Deciphering the Mechanism of Enzymatic Methane Synthesis

Methane is the simplest organic compound with the highest energy content of any carbon-based fuel. Methane accounts for almost a quarter of U.S. energy consumption, with one-half of homes using natural gas as their heating fuel. Interestingly, more than 90% of all methane on earth is produced biogenically by methanogens which are responsible for enzymatic synthesis of 1 billion tons of methane per year. Thus, understanding the biosynthesis of methane is imperative from basic energy, economic and environmental perspectives. Methane is formed by one of the few Ni containing cofactors (F_{430}) as part of the active site of methyl coenzyme M reductase (MCR), a strictly anaerobic enzyme present in methanogenic archaea. MCR catalyzes the reaction of methyl-coenzyme M (CH_3−SCoM) with coenzyme B (HSCoB) to form methane and the heterodisulfide CoMS−SCoB.

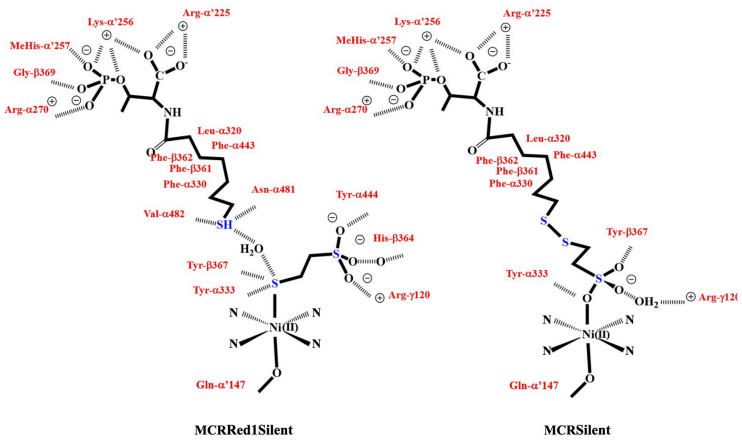

Fig 1. (left) Schematic representation of HSCoB and HSCoM bound in MCRred1silent and (right) CoMS−SCoB bound to MCRsilent.

In this collaborative study, the authors have uncovered a unique six-coordinate Ni(I) complex that is formed in the reverse reaction. A six-coordinate Ni(I) compound is unprecedented for biological systems and this study expands our understanding of the electronic structures that can be assumed by Ni centers during catalysis. Additionally, the unique binding of substrate to Ni supports a previously unsuspected long-range electron transfer mechanism during methanogenesis by MCR (see details below).

Detailed Description

The F_{430} is cofactor is buried at the end of a 30 Å long substrate channel and is anchored via a combination of electrostatic and H-bonding interactions between the carboxylate side-chains and the protein backbone. Both substrates enter the substrate channel in a strict order with CH_3−SCoM first followed by HSCoB resulting in a productive complex. Homolytic cleavage of the C-S bond in CH_3-SCoM, is then initiated by the reduced Ni(I) form to generate a CH_3• (methyl radical) that then abstracts the H from HSCoB forming methane. This is followed by S−S bond fusion and regeneration of the Ni(I) active site. Crystal structures on the oxidized Ni(II) form (MCRred1silent) have shown that substrate analogues HSCoM with HSCoB bind in the substrate alcove with the S of HSCoM forming a S-Ni bond to F_{430}. This methyl radical will go on to generate a CoBS• radical on H abstraction. However, such binding, if

catalytically relevant, leaves more than a 6 Å distance between the CoBS• and the bound S-atom of HSCoM posing a structurally "unreachable" conundrum for facile recombination to form CoMS-SCoB . Interestingly, the CoMS−SCoB binds to the Ni(II) center with its sulfotate oxygen (Figure 1) in the MCRsilent form. Since the published MCR mechanisms extrapolate structural details from the oxidized Ni(II) forms to the active Ni(I) form, such extrapolations are called to question in the face of the distance conundrum.

