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Methane—make it or break it:

Biochemical data resolve the controversy over how methanogenic archaea produce methane

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About 500 to 600 million metric tons of methane, a potent greenhouse gas, are emitted annually world-wide; ~69% of this methane is produced biologically by anaerobic archaea known as methanogens (1). In some environments, methane emissions are partly offset by anaerobic methane oxidizing archaea (ANME) and aerobic methanotrophic bacteria (1). In methanogens, the methyl-coenzyme M reductase (MCR) enzyme uses a nickel-containing cofactor (F_{430}) to catalyze the final step of methane synthesis (see the figure, panels A and B) (2, 3). The MCR reaction is reversible, and MCR may also catalyze the first step of methane oxidation by ANME (2). The MCR catalytic cycle begins with F_{430} in the reduced Ni(I) form (4), but what happens next has been unclear. On page 953 of this issue, Wongnate *et al.* (5) report evidence for a Ni(II)-thiolate intermediate.

MCR is notoriously difficult to study because the active Ni(I) state—termed MCR_{red1} —is extremely sensitive to oxygen (4). Various states of MCR with and without substrates and in different oxidation states have been characterized, but it is unclear which are relevant to catalysis (3, 6–8). Two possible mechanisms have been investigated (2, 3). The distinguishing feature of the two mechanisms is the nature of the first intermediate. In mechanism I, an organometallic methyl-Ni(III) intermediate is formed (MCR_{Me}); in mechanism II, a Ni(II)-thiolate ($MCR_{ox1-silent}$) and a methyl radical are produced (see the figure, panel C).

The two Ni species should be distinguishable via spectroscopic methods. In particular, MCR_{Me} contains an unpaired electron, which will give rise to an electron paramagnetic resonance (EPR) signal, whereas $MCR_{ox1-silent}$ is EPR-silent. The problem lies in observing such signals (or the lack thereof) before this first intermediate reacts further. Wongnate *et al.* overcome this obstacle by using a coenzyme B (CoBSH) (see the figure) analog that is one carbon atom shorter (CoB_6SH). This modification slows the reaction rate sufficiently to observe the first intermediate (9).

The authors first used stopped-flow and rapid chemical-quench methods to compare the rates of MCR_{red1} disappearance and of methane synthesis. They found that the two processes occur at the same rate. This observation is inconsistent with mechanism I, which involves formation of the comparatively stable methyl-Ni(III) intermediate. Instead, the kinetics support mechanism II, in which the methyl radical would react rapidly to form methane.

With these data in hand, Wongnate *et al.* took advantage of the slower reactivity of CoB₆SH to trap and characterize the elusive intermediate. They generated rapid freeze-quench samples at various time points during the first minute of the reaction and collected EPR spectra. The result is clear: The intensity of the EPR signal from MCR_{red1} decreases by 90% at a rate similar to that of methane synthesis, without the appearance of a new signal attributable to Ni(III). Thus, the intermediate is EPR-silent, consistent with a Ni(II)-thiolate (mechanism II) and not a methyl-Ni(III) (mechanism I) or any other Ni(III) species.

To confirm the intermediate's identity, the authors analyzed the same samples with another spectroscopic technique, magnetic circular dichroism (MCD). The MCD spectra of the starting MCR_{red1} and many other forms of MCR are distinct (8, 10). The spectrum of the intermediate closely resembles that of the MCR_{ox1-silent} state, in which Ni is coordinated by the thiolate group of coenzyme-M (SCoM) (see the figure, panel C) (7, 10). This direct evidence for a Ni(II)-thiolate species is further bolstered by combined computational and experimental thermodynamic analyses that strongly favor mechanism II.

Identification of the intermediate ends more than two decades of controversy and sets the stage for building a consensus MCR mechanism. Several key questions remain unanswered, however. For example, can radical intermediates in the early stage of the reaction be observed? The EPR data show that small amounts of a radical do form as MCR_{red1} disappears, but it is unclear how this species compares with previously reported radicals (11). Other previous observations must also be reconciled with the new results (11, 12). Looking beyond the first step of the reaction, the changes that bring SCoM bound to F₄₃₀ close enough to CoBSH (see the figure, panel B) to react remain unclear. Finally, does anaerobic methane oxidation proceed via the reverse mechanism, and, if so, what factors influence the direction of the reaction?

The answers to these questions will affect efforts to design biomimetic catalysts, engineered enzymes for methane oxidation, and commercially viable synthetic biological pathways (13). We are one step closer to knowing how to make methane; will we also be able to break it?

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Figure. How methanogens make methane

MCR is the key enzyme in methane synthesis by methanogens. **(A)** The crystal structure of MCR from *Methanothermobacter marburgensis* PDB code 1MRO) is in the $\text{MCR}_{\text{ox1-silent}}$ state, where the Ni atom in cofactor F_{430} binds to the thiolate group of SCoM. **(B)** In the reaction catalyzed by MCR, methyl-SCoM reacts with CoBSH to form and methane and the CoBS-SCoM heterodisulfide. MCR is believed to also catalyze the reverse reaction in ANME. **(C)** The exact mechanism by which the MCR reaction proceeds has been unclear. Two main mechanisms have been proposed. Mechanism I proceeds via a methyl-Ni(III)

intermediate, whereas mechanism II involves a Ni(II)-thiolate intermediate. Wongnate *et al.* now provide convincing evidence for mechanism II.

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