ABSTRACT: Methyl-coenzyme M reductase (MCR) catalyzes the terminal step in the formation of biological methane from methyl-coenzyme M (Me-SCoM) and coenzyme B (CoBSH). The active site in MCR contains a Ni-F430 cofactor, which can exist in different oxidation states. The catalytic mechanism of methane formation has remained elusive despite intense spectroscopic and theoretical investigations. On the basis of spectroscopic and crystallographic data, the first step of the mechanism is proposed to involve a nucleophilic attack of the NiI active state (MCRred1) on Me-SCoM to form a NiIII—methyl intermediate, while computational studies indicate that the first step involves the attack of NiI on the sulfur of Me-SCoM, forming a CH3 radical and a NiII—thiolate species. In this study, a combination of Ni K-edge X-ray absorption spectroscopic (XAS) studies and density functional theory (DFT) calculations have been performed on the NiII (MCRred1), NiIIII (MCRred1-silent), and NiIII—methyl (MCRMe) states of MCR to elucidate the geometric and electronic structures of the different redox states. Ni K-edge EXAFS data are used to reveal a five-coordinate active site with an open upper axial coordination site in MCRred1. Ni K-pre-edge and EXAFS data and time-dependent DFT calculations unambiguously demonstrate the presence of a long Ni—C bond (~2.04 Å) in the NiIII—methyl state of MCR. The formation and stability of this species support mechanism I, and the Ni—C bond length suggests a homolytic cleavage of the NiIII—methyl bond in the subsequent catalytic step. The XAS data provide insight into the role of the unique F430 cofactor in tuning the stability of the different redox states of MCR.

Methyl-coenzyme M reductase (MCR)1 from methanogenic archaea (1) catalyzes the terminal step in biological methane synthesis. Using coenzyme B (CoBSH) as the two-electron donor, MCR reduces methyl-coenzyme M (methyl-SCoM) to form methane and the heterodisulfide product, CoBS—SCoM (2, 3). MCR contains an essential redox active nickel tetrapyrrolic cofactor called coenzyme B (CoBSH) at its active site (4, 5), which is active in the reduced NiI state (MCRred1). All of the biologically generated methane, amounting to 1 billion tons per annum globally, is formed by MCR. Furthermore, recent evidence indicates that anaerobic methane oxidation is also catalyzed by MCR and occurs by a reversal of the methane synthesis reaction (6, 7). The central role of MCR in the synthesis of this important fuel, which

is also a potent greenhouse gas, makes it important to understand the catalytic mechanism of methane formation.

Two limiting mechanisms for MCR-catalyzed methane formation have been proposed (Figure 1). In mechanism I, based on the NiII crystal structure and model chemistry (8–11), NiI performs an S2 attack on the methyl group of methyl-SCoM to form a NiIII—methyl intermediate that undergoes one-electron reduction to form a NiII—methyl species, which then undergoes protonation to form methane, and a CoMS' radical. Condensation of the CoMS' radical with CoBSH generates a CoBSSCoM' radical anion that reduces NiII to the active NiI state. Mechanism II, which is based on density functional theory calculations (12, 13) and considers NiIII not to be a feasible intermediate, proposes that NiI—MCRred1

1 This work has been supported by NIH Grant 1P20RR17675 to S.W.R. We are grateful for funding from a Department of Energy grant (DE-FG02-08ER15931) to S.W.R.

2 To whom correspondence should be addressed. R.S.: e-mail, ritis@slac.stanford.edu; phone, (650) 926-4621; fax, (650) 926-4100.

3 University of Michigan.

Abbreviations: CoBSH, coenzyme B; DFT, density functional theory; ENDOR, electron nuclear double resonance; EPR, electron paramagnetic resonance; EXAFS, extended X-ray absorption fine structure; MCR, methyl-coenzyme M reductase; methyl-SCoM, methyl-coenzyme M; PDB, Protein Data Bank; TD-DFT, time-dependent density functional theory; XAS, X-ray absorption spectroscopy.
reacts with the sulfur of Me-SCoM to generate a methyl radical and a Ni\textsuperscript{II}–thiolate complex. The methyl radical is proposed to abstract a hydrogen atom from CoBSH to generate a CoBS\textsuperscript* radical that reacts with bound CoM to generate Ni\textsuperscript{II} and the same CoBS–SCoM\textsuperscript* radical anion proposed in mechanism I. Finally, reduction of Ni\textsuperscript{II} and generation of the CoBS–SCoM product occur as in mechanism I. The major distinction between the two mechanisms lies in the intermediate generated in the first step of catalysis: Ni\textsuperscript{III}–methyl or methyl radical and Ni\textsuperscript{III}–SCoM. However, so far, none of the proposed intermediates have been trapped during reactions with the natural substrates; presumably, these intermediates form and decay too fast to be observed by stopped-flow and rapid freeze quench EPR methods.

