

Supporting Information

Novikov and Copley 10.1073/pnas.1304923110

SI Materials and Methods

Reactions of pyruvate were carried out by loading a 1-mL slurry containing powdered minerals, pyruvate and, in some cases, NH_4Cl , into a small Teflon tube under an argon atmosphere in a glovebox. A small alumina ball was added to provide mixing during the incubation. The tube was placed inside the reactor vessel. The vessel was closed and removed from the glovebox. Gases (H_2S , H_2 , and CO_2) were introduced sequentially into the headspace above the Teflon tube. The vessel's contents were pressurized to 11,000 psi (750 atm) for experiments carried out at room temperature or 8,000 psi (544 atm) for experiments carried out at 110 °C by turning the bronze nut, which pushes the Teflon plug down, compresses the gas in the headspace to the pressure indicated on the pressure gauge, and closes the Teflon tube. An electrical heating coil was placed around each vessel and then a pair of vessels was fastened to a rotisserie that rocked the vessels for 24 h. At the conclusion of the incubation, the slurry was removed from the reaction vessel, diluted to 5 mL, and immediately transferred to a glovebox. The pH was adjusted to 9.5–10 to precipitate dissolved metal ions. After filtration, the solution was analyzed by NMR spectroscopy and mass spectrometry. Further details are provided in *SI Materials and Methods*.

Mineral Samples. FeS (pyrrhotite) was purchased (Aldrich) as a $\sim 1\text{-}\mu\text{m}$ powder and used as provided. Troilite was synthesized from elemental Fe and S (99.995% purity) under vacuum in a sealed quartz ampule. Other mineral samples were acquired from Estes Park Rock Shop and Kelly's Rocks (<http://www.kellysrocks.com/index.cfm>). Minerals that were subject to oxidation upon exposure to air were ground in a glovebox outfitted with calibrated oxygen sensor under argon (< 1 ppm of O_2) in a diamond disk mill in simulated vent water (pyrite) or in an alumina ceramic ball mill with distilled water (pyrite, pyrrhotite, chalcopyrite, marcasite, sphalerite, and arsenopyrite).

All mineral samples were subjected to X-ray diffraction analysis followed by automated matching to known mineral diffraction patterns and to trace element analysis by inductively coupled plasma mass spectrometry. Matrix elements for all mineral samples except FeS, pyrite and chalcopyrite were also analyzed using an ARL 3410+ inductively coupled optical emission spectrometer (Table S6). The surface area of selected ground samples was estimated by nitrogen or krypton adsorption on a Micromeritics TriStar II 3020 by Material Synergy laboratory.

Construction of a Reaction Vessel for High-Pressure Reactions. We constructed reaction vessels that allowed us to carry out reactions under simulated hydrothermal vent conditions (Fig. S1). Our first vessels were made of 316 alloy stainless steel. However, after a year of use, the internal surface of these vessels showed significant corrosion. Subsequently, vessels were constructed from high-strength titanium grade 5 alloy (6% Al, 4% V, 90% Ti).

High-Pressure Reactions in the Presence of Mineral Catalysts. The reactor vessel was assembled and loaded under argon in a glovebox. Reagents were loaded into custom disposable Teflon test tubes under argon in a glovebox. Reaction mixtures consisted of a freshly prepared solution of sodium pyruvate (20 mM) and, in some cases, NH_4Cl (70 mM), in 0.5-mL degassed water or simulated vent fluid, the latter containing 300 mM NaCl, 30 mM KCl, and 40 mM CaCl_2 . The mineral powder or slurry (100 mg/mL) was added along with a 1/8 or 3/16 in. alumina ball to allow mixing. (The alumina ball was highly polished to minimize its

surface area and thus its contribution to catalysis.) The reaction volume was brought to 1.0 mL by addition of extra water or simulated seawater. Because the presence of salts had little effect on the reaction, most reactions were carried out in water rather than simulated seawater. The Teflon tube was carefully placed in the bottom of the cylinder, leaving a headspace volume of about 0.3 mL between the top of the tube and the Teflon stopper. The bottom of the cylinder was plugged and the closed vessel was removed from the glovebox. The sealing nuts on the vessel were fully tightened using a torque wrench. Degassed water (0.5 mL) was introduced above the movable Teflon stopper and then the plunger was pushed into the cylinder, displacing the excess water through a secondary relief valve. After the piston was set at the desired initial position, the secondary relief valve was closed.

