VIP Enzymatic C–H Activation

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Intermediates in the Catalytic Cycle of Methyl Coenzyme M Reductase: Isotope Exchange is Consistent with Formation of a σ-Alkane–Nickel Complex**

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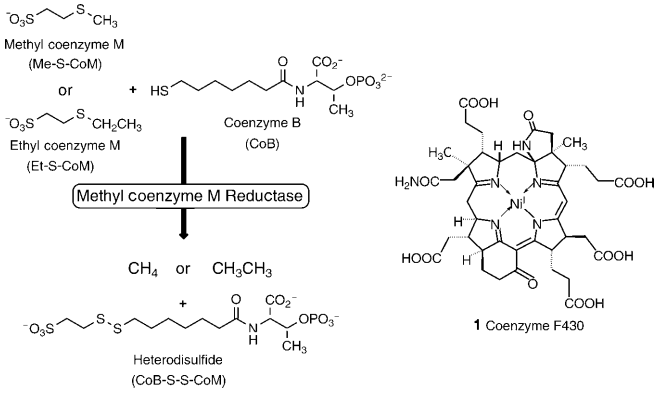
Methyl coenzyme M reductase (MCR) is the key enzyme that catalyzes the last step of methane formation in all methanogenic archaea.[1] It converts the two substrates methyl coenzyme M (CH_3-S-CoM) and coenzyme B (CoB-SH) into methane and the corresponding heterodisulfide (CoB-S-S-CoM) (Scheme 1).

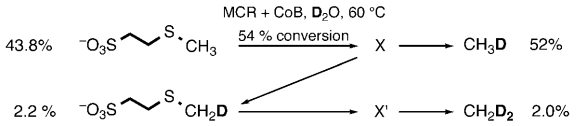


Scheme 1. The reaction catalyzed by methyl coenzyme M reductase in methanogens and the structure of the prosthetic group F430. The natural substrate, methyl coenzyme M, is converted into methane, and the non-natural substrate ethyl coenzyme M into ethane.

MCR consists of three protein chains arranged in a C_2-symmetric $\alpha_2\beta_2\gamma_2$ complex[2] with two active sites, each containing one molecule of the nickel hydrocorphinate F430 (**1**).[3] The nickel center must be in the nickel(I) oxidation state for the enzyme to be active,[4] and the fourth hydrogen of the product CH_4 originates from the medium.[5] With substrate analogues and inhibitors, different enzyme states containing Ni–H,[6] Ni–C,[7] and Ni–S[8] bonds have been characterized by EPR spectroscopy. However, no intermediates along the catalytic pathway could be observed to date, and the reaction mechanism is thus still unknown.

Herein, we report that MCR catalyzes the incorporation of deuterium from the medium not only into the product methane but also into the substrate. Studies with stable isotopes show the existence of an intermediate in which the carbon–sulfur bond is broken and through which the carbon-bound hydrogen atoms of the *S*-alkyl group of the substrate can exchange with solvent-derived deuterium.

Methane generated in assays with purified enzyme (MCR-I), CH_3-S-CoM, and CoB-SH in deuterated medium was analyzed by ^{1}H NMR spectroscopy. We found that not only CH_3D (the expected isotopologue), but also a significant amount of CH_2D_2 was formed (see the Supporting Information, Figure S1.1).[5c,9] To determine whether this double labeling was the consequence of deuterium incorporation into the substrate, the remaining CH_3-S-CoM was analyzed by ^{1}H NMR spectroscopy before full conversion. We found that deuterium is indeed introduced into the methyl group of methyl coenzyme M. After 54% conversion (for conditions and spectra, see the Supporting Information, Section 1.2), the remaining substrate contained 4.8% CH_2D-S-CoM.[10] (Scheme 2).



Scheme 2. Double incorporation of deuterium into the product methane and exchange of deuterium into the remaining substrate methyl coenzyme M catalyzed by MCR in D_2O.

