Coenzyme B Induced Coordination of Coenzyme M via Its Thiol Group to Ni(I) of F$_{430}$ in Active Methyl-Coenzyme M Reductase

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Methane is formed in methanogenic archaea by the reduction of methyl-coenzyme M (CH$_3$−S−CoM) with coenzyme B (HS−CoB) to CH$_4$ and the heterodisulfide CoM−S−S−CoB.[1] This reaction is catalyzed by methyl-coenzyme M reductase (MCR), which is composed of three different subunits in an $\alpha_2\beta_2\gamma_2$ arrangement and which contains tightly bound 2 mol of the nickel porphinoid F$_{430}$. Crystal structures of the 300 kDa enzyme with and without coenzymes or product bound have been resolved to 1.16 Å.[2] They were, however, only obtained for the enzyme in the inactive Ni(II) state. For the enzyme to be active, the prosthetic group has to be in the Ni(I) oxidation state,[3−5] which is rapidly lost by autoxidation of the Ni(I).[6] Therefore, it has not been known until now how within the active enzyme the active site Ni(I) interacts with the substrates.

Active MCR exhibits an axial EPR signal MCR$_{red1}$ derived from Ni(I) which does not change significantly when CH$_3$−S−CoM alone or together with HS−CoB are added to the active enzyme, showing that although an enzyme−substrate−complex is formed, there is no detectable direct interaction of the substrates with the Ni(I) of the prosthetic group.[7] The XAS data of the active enzyme are also the same in the absence and presence of the substrates.[8]

The reduction of CH$_3$−S−CoM catalyzed by MCR is inhibited by coenzyme M (HS−CoM), inhibition being reversible and competitive to CH$_3$−S−CoM. In the presence of only HS−CoM, the enzyme shows the axial MCR$_{red1}$ EPR signal. In the presence of both HS−CoM and HS−CoB, however, the axial signal is partially converted into the highly rhombic EPR signal MCR$_{red2}$.[7] On the basis of ^{1}H and ^{14}N data obtained from electron nuclear double resonance (ENDOR) and hyperfine sublevel correlation spectroscopy (HYSCORE) measurements, it was proposed that in the MCR$_{red2}$ state HS−CoM is axially coordinated to Ni(I).[9] Here we report experiments with ^{33}S-labeled HS−CoM, proving that the thiol group of HS−CoM coordinates to the Ni(I) ion of F$_{430}$.

[2-^{33}S]-coenzyme M (**4**) (Scheme 1) was synthesized in a one-pot procedure starting from elemental sulfur ([^{33}S$_8$]-S$_8$)[10] and potassium cyanide[11] according to Scheme 1.[12]

Figure 1 shows the X-band EPR spectra of MCR$_{red2}$−HS−CoM (^{32}S (99.25%) with nuclear spin $I = 0$, ^{33}S (0.75%) with $I = {}^3/_2$) and MCR$_{red2}$−H^{33}S−CoM. For a better comparison, the signals of MCR$_{red1}$ were subtracted from the red1/red2 mixture normally shown by these preparations. The EPR spectrum of ^{33}S-labeled MCR$_{red2}$ shows a pronounced line broadening at the high-field feature corresponding to the g_3 principal value. This is a strong indication for the presence of a large ^{33}S hyperfine coupling along this principal axis direction. No significant broadenings are observed

