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Minireview

New perspectives on anaerobic methane oxidation

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Summary

Anaerobic methane oxidation is a globally important but poorly understood process. Four lines of evidence have recently improved our understanding of this process. First, studies of recent marine sediments indicate that a consortium of methanogens and sulphate-reducing bacteria are responsible for anaerobic methane oxidation; a mechanism of 'reverse methanogenesis' was proposed, based on the principle of interspecies hydrogen transfer. Second, studies of known methanogens under low hydrogen and high methane conditions were unable to induce methane oxidation, indicating that 'reverse methanogenesis' is not a widespread process in methanogens. Third, lipid biomarker studies detected isotopically depleted archaeal and bacterial biomarkers from marine methane vents, and indicate that Archaea are the primary consumers of methane. Finally, phylogenetic studies indicate that only specific groups of Archaea and SRB are involved in methane oxidation. This review integrates results from these recent studies to constrain the responsible mechanisms.

Introduction

Methane oxidation in anoxic environments is microbially mediated and of global significance. Methane is an important trace gas in the atmosphere, and methane oxidation in anoxic waters and marine sediments serves as an important control on the flux of methane to the atmosphere. Methane produced in deep sediment diffuses upwards and is consumed by a population of prokaryotes through an undefined process in which sulphate acts as the terminal oxidant. This process is estimated to consume methane equivalent to 5–20% of the net modern atmospheric methane flux (20–100 $\times$ 10^{12} g $year^{-1}$). A variety of different environments exist in which sulphate-dependent methane oxidation (SDMO) is thought to occur, including recent marine sediments, methane seeps and vents, anoxic waters, soda lakes and deep continental margin sediments. Despite extensive study, the mechanism of SDMO is not understood, and no organisms have been isolated capable of explaining environmental observations.

A recent hypothesis postulates that methanogens (Archaea) operate in reverse to oxidize methane and produce hydrogen. Sulphate reducers then use the hydrogen generated from methane, thereby maintaining conditions that allow methane oxidation to proceed exergonically. Recent advances based on lipid biomarker isotope analysis and culture-independent identification also indicate that an association of Archaea and sulphate-reducing bacteria (SRB) is responsible for the observed oxidation of methane. The present review analyses new data that pertain to the mechanism and environmental importance of SDMO, and provides a new perspective on this enigmatic process. In particular, we review evidence contrary to the reverse methanogenesis hypothesis and explore alternative mechanisms to explain environmental and experimental observations.

Background

SDMO has been reviewed previously by Alperin and Reeburgh (1984), Hoehler *et al.* (1994) and Hoehler and Alperin (1996). Three lines of evidence have traditionally been used to support the existence of SDMO: (i) diagenetic (advection–diffusion–reaction) models of methane concentration profiles in anoxic sediments and water columns (Barnes and Goldberg, 1976; Reeburgh, 1976; Martens and Berner, 1977; Reeburgh and Heggie, 1977; Alperin and Reeburgh, 1984); (ii) tracer measurements using $[^{14}C]$-CH_4, $[^{3}H]$-CH_4 or $[^{35}S]$-SO_4^{2-} (Reeburgh, 1980; Devol and Ahmed, 1981; Iversen and Blackburn, 1981; Devol, 1983; Iversen and Jørgensen, 1985; Iversen *et al.*, 1987; Ward *et al.*, 1987; 1989; Alperin, 1989; Reeburgh *et al.*, 1991; Joye *et al.*, 1999); and (iii) stable isotope distributions (Oremland and DesMarais, 1983; Whiticar *et al.*, 1986; Oremland *et al.*, 1987; Alperin *et al.*, 1988; Blair and Aller, 1995; Martens *et al.*, 1999). Methane oxidation has been

