SUPPORTING INFORMATION

Structural Insight into Methyl-Coenzyme M Reductase Chemistry using Coenzyme B Analogues[‡]

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[‡] Co-ordinates and structure factors have been deposited in the Protein Data Bank as entry 3m32 (MCR_{CoBSH} + methyl-SCoM)

MATERIALS AND METHODS

Single Crystal UV-visible Microspectrophotometry. Data were collected on a microspectrophotometer from 4DX Systems AB, with a Zeiss CLX500 xenon lamp light source, spectrograph (Thermo-Oriel, CT, USA) and an DV401A CCD detector (Andor Technology, Ireland). For the photoreduction studies spectra were collected off-line at the synchrotron beamline following X-ray data collection, *i.e.* the microspectrophotometer was set up adjacent to the beamline rather than integrated into the X-ray data collection hardware. An Oxford Instruments Cryojet was used for crystals at 100K, and those at room temperature were mounted in quartz capillaries (1). For crystals used in structure determination, the UV-visible spectra were collected in the Kahlert Structural Biology Laboratory, University of Minnesota, after returning from X-ray data collection at BioCARS 14-BM-C, Advanced Photon Source (APS), Argonne National Laboratory, Argonne, IL, USA (Beamline 14-ID-B, BioCARS). An Oxford Instruments Cryojet was used to keep the crystals at 100K.

X-ray Photoreduction. In a separate set of experiments, crystals in the Ni(II)-MCR_{silent} state were subjected to X-irradiation at the APS (Beamline 14-ID-B, BioCARS). The Ni(II)-MCR_{silent} crystals were obtained by sitting drop vapor diffusion anaerobically at 4 °C by using the crystallization conditions described in the main text. For comparative single crystal UV-visible microspectrophotometry, green Ni(I)-MCR_{red1} crystals were moved out of the anaerobic chamber and exposed to oxygen. The change in color to yellow confirmed formation of the Ni(II) state in the crystals, and the crystals were cryoprotected in reservoir solution containing 25 % (v/v) PEG 400 by soaking for 2-5 minutes before cryocooling in liquid nitrogen in the anaerobic chamber.

Each crystal was irradiated for 1 minute, then a further 4 minutes and then a further 5 minutes (total 10 minutes), and single crystal UV-visible spectra recorded at each of these timepoints. Crystals were subjected to three different conditions; a wavelength of 1.608 Å (7.709 keV) to maximize photoelectron generation, either at (1) room temperature or (2) cryocooled at 100K, and (3) room temperature at the Ni K-edge (8.33 keV, 1.489 Å).

X-ray crystallography of the MCR-heterodisulfide product complex ($MCR_{CoBSH} + methyl-SCoM$). The methodology was identical to that described in the main text except for the following: The product complex crystals were obtained through co-crystallization from 2 μ l enzyme solution containing 124 μ M MCR_{red1} , 10 mM methyl-SCoM and 0.93 mM CoBSH incubated with 2 μ l of reservoir solution (100 mM Hepes-Na, pH 7.5, 150 mM $(Mg(CH_3COO)_2)$, 22 % PEG 400).

The library file in CCP4 for the heterodisulfide was incorrect, and so was modified in Monomer Library Sketcher in the CCP4 program suite by comparison with schematic drawings from Grabarse *et al.* (2). As well as using the positions of CoBSH and HSCoM from the published Ni(II)-MCR crystal structures (PDB codes 1hbn, 1hbo, 1hbu) as guides, the heterodisulfide position was also used (PDB code 1hbm) to determine relative occupancies of each species (2).

RESULTS AND DISCUSSION

Redox Changes. By double integration of the EPR spectra, the MCR samples used for crystallization were determined to be ~78% MCR_{red1} (Figure S11A), which matched that determined by UV-visible spectroscopy. At the time the MCR crystals were mounted for X-ray data collection and cryocooled in liquid nitrogen in the anaerobic chamber, their color (as assessed by eye) still appeared green, suggesting that a significant proportion of the $\text{Ni(I)-MCR}_{\text{red1}}$ state remained. Furthermore, MCR samples used in crystallization were incubated in EPR tubes under conditions identical to those of the crystallization trials, and then frozen at the time the crystals were mounted. EPR spectroscopy demonstrated that only 10-20% of the spin had been lost during the time from setting up the crystallization to crystal mounting (Figure S11B). However, by the time crystals were transferred to the goniometer at the synchrotron prior to X-ray data collection, they appeared yellow, suggesting that a significant conversion of Ni(I) to the inactive Ni(II) state had occurred during the course of storage, transport, and transfer. This was confirmed following X-ray data collection by single crystal UV-visible microspectrophotometry. The post-X-ray UV-visible spectra for all crystals used in the X-ray diffraction experiments had a dominant peak at 420 nm corresponding to $\text{Ni(II)-MCR}_{\text{red1-silent}}$ with very little to almost no evidence of $\text{Ni(I)-MCR}_{\text{red1}}$ absorption at 385 nm (Figure S3). Therefore most, if not all, of the active component of MCR in the crystals had been oxidized to the Ni(II) state.

