The Expanding Role of Methyl-Coenzyme M Reductase in the Anaerobic Functionalization of Alkanes

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The functionalization of unactivated C–H bonds is one of the most challenging reactions performed by enzymes. Because of the extreme low-acidity of the hydrogens that must be removed, these reactions are catalyzed exclusively by radical-mediated processes, necessitating the cleavage of C–H bonds exhibiting homolytic bond-dissociation energies as high as 105 kcal/mol.[1] Under oxic conditions, enzymes employ a broad spectrum of metallocofactors that couple the reduction of oxygen to the generation of a potent oxidant that can cleave unactivated C–H bonds by abstracting target hydrogen atoms (H•).[1] Under anoxic conditions, the choices are more limited. Until recently, it was believed that, with the exception of methane, alkanes were metabolized primarily through fumarate addition to afford alkylsuccinates, although other less well characterized systems have recently been described.[2] These alkylsuccinate synthases are glycyl radical enzymes (GREs), which carry a stable glycyl radical cofactor that reversibly generates a protein cysteinyl radical that abstracts a target H• from a substrate. The glycyl radical cofactors are installed by glycyl radical activases, which are radical S-adenosylmethionine (SAM) enzymes that catalyze the reductive cleavage of SAM to a 5'-deoxyadenosyl 5'-radical (5'-dA•). The 5'-dA• generates the glycyl radical by stereoselectively abstracting H• from a target glycyl residue of a GRE.[1]

Although there is evidence that methane can be metabolized by fumarate addition in some organisms,[2] the predominant mechanism is through a reversal of the last step in methanogenesis (**Figure 1**), which is catalyzed by methyl-coenzyme M (CoM) reductase (MCR). Recently, however, substantial evidence has shown that MCR-like enzyme systems can also oxidize butane, and most recently, ethane.[3] The initial observation of anaerobic ethane oxidation in cold marine seeps occurred nearly 15 years ago, but it has taken 10 years to isolate the microorganisms involved in this process.[4] Ethane, like methane, is chemically inert and contributes to the global carbon