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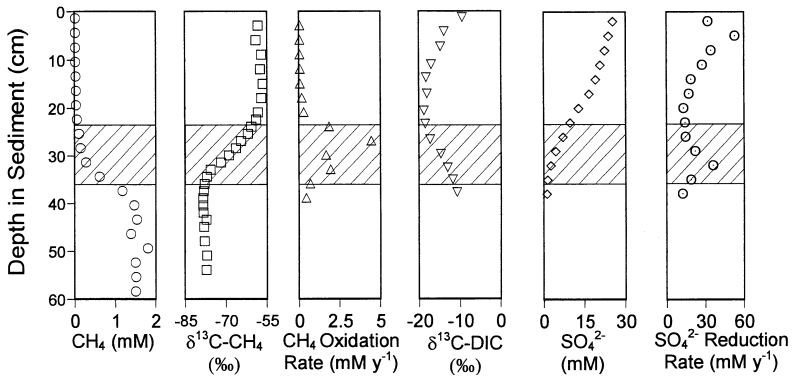

Fig. 1. Sediment depth profiles of CH_4 concentration (○, mM_{pw}), [$\delta^{13}C$]-CH_4 (□, ‰), CH_4 oxidation rate (Δ, mM_{pw} CH_4 $year^{-1}$), [$\delta^{13}C$]-DIC (dissolved inorganic carbon, ▽, ‰), SO_4^{2-} concentration (◇, mM_{pw}), SO_4^{2-} reduction rate (⊙, mM_{pw} SO_4^{2-} $year^{-1}$) in sediments of Skan Bay, Alaska. Values for CH_4 concentration analysis and [$\delta^{13}C$]-CH_4 analysis were taken by D. Valentine during an August 1997 cruise. Values for CH_4 oxidation rate, [$\delta^{13}C$]-DIC, SO_4^{2-} concentration, and SO_4^{2-} reduction rate were taken from Alperin, 1989). The depth of zero represents the sediment–water interface. The zone of anaerobic methane oxidation is highlighted by hatched rectangles and extends from approximately 25 to 35 cm depth.

studied extensively in the anoxic sediments of Skan Bay, Alaska (see Fig. 1).

Despite extensive study of SDMO, the organisms responsible have not been isolated, and the process remains a puzzle. Numerous bacterial isolates conserve energy from methane oxidation, but all use molecular oxygen to activate the very stable methane molecule (Hanson and Hanson, 1996). SDMO occurs in environments completely devoid of oxygen, and all evidence indicates that aerobic methanotrophs are not involved. The oxidation of methane by pure cultures of anaerobes has been reported in both SRB (Davis and Yarborough, 1966; Iversen, 1984) and methanogens (Zehnder and Brock, 1979; 1980; Harder, 1997). However, the observed methane oxidation occurred as only a small fraction of the total metabolism, and such mechanisms are unable to account for the net methane oxidation observed in the environment.

Reverse methanogenesis and H_2 syntrophy

Hoehler *et al*. (1994) used field and laboratory studies to suggest that SDMO is mediated by a consortium of methanogens and SRB through a process of 'reverse methanogenesis'. Hoehler *et al*. (1994) proposed that a methanogen–sulphate reducer consortium provided a biochemically feasible mechanism for net anaerobic methane oxidation and was consistent with seasonal variations observed in Cape Lookout Bight. The hypothesis was also attractive for several reasons: it was consistent with all previous field, tracer, stable isotope and modelling studies, with results from inhibition experiments, it required no new organism, and it was more credible energetically than previously proposed reaction schemes.

Hydrogen syntrophy is hypothesized to form the basis of the methanogen–sulphate reducer consortium. Hydrogen is known to be a competitive substrate in anaerobic environments. Its concentration represents a dynamic steady state and is indicative of the dominant terminal electron-accepting process (Lovley and Phillips, 1987; Lovley and Goodwin, 1988; Hoehler *et al*., 1998). The maintenance of low H_2 allows for syntrophic oxidation of organic material through the process of interspecies H_2 transfer (Wolin, 1982; Schink, 1997). Using results from field and laboratory studies, Hoehler *et al*. (1994) hypothesized that, under sufficiently low H_2, methanogens reverse their metabolism and mediate the net reversal of methanogenesis (acting as methane oxidizers; MO, eqn 1), using water as the terminal electron acceptor. The H_2 is removed efficiently and maintained at low concentrations by SRB (eqn 2) operating in a syntrophic association with the methanogens (Hoehler and Alperin, 1996). The sulphate reducers are more efficient at using H_2 as an electron donor; thus, they can create conditions that thermodynamically favour the oxidation of methane. The net reaction of the syntrophic association (eqn 3) yields approximately -25 kJ mol^{-1} methane oxidized. The available energy can be shared by the two partners in the association, and the H_2 level determines the partitioning of energy between the two organisms.

