riftia tube worms, galatheid crabs, and *Calyptogena magnifica* clams, as well as other organisms, are present at the Galápagos site and the East Pacific Rise hydrothermal vents at 21°N. The mechanism by which these benthic organisms migrate from one isolated deep-sea hydrothermal vent site to another is likely to be in the form of a planktonic larval stage (26).

The most commonly observed and morphologically conspicuous microorganisms found at the Galápagos hydrothermal vent systems have been described (27). These microorganisms and those found at the East Pacific Rise participate in a diverse number of microbial processes; they include sulfur-oxidizing, metal-oxidizing, and methane- and methylenamine-oxidizing bacteria, as well as the thermophilic methanogen *M. jannaschii* (28). Since the macrocycle 1a appears to occur only in *M. jannaschii*, this molecule can potentially be used as a species-specific marker to assess this microbe's contribution to the archaeabacterial community in the hydrothermal vent systems. In addition, the use of this molecular marker and the other glycerol ethers should enable us to determine whether the archaeabacterial community is relatively homogeneous at geographically distant and isolated hydrothermal vent sites. Our screening does not preclude the possibility that other hydrothermal vent archaeabacteria biosynthesize the macrocyclic ether 1a, but certainly the absence of 1a among the glycerol ethers in hydrothermal vent organic material would rule out the presence of *M. jannaschii*.

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References and Notes


The Exclusion of D2O from the Hydration Sphere of FeSO4 • 7H2O Oxidized by Thiobacillus ferroxidans

Abstract. Infrared spectra demonstrate that neither FeSO4 • 7H2O nor its bacterial or abiotic hydrated oxidation products incorporate deuterium in acid D2O solutions. Deuterium exchange occurred as bridging OD when bacterially oxidized iron was precipitated from D2O solutions as ferric hydroxysulfates. The exclusion of deuterium depended upon the stabilization of aquated Fe(II) and Fe(III) complexes by sulfate ions in outer-sphere coordination and is consistent with the requirement and postulated role of sulfate in iron oxidation by Thiobacillus ferroxidans.

Despite the recognized importance of metal ions to living systems, often overlooked is the fact that all biologically important cations are present in aqueous solution as hydrated species. The chemical reactivity of a cation may depend upon molecular exchange between its primary hydration sphere and other molecules in solution (I), yet very little is known about how the biological activity of a metal ion relates to the coordinated water molecules. I present evidence that oxidation of iron by a chemolithotrophic bacterium is dependent upon the existence in acid solution of a Fe(II) hydrate complex stabilized by SO42−. The organism, *Thiobacillus ferroxidans*, is important in the natural leaching of pyritic minerals and in biowhydrometallurgical technology (2).

Although rates for the exchange between solvent water molecules and water molecules in the hydration sphere of aquated ions differ widely, even the most tightly bound are reported to exchange at measurable rates (I, 3). For example, one of the slowest reported is hexaaquated Rh(III), in solution with perchlorate ions (t1/2 = 33 hours) (4); more common values are rates like 10−6 second for hexaaquated Fe(II) ammonium sulfate or hexaaquated Co(II) (5).

A slower range of exchange rates would be expected with D2O as solvent than with H2O, since the energy required for breaking deuterium bonds is generally thought to be greater than for hydrogen bonds (6). However, isotopic effects alone would not prevent the formation of deuterated complexes, at measurable rates, during incubation of soluble hydrated complexes in 95 percent D2O solutions (7). It was extraordinary, therefore, to find that hydrated Fe(III) precipitates derived from FeSO4 • 7H2O in 95 percent D2O solutions, acidified to permit oxidation by T. ferroxidans, did not contain demonstrable amounts of deuterium.

Earlier investigation had shown that oxidation by the acidophilic chemolithotrophic bacterium *T. ferroxidans* precipitated an amorphous hydrated Fe(III) sulfate from acidic Fe(II) sulfate solutions (8). This precipitate was similar in composition and infrared spectrum to an amorphous Fe(III) sulfate (2Fe2O3 • SO4 • mH2O) described by Margulis et al. (9). In an effort to characterize this material and determine if the
precipitates contained bridging OH groups, I carried out the oxidation of FeSO\textsubscript{4} \cdot 7H\textsubscript{2}O in acidified D\textsubscript{2}O solution. Although the products of bacterial oxidation were of particular interest, infrared spectroscopic comparisons were made of sediments obtained from abiotically as well as bacterially oxidized iron in acid D\textsubscript{2}O or H\textsubscript{2}O solutions (Fig. 1).