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Scheme 1. Synthesis of [2-^{33}S]-Coenzyme M^a
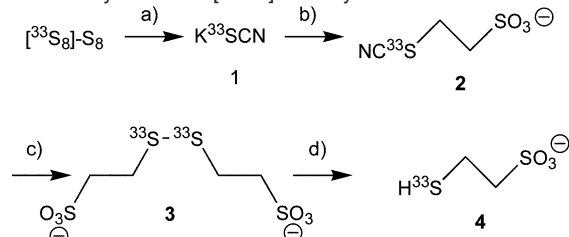
^{a} Conditions: (a) N$_2$, KCN, EtOH, 4 h, reflux. (b) N$_2$, BrCH$_2$CH$_2$SO$_3$Na, DMF, 4 h, 120 °C. (c) (1) N$_2$, K$_2$CO$_3$, H$_2$O, 1 h, 60 °C; (2) H$^+$, NH$_3$. (d) N$_2$, DTT, H$_2$O, 30 min. Overall yield from [^{33}S$_8$]-S$_8$, 87% (NMR); after final purification for MCR-assay, 44%.
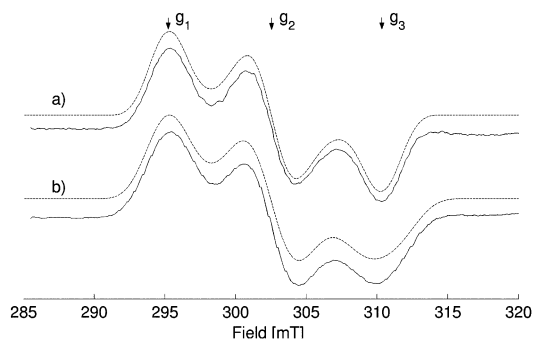
Figure 1. X-band EPR spectra of MCR in the MCR$_{red2}$ state (coenzyme M inhibited enzyme in the presence in coenzyme B). (a) With HS−CoM. (b) With H^{33}S−CoM. Solid lines: experimental spectra after subtraction of MCR$_{red1}$ signals. Dashed lines: simulations, spectrum b used the same spin Hamiltonian parameters as in (a) but with the addition of a ^{33}S hyperfine interaction, $|A_{1,2}| = 20$ MHz, $|A_3| = 35$ MHz. Experimental conditions: 77 K, modulation amplitude 0.6 mT, microwave frequency 9.45 GHz (Supporting Information contains further details).

at g_1 and g_2. From spectral simulations, the ^{33}S hyperfine coupling along g_3 is estimated to be roughly $|A_3| = 35$ MHz, with upper limits along g_1 and g_2 of $|A_{1,2}| = 25$ MHz.

In contrast to MCR$_{red2}$, when either the ox1 or the red1 form of MCR is incubated in the presence of H^{33}S−CoM, no significant line broadenings are observed in the EPR spectra. This shows that any interaction with ^{33}S in these two forms is small (as compared to the EPR spectral resolution).

A clear-cut proof of the coordination of HS−CoM to Ni(I) is obtained from HYSCORE spectra measured at Q-band[13] at the low-field end (g_1 value) of the EPR spectrum. Figure 2a and 2b shows the single-crystal-like HYSCORE spectra of MCR$_{red2}$−HS−CoM and MCR$_{red2}$−H^{33}S−CoM at g_1 which are free of contributions from other paramagnetic species of MCR. The additional peaks observed in Figure 2b originate from ^{33}S interactions (labeled in Figure 2b). The two cross-peaks in the (− +)-quadrant at (−10.8, 31.8) MHz and (−31.8, 10.8) MHz are assigned to triple-quantum transitions

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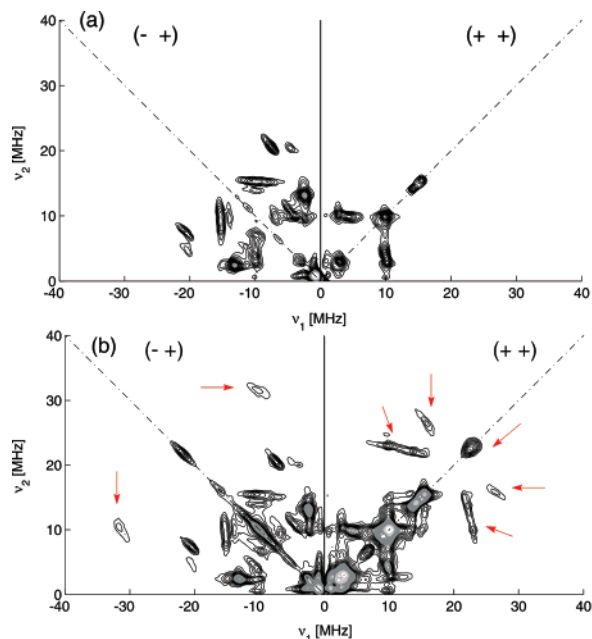
Figure 2. Q-band HYSCORE spectra recorded at 25 K of MCR in the MCR$_{red2}$ state; observer position at g_1. (a) With HS$-$CoM. (b) With H^{33}S$-$CoM. The arrows identify peaks that originate from ^{33}S interactions. The Supporting Information contains further details.