$$CH_4 + 2H_2O \rightarrow CO_2 + 4H_2 \quad \text{(MO)} \quad (1)$$

$$SO_4^{2-} + 4H_2 + H^+ \rightarrow HS^- + 4H_2O \quad \text{(SRB)} \quad (2)$$

$$SO_4^{2-} + CH_4 \rightarrow HCO_3^- + HS^- + H_2O \quad \text{(Net)} \quad (3)$$

Metabolic reversibility like that hypothesized in eqn 1 is known to occur among anaerobic microbes. Hydrogen acts as a thermodynamic trigger for metabolic reversal in the '*Reversibacter*', a homoacetogen capable of converting acetate to CO_2 and H_2 under low H_2 (Zinder and Koch, 1984; Lee and Zinder, 1988). Furthermore, some methanogens are capable of oxidizing methane to CO_2 during growth,

Table 1. Selected ^{13}C-depleted lipids from environments associated with anaerobic methane oxidation.

Lipid	Known source	Santa Barbara Basin[a] (‰)	Eel River Basin[b] (‰)	Marmorito Carbonate[c] (‰)	Napoli[d] (‰)	Hydrate Ridge[e] (‰)
Archaeol	Archaea	−119	−100	–	−76.2	–
sn-2-hydroxyarchaeol	Methanosarcinales	−128	−110	–	–	–
Hydroxyarchaeols	*Archaea*	–	–	–	−77[f]	–
Crocetane	Unknown (Archaea?)	−119	–	−108.3	–	−117.9
Phytanol	Archaea	–	–	−108.5	–	–
Biphytanediol	Archaea	–	–	–	−77	–
PMI	Archaea	−129	–	−105.5	−64.8	−123.8
PMI:4	Archaea	–	–	–	–	−107.3
Monoalkylglycerolethers	Deeply branched bacteria	−104[g]	–	–	–	–
n-hexadecan-1-ol	Bacteria (SRB)	–	–	−87.6[h]	–	–
Fatty acids (C_{14}-C_{18})	Bacteria (SRB)	−67[i]	–	–	–	–
Iso/anteiso fatty acids	Bacteria (SRB)	–	–	–	−68	–
Diplopterol	Bacteria	–	–	–	−60	–

–, indicates that values were either not reported or are not included here.
a. Seep environment (Hinrichs *et al.*, 2000).
b. Seep environment (Hinrichs *et al.*, 1999).
c. Ancient seep environment (Thiel *et al.*, 1999).
d. Cold seep environment (Pancost *et al.* 2000).
e. Core SO109-1 TVG 41-2 (Elvert *et al.*, 1999).
f. Both *sn*-2 and *sn*-3-hydroxyarchaeol were present.
g. Average $\delta^{13}C$ of all monoalkylglycerolethers found.
h. Additional lipids were found with similar structures and isotope values.
i. Average $\delta^{13}C$; several fatty acids were identified with isotope compositions as light as −114‰.

although the rate of methane oxidation is always a fraction of the production rate (Zehnder and Brock, 1979; 1980; Harder, 1997).