The protocol for bacterial oxidation consisted of incubating 20 ml of 0.1M FeSO\textsubscript{4} \cdot 7H\textsubscript{2}O in H\textsubscript{2}O or 95 percent D\textsubscript{2}O, containing 3.0 \times 10\textsuperscript{10} washed cells of the Leathen strain of T. ferrooxidans. The pH of the solution was adjusted with concentrated H\textsubscript{2}SO\textsubscript{4} to between 2.2 and 2.5, corresponding to a pD range between 2.6 and 2.9 in D\textsubscript{2}O solutions (10). Approximately 21 percent of the iron in D\textsubscript{2}O solutions was oxidized by the bacterial suspension in 30 hours of continuous shaking at 20°C. In that time, the amount of iron oxidized in D\textsubscript{2}O was 68 percent of that in H\textsubscript{2}O. After 48 hours, sufficient hydrated Fe(III) sulfate had precipitated to be recovered for analysis (11). After the first crop of precipitate had been removed by centrifugation, supernatant...
solutions were passed through 0.22-μm filters to remove bacterial cells and terminate oxidation. Amorphous Fe(III) sediments that continued to form in the bacteria-free D2O filtrates, over a period of 14 months, lacked deuterium and were uniform in infrared spectral characteristics (Fig. 1).

Abiotic oxidation of FeSO₄ · 7H₂O in acid D₂O solutions, accomplished either by heating to 80°C or by the dropwise addition of 30 percent H₂SO₄, caused rapid precipitation of Fe(III) as an amorphous hydrated sulfate similar to that formed by bacterial oxidation. This was contrary to earlier findings (6), which I again confirmed, that abiotic oxidation in H₂O produced precipitates with infrared bands indicative of admixture with αFeOOH. However, D₂O sediments formed after abiotic oxidation lacked the distinctive librational OH bands of αFeOOH and bands due to deuterium incorporation (Fig. 1).

In contrast, when filtrates of supernatant solutions, derived from bacterial oxidation of iron in D₂O, were treated with solid (NH₄)₂SO₄ to form ammoniojarosite, (NH₄)₂Fe(SO₄)₂(OH)₆, deuterium incorporation did occur as evidenced by strong OD stretch, ND stretch, and OD bridging frequencies (Fig. 2). Continued precipitation of ammoniojarosite from D₂O produced sediments with more complete replacement of protium by deuterium (Fig. 2C).

Sodium ions are not as efficient as monovalent cations of larger radii in forming jarosites in bacteri ally oxidized iron solutions (7, 12). A D₂O solution of bacterially oxidized FeSO₄ · 7H₂O, treated with solid sodium sulfate, produced a brown precipitate that consisted mainly of the amorphous hydrated Fe(III) sulfate, as shown by its fibrous microstructure under scanning electron microscopy. This was confirmed by infrared spectroscopy (Fig. 3) and sodium analysis, which showed that the material contained very little deuterium or sodium jarosite. In contrast, the addition of solid NaCl to bacterially oxidized iron in D₂O solution yielded a yellowish precipitate that had the infrared spectrum of a ferric hydroxysulfate (Fig. 3C), with OD present instead of bridging OH (Fig. 3). This result was unexpected because the addition of chloride ions to H₂O solutions of bacterially oxidized Fe(III) sulfates generally had yielded amorphous sediments rather than crystalline Fe(III) hydroxysulfates (8). Scanning electron microscopy, x-ray diffraction, and flame photometric analysis indicated that the substance was a crystalline sodium ferric hydroxysulfate similar to natrojarosite. However, the amount of iron in the precipitate (~26 percent) was less than expected for natrojarosite.

The absence of deuterium exchange, either during or after oxidation of FeSO₄ · 7H₂O, indicated that the hydration spheres of both the Fe(II) and Fe(III) complexes were unexpectedly stable in the acid D₂O systems that I studied. This result suggested that outer-sphere O₂²⁻ (as associated with Fe(II) or Fe(III) complexes) not only prevented the replacement of H₂O molecules by D₂O but also prevented the exchange of deuterons and OD⁻ with protons and OH⁻ that arise from dissociation of water. This interpretation was corroborated by infrared spectra of FeSO₄ · H₂O, formed by forced-draft evaporation of FeSO₄ · 7H₂O in acid D₂O solution. The infrared spectrum of the monohydrate formed (Fig. 3A) after several months of equilibration of the heptahydrate in D₂O at room temperature, showed very little deuterium present. This recalcitrance to deuterium exchange persisted despite changes in the coordination sphere of Fe(II) during conversion of the heptahydrate to the monohydrate, particularly, the introduction of outer-sphere (T₄) SO₄²⁻ into an inner coordination site, as shown by its conversion to C₃₄ symmetry (15).

Earlier reports of SO₄²⁻ stabilizing the coordinated water of Fe(II) hydrates (13, 14) and other hydrates of divalent cations (13, 15) give no indication that the stability noted would have the effect of excluding solvent exchange. However, one may suppose that deuterium exclusion signifies the existence of an exchange barrier greater than that usually attributed to a deuterium isotope effect (that is, due to the strength of deuterium bonding between heavy water molecules in the solvent phase and their reduced mobility as compared to that of H₂O).

