Methane Oxidation

DOI: 10.1002/anie.201002967

Functionalization of Methane in Anaerobic Microorganisms

*Rudolf K. Thauer**

metabolism · methane monooxygenase · methyl coenzyme M reductase · nickel · NO dismutation

Approximately 140 Gt (1 Gt = 10^{15} g) of biomass (70 Gt carbon) are formed globally each year from CO_2 via oxygenic photosynthesis (net primary production). Of this amount, 2–3% end up in anaerobic environments that not only lack O_2 but are also deficient in other electron acceptors with a high redox potential, such as nitrate, manganese(IV), iron(III), and sulfate. Such environments include freshwater sediments, swamps, paddy fields, landfills, the intestinal tract of ruminants and termites, and deeper layers of marine sediments. In these methanogenic environments, the biomass is fermented to methane, which involves anaerobic bacteria, protozoa and/or fungi, and methanogenic archaea, yielding approximately 1 Gt CH_4 per year. Recalcitrant biomass components, such as lignin, require up to thousands of years to be completely fermented. Methane is also formed from biomass thermogenically and from carbonate geochemically. Methane has built up to large deposits over millions of years; the quantities trapped in methane hydrates alone are estimated to be up to 10000 Gt (Figure 1).

It is estimated that approximately 1 Gt of methane per year diffuses into oxic environments and is oxidized there by aerobic bacteria to CO_2 (0.6 Gt per year) or escapes into the atmosphere (0.5–0.6 Gt per year), where most of the atmospheric methane is photooxidized to CO_2. Only 0.03 Gt of methane per year is removed from the atmosphere by aerobic bacteria that live in soil and water (Figure 1).[1]

Since the mid 1960s, evidence from biogeochemical studies has accumulated that methane is also oxidized to CO_2 in anoxic environments by microorganisms. This evidence was, however, received with scepticism by the scientific community, as it was generally believed that an activated oxygen species is necessary to functionalize methane in living cells. The reasoning was that the dissociation energy of the C–H bond in methane (439 kJ mol^{-1}) exceeds that of the X–H bond in other biomolecules, except for the dissociation energy of the O–H bond in H_2O (497 kJ mol^{-1}) and in other oxygen-derived species. It was only in the last 10 years that evidence was provided for the anaerobic oxidation of methane (AOM) by microorganisms. In the meantime, there is no longer any doubt that methane can be used by anaerobes to fuel their energy metabolism, by using sulfate, Mn^{IV}, Fe^{III}, or nitrite as the terminal electron acceptor (Figure 1). Estimates suggest that 0.3 Gt CH_4 per year are oxidized by anaerobes. AOM occurs where methane is the only available electron donor, and suitable electron acceptors such as nitrate, nitrite, Fe^{III}, Mn^{IV}, or sulfate are present. The most abundant anaerobic