Much of our understanding of the structure and mechanism of MCR catalysis is based on crystal structures of various inactive Ni\textsuperscript{II} states (MCR\textsubscript{silent}, MCR\textsubscript{ox1–silent}, and MCR\textsubscript{red1–silent}) (8, 10, 14), since the crystal structures of the active states of MCR have not been reported. In all states, the central Ni atom is coordinated by four corphin ring nitrogens and a lower axial glutamine oxygen atom. The upper axial ligands in MCR\textsubscript{ox1–silent} and MCR\textsubscript{silent} are the thiol group of coenzyme M and the sulfonate oxygen of the heterodisulfide product, respectively (8, 10, 14). In MCR\textsubscript{red1–silent}, two structures have been proposed: a five-coordinate site lacking an upper axial ligand and a six-coordinate site with the thiol group of coenzyme M as the upper axial ligand (10). EXAFS studies have also been performed on various Ni\textsuperscript{II} forms of MCR, which are consistent with crystal structures; however, high-k EXAFS studies have not been reported (8, 15).

In this study, Ni\textsuperscript{I} and Ni\textsuperscript{III}–methyl species, which are the starting state and a putative intermediate state in the MCR-catalyzed reaction, respectively, have been trapped and characterized. Although a Ni\textsuperscript{III}–methyl intermediate has not yet been identified during the reaction with the natural substrate Me-SCoM, its formation has been demonstrated in the reaction of MCR\textsubscript{red1} with methyl bromide (16) and methyl iodide (17). Analogous Ni\textsuperscript{III}–alkyl species are formed by the reaction of MCR\textsubscript{red1} with corresponding alkyl halides (18–20). Furthermore, this species has been shown to react with HSCoM (and other thiolates) to generate the Ni\textsuperscript{II}–MCR\textsubscript{red1} state and methyl-SCoM (or other alkyl thioethers). The similarity between the rate constants for methane formation from the MCR\textsubscript{Me} species with HSCoM and CoBSH (1.1 s\textsuperscript{-1}) and the steady-state k\textsubscript{cat} for methane formation from natural substrates (4.5 s\textsuperscript{-1} at 25 °C) is consistent with the catalytic intermediacy of the methyl–Ni species (17). Thus, there is significant evidence supporting the catalytic relevance of the MCR\textsubscript{Me} intermediate. Although the crystal structure of active MCR\textsubscript{red1} is not known, much of our understanding about the active site structure in MCR\textsubscript{red1} comes from Ni K-edge EXAFS studies. Two structures have been proposed: a five-coordinate site with an open upper axial ligand (8) and a six-coordinate site with an oxygen atom as the upper axial ligand (15). However, in both studies, the k range for the reported EXAFS data was 2–12 Å\textsuperscript{-1}, limiting the resolution (±0.16 Å) and the ability to identify and distinguish the equatorial and axial ligands.

The goal of this study was to determine high-resolution structures of the active Ni\textsuperscript{II} state with accurate metrical parameters. Although direct structural information about the Ni\textsuperscript{III}–methyl state is not available, EPR, ENDOR, and HYSPECTROscopy studies have determined the electronic structure of the active site Ni center (16, 17). These studies describe MCR\textsubscript{Me} to be formally Ni\textsuperscript{III} with a methyl–Ni bond formed by the oxidative addition reaction of methyl iodide with the Ni\textsuperscript{II}–MCR\textsubscript{red1} complex. The presence of a large 13C hyperfine coupling indicates that the methyl group is coordinated to the paramagnetic Ni\textsuperscript{III} center by a covalent bond, with a Ni–C bond distance of approximately 1.9–2.0 Å (16, 17), although the actual bond distance cannot be precisely determined by analysis of these hyperfine couplings. Similarly, EPR studies on the adduct between propane-sulfonate and MCR demonstrated the presence of a Ni(III)–C bond with an approximate bond distance of 2 Å (21). The active site geometry of the MCR\textsubscript{Me} state has not been determined by any structure determination technique.