We designed experiments aimed at testing the 'reverse methanogenesis' hypothesis, which involved screening pure cultures of several methanogens for H_2 production under conditions of low H_2 and high CH_4. A H_2 removal technique was developed based on the principle of gas sparging (Valentine *et al.*, 2000), and *Methanosaeta concilii*, *Methanosaeta thermophila*, *Methanobacterium* strain *Marburg and Methanosarcina barkeri* were screened for H_2 production/CH_4 oxidation potential. Although most cultures produced H_2 initially, production was not sustained, and methane oxidation was never observed (Valentine, 2000). Similar studies have also been performed by other researchers using other methanogens with similar results (J. Harder, personal communication).

New evidence pertaining to anaerobic methane oxidation

Isotopically (^{13}C) depleted lipid biomarkers

Methane in marine environments is generally isotopically light, with values ranging from −50 to −90‰ (Whiticar, 1999) relative to the Pee Dee Belemnite standard. Processes that oxidize methane are expected to yield catabolic and anabolic products, which are also depleted in ^{13}C. Several recent studies have focused on determining the isotopic compositions and molecular structures of specific lipids associated with SDMO (Table 1). The extremely light carbon isotope signatures of the observed lipids serves as compelling evidence that methane-derived carbon is entering the anabolic pathways of the organisms that produce the lipids, and also indicates that these organisms are directly involved in SDMO. Many of the lipids are so isotopically light that a substantial isotope fractionation (≈60‰) must still occur even if methane is the initial carbon source.

The majority of ^{13}C-depleted lipids that have been observed are thought to be unique to Archaea, and are commonly used as biomarkers of archaeal metabolism (De Rosa *et al.*, 1986; Koga *et al.*, 1993; Table 1). The predominance of archaeal lipids indicates that Archaea are the primary consumers of methane. However, different environments seem to contain different specific lipids, indicating that several distinct populations of Archaea may be involved in SDMO (Hinrichs *et al.*, 1999; 2000; Thiel *et al.*, 1999; Pancost *et al.*, 2000). Many of the observed lipids are known to be produced by the *Methanosarcinales* (a genus of mainly methylotrophic methanogens), although some lipids are produced more broadly within the Archaea, and the producers of some lipids remain unknown. Some ^{13}C-depleted lipids generally associated with Bacteria have also been observed along with archaeal lipids (Thiel *et al.*, 1999; Hinrichs *et al.*, 2000; Pancost *et al.*, 2000). The putative bacterial lipids are especially prevalent in SRB (Kaneda, 1991) and have been found with isotopic signatures intermediate between the source methane and the archaeal lipids. Such lipids are probably produced by organisms that are indirectly involved in SDMO, and may possibly be anabolic products of syntrophic SRB.

Culture-independent evidence

Culture-independent identification techniques have recently been applied to the problem of anaerobic methane oxidation. Hinrichs *et al*. (1999) analysed small-subunit ribosomal RNA (16S rRNA) sequences from a methane seep in the Eel River Basin, a site at which SDMO is thought to occur. The results of this study found a mixture of Bacteria and Archaea. The Bacteria present were mainly related to known anaerobes including SRB. The Archaea consisted primarily of a novel group (ANME-1, probably representing a new archaeal genus) peripherally related to the *Methanosarcinales*, as well as novel species of *Methanosarcinales*. Isotopic analysis of lipid biomarkers in the same sediments (Table 1) provides circumstantial evidence linking the novel archaeal phylotypes to methane oxidation through both the lipid structures and the isotopic signatures.

More recent 16S rRNA studies of methane hydrates, borehole fluid and high-methane sediments have also found sequences from ANME-1, but *Methanosarcinales* are found to predominate over ANME-1 in each of the environments sampled (B. Lanoil *et al*., unpublished data). The *Methanosarcinales*-related clones found by B. Lanoil and colleagues are most closely related to the *Methanosaeta*, although some are more deeply branching within the *Methanosarcinales*. Additional evidence from the Black Sea also supports the possibility that *Methanosarcinales* are involved in anaerobic methane oxidation. Microscopic analysis of microbial communities associated with methane seep carbonates (in which SDMO is thought to occur) indicated that the dominant populations strongly resemble the *Methanosaeta* (Pimenov *et al*., 1997). Phylogenetic (16S rRNA) analysis of Black Sea anoxic waters also reveal the presence of *Methanosarcinales* (Vetriani and Kerkhof, 1999); methane oxidation has been observed in the anoxic waters of the Black Sea (Reeburgh *et al*., 1991).