In a recent study the Ragsdale group at the University of Michigan in collaboration with Dr. Ritimukta Sarangi at SSRL have combined near-infrared (NIR), XAS, and EPR results to characterize the active Ni(I) state of MCR. The experimental data were combined with QM-MM and TD-DFT studies (Simone Raugui, PNNL) on the chemical models to successfully describe the binding details. Ni K-edge XAS and EXAFS measurements were performed on BL 7-3 on the active Ni(I) state bound to CoMS−SCoB and CoMS−$SCoB_6$, which is formed by the reaction of CH_3−SCoM and the slower substrate $HSCoB_6$ to reveal that CH_3−SCoM binds to the active Ni(I) state of MCR through its sulfonate ligand, in the process forming a hexacoordinate Ni(I) complex (Figure 2).

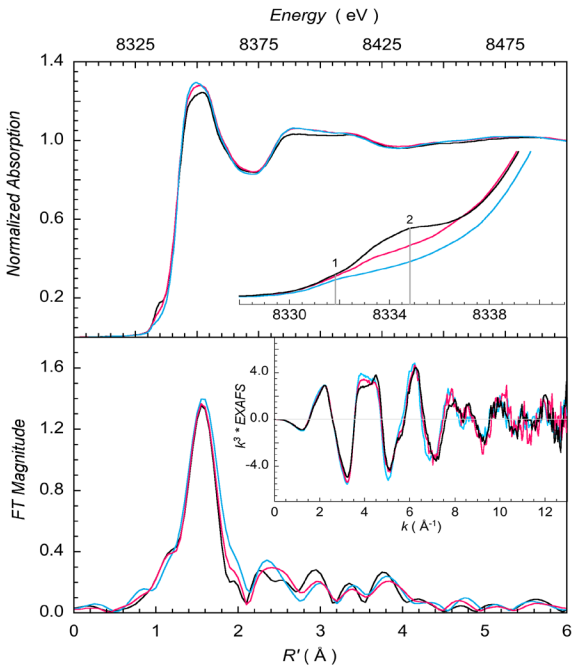

Fig 2. (top) Ni K-edge XAS data for the active Ni(I) form bound to MCRred1 showing pre-edge changes upon CoMS−SCoB binding to the Ni(I) center. (bottom) A comparison of EXAFS data reveals a unique Ni(I) hexacoordiate active site.

This is remarkable, since Ni(I) complexes are usually four or five-coordinate and a six-coordinate Ni(I) is unprecedented in metalloenzyme active sites. The proposed binding mode addresses and allows the authors to provide a possible resolution to the difficult recombination of the CoBS radical with the Ni−SCoM species over a distance of 6.4 Å to generate the heterodisulfide product. The binding of CoMS−SCoB through the sulfonate also provides a viable mechanism of C−H bond activation going in the reverse direction in the situation where the disulfide bond is unable to get closer to the Ni(I) due to the anchoring of the substrate at the top of the channel by electrostatic residues. This revised mechanism obviates the need for substantial active site rearrangements that may involve large thermodynamic and kinetic penalties and provides a unifying solution based on long range electron transfer from the Ni(I) to the substrate. The study was highlighted in JACS Spotlight as "groundbreaking clarification of Ni(I)–sulfonate binding" (DOI: 10.1021/jacs.1c03484).

Primary Citation

Nickel–Sulfonate Mode of Substrate Binding for Forward and Reverse Reactions of Methyl-SCoM Reductase Suggest a Radical Mechanism Involving Long-Range Electron Transfer
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References

1. DeLong, E. F., Nature 2000, 407, 577-79.
2. Atreya, S. K.; Mahaffy, P. R.; Wong, A., Methane and related trace species on Mars: Origin, loss, implications for life, and habitability. Planetary and Space Science 2007, 55, 358–369.
3. Lashoff, D. A.; Ahuja, D., Relative contributions of greenhouse gas emissions to global warming. Nature 1990, 344, 213-242.
4. Ermler, U.; Grabarse, W.; Shima, S.; Goubeaud, M.; Thauer, R. K., Crystal structure of methyl-coenzyme M reductase: the key enzyme of biological methane formation. Science 1997, 278 (5342), 1457-62.
5. Wongnate, T.; Ragsdale, S. W., The reaction mechanism of methyl-coenzyme M reductase: how an enzyme enforces strict binding order. J Biol Chem 2015, 290 (15), 9322-34.
6. Wongnate, T.; Sliwa, D.; Ginovska, B.; Smith, D.; Wolf, M. W.; Lehnert, N.; Raugei, S.; Ragsdale, S. W., The radical mechanism of biological methane synthesis by methyl-coenzyme M reductase. Science 2016, 352 (6288), 953-8.