In the work described here, Ni K-pre-edge and edge X-ray absorption spectroscopy and EXAFS investigations have been combined with time-dependent density functional theory (DFT) calculations to determine the local geometric structure at the active sites in MCR\textsubscript{red1–silent}, MCR\textsubscript{red1}, and MCR\textsubscript{Me}. The ~0.1 Å resolution (k = 17 Å\textsuperscript{-1}) EXAFS data presented here provide a precise description of the active site geometry in the active MCR\textsubscript{red1} state with the ability to differentiate between the axial Ni–O(Gln) bond distance and the Ni–N(F\textsubscript{320}) distances. This ability to discriminate the axial and equatorial bond distances was not present in previously reported EXAFS data, which extended up to k = 12 Å\textsuperscript{-1} (8, 15). The results presented in this study also provide the first experimentally determined atomic-level description of the Ni center in the proposed intermediate methyl–Ni state with precise Ni–first neighbor bond distance measurements. The trends in the Ni K-pre-edge and edge energy positions are used to determine the changes in bonding and ligand field. Together, the XAS and EXAFS studies are used to provide insight into the electronic structures of the active sites and how they relate to the mechanism of formation of methane by MCR.

**EXPERIMENTAL PROCEDURES**

**Sample Preparation. (i) Material and Organisms. Methanothermobacter marburgensis** was obtained from the Oregon Collection of Methanogens catalogue as OCM82. All buffers, medium ingredients, and other reagents were acquired from Sigma-Aldrich and, unless otherwise stated, were of the highest purity available. Solutions were prepared using nanopure deionized water. N\textsubscript{2} (99.98%), H\textsubscript{2}/CO\textsubscript{2} (80%/20%), and ultra-high-purity (UHP) H\textsubscript{2} (99.999%) were obtained from Cryogenic Gases (Grand Rapids, MI). Ti(III) citrate solutions were prepared from a stock solution of 200 mM Ti(III) citrate, which was synthesized by adding sodium citrate to Ti(III) trichloride (30 wt % solution in 2 N hydrochloric acid) under anaerobic conditions and adjusting the pH to 7.0 with sodium bicarbonate. The concentration of Ti(III) citrate was determined routinely by titrating against a methyl viologen solution.

**Ni K Edge X-ray Absorption Spectroscopy on MCR\textsubscript{red1} and MCR\textsubscript{Me}**

Biochemistry, Vol. 48, No. 14, 2009 3147
added at a flow rate of 1 mL/min during the entire growth period. MCRred1 was generated in vivo and purified as described previously (18). This purification procedure routinely generates ~70% MCRred1 as determined by UV–visible and EPR spectroscopy.

(iii) Preparation of MCRred1 and MCRMe. Samples. MCRred1 was prepared in 50 mM Tris-HCl (pH 7.6) containing 30% glycerol. The MCRMe samples were prepared in the anaerobic chamber by incubating MCRred1 with excess methyl iodide in 50 mM Tris-HCl (pH 7.6). The reaction mixture was split into three aliquots. One sample was transferred to a cuvette to monitor the conversion of the NiII–MCRred1 complex to NiIII/NiIV by UV–visible spectroscopy; a second aliquot was frozen in liquid nitrogen in an EPR tube to measure the concentration of MCRMe species, and a third sample was loaded in 1 mm lucite cells with 37 μm Kapton windows for X-ray absorption studies, frozen in liquid nitrogen, and maintained under liquid N₂ conditions until data were collected. An important point to note is that the reaction of MCRred1 to MCRMe always goes to at least 100% conversion, if not more. This observation has been made repeatedly, and in these studies, we observed a 3% increase in the MCRMe concentration compared to the starting MCRred1 concentration that was used.