Alternative mechanisms for anaerobic methane oxidation

Several recent studies raise questions about reverse methanogenesis (Hoehler *et al*., 1994) as the mechanism responsible for SDMO. First, repeated attempts to induce reverse methanogenesis using low H_2 and high CH_4 have proved unsuccessful (Valentine, 2000; J. Harder, personal communication). Secondly, the thermodynamic yield of reverse methanogenesis allows only -25 kJ mol^{-1} (CH_4) to be shared by an Archaea and a SRB (assuming typical sediment concentrations), a value below the commonly accepted biological energy quantum (≈ -20 kJ mol^{-1} per organism; Schink, 1997). Thirdly, specific ^{13}C-depleted bacterial lipids (probably from SRB) have been found in samples with a mixture of light archaeal lipids and heavier bacterial lipids (Thiel *et al*., 1999; Hinrichs *et al*., 2000; Pancost *et al*., 2000); without interspecies carbon transfer, it is difficult to explain why some bacterial lipids are found to be isotopically depleted, whereas others in the same sample are not. Finally, phylogenetic and microscopic evidence indicates that Archaea related to the *Methanosarcinales* (possibly closely related to the *Methanosaeta*) may be responsible for methane oxidation in some environments (Pimenov *et al*., 1997; Hinrichs *et al*., 1999; B. Lanoil *et al*. unpublished data); many *Methanosarcinales* are unable to use H_2 during methanogenesis, and tend to grow from methylated compounds and acetate instead.

We explored two alternative mechanisms for SDMO to account for the new findings. Mechanisms were sought that allow for greater thermodynamic yields for the organisms involved, involve interspecies carbon transfer, require no novel biochemical pathways and are consistent with all previous observations. One such mechanism that matches all these requirements involves the formation of acetic acid and H_2 from two molecules of methane (eqn 4) by methane-oxidizing Archaea (MO) with subsequent consumption of H_2 and acetic acid (eqns 5 and 6) by SRB:

$$2CH_4 + 2H_2O \rightarrow CH_3COOH + 4H_2 \quad \text{(MO)} \quad (4)$$

$$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O \quad \text{(SRB)} \quad (5)$$

$$CH_3COOH + SO_4^{2-;} \rightarrow 2HCO_3^- + HS^- + H^+ \quad \text{(SRB)} \quad (6)$$

$$2CH_4 + 2SO_4^{2-} \rightarrow 2HCO_3^- + 2HS^- + 2H_2O \quad \text{(Net)} \quad (7)$$

The net reaction (eqn 7) is simply twice the reaction normally associated with anaerobic methane oxidation (eqn 3). The coupling of two methane molecules to form acetic acid allows for energy conservation from the net process (eqn 7), which would normally be reduced (i.e. divided by two to equal eqn 3) for thermodynamic calculations. The net reaction (eqn 7) allows for twice as much free energy as the mechanism of reverse methanogenesis (eqn 3), although the energy must be split three ways instead of two.

A second alternative mechanism has been considered previously (Zehnder and Brock, 1980; Hoehler *et al*., 1994) and involves the formation of acetate from CO_2 and CH_4 (a reversal of aceticlastic methanogenesis) by a methane-oxidizing Archaea (eqn 8). Acetate would then be consumed by a SRB (eqn 9) acting in a syntrophic association with the methane oxidizer:

$$CH_4 + HCO_3^- \rightarrow CH_3COO^- + H_2O \quad \text{(MO)} \quad (8)$$

$$CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^- \quad \text{(SRB)} \quad (9)$$

$$CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O \quad \text{(Net)} \quad (3)$$

The net reaction (eqn 3) does not show the fixation of

Table 2. Thermodynamics of two proposed mechanisms for sulphate-dependent methane oxidation.