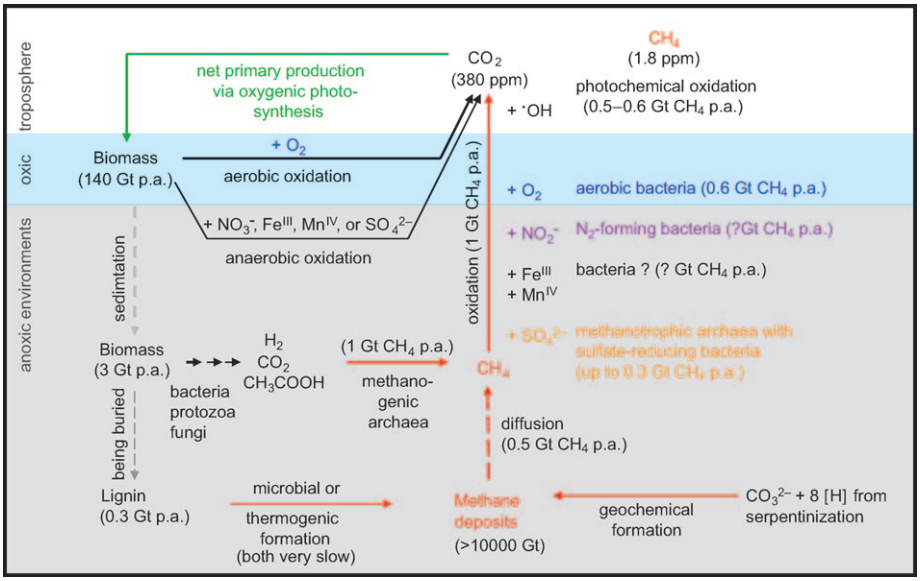

Figure 1. The global methane cycle,[9,10] highlighting the microorganisms that catalyze the anaerobic oxidation of methane (AOM) with nitrite (magenta) and sulfate (orange). p.a. = per year. For details, see text.

[*] Prof. Dr. R. K. Thauer
Max Planck Institute for Terrestrial Microbiology
Karl-von-Frisch-Strasse 10, 35043 Marburg (Germany)
E-mail: thauerr@mpi-marburg.mpg.de

process appears to be AOM with sulfate (up to 0.3 Gt per year). However, the biochemistry involved in AOM is still unclear.

Two recent papers in *Nature* indicate that there are at least two different mechanisms of AOM: one in methanotrophic N_2-forming bacteria that surprisingly involves a methane monooxygenase-related copper enzyme,[2] and the other in methanotrophic archaea that involves the key nickel enzyme of methanogenesis, namely methyl coenzyme M reductase.[3]

In 2006, the first report appeared on a mixed culture of bacteria and archaea that grow slowly on methane and nitrite under strictly anaerobic conditions, with CO_2 and N_2 as the end products formed ($3\,CH_4 + 8\,NO_2^- + 8\,H^+ \rightarrow 3\,CO_2 + 4\,N_2 + 10\,H_2O$; $\Delta G^{o\prime} = -928$ kJ (mol CH_4)$^{-1}$.[4] Upon continuous cultivation, the archaea disappeared, and the main remaining bacterial population represented a novel lineage in the bacterial kingdom.[5,6] The complete genome of the apparently anaerobic denitrifying bacterium was assembled and found to harbor all genes involved in the well-established aerobic pathway of methane oxidation ($CH_4 \rightarrow CH_3OH \rightarrow CH_2O \rightarrow \rightarrow \rightarrow HCOOH \rightarrow CO_2$), whereas it lacked important genes for nitrogen production from nitrite, namely those for nitrous oxide reductase, which catalyzes the reduction of N_2O to N_2.[2] Evidence was provided that the aerobic pathway of methane oxidation is expressed, and isotopic labeling studies revealed that the organism catalyzes the conversion of 2 NO to N_2 and O_2 ($\Delta G^o = -173$ kJ (mol N_2)$^{-1}$. The O_2 thus anaerobically generated is subsequently used to oxidize CH_4 to CH_3OH, as catalyzed by a membrane-associated particulate methane monooxygenase (pMMO), which is a copper enzyme. At least this is the way the authors interpreted their findings, which altogether look very convincing.

But there remain some open questions. The enzyme catalyzing the dismutation of 2 NO to N_2 and O_2 has not yet been identified, and it has not yet been completely ruled out that the pMMO in the methanotrophic anaerobe catalyzes the oxidation of methane to methanol with NO without free O_2 being an intermediate. Due to exchange activities between NO_2^- and H_2O and between NO and NO_2^-, it was not possible to show that the oxygen atom in CH_3OH is derived directly from O_2. Furthermore, it has also not been possible to grow the bacterium on methane and O_2, even at very low O_2 concentrations. Therefore, there remains a caveat. Nonetheless, the results are a major finding in the area of microbiology and biochemistry. Both an enzyme catalyzing the dismutation of 2 NO to N_2 and O_2 and a pMMO using NO instead of O_2 in the monooxygenation reaction would be something completely new and unexpected.