The non-natural substrate ethyl coenzyme M is converted by MCR, giving ethane,[11] although about 200 times[11c] more slowly than the natural substrate Me-S-CoM gives methane. Investigation of deuterium incorporation into Et-S-CoM under the conditions used for Me-S-CoM revealed that deuterium is introduced at both carbon centers of the *S*-ethyl group. After 2 min reaction time, only about 1% of the substrate had been converted into ethane, but the remaining substrate contained 9.0% of CH_3CHD-S-CoM and 15.2% CH_2DCH_2-S-CoM. After 32 min, a mixture of 11 isotopologues could be detected (containing, for example, 10.8% CHD_2CD_2-S-CoM and 4.9% CD_3CHD-S-CoM). Figure 1

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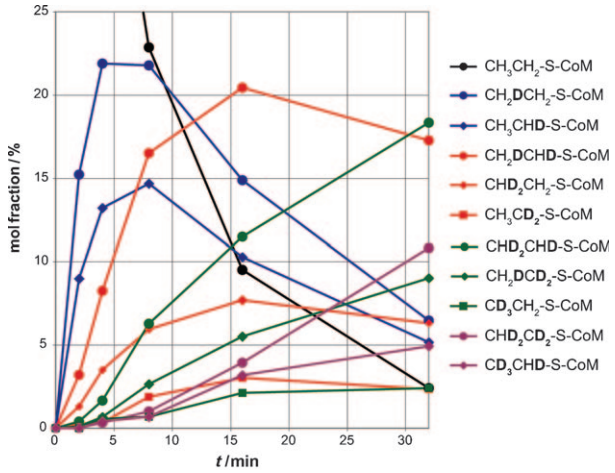


Figure 1. Formation of the 11 isotopologues of ethyl-S-CoM that are detectable by ^{1}H NMR spectroscopy as a function of incubation time with MCR-I in deuterated medium at 60 °C. Molar fractions were determined from ^{1}H{^{2}H} NMR spectra. CD_3CD_2SCoM is not observed in the ^{1}H NMR spectrum. (See the Supporting Information for experimental conditions.)

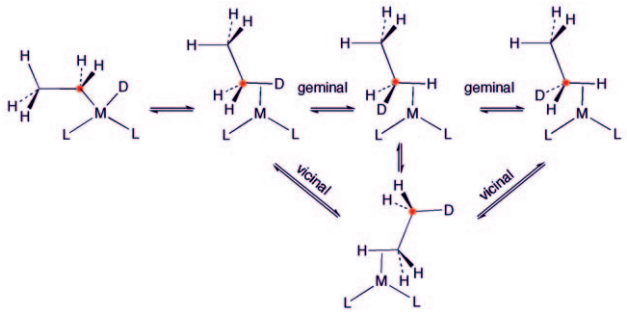
shows the molar fractions of Et-S-CoM isotopologues as a function of time.

As direct deuterium incorporation into the methyl group of ethyl-S-CoM was considered to be mechanistically unreasonable, we tested for putative scrambling of the two carbon centers within the ethyl group using a ^{13}C label. In experiments with $CH_3{}^{13}CH_2$-S-CoM in non-deuterated medium, the ^{13}C label was nearly statistically distributed within the ethyl group after incubation for 32 min. The scrambling process showed apparent first-order kinetics, with a half-life time of about 4 min (see the Supporting Information, Figure S1.3).

To correlate deuterium incorporation with carbon scrambling, experiments with $CH_3{}^{13}CH_2$-S-CoM were run in deuterated medium. Analysis after 2 min reaction time showed the following molar fractions of monodeuterated isotopologues: $CH_3{}^{13}CHDS$-CoM (8.2 %); $CH_2D{}^{13}CH_2$-S-CoM (0.0 %); $^{13}CH_3CHD$-S-CoM (0.0 %); $^{13}CH_2DCH_2$-S-CoM (12.9 %). After 8 minutes, the doubly deuterated species were found at molar fractions of $CH_3{}^{13}CD_2$-S-CoM (1.18 %), $^{13}CHD_2CH_2$-S-CoM (3.43 %), and $^{13}CH_2DCHD$-S-CoM (4.71 %), $CH_2D{}^{13}CHD$-S-CoM (4.84 %), which is close to the statistically expected ratios of 1:3 and 1:1, respectively (Supporting Information, Figure S1.5).