with $\Delta m_I = 3$. For a simplified system with an isotropic g tensor and an axial hyperfine tensor, the two triple-quantum frequencies can be written to first order as

$$\nu_{TQ}^{(+/-)} = 3\left[\left(\pm\frac{A_\perp}{2} + \nu_I\right)^2 \sin^2 \beta + \left(\pm\frac{A_\|}{2} + \nu_I\right)^2 \cos^2 \beta\right]^{1/2}$$

with the hyperfine principal values $A_\perp$ and $A_\|$, the nuclear Zeeman frequency ν_I, and the angle β between the $A_\|$ principal axis and the static magnetic field vector $\mathbf{B}_0$. For a nuclear quadrupole interaction that is small as compared to the hyperfine interaction, these frequencies are to first-order independent of the nuclear quadrupole interaction and differ by $6\nu_I$ for $\beta = 0, 90°$. In Figure 2b, the observed splitting of 20.9 MHz is slightly smaller than $6\nu_I = 21.7$ MHz, indicating that the orientations selected in this experiment are close to the principal axis of $A_\perp$. The ^{33}S hyperfine coupling A along g_1 can easily be estimated from the equation

$$\nu_{TQ}^{(+)2} - \nu_{TQ}^{(-)2} = 18\nu_I(a_{iso} + T(3\cos^2\beta - 1)) = 18\nu_I A$$

where a_{iso} is the isotropic hyperfine coupling, and T is the dipolar coupling.[14] Along g_1, we then find $|A| = 13.8$ MHz, and for the principal value $|A_\perp|$ we estimate a coupling of about 15 MHz.

Several additional peaks are observed in the $(+ +)$-quadrant of Figure 2b. The strong diagonal peak at 23 MHz is most probably a sulfur double-quantum transition ($\Delta m_I = 2$), and the cross-peaks represent correlations between sulfur transitions and/or nitrogen$-$sulfur combination transitions. An unequivocal assignment of all of the new peaks observed in the ^{33}S sample is difficult because the HYSCORE spectrum could only be observed along g_1. This is because the large anisotropy of the ^{33}S hyperfine coupling broadens the peaks beyond detection as soon as the B_0 observer field in the HYSCORE experiments is shifted to higher values.

The combination of EPR and HYSCORE data proves that, in the MCR$_{red2}$ state, HS$-$CoM is directly coordinated to the Ni(I) ion. An estimate of the spin density on ^{33}S can be obtained from the hyperfine tensor and by considering the relative signs of the principal values. Assuming three positive principal values [$(A_1, A_2, A_3) = (15, 15, 35)$ MHz] yields an isotropic part of $a_{iso} = 21.7$ MHz and a dipole part of $(-6.7, -6.7, 13.4)$ MHz. For a hyperfine tensor [$(A_1, A_2, A_3) = (-15, -15, 35)$ MHz], the isotropic part is $a_{iso} = 1.7$ MHz, and the dipole part is $(-16.7, -16.7, 33.4)$ MHz. In the first case, a spin density of 0.6% in the s-orbital is estimated from the isotropic part, and a spin density of 7% in a 3p-orbital is estimated from the dipolar part.[15] For the second case, the corresponding values are 0.05% (s-orbital) and 17% (3p-orbital). In either case, the large spin density on the sulfur ligand is further proof that the ground state of MCR$_{red2}$ has a relatively high percentage of d$_{z^2}$ character, and the large hyperfine coupling A_3 corroborates the proposal that the g_3 principal axis is perpendicular to the macrocycle.[9]

On the basis of the finding that the HS$-$CoM interaction is dependent on the presence of HS$-$CoB in MCR$_{red2}$, we propose that HS$-$CoB is not only required as a second substrate,[16] but also to induce a change forcing the real substrate, CH$_3$$-S-$CoM, and Ni(I) of the prosthetic group to interact in the active enzyme MCR$_{red1}$.

Supporting Information Available: Analytical data of [2-^{33}S]-coenzyme M (4, NH$_4^+$-form) and experimental procedures (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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