Reaction	ΔG^{o}	$\Delta G^{o/a}$ (pH, T)	$\Delta G'^{ab}$	Eqn
$CH_3COO^- + H^+ \rightarrow CH_3COOH$	At equilibrium	At equilibrium	0[c]	
Mechanism 1				
$2CH_4 + 2H_2O \rightarrow CH_3COOH + 4H_2$	+166.6	+169.4	−14.4[d]	4
$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O$	−152.2	−154.1	−17.8[d]	5
$CH_3COOH + SO_4^{2-} \rightarrow 2HCO_3^- + HS^- + H^+$	−47.6	−43.3	−18.5	6
$2CH_4 + 2SO_4^{2-} \rightarrow 2HS^- + 2HCO_3^- + 2H_2O$	−33.2	−28.0	−50.7	7
Mechanism 2				
$CH_4 + HCO_3^- \rightarrow CH_3COO^- + H_2O$	+31.0	+28.5	+5.3	8
$CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^-$	−47.6	−42.4	−30.6	9
$CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$	−16.6	−14.0	−25.4	10

a. Calculations corrected for typical summer conditions in sediments of Skan Bay, AK (T = 4°C, pH = 7.2).
b. Calculations used chemical concentrations of: CH_4 (2.2×10^5 Pa), SO_4^{2-} (2 mM), HS^- (1 mM), acetate (3 μM), acetic acid (11 nM), HCO_3^- (32 mM), H_2 (3.16×10^{-2} Pa) (Shaw *et al.*, 1984; Alperin, 1989).
c. H_2 levels have not been accurately quantified in Skan Bay sediments; the value of 3.16×10^{-2} Pa was chosen as a reasonable estimate based on the work of Hoehler *et al.*, 1998).
d. Equilibrium is assumed for the acid–base reaction involving acetate ($pK_a = 4.75$), allowing for calculations of acetic acid concentration.Standard thermodynamic values and equations were used for all calculations (Thauer *et al.*, 1977; Atkins, 1994). All free energy yields are given in kJ reaction^{-1}.

bicarbonate to form acetate, as that carbon is later reoxidized by the SRB.

To determine the feasibility of the proposed mechanisms, we calculated the free energy change of each reaction for the conditions present within the methane oxidation zone in sediments of Skan Bay, Alaska (Table 2). We feel that the mechanism involving the formation of acetic acid from two methane molecules (eqns 4–7) is advantageous over the other hypotheses, as it allows for more energy for each organism involved, and better explains why some acetate-using SRB may contain isotopically depleted lipids. Lipids formed by Archaea producing acetate from methane and bicarbonate (eqn 8) are likely to have isotopic values intermediate between methane and ambient bicarbonate. Also, the coupling of two methane molecules to form acetate is biochemically feasible (Valentine, 2000), requires no new organisms and is consistent with previous field and laboratory studies.

The thermodynamics of acetate production from methane and bicarbonate (eqn 8) are poor under the conditions assumed in Table 2, but would be more favourable under conditions of high methane (i.e. deep methane vents), in microenvironments surrounded by SRB, or if acetic acid was the molecule transferred between species (as in eqns 4 and 6). The production of acetic acid (not acetate) from methane and carbon dioxide under 50 atm of methane (otherwise assuming the conditions of Skan Bay sediments) would yield −12.9 kJ mol^{-1}. Such a thermodynamic yield is still below the biological energy quantum of ≈ −20 kJ mol^{-1} and is well below the yield of −28.7 kJ mol^{-1} for the production of acetic acid and H_2 from two methane molecules (eqn 4) under identical conditions.

