigated may be high enough to render the slowly accumulating "anaerobic prebiotic broth" a geochemical impossibility. For the alternative theory of a pyrite-pulled

Table 4. Formation of phenylpropionate and cinnamate from phenylpyruvate in the presence of FeS and H_2S

Run	Starting materials, mmol: FeS	H_2S	PhPy	Products, μmol: 1 day Ci	PhPr	PhPy	5 days Ci	PhPr	PhPy	12 days Ci	PhPr	PhPy
1		2	0.4	3.9	0	106	6.6	0	26	9.7	0	16
2	2		0.4	0.1	0	242	0.1	0	252	0.13	0	250
3	2	2	0.4	1.4	2	162	1.2	21.5	64	1.0	96	68

PhPy, phenylpyruvate; Ci, cinnamate; PhPr, phenylpropionate.

chemo-autotrophic origin of life the reactions reported here have the opposite significance; they support this hypothesis, and they open a vista onto an unusual aqueous organic chemistry on pyrite surfaces.

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