This distribution demonstrates that deuterium is introduced only at the carbon center bound to sulfur in the substrate and that deuterium incorporation into the methyl group is a consequence of the carbon scrambling. (A plot showing the time dependence of all observed isotopologues of the ^{13}C-labeled substrate is given in the Supporting Information, Figure S1.4.)

The same pattern of isotope exchange has been observed by Periana and Bergman with a hydridoethylrhodium complex $[Cp^*(PMe_3)Rh(Et)(D)]$.[12] They found that the [1-^{13}C]ethyl deuteride rearranges to the [1-^{13}C, 1-^{2}H]ethyl hydride upon warming to −80 °C. On further warming to −25 °C, rearrangement of the α-^{13}C label to the β carbon of the ethyl ligand was observed whereby the deuterium remained attached to the labeled carbon during the vicinal bond shift (Scheme 3).

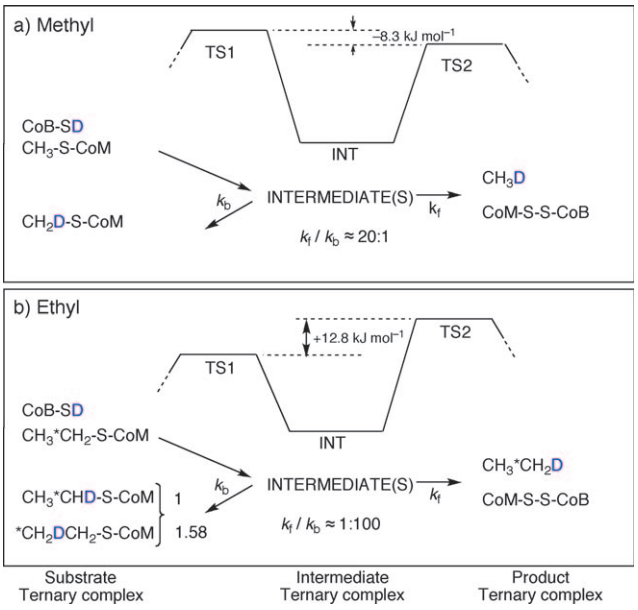


Scheme 3. Bond shifts via a σ-complex as proposed by Bergman[12] for a [(H)(Et)RhCp*PMe_3] complex and consistent with the pattern of isotope exchange with $CH_3{}^{13}CH_2$S-CoM in D_2O catalyzed by MCR.

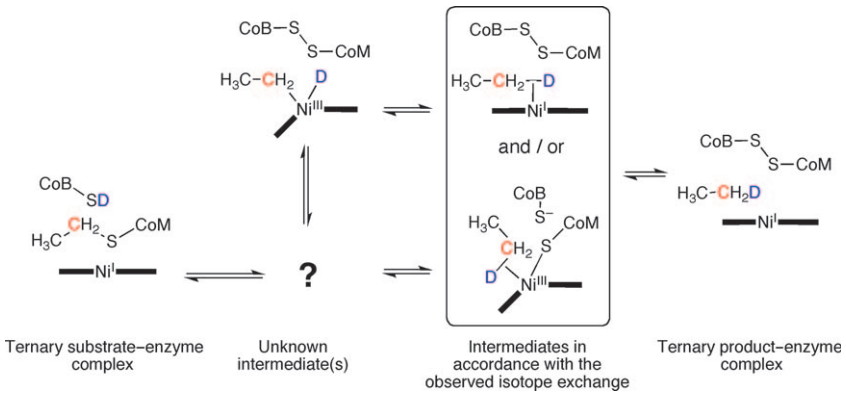
We have recently shown that the MCR-catalyzed reaction is indeed reversible.[13] In a competitive experiment, methane was converted into Me-S-CoM with 0.011 U mg^{-1}, whereas ethane was converted into Et-S-CoM with 0.00074 U mg^{-1} (for details, see the Supporting Information). These reverse reactions are much slower than the isotope exchange described herein (ca. 1 U mg^{-1} for Me-S-CoM and 10 U mg^{-1} for Et-S-CoM). Therefore, the formation of CH_2D_2 from Me-S-CoM in D_2O is mainly due to deuterium exchange into the substrate. Isotope exchange occurs along the catalytic pathway before the products are formed and at least one intermediate must exist, which either reacts back to the substrates (with the possibility of isotope exchange) or forward to give the products. Scheme 4 illustrates the minimal reaction profile taking into account the ratio of substrate deuteration versus product formation for the two substrates.

