Inorganic Chemistry in Marine Sediments

Wolfgang Buckel*

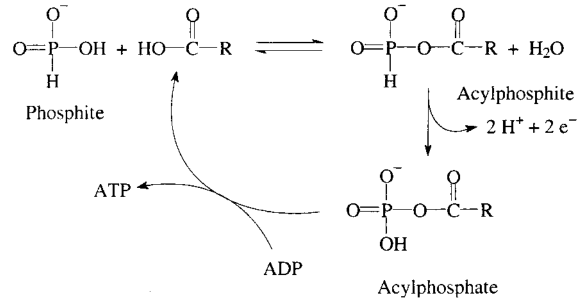
Several anaerobic microorganisms exploit inorganic energy sources and are collectively termed lithotrophs. The best known organisms of this type are acetogenic bacteria and methanogenic archaea, which are able to reduce CO_2 with molecular hydrogen to acetate or methane, respectively.[1] Instead of CO_2, marine organisms usually use sulfate (S^{VI}) as an electron acceptor, which is reduced by eight electrons to sulfide (S^{-II}). These sulfate reducers are able to grow at partial hydrogen pressures lower than that required by methanogens and acetogens. In sea water with 20 mM sulfate, the sulfate reducers are superior to the methanogens, which predominate in sulfate-deficient freshwater swamps and sediments. Sulfate reducers are metabolically much more versatile than methanogens. Besides hydrogen they are able to oxidize various organic acids such as lactate, fatty acids, dicarboxylic acids, and benzoate either to acetate or completely to CO_2.[2]

Recently, phosphite, a new inorganic electron donor was introduced into microbiology. Bernhard Schink, Universität Konstanz, Germany, and Michael Friedrich, Max-Planck-Institut für terrestrische Mikrobiologie in Marburg, Germany, isolated sulfate-reducing bacteria from sediments of the Canale Grande in Venice that are capable of oxidizing phosphite (P^{III}) to phosphate (P^{V}) [Eq. (1)].[3] The strictly

$$4\,HPO_3^{2-} + SO_4^{2-} + 2\,H^+ \longrightarrow 4\,HPO_4^{2-} + H_2S \qquad (1)$$

anaerobic organisms (strains FiPS3 and FiPS5), which perform this purely inorganic fermentation or sulfate respiration, grow with a doubling time of three days and require no organic compounds for the synthesis of cellular material. They are able to assimilate CO_2 and are therefore called lithoautotrophs. According to the sequence of their 16S ribosomal RNA, these organisms are classified as members of the δ-group of the Gram-negative proteobacteria; most sulfate reducers belong to this group. Only a few sulfate reducers are found among the archaea and the Gram-positive bacteria.

The smooth microbial oxidation of phosphite by the unusual oxidant sulfate is surprising because the nonenzymatic oxidation of phosphite to phosphate with various agents only occurs slowly and exhibits complex kinetics.[4] The considerably high free energy of phosphite oxidation of -364 kJ per mol sulfate is sufficient to yield 5 mol ATP from ADP and inorganic phosphate. Under the irreversible conditions of the living cell about $-70\,\mathrm{kJ\,mol^{-1}}$ of metabolic energy are generally required for the synthesis of 1 mol ATP.[5] Whereas sulfate reduction itself leads to a maximum of 1 mol ATP, it is doubtful, however, whether the extremely low redox potential of the HPO_4^{2-}/HPO_3^{2-} pair ($E_0' = -690$ mV) can be used for the formation of electrochemical proton gradients ($\Delta\mu(H^+)$) to generate additional ATP through the standard mechanisms. The oxidative formation of ATP seduces me to propose a novel type of stepwise substrate phosphorylation. Reaction of phosphite with a carboxylate of an enzyme could yield an acylphosphite, consecutive oxidation of which might afford an acylphosphate, whose energy-rich phosphate residue ($\Delta G^{\ominus\prime}_{\mathrm{hydrolysis}} \approx -50\,\mathrm{kJ\,mol^{-1}}$) is readily transferred to ADP thus generating ATP (Scheme 1).[5][**] The thermodynamically unfavorable formation of acylphosphite can be "pulled over" by the strongly exergonic oxidation with sulfate ($\Delta E_0' = 470$ mV or $\Delta G^{\ominus\prime} = -91$ kJ per mol phosphite). In glycolysis (Embden–Meyerhof pathway) the energy-poor thiosemiacetal, formed by addition of glyceraldehyde-3-phosphate to a specific cysteine residue of the enzyme glyceraldehyde-3-phosphate dehydrogenase, is oxidized to an energy-rich thiol ester in an analogous fashion. Phosphorolysis leads to 3-phosphoglceroyl-1-phosphate, whose energy rich 1-phos-



Scheme 1. Novel stepwise substrate phosphorylation by oxidation of acylphosphite to acylphosphate.

---

[*] Prof. Dr. W. Buckel
Laboratorium für Mikrobiologie, Fachbereich Biologie
Philipps-Universität
35032 Marburg (Germany)
Fax: (+49) 6421-2828979
E-mail: buckel@mailer.uni-marburg.de

[**] In biochemistry an energy-rich compound is defined as: $\Delta G^{\ominus\prime}_{\mathrm{hydrolysis}} \leq -30\,\mathrm{kJ\,mol^{-1}}$.

phate is transferred to ADP in a reaction catalyzed by 3-phosphoglycerate kinase. Whether the oxidation of phosphite is linked to the formation of ATP [Eq. (1)], could be investigated by measuring the cell yield (cell mass per mol sulfate reduced). Under autotrophic (CO_2-assimilating) growth conditions, 2–3 g dry cells are produced per mol of ATP. A preliminary estimation of the cell yield indeed suggests an involvement of phosphite oxidation in the synthesis of ATP (B. Schink, M. Friedrich, personal communication).