Those who were skeptical that methane can be anaerobically oxidized by microorganisms might now find themselves justified: molecular oxygen as oxidant in AOM after all? This may be true in the case of AOM with nitrite, but not in the case of AOM with sulfate ($CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$ $\Delta G^{o\prime} = -17$ kJ mol^{-1}), where for thermodynamic reasons, methanol as intermediate can be excluded.[7] All the available evidence indicates that AOM with sulfate proceeds in reverse of methanogenesis, with a methyl coenzyme M reductase homologue catalyzing the activation of methane.[7,8] It has now been shown[3] that the nickel enzyme purified from a methanogen can catalyze the oxidation of methane to methyl coenzyme M ($CH_4 + CoM{-}S{-}S{-}CoB \rightarrow CH_3{-}S{-}CoM + HS{-}CoB$; $\Delta G^{o\prime} = 30 \pm 10$ kJ mol^{-1}) with apparent K_m and V_{max} values that are consistent with the values estimated for cultures catalyzing AOM with sulfate. Again there remains a caveat: AOM with sulfate is catalyzed by mixed cultures of archaea related to methanogens and of sulfate-reducing bacteria, and it has not yet been possible to show that the archaea containing the methyl coenzyme M reductase can catalyze the oxidation of methane in the absence of the sulfate-reducing bacteria. There is therefore still room for surprises.

Received: May 17, 2010
Published online: July 29, 2010

[1] The concentration of methane in the troposphere has increased over the past 100 years from 0.9 to 1.8 ppm, which is of concern as methane is a potent greenhouse gas. Reasons for the increase are changes in agricultural use of the land (e.g. more ruminants, more rice fields), but also increased CH_4 emissions associated with the energy industry, such as gas-pipe leaks and biomass fires, which add up up to 0.2 Gt methane per year (these are included in the 0.5–0.6 Gt CH_4 that are oxidized annually in the troposphere photochemically; Figure 1).
[2] K. F. Ettwig, M. K. Butler, D. Le Paslier, E. Pelletier, S. Mangenot, M. M. M. Kuypers, F. Schreiber, B. E. Dutilh, J. Zedelius, D. de Beer, J. Gloerich, H. J. C. T. Wessels, T. vanAlen, F. Luesken, M. L. Wu, K. T. van de Pas-Schoonen, H. J. M. Op den Camp, E. M. Janssen-Megens, K. J. Francoijs, H. Stunnenberg, J. Weissenbach, M. S. M. Jetten, M. Strous, *Nature* **2010**, *464*, 543 – 548.
[3] S. Scheller, M. Goenrich, R. Boecher, R. K. Thauer, B. Jaun, *Nature* **2010**, *465*, 606 – 609.
[4] A. A. Raghoebarsing, A. Pol, K. T. van de Pas-Schoonen, A. J. P. Smolders, K. F. Ettwig, W. I. C. Rijpstra, S. Schouten, J. S. S. Damste, H. J. M. Op den Camp, M. S. M. Jetten, M. Strous, *Nature* **2006**, *440*, 918 – 921.
[5] K. F. Ettwig, S. Shima, K. T. van de Pas-Schoonen, J. Kahnt, M. H. Medema, H. J. M. Op den Camp, M. S. M. Jetten, M. Strous, *Environ. Microbiol.* **2008**, *10*, 3164 – 3173.
[6] K. F. Ettwig, T. vanAlen, K. T. van de Pas-Schoonen, M. S. M. Jetten, M. Strous, *Appl. Environ. Microbiol.* **2009**, *75*, 3656 – 3662.
[7] R. K. Thauer, S. Shima, *Ann. N. Y. Acad. Sci.* **2008**, *1125*,158 – 170.
[8] K. Knittel, A. Boetius, *Annu. Rev. Microbiol.* **2009**, *63*, 311 – 334.
[9] R. K. Thauer, A. K. Kaster, H. Seedorf, W. Buckel, R. Hedderich, *Nat. Rev. Microbiol.* **2008**, 6, 579 – 591.
[10] R. Conrad, *Environ. Microbiol.* **2009**, *1*, 285 – 292.