In the intermediate ternary complex, the C–S bond is replaced by a C–D bond[14] and therefore all C–H(D) bonds of the prospective alkane are already set up, although the exact binding mode is not known. However, our experimental findings are consistent with the formation of a σ-coordinated alkane as an intermediate (Scheme 5). In contrast to the mechanisms proposed earlier,[1a,15] which assumed involvement of a single axial coordination site on the nickel, a mechanism via nickel(hydrido)(alkyl) and nickel(σ-alkane) intermediates such as proposed herein requires the availability of two adjacent coordination sites. From EPR studies with substrate analogues, it is known that the enzyme undergoes a major conformational change upon binding of the second substrate,[16] and MCR-species with a hydride and a thiolate coordinated to the nickel center along with the coordinatively distorted hydrocorphin ligand have been characterized.[6,8] Therefore, a second adjacent coordination site may well be accessible.

In the reverse reaction, the activation of the strong C–H bond of methane, a nickel(σ-alkane) complex as the first intermediate is more in line with chemical precedence for C–H activation at transition metals[17] than, for example, the very endothermal abstraction (70 kJ mol^{-1}) of a hydrogen



Scheme 4. Reaction profiles of a) methyl coenzyme M and b) the non-natural substrate ethyl coenzyme M. From the ratio of deuterium incorporation versus product formation, the indicated energy differences are calculated.



Scheme 5. Hypothetical intermediates in MCR-catalyzed reactions. For clarity, the intermediates are drawn with the non-natural substrate ethyl coenzyme M labeled with one ^{13}C (drawn in red) in deuterated medium. The ligand of coenzyme F430 is shown schematically as bold lines. The bend in the Ni^{III}-intermediates symbolizes two *cis* coordination sites and a distorted equatorial macrocycle.

atom from methane by a thiyl radical. The latter process would be required in the reverse reaction if it would proceed along the mechanism proposed by Siegbahn and co-workers on the basis of DFT calculations.[15c,18] The observed pattern of exchange in the forward reaction is also in qualitative agreement with the radical substitution pathway calculated by Siegbahn (see the Supporting Information, Figure S1.6). However, the observed relative rates of product formation and exchange into substrate are clearly not.

In summary, we have shown that the MCR-catalyzed reaction proceeds via a pathway with at least one intermediate in which the carbon–sulfur bond of the substrate is broken and through which the carbon-bound hydrogen atoms of the *S*-alkyl group of the substrate can exchange with solvent-derived deuterium according to a pattern that has also been observed in [(H)(alkyl)RhCp*(L)] complexes. This reactivity is the case for both the native substrate and the non-natural substrate ethyl-S-CoM. We propose considering (σ-alkane)-Ni F430 and (H)(alkyl)Ni F430 complexes as intermediates for both methane formation and anaerobic methane activation (Scheme 5). However, with the exception of the CH_4 activation by $Ni(H)(OH)^+$ in the gas phase observed by Schlangen and Schwarz,[19] no non-enzymatic example of this type of reactivity catalyzed by a late transition metal with an odd number of electrons (such as nickel(I), d^9) is currently known. Hopefully, the biological importance of the MCR-catalyzed reactions and the current interest in mild C–H activation will prompt in-depth chemical studies of this type of odd-electron transition metal centers as catalysts.

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