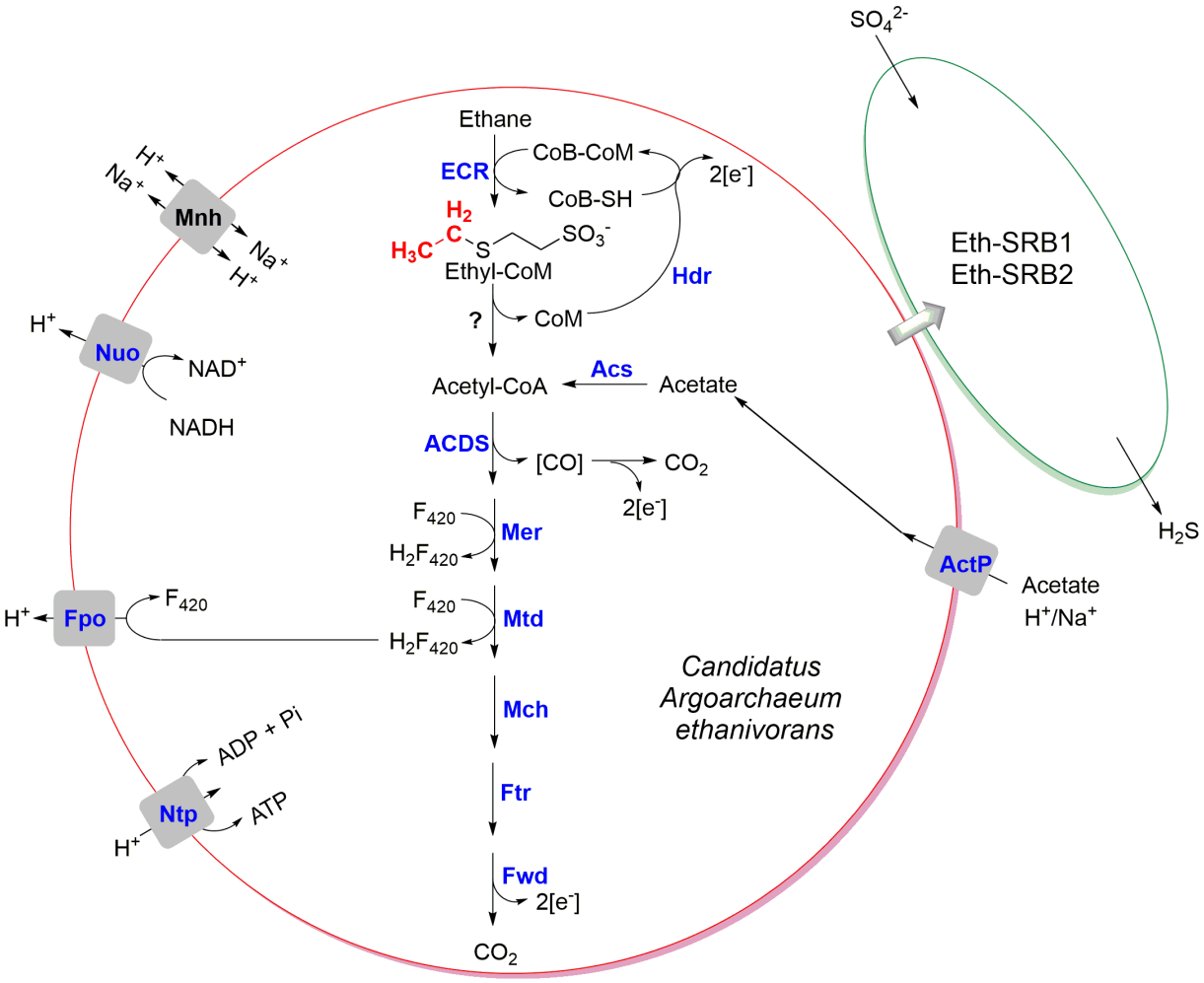

Figure 1. Predicted pathway for the oxidation of ethane by *Ca. A. ethanivorans* coupled to sulfate reduction by Eth-SRB1 and Eth-SRB2. All depicted reactions are hypothesized to be present in the genome of *Ca. A. ethanivorans*, and the ones in blue were identified by proteomic analyses. The abbreviations for the enzymes shown are as follows: ethyl-CoM reductase, ECR; acetyl coenzyme A decarbonylase/synthase, ACDS; N^5, N^{10}-methylenetetrahydromethanopterin reductase, Mer; methylenetetrahydromethanopterin dehydrogenase, Mtd; methylenetetrahydropterin cyclohydrolase, Mch; formylmethanofuran: tetrahydromethanopterin formyltransferase, Ftr; formylmethanofuran dehydrogenase, Fwd; acetate/cation symporter, ActP; acetyl coenzyme A synthase, Acs; multicomponent Na^+:H^+ antiporter, Mnh; NADH-quinone oxidoreductase, Nuo; $F_{420}H_2$ dehydrogenase, Fpo; vacular/archaeal (V/A)-type H^+/Na^+-transporting ATPase, Ntp; and heterodisulfide reductase, Hdr.

cycle. However, little is known about the anaerobic oxidation of ethane with respect to the microorganisms and metabolic processes involved. Recently, Chen, *et al.* have reported the successful isolation of a mixed culture of ethane-oxidizing and sulfate-reducing microorganisms by exclusively feeding ethane to sediment cultures taken from a marine cold seep and culturing them at 12 °C over the course of several years while monitoring for sulfide production.[4] Genomic analysis of the mixed culture allowed them to identify a predominate microorganism of archaeal lineage and two sulfate-reducing Deltaproteobacteria, Eth-SRB1 and Eth-SRB2.[4] Due to the slow-growing nature of the archaeal species, Chen, *et al.* designated this new strain *Candidatus Argoarchaeum ethanivorans*, meaning slow-growing archaeon capable of ethane oxidation.[4] Phylogenetic analysis of *Ca. A. ethanivorans* revealed that it is related to anaerobic methanotrophic archaea (ANME) and additional Methanosarcinales.[4]

Several microscopic techniques were employed to characterize the morphology of the mixed culture and to differentiate *Ca. A. ethanivorans* from Eth-SRB1 and Eth-SRB2. First, standard light microscopy showed that *Ca. A. ethanivorans* exists as small cocci with a diameter of 0.5 μm, while Eth-SRB1 and Eth-SRB2 exist as slightly curved rods and ellipsoids. DAPI (4',6-diamidino-2-phenylindole) staining of the nucleic acids coupled with helium ion microscopy suggested that *Ca. A. ethanivorans* uses budding for cell division, given that overlays of images from DAPI staining showed the presence of nucleic acids within the budding structures observed from helium ion microscopy.[4] Moreover, the microscopic images suggested that *Ca. A. ethanivorans* associates physically and tightly with Eth-SRB1 and Eth-SRB2, further suggesting a syntrophic relationship.