X-ray Absorption Spectroscopy. Ni K-edge X-ray absorption spectra of MCRred1–silent, MCRred1, and MCRMe were measured at the Stanford Synchrotron Radiation Laboratory on 16-pole, 2.0 T, wiggler beamline 9-3. A liquid N₂-cooled Si(220) double-crystal monochromator was used for energy selection. A Rh-coated harmonic rejection mirror and a cylindrical Rh-coated bent focusing mirror were used. An Oxford Instruments CF1208 continuous-flow liquid He cryostat was used to maintain the sample temperature at ~10 K throughout the course of data measurement. Data were measured up to k = 18 Å⁻¹ in fluorescence mode using a Canberra Ge-30 element array detector. Internal energy calibration was accomplished by simultaneous measurement of the absorption of a Ni foil placed between two ionization chambers situated downstream of the sample. The first inflection point of the Ni foil was assigned to 8331.6 eV. All samples were closely monitored for photoreduction. However, the Ni active site in all three states of the protein was resistant to photoreduction. Spectra presented here are a 10-scan, 24-scan, and 28-scan average for MCRred1–silent, MCRred1, and MCRMe, respectively. The energy-calibrated data were averaged and processed by fitting a second-order polynomial to the pre-edge region, which was subtracted from the entire spectrum as background using Pyspline (22). A three-region spline function of order 2,3 and 3 was used to model the background atomic absorption and subtracted from the spectrum. Normalization was accomplished by dividing the entire spectrum by a polynomial of order 1, which was fit to the postedge region. The experimental threshold energy was chosen to be 8340 eV (8, 15). The intensities and energies of the pre-edge transitions were quantitated by performing least-squares refinement using EDG–FIT (23). The pre-edge features were modeled by using 1:1 Gaussian/Lorentzian Pseudo-Voigt line shapes to simulate the convolution of instrument and core-hole lifetime broadening. Additional Pseudo-Voigt line shapes were also required to mimic the rising-edge transition and shoulders in the edge region. The data were fit over two different energy ranges: 8125–8135 and 8127–8140 eV. The least-squares error and a comparison of the second derivatives of the data and fit were used to estimate the goodness of the fit. Standard deviations in energy position and intensity were used to quantitate the errors in these parameters. Theoretical EXAFS phase and amplitude parameters were calculated using FEFF (Macintosh version 8.4) (24–26) and the published crystal structure of MCRred1–silent (PDB entry 1MRO) (27) as the initial starting model. Data were fit using EXAFSPAK (23). The metrical parameters obtained by fitting the data indicated significant differences in the local structures of MCRred1, MCRred1–silent and MCRMe around the central absorbing Ni atom. On the basis of these preliminary fits, a new set of theoretical EXAFS signals, χ(k), were calculated, and the data for MCRred1 and MCRMe were refit using the new theoretical parameters generated from their individual refined models. The structural parameters varied during the fitting process were restricted to the bond distance (R) and the bond variance (σ²), which is related to the Debye–Waller factor, resulting from a combination of static and dynamic disorder (due to thermal motion) between the absorber and scatterer pair. The nonstructural parameter, ΔE₀, was also allowed to vary but was restricted to a common value for every component in a given fit. Coordination numbers were systematically varied in the course of a fit but were fixed within a given fit. In the case of MCRred1 and MCRMe, which were estimated to have 36 and 33% of the MCRred1–silent decay product using UV–vis spectroscopy, respectively, partial coordination numbers were also explored during the course of the fit. It should be noted that the EXAFS fits to the data were performed between k = 2 and 17 Å⁻¹. Since, the Fourier transform intensity, I(k), is significant between 1.5 and 4 Å, the number of independent parameters is 26 (using the formula 2δkδR/π + 2) (28). The maximum number of independent parameters used in the fits is 13, which is lower than the number of maximum allowed independent parameters.

DFT Calculations. Gradient-corrected (GGA), spin-unrestricted density functional theory calculations were performed using the Gaussian03 (29) package on a 32-CPU Linux cluster. Geometry optimizations were performed in each case. The B3LYP (30–32) hybrid functional and the following basis sets were employed: triple-ζ 6-311+G* (33–35) on Ni, 6-311G* (33–35) on S, and 6-31G* (36–38) on O, C, H, and N. The input structures were based on the published crystal structure and the EXAFS best-fit results presented herein. The transaxial glutamine ligand was fixed at the EXAFS distance for MCRred1 DFT calculations. Time-dependent DFT calculations were performed with the electronic structure program ORCA (39, 40) to calculate the energies and intensities of the Ni 1s → 3d pre-edge transitions. Single-point ground-state calculations were performed using the geometry-optimized coordinates obtained from the Gaussian03 package. The BP86 functional and the following basis sets were employed: CP(PPP) (41, 42) on Ni (core properties basis set as implemented in ORCA) and TZVP (43, 44) on N, C, H, O, and S. Tight convergence criteria was imposed on all calculations. The calculated energies and intensities were convoluted with a Gaussian function with half-widths of 1.4 eV (45) to account for core-hole and instrument broadening. Calculations were performed in a dielectric continuum using the conductor-like screening
Ni K-Edge X-ray Absorption Spectroscopy on MCR\textsubscript{red1} and MCR\textsubscript{Me}