The vessel was attached to a stainless steel gas-delivery system. The amount of each gas needed was calculated using the nonideal gas equation: $PV = ZNRT$, in which P is pressure in Pascals, V is the headspace volume in cubic meters, Z is the gas-specific compression coefficient (0.85 for H_2S , 0.84 for CO_2 and 1.00 for H_2), N is the number of moles, R is the universal gas constant, and T is temperature in degrees.

The system was purged with the first gas used (typically H_2S) and then the desired pressure of the gas was set in the system. The gas was introduced to the headspace over the reaction mixture by briefly opening the valve on the vessel. Excess gas was released by closing the reactor gas valve and opening the gas line purge valve. The system was then purged with the next gas (typically H_2) and the process was repeated until all desired gases had been introduced into the vessel. The process of adding gases to the desired pressures usually took less than 2 min. During this short time, the introduced gases did not dissolve significantly, so the number of moles of each gas present could be accurately calculated based upon the differences in headspace pressures before and after addition of each gas.

The vessel's contents were pressurized to 11,000 psi (750 atm) for experiments carried out at room temperature or 8,000 psi (544 atm) for experiments carried out at 110 °C by turning the bronze nut, which pushes the Teflon plug down, compresses the gas in the headspace to the pressure indicated on the pressure gauge, and closes the Teflon tube. An electrical heating coil was placed around each vessel and then a pair of vessels was fastened to a rotisserie that rocked the vessels over an arc of nearly 360 degrees so that the alumina ball placed inside would roll back and forth every 10 seconds, thus stirring the contents of the vessels. In the first hour the pressure decreased to about 10,000 psi for reactions carried out at room temperature, most likely to dissolution of gases in the liquid and adsorption on the mineral, and the temperature rose transiently to about 35 °C (for reactions carried out at room temperature).

Recovery and Identification of Products. Reaction mixtures were recovered from the reactor and diluted to ~ 5 mL with distilled water. A few boiling chips were added and the slurry was immediately introduced into a glovebox antechamber. Dissolved gases were removed by three cycles of evacuation and purging with argon. The pH of the solution was measured at this point. The slurry was then transferred into the glove box and 2% aq NaOH was added to raise the pH to 9.5–10 to remove soluble metal ions leached from the minerals by precipitation as metal hydroxides. This step is particularly important for materials containing paramagnetic metal ions that interfere with acquisi-

tion of NMR data. For Fe^{2+} -containing minerals, it is essential to carry out this step in the absence of O_2 . At basic pH, Fe^{2+} is rapidly oxidized to Fe^{3+} , which binds strongly to organic acids, causing precipitation and/or formation of soluble but paramagnetic complexes for which NMR characterization was impossible. Subsequently, the slurry was filtered through a glass filter [25 mm diameter, porosity 4–5.5 (fine)] to remove precipitated metal hydroxides and the pH was adjusted to 7.5 ± 0.1 with 0.2% aqueous trifluoroacetic acid. The solution was removed from the glovebox. Aliquots of this solution were diluted 100-fold with 50% methanol in water and injected onto an electrospray (ESI⁻) mass spectrometer. The remainder of the solution was prepared for NMR analysis by removing the water by rotary evaporation at 40 °C. The residue was dissolved in D_2O (1 mL) and the solution was evaporated again. The residue was again dissolved in D_2O (0.6 mL), and a solution of sodium *p*-trifluoromethyl benzoate (1 mg/mL, pH = 7.5, 0.1 mL) was added to serve as an internal standard.