Proteomic and genomic analyses of *Ca. A. ethanivorans* found that the organism contains homologs of many of the same proteins involved in the production of methane via methanogenesis

(**Figure 1**). The most striking enzyme identified included all the subunits of MCR: Mcrα, Mcrβ, and Mcrγ. Furthermore, ethyl-CoM, but not methyl-CoM, was identified in the cell extracts of the mixed culture containing *Ca. A. ethanivorans* and Eth-SRB1 and Eth-SRB2. This result suggested that the encoded MCR converts ethane to ethyl-CoM rather than methane to methyl-CoM. The ethyl-CoM is hypothesized to be oxidized to CO_2 via reverse methanogenesis (**Figure 1**).[4] MCR is the terminal enzyme in methanogenesis (or the first in anaerobic methane oxidation, AOM) and is responsible for the release of methane. MCR uses three cofactors to perform this reaction: CoM, coenzyme B (CoB), and a Ni-containing tetrapyrrole, F_{430} (**Figure 2A**). The proposed mechanism for methane oxidation by MCR initiates with the attack of an F_{430}-Ni(I) species onto the sulfur of methyl-CoM, inducing the homolytic cleavage of the

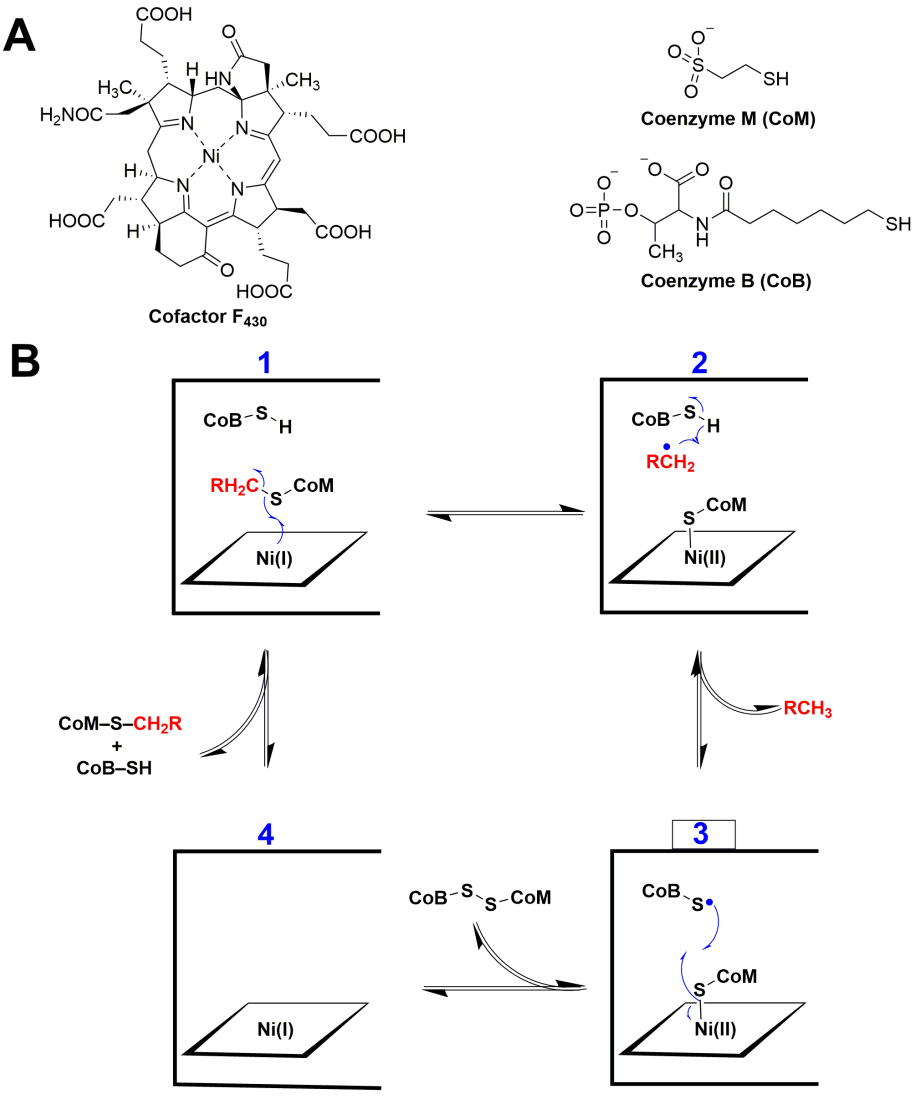

Figure 2. Required coenzymes for MCR (A) and proposed mechanism for MCR (B), where R can represent H or CH_3 for either methane or ethane.

C–S bond and formation of a methyl radical and an F_{430}-Ni(II)-thiolate(CoM) intermediate (**Figure 2B, step 1**). The nascent methyl radical abstracts a hydrogen atom from the CoB thiol, generating methane and a CoB thiyl radical (**Figure 2B, step 2**). The CoB thiyl radical attacks the sulfur of the F_{430}-Ni(II)-thiolate(CoM) intermediate, affording an F_{430}-Ni(I) species and releasing a CoB/CoM heterodisulfide (CoB-S-S-CoM) (**Figure 2B, step 3**).[5] The addition of CoB and methyl-CoM to this form of the enzyme allows for another round of turnover (**Figure 2B, step 4**). It is likely that the same mechanism proposed for MCR could be used for ethane and even additional short-chain alkanes such as propane and butane.