**RESULTS**

*Ni K-Edge XAS.* The normalized Ni K-edge XAS spectra of MCR\textsubscript{red1}, MCR\textsubscript{red1-silent}, and MCR\textsubscript{Me} are shown in Figure 2 (46). The inset shows the expanded spectra of the second derivative of the pre-edge region. The MCR\textsubscript{red1-silent} data presented here agree well with the previously published XAS data of Duin et al. (8). The pre-edge feature observed at \(\sim 8332\) eV occurs due to an electronic dipole-forbidden quadrupole-allowed transition from the Ni 1s orbital to \(\sim 2.0\) Å, which is absent in both MCR\textsubscript{red1} and MCR\textsubscript{Me}. This formally forbidden transition gains intensity due to devation of the absorbing Ni center from centrosymmetry (49). The pre-edge energy position dominantly reflects the change in the absorbing Ni center from centrosymmetry (49). The pre-edge energy position of MCR\textsubscript{red1} relative to MCR\textsubscript{red1-silent} and MCR\textsubscript{Me} at 8331.5, 8332.0, and 8332.6 eV, respectively (Table 1), indicating an increase in ligand field (LF) felt by the absorbing Ni atom and shifts to a higher energy with an increase in LF (50, 51). Least-squares fits reveal that the pre-edge transitions for MCR\textsubscript{red1}, MCR\textsubscript{red1-silent}, and MCR\textsubscript{Me} occur at 8331.5, 8332.0, and 8332.6 eV, respectively (Table 1), indicating an increase in ligand field on going from MCR\textsubscript{red1} to MCR\textsubscript{red1-silent} to MCR\textsubscript{Me}. In addition to the shift in the pre-edge energy position, an increase in the pre-edge intensity is observed for MCR\textsubscript{Me} relative to MCR\textsubscript{red1} and MCR\textsubscript{red1-silent} (Table 1), indicating a significant increase in the level of 4p mixing in MCR\textsubscript{Me}. A comparison of the Ni K-rising-edge spectra of MCR\textsubscript{red1}, MCR\textsubscript{red1-silent}, and MCR\textsubscript{Me} is shown in Figure 2. The rising-edge energy positions (approximated to the first inflection points) for MCR\textsubscript{red1}, MCR\textsubscript{red1-silent} and MCR\textsubscript{Me} occur at 8341.0, 8342.2, and 8342.4 eV, respectively.

EXAFS. A comparison of the \(k^3\)-weighted Ni K-edge EXAFS for MCR\textsubscript{red1-silent} (red), MCR\textsubscript{red1} (green), and MCR\textsubscript{Me} (blue) and their corresponding Fourier transforms.

![FIGURE 2: (A) Normalized Ni K-edge XAS spectra of MCR\textsubscript{red1-silent} (red), MCR\textsubscript{red1} (green), and MCR\textsubscript{Me} (blue). The inset shows the expanded second-derivative spectrum. (B) First-derivative spectrum showing the edge inflection points.](Image)

**FIGURE 3:** Comparison of the \(k^3\)-weighted Ni K-edge EXAFS for MCR\textsubscript{red1-silent} (red), MCR\textsubscript{red1} (green), and MCR\textsubscript{Me} (blue) and their corresponding Fourier transforms.