Photoelectrons generated from oxidation of solvent in the intense X-ray beam can often reduce redox centers. At the wavelength of 0.973 Å where the data were collected, there was no evidence of reduction of Ni(II) to Ni(I) in our crystals. However, maximal photoelectrons are known to be generated at longer X-ray wavelengths (> 1.5 Å), and this fact was used by Schlichting *et al.* to visualize ferryl heme in cytochrome P450cam crystals through addition of an X-ray generated photoelectron to a cytochrome P450-camphor- O_2 ternary complex (3). Earlier work had demonstrated that the $\text{Ni(II)-heterodisulfide}$ complex ($\text{MCR}_{\text{silent}}$) could be cryoreduced by γ -irradiation at 77 K, followed by annealing at 262 K to generate $\text{Ni(I)-MCR}_{\text{red1}}$, although only at ~15% yield (4). Therefore, crystals not used for X-ray diffraction data collection were exposed to high intensity X-irradiation at longer

wavelengths, including at the Ni K-edge (8.33 keV, 1.489 Å), to see if reduction of Ni(II)-MCR_{silent} to Ni(I)-MCR_{red1} could be achieved. However, there was no noticeable effect on the single crystal UV-visible spectra indicating that the X-irradiation did not convert the Ni(II)-MCR_{silent} form to the Ni(I)-MCR_{red1} state (data not shown). Even at room temperature, where photoelectrons and radicals can propagate easily through the sample, there was no evidence of conversion of Ni(II) to Ni(I).

MCR_{CoBSH + methyl-SCoM} crystal structure. The X-ray data collection, processing, refinement and model building statistics are given in Table S1. The 1.35 Å resolution electron density was most consistent with 50 % heterodisulfide product (CoBS-SCoM) (Figure S6) and a background of 50 % CoBSH and HSCoM (Table S1). Although the nickel had been oxidized to Ni(II) by the time X-ray data were collected, this crystal was the result of co-crystallization of a predominantly Ni(I)-MCR_{red1} solution with CoBSH and methyl-SCoM substrates. This indicates that at least 50% of the enzyme was in the active Ni(I) state at the start of crystallization, and this is supported by the EPR data (Figure S11) and the initial green color of the crystals. A previous MCR crystal structure to 1.8 Å resolution also contained the heterodisulfide product at high yield (2). This was of the MCR_{silent} state, which is generated by growing and harvesting the protein in an H₂/CO₂ (80%/20%) atmosphere (Scheme S1). MCR purified anaerobically from these conditions contains the inactive Ni(II) state with the heterodisulfide bound in the substrate channel. The heterodisulfides from these two structures (PDB code 1hbm and the MCR_{CoBSH + methyl-SCoM} structure presented here) overlay well (rmsd 0.125 Å) indicating that the heterodisulfide product can be generated both *in vivo* and *in vitro*. As in the previously described MCR_{silent} structure, we also observed that the CoB-portion of the product remains in an almost identical position to CoBSH (as observed in the MCR_{ox1-silent} and MCR_{red1-silent} structures (2, 5)), whilst the CoM-portion has moved so that one oxygen of the sulfonate group interacts with the Ni(II) at a distance of 2.1 Å. Hydrogen bonds from both Tyr α 333 and Tyr β 367 hydroxyls to sulfonate oxygens are present, as also observed in the previous 1hbm MCR_{silent} crystal structure (2, 5). The coordinates and structure factors have been deposited in the Protein Data Bank as entry 3m32.

All the crystal structures in this work are of MCR in the inactive Ni(II) state, although when the crystals were cryocooled for X-ray data collection they were still predominantly MCR_{red1}, the active Ni(I) state. The presence of heterodisulfide product in the structure obtained by co-crystallization of MCR_{red1} with the natural substrates CoBSH and methyl-SCoM (MCR_{CoBSH + methyl-SCoM}) corroborates the EPR data (Figure S11A) and the qualitative green color of the crystals when cryocooled, indicating that the crystals were obtained by co-crystallization of a predominantly Ni(I)-MCR_{red1} solution with the CoB_xSH analogues. Attempts to reduce the nickel center back to Ni(I) using the photoelectrons generated from solvent by X-irradiation were unsuccessful.

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Table S1: X-ray Data Collection, Processing and Refinement Statistics for MCR_{CoBSH} + methyl-SCoM.