In nature, phosphorus almost exclusively occurs in the oxidation state +V, whereas all the oxidation states of nitrogen from +V to −III are known in biology. The discovery of the biological phosphite oxidation raises the question of whether there is a phosphorus cycle analogous to the nitrogen cycle. One can find almost nothing in the literature on the reduction of phosphate to phosphane (PH_3), which has been detected in trace amounts in anoxic sediments and even in human faeces.[6] St. Elmo's fire is caused by self-igniting diphosphane, though the origin of this phosphane remains enigmatic. The lower oxidation states of phosphorus formally occur in biological molecules with C–P and C–P–C bonds, which are synthesized from phosphoenolpyruvate. Phosphinotricin [2-amino-4-(hydroxymethylphosphinoyl)butyric acid], a herbicide that inhibits glutamine synthetase, contains a phosphorus atom, which is directly connected to two carbon atoms. Hydrolysis of phosphinotricin could yield phosphinic acid (phosphinate, +I), which at 140 °C under acidic conditions disproportionates to phosphonic acid (phosphite, +III) and phosphane (−III).[7] An organism containing enzymes, which catalyse this disproportionation at lower temperatures and at neutral pH, is certainly present in nature. If one put these reactions together, a biological phosphorus cycle is formed: Phosphoenolpyruvate (+V) → phosphinotricin (+I) → phosphinate (+I) → phosphane (−III) + phosphite (+III) → phosphate (+V). The phosphane is also oxidized by air to phosphate.

At the time of the origin of life, about 3.8 billion years ago, phosphites could have been more important than today. Phosphites could have originated from volcanoes or may have been introduced to earth by meteorites. At this time anaerobic inorganic fermenters, such as the phosphite-oxidizing sulfate reducers, could have formed the predominating class of organisms. Perhaps in an iron–sulfur world,[8] the first energy-rich acylphosphates for ATP synthesis were generated by the oxidation of acylphosphites.

In October 2000, only four months after the publication of the paper describing phosphite oxidation, the sulfate reducers again attracted attention. Antje Boetius et al.[9] from the Max-Planck-Institut für marine Mikrobiologie, Bremen, Germany, and the GEOMAR Forschungszentrum für marine Geowissenschaften, Kiel, Germany, reported very large sulfate reduction rates where methane hydrate enters upper anoxic levels of the sea floor, for example in the Pacific Ocean at depths of 600–800 m near the Oregon coast. Analysis of the microbial population with DNA-probes showed that methanogenic archaea were surrounded by sulfate reducers of the δ-subgroup of proteobacteria. Although biochemical data are lacking, there is evidence that the methanogens catalyze the oxidation of methane to H_2 and CO_2 or acetate by reversion of their "normal" methane-forming pathway. The thermodynamically very unfavorable anaerobic methane oxidation is only made possible by the presence of sulfate reducers, which keep the partial pressure of H_2 and, if applicable, the acetate concentration at extremely low values. Under standard conditions the oxidation of methane by sulfate yields only small amounts of free energy ($\Delta G^{\circ\prime} = -30\,\mathrm{kJ\,mol^{-1}}$, [Eq. (2)]), from which two organisms have to thrive. In reality the free energy ΔG certainly is higher, because dissolved methane hydrate leads to locally high methane concentrations, and the H_2S generated will be rapidly consumed by sulfate-oxidizing bacteria sitting on the oxic top of the sea floor. The importance of the work of Boetius et al. lies in that it provides the first concrete indications of the organisms, which participate in anaerobic methane oxidation.

$$CH_4 + SO_4^{2-} + 2\,H^+ \longrightarrow CO_2 + H_2S + 2\,H_2O \qquad (2)$$

---

[1] R. K. Thauer, *Microbiology* **1998**, *144*, 2377–2406.

[2] W. Buckel in *The Biology of Procaryotes* (Eds.: J. W. Lengeler, G. Drews, H. G. Schlegel), Thieme, Stuttgart, **1999**, pp. 278–326.

[3] B. Schink, M. Friedrich, *Nature* **2000**, *406*, 37.

[4] F. A. Cotton, G. Wilkinson, C. A. Murillo, M. Bochmann, *Advanced Inorganic Chemistry*, Wiley, New York, **1999**, p. 412.

[5] R. K. Thauer, K. Jungermann, K. Decker, *Bacteriol. Rev.* **1977**, *41*, 100–180.

[6] M. L. Ehrlich, *Geomicrobiology*, Marcel Dekker, New York, **1994**, pp. 241–261.

[7] *Römpp Chemie Lexikon*, *Vol. 4*, Thieme, Stuttgart, **1995**, p. 3381.

[8] G. Wächtershäuser, *Prog. Biophys. Mol. Biol.* **1992**, *58*, 85–201.

[9] A. Boetius, K. Ravenschlag, C. J. Schubert, D. Rickert, F. Widdel, A. Gieseke, R. Amann, B. B. Jørgensen, U. Witte, O. Pfannkuche, *Nature* **2000**, *407*, 623–626.

1433-7851/01/4008-1418 $ 17.50+.50/0