*Table 1: Ni K-Pre-Edge and Edge Energy Positions and Intensities.*

| protein       | pre-edge energy (eV) | pre-edge intensity \((\times 10^{-2})^b\) | edge inflection (eV) |
|---------------|----------------------|-------------------------------------------|----------------------|
| MCR\textsubscript{red1} | 8331.5 ± 0.04        | 5.3 ± 1.8^c                                 | 8341.0                |
| MCR\textsubscript{red1-silent} | 8332.0 ± 0.02      | 5.1                                        | 8342.2                |
| MCR\textsubscript{Me}      | 8332.6 ± 0.02        | 15.7                                       | 8342.4                |

^a^ The spectral broadening is \(\sim 1.4\) eV. ^b^ The errors in pre-edge energy position obtained from a statistical analysis over several best fits are given. The systematic error due to monochromator energy drift and calibration is less than 0.04 eV. ^c^ The error in total intensity estimation due to data processing and statistical estimation of the standard deviation is \(\pm 0.5 \times 10^{-2}\). The error in intensity estimation of MCR\textsubscript{red1} is higher due to the presence of low-lying edge transitions.
corrected Fourier transforms (FTs), and the corresponding fits are presented in panels A–C of Figure 4. The EXAFS best-fit parameters are listed in Table 2. In all fits, the addition of a shell was justified on the basis of a significant decrease in the error function. A complete shell-by-shell analysis is presented in the Supporting Information. The first shell of the EXAFS data for MCRred1–silent was fit with four Ni–N components at 2.09 Å, one Ni–S component at 2.41 Å, and one Ni–O component at 2.26 Å. The second and third shells (2.0–4.5 Å region in the FT spectra) were fit with single-scattering (SS) and multiple-scattering (MS) components from the corphin ring. Since the MCRMe sample was contaminated with ~33% of the MCRred1–silent decay form, a 0.33 Ni–S component was included and proved to be necessary for obtaining a good fit. In the case of MCRMe, the first shell of the EXAFS data was fit with five Ni–N components at 2.08 Å and a weak Ni–O component at 2.32 Å. Split first-shell fits were also performed which resulted in a best fit with one Ni–O component at 2.32 Å. However, the error value did not improve significantly to justify the addition of two independent parameters resulting from the split first shell. The second and third shells were fit with single-scattering (SS) and multiple-scattering (MS) components from the corphin ring. Since the MCRMe sample was contaminated with ~33% of the MCRred1–silent decay form, a 0.33 Ni–S component was included and proved to be necessary for obtaining a good fit. For all three data sets, the second and third shell were fit using the same number and type of SS and MS components.

**ANALYSIS**

**Ni K-Pre-Edge TD-DFT Calculations.** The geometric and electronic structure of MCRred1–silent has been well characterized using X-ray diffraction and optical spectroscopic techniques, which indicate a six-coordinate high-spin S = 1 d8 NiII species with a d2z2 ground state (27, 55, 56). Thus, it is expected that two 1s → 3d transitions corresponding to the two singly occupied d2z2 and d2z2–y2 orbitals should be present. However, the spectral broadening at the Ni K-edge is ~1.4 eV which does not allow the two pre-edge features to be well-resolved. In addition, the overlap of the pre-edge with the intense rising-edge transition renders it impossible to separate out the two 1s → 3d transitions. Thus, the average energy of the two 1s → 3d transitions is determined to be 8332.0 eV.

The one-electron reduced MCRred1 form is an S = 1/2 d9 species with a d3z2–y2 ground state, indicating that the d2z2 hole becomes occupied upon reduction and loss of the upper axial ligand (I, 57, 58). The pre-edge energy position in MCRred1 is shifted to lower energy (by 0.6 eV) compared to MCRred1–silent. This is consistent with a decrease in LF associated with the decrease in the number of ligands from six (MCRred1–silent) to five (MCRred1) (see Table 2). It is important to note here that although the ligand field in MCRred1 has decreased relative to that in MCRred1–silent, the energy position (8331.5 eV) is comparable to those of other covalent NiII compounds (59, 60), indicating a strong ligand field at the Ni active site exerted due to the presence of the equatorial F430 cofactor. In MCRred1–silent, the axial bond distances are longer by ~0.04 Å, which might suggest that the pre-edge energy position of MCRred1–silent should be lower than in MCRred1. However, the presence of a strong Ni–S...
lignant in MCR\textsubscript{red1}–silent compensates for the increase in ligand field in MCR\textsubscript{red1} due to shortening of the equatorial Ni–N bonds (see Table 1). This increases the ligand field felt at the Ni center in MCR\textsubscript{red1}–silent and shifts the pre-edge to a higher energy than in MCR\textsubscript{red1}. The rising-edge spectrum of MCR\textsubscript{red1} shows two transitions at 8333.5 and 8334.9 eV. These transitions are usually most intense in four-coordinate square planar complexes in ideal D\textsubscript{4h} geometry and lose intensity as the site symmetry deviates from D\textsubscript{4h} (61, 62). These intense edge features can result from the following effects: long-range multiple-scattering effects that are enhanced at the rising edge due to the larger photoelectron mean free path (63) and a formally forbidden two-electron shakedown process which becomes allowed in the excited state (64, 65). While the shakedown transition is enhanced for compounds that have strong charge transfer transitions or are very covalent (50, 66), both effects are enhanced in the D\textsubscript{4h} symmetry, in which the central atom lies in the plane of the equatorial ligands. The presence of the low-lying edge transition only in the MCR\textsubscript{red1} form indicates that the Ni\textsuperscript{i} center is closer to the corphin plane relative to the MCR\textsubscript{red1}–silent or MCR\textsubscript{Me} forms.