Data collection and processing statistics	
Measured reflections	2443766
Unique reflections	474767
Resolution (Å) ^a	50.0-1.35 (1.40-1.35)
Completeness (%) ^a	93.2 (81.2)
Rsym (%) ^{a, b}	6.3 (27.6)
I/σI ^a	21.1 (5.5)
Space group	P2 ₁
Refinement and model building statistics	
Resolution (Å) ^a	20.28-1.35 (1.38-1.35)
No. of reflection in working set ^a	450864 (24362)
No. of reflection in test set ^a	23861 (1353)
R-work (%) ^c	14.00
R-free (%) ^d	15.68
ESU (Å) Rwork/Rfree	0.049/0.049
No. protein atoms	19913
No. co-factor and co-enzyme atoms	236
No. ligand atoms	41
No. water molecules	2475
RMS	
bond lengths (Å)	0.034
bond angles (deg.)	2.775
Ramachandran plot (%)	
favored	97.8
allowed	2.1
disallowed	0.1
Average B-factor (Å ²)	
protein	13.20
co-factor F430 and co-enzymes	8.32
ligands	32.50
solvent atoms	24.10

over all	14.37
Occupancy of HSCoM per active site (%) ^e	50/50
Occupancy of CoBSH per active site (%) ^e	50/50
Other molecule, occupancy per active site (%) ^e	Heterodisulfide, 50/50
^a Values in brackets correspond to the highest resolution shell. ^b $R_{sym} = \frac{\sum_{hkl} \sum_{j=1}^N I_{hkl} - I_{hkl}(j) }{\sum_{hkl} N \cdot I_{hkl}}$, sum over all reflections and all observations N, with $I_{hkl}(j)$ intensity of the j^{th} observation of reflection hkl and I_{hkl} mean intensity of the reflection hkl. ^c $R_{-work} = \frac{\sum F_o - F_c }{\sum F_o }$, where $ F_o $ = observed structure factor amplitude and $ F_c $ = calculated structure factor amplitude. ^d R-free, R-factor based on 5% of the data excluded from refinement. ^e Occupancy in percent of model in each of the two crystallographically independent active sites in the ASU

Figure S1. Example using the $\text{MCR}_{\text{CoB8SH}}$ data to show how the Fo-Fc difference electron density map was used to elucidate the occupancy of CoBSH and the analogue; (A) Initial map before refinement fits well with CoB₈SH. (B) Positive and negative Fo-Fc difference electron density peaks after refinement with 100 % CoB₈SH as a model. (C) In final model with 50% CoB₈SH and 50% CoBSH there is no Fo-Fc difference electron density present at a contour of 4σ . $2\text{Fo}-\text{Fc}$ electron density map (contoured at 1σ) is shown as a blue mesh. Positive and negative peaks in the Fo-Fc difference electron density map (contoured at 4σ) are shown as a green and red mesh, respectively. The protein is drawn as cartoon. Coenzyme F₄₃₀, HSCoM, CoB₈SH and CoBSH are drawn as stick and colored by atom (carbon: F₄₃₀ dark grey; HSCoM medium grey; CoB₈SH light blue; CoBSH light green). The nickel is displayed as a green sphere. The figure was generated using PyMOL (<http://www.pymol.org/>).

Figure S2. Stereo image of 2Fo-Fc electron density quality for the MCR_{CoB5SH} data. 2Fo-Fc electron density (contoured at 1 σ) is shown as a mesh, colored green (F₄₃₀), red (HSCoM) and blue (amino acids). Models are drawn as stick and colored by atom (carbon: F₄₃₀ dark grey; HSCoM medium grey; amino acids white). The nickel is displayed as a green sphere. The figure was generated using PyMOL (<http://www.pymol.org/>).

Figure S3. UV-visible spectra. (A) Solution spectrum of Ni(I)-MCR_{red1} used in anaerobic crystallization (solid line), and the same solution after being exposed to air and being oxidized to Ni(II) (dotted line). (B) Single crystal spectra (offset for clarity) collected on the crystals in this study following X-ray data collection. (1) Oxidized Ni(II)-MCR_{red1}-silent crystal; (2) MCR_{HSCoM}; (3) MCR_{CoB5SH}; (4) MCR_{CoB6SH}; (5) MCR_{CoB8SH}; (6) MCR_{CoB9SH}; (7) MCR_{CoBSH} + methyl-SCoM.