Separation of Reaction Products by Silica Chromatography. When reaction mixtures were complex, we separated the reaction products by silica gel chromatography before characterization by NMR spectrometry and mass spectrometry. The reaction mixtures were recovered from the reactor and diluted to ~5 mL with distilled water. The slurry was filtered through a 0.2- μm nylon filter. The filter was washed with 2 mL of water and 2 mL of methanol. The filtrate was brought to 10 mL. A 1 mL aliquot was set aside and prepared for ^1H -NMR as described above. The remaining solution was mixed with 1 g of silica and the solvent was removed by rotary evaporation at 30 °C until a free-flowing

powder was obtained. This powder was loaded onto a silica column (7 g silica) that had been pre-equilibrated with dichloromethane:acetic acid (97:3 by volume). The column was eluted with a gradient from 97:3–0:100 dichloromethane:acetic acid) in a total volume of 600–800 mL. Twelve to fourteen fractions (50 mL each) were collected. The column was then washed with 100 mL 1-butanol:acetic acid:water (50:25:25 by volume). Solvent was removed from all fractions by rotary evaporation at 40 °C. The residues were dissolved in D_2O (0.7 mL), the internal standard (sodium *p*-trifluoromethyl benzoate in D_2O) was added, and the various fractions were subjected to analysis by NMR. An aliquot (0.1 mL) of each fraction was diluted with CH_3OH and subjected to analysis by electrospray ionization mass spectrometry.

Characterization of Reaction Products. Reaction components were analyzed by ^1H -, ^{13}C -, 2D correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC) NMR. Peak assignments were confirmed when possible by addition of small amounts of authentic standards to the reaction mixture. Individual components were quantified by comparison of ^1H -NMR peak areas with those of an internal standard (sodium *p*-trifluoromethyl benzoate). The limit of detection of compounds by ^1H -NMR was about 50–70 μM on a Varian Inova 500-MHz spectrometer (equipped with an NTR 5-mm probe), so we could detect products recovered with a yield as low as 0.1%. Typically we could account for >80% of the initial pyruvate by quantitation of identified products and remaining pyruvate.

1. Ludwig EE (2001) *Applied Process Design for Chemical and Petrochemical Plants* (Gulf Professional Publishing, Boston).

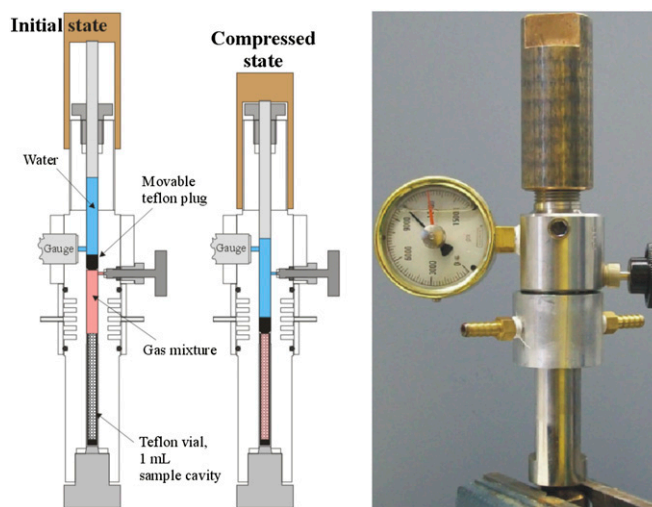


Fig. S1. The reactor used for hydrothermal reactions.