The presumed transfer of acetic acid instead of acetate (eqns 4–7) represents a 'whole cell' perspective on the bioenergetics of metabolism. Given the low energy yield of SDMO, it is unlikely that metabolic energy is used actively to import or export acetate. Instead, it is anticipated that acetic acid will pass through the membrane in the undissociated form (Thauer *et al.*, 1977). The transfer of acetic acid (not acetate) does not alter the net thermodynamics of methane oxidation, but it does alter the partitioning of energy between the various organisms involved as a result of concentration differences between acetate and acetic acid. If acetate is the species transferred between organisms, the thermodynamic yield of the methane-oxidizing Archaea (according to eqn 8) would be lower, and the yield for acetate-consuming SRB would increase correspondingly. However, interspecies acetate transfer could occur in structured microenvironments in which SRB maintain acetate concentrations below the level in the surrounding pore water; in such a case, the energetics of acetate transfer might be similar to those of acetic acid transfer.

The hypotheses presented here can be tested experimentally, both in the laboratory and in the field. Laboratory experiments involving mixed cultures of H_2 and acetate-using SRB can be used to screen known methanogens by feeding the cultures sulphate and methane. Direct methane consumption could be observed in such cultures. Various isotopic techniques can also be used to test this hypothesis in environmental samples. Given the light isotopic signature of methane in marine systems, one could analyse the isotopic signature of porewater acetate in a well-defined methane-oxidizing sediment. Abnormally isotopically light acetate would be an indication of the validity of this mechanism. However, if isotopically heavy sources of acetate contribute to the acetate pool, a light signal could be diluted. This may be the case when methane oxidation only accounts for a fraction of sulphate reduction. Methane vent sites may provide a good location to test this hypothesis, as methane oxidation seems to be the dominant form of

metabolism, and the isotopic signature of acetate is more likely to reflect the methane source. Isotope tracer incubation experiments could also be performed to track methane through dissolved acetate (or other intermediates). Any isotope of carbon or hydrogen could be used to perform such experiments by incubating sediments with labelled methane, separating or isolating acetate from the porewater and analysing the isotopes of acetate.

Problems and prospects

Thermodynamic insights

One factor frequently overlooked in discussions of SDMO is how the thermodynamics and kinetics of the process vary between the different sorts of environments in which it occurs. Differences in methane and sulphate concentrations tend to drive such variations. Vent sites have methane partial pressures proportional to depth, often greater than 50 atm, whereas methane in recent sediments, such as those of Skan Bay, Alaska, generally ranges from 0.2 to 2.0 atm. Methane partial pressure in anoxic waters, such as those of the Black Sea, are generally < 0.01 atm. Given typical conditions, free energy yields for methane oxidation (according to eqn 3) in these environments range from -35 kJ (vents) to -25 kJ (sediments) to -22 kJ (anoxic waters). Variations in the partial pressure of methane will also significantly influence the kinetics of methane uptake. At low methane levels, it is difficult to bind and activate the stable methane molecule, whereas at high levels, a methane-binding enzyme may not require a high affinity for substrate. Along with other interenvironmental differences, including pH, temperature, salinity and pressure, differences in thermodynamics and kinetics may select for different methane-oxidizing communities at different sites. The variety of different lipid biomarkers found at different sites (Table 1) seems to be consistent with such an assessment.

SDMO is coupled to energy conservation

Numerous attempts to enrich for methane-oxidizing anaerobes have failed. Coupled with the poor thermodynamic yield of SDMO, this has led to the idea that methane oxidation is a co-metabolic activity, and that the responsible organism(s) do not conserve energy from the process (Schink, 1997) (i.e. organisms cannot be enriched based on co-metabolic activity). Evidence from natural settings seems to indicate that this is not the case. The strongest evidence comes from comparing rates of methane oxidation with rates of sulphate reduction for a given setting. In several marine sediments containing little organic material, the depth-integrated rates of methane oxidation are equal to the depth-integrated rates of sulphate reduction, indicating that all sulphate reduction is coupled to methane oxidation (Iversen and Jørgensen, 1985; Niewohner *et al*., 1998; Borowski *et al*., 1999). In such environments, there is no opportunity for co-metabolism, as there is no available organic material, and sulphate (the sole terminal electron acceptor available in the sediments) reduction is quantitatively linked to methane oxidation. Further, the high abundance of specific ^{13}C-depleted lipids from different methane-oxidizing environments indicates that methane is the sole carbon source during growth of these populations, with no other carbon entering the lipid-synthesizing anabolic pathways. Methane oxidation rate measurements rely on methane conversion to CO_2, further indicating that methane oxidation is primarily a dissimilatory process. In addition, there may be more energy available for methane oxidation than previously realized, as discussed above. Taken together, the rate, isotope and thermodynamic evidence strongly indicate that methane oxidation is an energy-conserving metabolic process for some Archaea.