Figure S4. Stereo images showing the modeling of CoB₆SH (50%) and CoBSH (50%) in the MCR_{CoB₆SH} data. (A) Final refined model. Average B-factor is 11.3 Å² for the CoBSH and CoB₆SH atoms from the first non-coincident carbon in the alkyl chain (C3) to the thiol. This matches the average B-factor for F₄₃₀ and protein residues in this part of the substrate channel. There is no Fo-Fc difference electron density present at a contour of 4σ. (B) Model in which 50% CoB₆SH is built as taking the path of the 50% CoBSH model and then refined. Average B-factor rises by 62% (18.2 Å²) for the same CoBSH and CoB₆SH atoms as in panel (A). 2Fo-Fc electron density (contoured at 1σ) is shown as a blue mesh. Positive and negative peaks in the Fo-Fc difference electron density map (contoured at 4σ) are shown as a green and red mesh, respectively. The protein is drawn as cartoon. CoB₆SH and CoBSH are drawn as stick and colored by atom (carbon: CoB₆SH pale yellow; CoBSH light green), with thiols represented by yellow spheres. F₄₃₀ and HSCoM are drawn as stick colored by atom (carbon: F₄₃₀ dark grey; HSCoM medium grey). The nickel is displayed as a green sphere. The figure was generated using PyMOL (<http://www.pymol.org/>).

Figure S5. Stereo image of the partially occupied HSCoM (50%) in the MCR_{CoB₆SH} data. 2Fo-Fc electron density (contoured at 1 σ) is shown as a blue mesh. The coenzymes are drawn in stick and colored by atom (carbon: F₄₃₀ dark grey; HSCoM medium grey; CoB₆SH pale yellow). Waters are displayed as red spheres. The nickel is displayed as a green sphere. The figure was generated using PyMOL (<http://www.pymol.org/>).

Figure S6. The active site and substrate channel of the MCR_{CoBSH} + methyl-SCoM crystal structure. 2Fo-Fc electron density map (contoured at 1 σ) is shown as a blue mesh. The protein is drawn as cartoon. Heterodisulfide is drawn as stick and colored by atom (carbon: purple). Coenzyme F₄₃₀ and HSCoM are drawn as stick colored by atom (carbon: F₄₃₀ dark grey; HSCoM medium grey). The nickel is displayed as a green sphere. The figure was generated using PyMOL (<http://www.pymol.org/>).

Figure S7. Stereo image of interactions involving the two conformers of Val α 482 observed in the MCR_{HSCoM} data. The dominant Val α 482 conformer observed when CoBSH is present, and the dominant conformer when CoBSH is absent, are drawn explicitly as white and maroon stick respectively. The protein residues interacting with the non-coincident Val α 482 side-chain methyl group are drawn explicitly as white stick, and interactions less than 3.8 Å are indicated by dashed lines colored by conformer. Unusually, Asp α 316 is protonated and uncharged, as evidenced by three hydrogen bonds to one of the carboxylate oxygens (the crystallization pH was 7.5). 2Fo-Fc electron density (contoured at 1 σ) is shown as a blue mesh. The protein is drawn as cartoon. CoBSH is drawn as stick and colored by atom (carbon: light green). Water W1 and waters present in the absence of CoBSH are displayed as red spheres. The figure was generated using PyMOL (<http://www.pymol.org/>).

Figure S8. Stereo image of the two conformations of the glycine rich loop seen in the $\text{MCR}_{\text{HSCoM}}$ data overlaid with the previously published alternative conformation. Models are drawn as stick and colored by atom (carbon: F_{430} dark grey; HSCoM, medium grey; CoBSH, light green; main loop conformer, white; alternative conformer, maroon; previously published alternative conformation, faded wheat). 2Fo-Fc electron density (contoured at 1σ) is shown as a blue mesh. Positive peaks in the Fo-Fc difference electron density map (contoured at 4σ), before modeling of the alternative conformation, are shown as a green mesh. The nickel is displayed as a green sphere. The figure was generated using PyMOL (<http://www.pymol.org/>).

Figure S9. Propagation of conformational changes in MCR_{HSCoM}. The overall protein is drawn as cartoon, with the C α traces for the parts of the protein with alternate conformers drawn as stick (main conformer, white; alternate conformer observed in MCR_{HSCoM} data, maroon). The coenzymes are drawn as stick and colored by atom (carbon: F₄₃₀ dark grey; HSCoM medium grey; CoBSH light green). The nickel is displayed as a green sphere. The figure was generated using PyMOL (<http://www.pymol.org/>).

Figure S10. Stereo image of the two different conformers of CoB₉SH. 2Fo-Fc electron density (contoured at 1 σ) is shown as a blue mesh. Positive peaks in the Fo-Fc difference electron density map (contoured at 4 σ), in the absence of the second conformer, are shown as a green mesh. The protein is drawn as cartoon. The coenzymes are drawn as stick and colored by atom (carbon: F₄₃₀ dark grey; HSCoM medium grey; CoB₉SH magenta). The nickel is displayed as a green sphere. The figure was generated using PyMOL (<http://www.pymol.org/>).

Figure S11. (A) EPR spectra of MCR_{red1} sample; (B) MCR_{red1} EPR signal intensity of protein solution during the time course of crystallization.

Scheme S1. Scheme of the characterized forms of MCR. Extension of Figure 3 from Ermler *et al.* (6).