Table S1. Products formed from pyruvate in the presence of 100 mM H₂, 20 mM H₂S and 100 mM CO₂

Mineral	Products (%)*						
	None	Pyrite (25 °C)	Pyrite (110 °C)	Arsenopyrite (25 °C)	Arsenopyrite (110 °C)	Pyrrhotite (Aldrich FeS) (25 °C)	Troilite [†] (syn. FeS) (25 °C)
Pyruvate remaining	78	3.7 ± 1.2	0	0	0	18.3 ± 4.1	50
Thiolactate	0	0	0	14	1	2.1 ± 0.7	0
Thiolactate persulfide	2.4	30 ± 4	25	0	0	1.4 ± 0.2	0
Thiolactate disulfide	2.6	39 ± 5	36	52	21	3.3 ± 1.0	2
Lactate	0	0.5 ± 0.1	0.5	4	4	38.3 ± 1.0	22
Propionate	0	0.1 ± 0.03	27	0.5	59	2.9 ± 0.5	0
Aldol product	0.2	0	0	0	0	0.9 ± 0.4	0
Reduced aldol prod.	0	0	0	0	0	0	13
Alanine	0	0	0	0	0	0	0
Missing	16.8	26 ± 5	11.5	31 ± 2	16	32.8 ± 2.2	13

*Figures given for product yields are the % of the initial pyruvate that was consumed to form the product. For example, conversion of 40% of 20 mM pyruvate to thiolactate disulfide would result in 4 mM thiolactate disulfide.

[†]This reaction was carried out in the presence of 133 mM H₂, 5 mM H₂S and 100 mM CO₂.

Table S2. Products formed from pyruvate in the presence of 70 mM NH₄Cl, 100 mM H₂, 20 mM H₂S, and 100 mM CO₂

Mineral	Products (%)*							
	None (25 °C)	FeS (25 °C)	None (110 °C)	Arsenopyrite (110 °C)	Pyrite (110 °C)	Sphalerite (110 °C)	Marcasite (110 °C)	Chalcocopyrite (110 °C)
Pyruvate remaining	28	16	20	0	0	50	0	0
Thiolactate	0	6	40	6	6	22	2	16
Thiolactate persulfide	16	0	0	0	0	0	0	0
Thiolactate disulfide	20	3	20	3	21	28	1	2
Lactate	0	37	0	23	18	1	8	1
Propionate	0	5	0	18	21	0	39	54
Aldol product	0	0	0	0	0	0	0	0
Reduced aldol prod.	0	0	0	0	0	0	0	0
Alanine	0	16	0	7	2	1	3	5
Missing	36	17	20	39	29	49	47	25

*Figures given for product yields are the % of the initial pyruvate that was consumed to form the product. For example, conversion of 40% of 20 mM pyruvate to thiolactate disulfide would result in 4 mM thiolactate disulfide.

Table S3. Effect of reagents that promote free radical reactions on the yields of thiolactate disulfide and thiolactate persulfide from reaction mixtures containing 20 mM pyruvate and 20 mM H₂S after incubation for 24 h at 25 °C

Additive (20 mM)	Thiolactate disulfide, %	Thiolactate persulfide, %	Thiolactate, %	Pyruvate, %	Total recovery, %
None	18	14	0	25	57
Hydroquinone	24	11	25	20	80
4-hydroxy-TEMPO	45	12	29	0	86

Product yields are expressed in terms of the percent of pyruvate converted to that product.

Table S4. Products formed from lactate and thiolactate disulfide in the presence of 100 mM CO₂, 133 mM H₂, and pyrrhotite (Aldrich FeS) for 24 h at 110 °C

Reactant	Remaining reactant, %	Propionate, %
Lactate	99	0
Thiolactate disulfide	85	15

Table S5. Characterization of minerals

Mineral	Most prominent trace elements, mg/kg								BET Surface area, m ² /g*
	Mn	Cu	Ni	Cr	Mo	P	As	Sn	
FeS (Aldrich)	550	380	230	160	150	130	59	33	1.51
	1500	250	53	50	30	28	19	15	
Troilite	1500	250	53	50	30	28	19	15	0.21
	1800	420	305	280	180	172	140	120	
Pyrite	1800	420	305	280	180	172	140	120	1.98
	1000	170	50	45	14	10	5.9	2.3	

*Five pressure points were used to calculate the Brunauer-Emmet-Teller (BET) surface area.