MCR\textsubscript{Me} has been shown to be a d\textsuperscript{3} species with an S = \textfrac{1}{2} ground state (17, 58). This electronic structure allows for a total of three Ni 1s \rightarrow 3d transitions contributing to the Ni K-pre-edge transition. It has been previously shown on the basis of EPR data that the ground state is d\textsubscript{3z\textsuperscript{2}-y\textsuperscript{2}}; thus, the three 1s \rightarrow 3d transitions are to the \beta d\textsubscript{z\textsuperscript{2}-y\textsuperscript{2}} and \alpha and \beta d\textsubscript{y\textsuperscript{2}} orbitals. The pre-edge feature for MCR\textsubscript{Me} has shifted by 1.1 eV to a higher energy compared to that of MCR\textsubscript{red1}, and the intensity has approximately quadrupled. This indicates a dramatic increase in ligand field and Ni 3d-4p mixing. The pre-edge energy position is higher than that for several other Ni\textsuperscript{III} complexes (pre-edge energy position of \textapprox 8332 eV) supporting the increase in ligand field (59, 60). In addition, the EXAFS results indicate that the first-shell coordination has increased from four to five on going from MCR\textsubscript{red1} to MCR\textsubscript{Me} (see Results). Together, the pre-edge and EXAFS data indicate that MCR\textsubscript{Me} has an additional strongly coor-

dinating axial light-atom ligand, consistent with previous EPR/ENDOR (16, 58) studies that show the axial ligand to be a methyl group. The presence of a Ni–C axial interaction is also expected to result in a dramatic increase in the Ni 3d\textsubscript{3z\textsuperscript{2}-y\textsuperscript{2}}-4p mixing and hence the 1s \rightarrow d\textsubscript{z\textsuperscript{2}} (\alpha and \beta) transition intensity; the pre-edge data directly indicate the presence of a methyl group as the upper axial ligand in MCR\textsubscript{Me}.

Interestingly, although the pre-edge energy position shifts to a higher energy with the transition from MCR\textsubscript{red1}–silent to MCR\textsubscript{Me}, the edge inflection points are very similar for the two species (Table 1). This shows that the Q\textsubscript{Ni} in MCR\textsubscript{Me} is similar to that in MCR\textsubscript{red1}–silent (67), indicating that the additional hole in MCR\textsubscript{Me} is spread over the entire corphin ring and the site is best described as [Ni(F\textsubscript{430})Me\textsuperscript{+}]\textsuperscript{−}, where F\textsubscript{430} represents the corphin ring and the positive charge is delocalized (see Discussion for further analysis of the charge distribution).

To support the Ni K-pre-edge analysis, TD-DFT calculations were performed on starting active site structures of MCR\textsubscript{red1}–silent, MCR\textsubscript{red1}, and MCR\textsubscript{Me}, which were generated by considering the crystal structure of MCR\textsubscript{red1}–silent and the EXAFS data presented herein. Selected DFT parameters are listed in Table 3. The calculated spectra on the geometry-optimized structures are presented in Figure 5. The calculated pre-edge energies are 8118.8, 8119.2, and 8119.6 eV for MCR\textsubscript{red1}, MCR\textsubscript{red1}–silent, and MCR\textsubscript{Me}. The trend in energy position is in reasonable agreement with the experimental data (see Table 1).

