Carbon and sulfur back flux during anaerobic microbial oxidation of methane and coupled sulfate reduction

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AUTHOR SUMMARY

Microbial redox processes such as the degradation of organic carbon to carbon dioxide in natural habitats are commonly believed to occur in a single direction. However, in the area of enzyme kinetics, bidirectionality is a long-established feature, with the reverse reaction being the more significant the closer the system operates to thermodynamic equilibrium (i.e., the lower the energy yield). Hence, the energy metabolism (catabolism) in organisms, viewed as a multiplicity of enzymatic systems, should, in principle, exhibit some reversibility if the overall energy yield is low—a situation common among strictly anaerobic microorganisms. Such reversibility should be expressed as a back flux from product to substrate. We tested this possibility using highly enriched consortia of marine archaea and bacteria that catalyze the anaerobic oxidation of methane (AOM) with sulfate. The energy gain from AOM (approximately -20 kJ mol^{-1}) is one of the lowest among metabolic processes fueling life. Expectedly, product radiolabeling with ^{14}C-bicarbonate and ^{35}S-sulfide showed the presence of back fluxes to the methane and sulfate pools during net AOM. Refined data evaluation revealed back fluxes up to 5% and 13%, respectively, of the net AOM rate. The existence of such back fluxes through the entire catabolism is a commonly overlooked kinetic and energetic aspect of microbial ecophysiology. It also has implications on the interpretation of isotope labeling experiments in the determination of in situ degradation rates and understanding of natural isotope patterns in anoxic habitats.

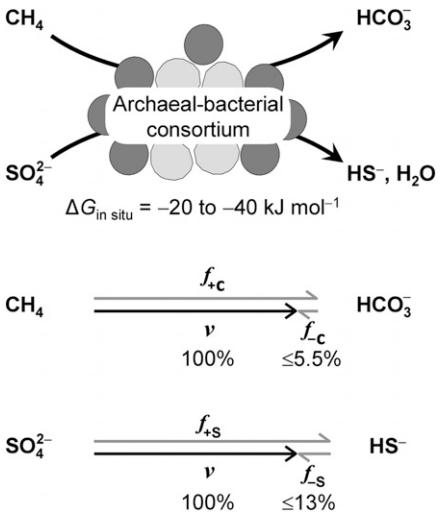

Fig. P1. Stoichiometry and fluxes during the AOM with sulfate by marine microbial consortia. The mode of coupling between methane oxidation and sulfate reduction is insufficiently understood. AOM has one of the lowest energy yields among reactions that fuel microbial life in natural habitats. The enzymatic reactions in AOM must, therefore, operate not far from equilibrium, favoring product back flux. This finding is illustrated (*Lower*) by means of reaction vectors (→, forward flux; ←, back flux; →, sum or net reaction; lengths indicate rates, the figure is not drawn with proportionality, and exact values are shown). The net rates (v; same for carbon and sulfur because of stoichiometry) are composed of forward and back fluxes (respective symbols: f_{+C}, f_{-C} for carbon and f_{+S}, f_{-S} for sulfur). In contrast to the net rates of methane oxidation and sulfate reduction, the respective forward and back fluxes can be independent of each other.

Although a catabolism with low-energy gain (a weakly exergonic catabolism) is common in strictly anaerobic microorganisms (1, 2), the possibility of product back fluxes existing in anaerobes has been treated in very few studies. Known examples are the conversion of added ^{14}C-methane to ^{14}C-carbon dioxide during net methane formation by various methanogenic archaea (3) and the conversion of added ^{35}S-sulfide to sulfate during net sulfate reduction with lactate by *Desulfovibrio* (4), a genus of sulfate-reducing bacteria. In these cases, the back flux of one element (C or S, respectively) through the corresponding pathway in the respective organism was shown.

Here, we present a refined quantitative study of the back fluxes of the products derived from both the electron donor and the electron acceptor. As an ecologically relevant example, we chose the AOM with sulfate, which is catalyzed by evolutionarily unrelated microorganisms of the archaea and regular bacteria (5) that seem to cooperate in microaggregates (consortia) with an as yet unknown mode of coupling (Fig. P1). AOM with sulfate is one of the least exergonic catabolic reactions sustaining life, the standard free energy change ΔG° being only -16.6 kJ mol^{-1}; free energy changes under in situ conditions can be only slightly more favorable (Fig. P1). Moreover, the low-energy yield is apparently shared between the two members of the consortia.

To test and quantify back fluxes of both carbon and sulfur during AOM, we used marine AOM consortia that were essentially free of nonliving particulate organic matter and had been highly enriched in vitro from anoxic sediment of two marine methane seep areas: Hydrate Ridge (HR; Cascadia Margin, Oregon, Northeast Pacific) and Isis Mud Volcano sediment (MV; Eastern Mediterranean Sea). The 1:1 stoichiometric conversion of methane and sulfate, according to Fig. P1, was confirmed by quantification of methane consumption and sulfide production as well as substrate-labeling experiments with $^{14}CH_4$ and $^{35}SO_4^{2-}$. In the absence of methane, methanogenesis and sulfate reduction because of use of endogenous compounds or dead cell carbon were not detectable. Hence, these cultures were ideally suited to study the flux of labeled inorganic carbon and sulfide into the pools of methane and sulfate, respectively.

To determine the back flux of sulfide to sulfate and bicarbonate to methane, we added ^{35}S-H_2S or ^{14}C-$NaHCO_3$ and

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measured the accumulated radioactivity of $^{35}SO_4^{2-}$ and $^{14}CH_4$, respectively. A refined formula for data evaluation yielded sulfur back fluxes (Fig. P1) of 7% (HR) and 13% (MV) of the net AOM rate. The determined carbon back fluxes were 3.2% (HR) and 5.5% (MV) of the net AOM rate (Fig. P1). The carbon back flux continued when AOM was prevented by omitting sulfate. In contrast, when AOM was prevented by omission of methane, the back flux from ^{35}S-H_2S to the sulfate pool was negligible.

Because individual reactions within the catabolism are less exergonic than the overall catabolic process, the particular reactions constituting the overall catabolism of the consortia must be even more reversible than determined in this study.

The existence of the reverse reaction of all products through the entire catabolism has consequences for the study of not only AOM but also other biogeochemical processes with low-energy yields in natural habitats. A low-energy habitat of present interest is the deep biosphere where energy-rich substrates have been depleted.

Microbially catalyzed back fluxes also have practical implications in the biogeochemical evaluation of habitat data. Microbial catabolic activity in natural habitats is usually measured by examining the flux of an isotope label added from the substrate to the product pool rather than by determining the net rate of product accumulation by chemical quantification. However, if back flux occurs, the label flux from the substrate is not identical to the microbial net rate. Accurate net rate determination by isotope labeling would have to include both forward and back flux measurement to yield the net rate. Moreover, detection of a reaction in situ by labeling does not necessarily indicate a net reaction occurring in the direction of label conversion; it may represent the back flux during the opposite reaction. Finally, the existence of reverse reactions may also be important for our understanding of stable isotope fractionation and the underlying mechanisms. Reversibility through an entire biochemical pathway (e.g., sulfate ⇌ sulfide) with particular consideration of low-energy conditions is usually not taken into account in the study of stable isotope patterns.

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