The role of SO₄²⁻ in stabilizing aquated Fe(II) and Fe(III) complexes in acid solution parallels the requirement for SO₄²⁻ in iron oxidation by T. ferrooxidans (16). Although this requirement is best satisfied by high concentrations of SO₄²⁻, other oxyanions such as PO₄³⁻, AsO₄³⁻, WO₄²⁻, and TeO₂²⁻ partially replace SO₄²⁻ and SeO₂²⁻ replaces it completely (17). The loose specificity of the bacterial requirement can now be understood to mean that T. ferrooxidans needs an oxyanion-stabilized hexaquated complex of Fe(II) as substrate for iron oxidation rather than a particular anion at high concentration.

Conceivably, oxyanions stabilize hydrates by hydrogen bonds which bridge coordinated water molecules of the iron complex (18). In contrast, anions such as chloride or nitrate, which inhibit iron oxidation by T. ferrooxidans, destabilize the required substrate complex by replacing SO₄²⁻ in the outer sphere. Such action would destruct the hydration sphere and lead to replacement of water molecules by other ligands. Similarly, cations suitable for interaction with the Fe(III) complex can release stabilizing hydrogen bonds and allow entry of bridging SO₄²⁻ and OH⁻ into the complex to replace water molecules and form jarosites.

In nature, iron and SO₄²⁻ occur together in acid solution wherever pyritic minerals are exposed to air and water. The reduced sulfides serve as reservoirs of chemical energy for the iron-oxidizing thiobacilli (2). As the iron sulfide minerals are leached by bacterial action, an aqueous environment results that contains, as major solutes, hydrated Fe(II) and Fe(III) with excess SO₄²⁻. The association of iron and sulfur is maintained in such environments by the activity of organisms such as T. ferrooxidans, which coprecipitate both elements by oxidizing Fe(II) in hydrated complexes stabilized by SO₄²⁻.

References and Notes

11. In most cases, iron precipitates were prepared for infrared analysis by sedimentation in centrifuge tubes, washed twice in H₂SO₄ (pH 2.5), twice with distilled water, and twice with acetone, and then dried in a forced-draft oven at 80°C. In order to check on the order of interaction, one bacterially oxidized sediment from D₂O was washed only in D₂O and was then dried with acetone. This did not alter the results; nor did the omission of washing in H₂SO₄ (pH 2.5) followed by washing in H₂O or washing only in D₂O followed by vacuum drying. All spectra were determined from samples incorporated in KBr disks under vacuum, by standard techniques, and then scanned with a Beckman Acculab 6 or a Perkin-Elmer 233-B infrared spectrophotometer.
Estradiol Fatty Acid Esters Occur Naturally in Human Blood

Abstract. Treatment of nonpolar ether extracts of human female blood with mild alkali produced more immunoassayable estradiol than the unhydrolyzed extract. Analysis of the serum extracts showed that the substance which released immunoreactive estradiol after hydrolysis has chromatographic properties identical to those of fatty acid esters of estradiol esterified at carbon 17. The physiological role of these previously unknown endogenous esters might be inferred from their structural similarity to synthetic drugs used therapeutically for their prolonged estrogenic action.

For over a half-century, alkyl and aryl esters of estradiol have been known as biologically potent steroids, generally considered to be "long-acting" estrogens (1–3). These esters, such as estradiol benzoate, propionate, cyclopentylpropionate, and valerate are synthetic drugs still used for prolonged estrogen stimulation (4–6). The most potent natural steroidal estrogen, 17β-estradiol (E2), exists not only as the "free" steroid, but in conjugated form. However, the conjugates are exclusively ionic, extremely water-soluble polar steroids linked to glucuronic acid or sulfate moieties (7, 8). To our knowledge, the natural occurrence of estrogenic steroids similar to the pharmacologically active, nonpolar alkyl esters of estradiol has not previously been shown. We now present evidence that alkyl esters of estradiol normally circulate in human blood and thus exist endogenously in nature.

An unusual nonpolar metabolite of estradiol was produced in our laboratory by in vitro incubations of several tissues (9). This compound, which could be converted back into estradiol by mild hydrolytic treatment, was named the lipidoid derivative of estradiol (LE2) to underscore its unique hydrophobic nature and uncertain structure. Subsequently, we isolated and identified LE2 as a family of fatty acid esters of estradiol esterified exclusively at carbon 17 (10). The experiments described here were designed to determine whether LE2 is present in human blood. Using immunoochemical and chromatographic techniques, we found that a nonpolar compound with properties identical to those of LE2, the carbon 17 esters of estradiol, exists in serum.

We reasoned that if LE2 is present in human blood, mild alkaline hydrolysis of an ether extract of serum should produce more estradiol than the untreated extract. Unlike LE2, other known conjugates of estradiol, such as sulfates and glucuronides, are neither extractable with ether (11) nor hydrolyzable with a weak base (12). Estradiol was measured by radioimmunoassay with an antiserum highly specific for estradiol (13). Esters representative of LE2, such as estradiol-17-stearate and estradiol-17-arachidonate, did not cross-react in this assay.

Serum samples (14) from males, cycling...
The Exclusion of D$_2$O from the Hydration Sphere of FeSO$_4$$
$$\cdot$$ 7H$_2$O Oxidized by *Thiobacillus ferrooxidans*

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