Assuming that organisms can grow from SDMO, why have all attempts at enrichment failed? There are a number of possible explanations for this, but one of the most likely is that the organisms involved have exceptionally slow growth rates because of their marginal thermodynamic yields. The probable syntrophic association will further decrease the growth rate. If the same mechanism is active in recent sediments and in seeps, then growth may be expected to occur much faster under the methane levels found at the seeps because of the more favourable thermodynamics. Enrichment attempts using proper inoculum and high pressures of methane (50+ atm) have a greater potential for success than standard roll-tube techniques.

Future studies

The mechanism behind anaerobic methane oxidation will remain a mystery until either representative organisms are cultured or known organisms are shown to perform methane oxidation in a manner consistent with field observations. Environmental studies have the potential to yield further insight into the mechanism and prevalence of the process. Highly integrated studies that include concentration, rate, phylogenetic, biomarker, microscopy and isotope analyses can simultaneously demonstrate metabolic activity, population abundance, bioenergetics and pathways of carbon flow for a given environment. These studies can be used to test the various hypotheses for methane oxidation, and the use of exogenous isotope tracers can be used to follow the immediate fate of methane in samples (i.e. to lipids, acetate or other intermediates).

Expanding the importance of SDMO

In addition to the importance of SDMO in present-day methane cycling, the process may have played an

important role in the early biogeochemical evolution of the earth. During the early Precambrian era, the sun was much fainter than today, yet the climate was warm. To explain this 'faint young sun paradox', it has been hypothesized that an abundance of greenhouse gases was present in the atmosphere of early earth (Kasting, 1997). Because CO_2 levels have been constrained by geological evidence (Rye *et al*., 1995), methane has been postulated to be the key component. If abundant methane and sulphate were present on the early earth, then SDMO may have played a key role in modulating the climate of early earth and may have been the primary sink for atmospheric methane. Evidence pertaining to such a hypothesis may be located in the geological record (in isotopes and biomarkers), in the biochemistry/genetics of the process (through the relationships of undiscovered enzymes/genes) and can be further understood through bioenergetic and kinetic studies of methane consumption in extant ecosystems.

Concluding remarks

SDMO is a process that occurs widely in anoxic marine systems and acts as a barrier for methane release to the water column and the atmosphere. Recent evidence indicates that the mechanism of methane oxidation involves methane-oxidizing Archaea and SRB, acting in a syntrophic association. Hydrogen appears to be a key intermediate in this syntrophic association, and we hypothesize that acetate is also involved. Although methane oxidation occurs in a variety of different settings and appears to be performed by several different organisms, there are indications that similar mechanisms may be active in all such settings. SDMO appears to be a fundamental metabolic process in some Archaea and is probably coupled to energy conservation and growth. Future field and laboratory studies will undoubtedly yield further insights into this important and enigmatic process.

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Note added in proof

A recent study of hydrate-containing marine sediments conducted by A. Boetius and colleagues (in press) describes an Archaea-SRB consortium apparently responsible for SDMO in this environment. Using 16S rRNA-targeted oligonucleotide probes and fluorescence *in situ* hybridization, the authors are able to visualize clusters of archaeal cells surrounded by layers of SRB. The specificity of the probes also indicates that the consortium consists of specific Archaea closely related to the *Methanosarcinales* and specific SRB closely related to *Desulfosarcina/Desulfococcoccus*. By demonstrating the close physical association of the consortium and the specific species involved, this study has added further insight to our understanding of SDMO.

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