Interestingly, unlike in methanotrophic archaea, *Ca. A. ethanivorans* was found to contain a homolog of N^5, N^{10}-methylenetetrahydromethanopterin reductase (Mer) and the membrane-associated heterodisulfide reductase. In particular, the heterodisulfide reductase makes it possible for the MCR reaction to be reversible,[3,4] allowing for the anaerobic oxidation of methane and other short-chain alkanes.[3] Like anaerobic methane oxidation, ethane oxidation in *Ca. A. ethanivorans* is dependent on the syntrophic relationship with the sulfate-reducing bacteria Eth-SRB1 and Eth-SRB2.[4] This conclusion is supported by the co-isolation of the two species from sediments, and the observation that *Ca. A. ethanivorans* lacks the required enzymes for sulfate reduction. In addition, there is an approximate 1:1 ratio of ethane consumed to sulfide produced in mixed cultures, further substantiating that the *Ca. A. ethanivorans* and Eth-SRB1 and Eth-SRB2 have a syntrophic relationship. Lastly, genomic analysis of Eth-SRB1 and Eth-SRB2 suggests that they contain the necessary multi-heme cytochromes that are important in the storage and transfer of electrons needed for sulfate reduction in other Deltaproteobacteria.[4] Taken together, these data suggest that Eth-SRB1 and Eth-SRB2 are indeed the sulfate reducing partners of *Ca. A. ethanivorans*.

In conclusion, this work has significantly expanded our understanding of anaerobic alkane functionalization. Future characterization of the ethane oxidizing pathway and other similar pathways for the oxidation of other short-chain alkanes will reveal more details about the process. For example, can one set of enzymes act on all short-chain alkanes, or are there specific enzymes responsible for the oxidation of a given alkane? It is exciting to finally reveal the microorganisms responsible for the anaerobic oxidation of ethane at marine hydrocarbon seeps, a study that took over 10 years to complete because of the slow-growing nature of these microorganisms under these conditions. This lesson in perseverance explains how unreactive carbon molecules can be used as fuel by living organisms growing under anoxic conditions.

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Funding Sources

This work was supported by the National Institutes of Health (GM122595 to SJB), the National Science Foundation (MCB1716686 to SJB) and the Eberly Family Distinguished Chair in Science to SJB. SJB is an investigator of the Howard Hughes Medical Institute.

Notes

The authors declare no competing financial interest.

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