Fits to the EXAFS data for MCR\textsubscript{Me} indicate the presence of a weak Ni–O component at 2.32 Å. Since the contribution of a relatively long first-shell light atom to the EXAFS data is usually weak, the fits cannot be definitive indicators of the presence of the long Ni–O component. Hence, to test for the presence of this long Ni–O (Gln) axial interaction, TD-DFT calculations were also performed on a MCR\textsubscript{Me} model with no axial glutamine ligand (MCR\textsubscript{Me}–NA). The calculations show that both the relative pre-edge energy and intensity are in worse agreement with the data for the MCR\textsubscript{Me}–NA model relative to the MCR\textsubscript{Me} model, strongly

Table 2: Ni K-Edge EXAFS Least-Squares Fitting Results\textsuperscript{a,b,c}

| path | MCR\textsubscript{red1}–silent | MCR\textsubscript{red1} | MCR\textsubscript{Me} |
|------|-------------------------------|------------------------|---------------------|
| Ni–N | 4.09 (4.07) 298               | 4.05 (4.07) 624        | 5.08 (5.03) 591     |
| Ni–O | 2.26 (2.23) 382               | 2.25 (2.23) 949        | 2.35 (2.31) 951     |
| Ni–S | 2.41 (2.40) 644               | 0.36 (2.40\textsuperscript{a}) 87 | 0.33 (2.40\textsuperscript{c}) 81 |
| Ni–C | 6.00 (5.98) 720               | 6.00 (5.98) 604        | 6.00 (5.98) 641     |
| Ni–CN | 3.20 (3.19) 288               | 3.21 (3.20) 415        | 3.23 (3.20) 291     |
| Ni–CN | 4.43 (4.40) 924               | 4.41 (4.40) 801        | 4.38 (4.35) 588     |

Table 3: Selected DFT Parameters

| model | bond distance (Å)\textsuperscript{a} | Löewdin charge | Mulliken population (%) |
|-------|--------------------------------------|-----------------|-------------------------|
| Ni–N\textsubscript{Me} | 2.06 (2.05) 2.07 2.07 2.07 2.07 | 0.21 (0.21) 0.21 0.21 0.21 0.21 | Ni(3d\textsubscript{y\textsuperscript{2}}+3d\textsubscript{z\textsuperscript{2}-y\textsuperscript{2}})\textsuperscript{a} |
| Ni–C | 2.09 (2.08) 2.09 2.09 2.09 2.09 | 0.22 (0.22) 0.22 0.22 0.22 0.22 | Ni(3d\textsubscript{y\textsuperscript{2}}+3d\textsubscript{z\textsuperscript{2}-y\textsuperscript{2}})\textsuperscript{a} |
| Ni–C | 2.04 (2.03) 2.04 2.04 2.04 2.04 | 0.23 (0.23) 0.23 0.23 0.23 0.23 | Ni(3d\textsubscript{y\textsuperscript{2}}+3d\textsubscript{z\textsuperscript{2}-y\textsuperscript{2}})\textsuperscript{a} |

\textsuperscript{a} The calculations were performed on simplified models (shown in Figure 6). \textsuperscript{b} The numbering of the Ni–N bonds is explained in Figure S2 of the Supporting Information. \textsuperscript{c} The charge on the entire corphin ring. \textsuperscript{d} The combined contribution of the antibonding Ni 3d\textsubscript{z\textsuperscript{2}-y\textsuperscript{2}} and 3d\textsubscript{y\textsuperscript{2}} to the valence orbitals. In the case of MCR\textsubscript{Me}, only 3d\textsubscript{z\textsuperscript{2}-y\textsuperscript{2}} character is present. \textsuperscript{e} Valence Ni 3d\textsubscript{y\textsuperscript{2}} and 3d\textsubscript{z\textsuperscript{2}-y\textsuperscript{2}} character due to back-bonding with filled \pi\textsuperscript{+}-type F\textsubscript{430} orbitals.
EXAFS results indicate that the active site in the equatorial Ni also indicates that the Ni cofactor exhibits a large spread in 2.31 Å. However, fits to the EXAFS data presented here - indicate Ni with the TD-DFT calculated spectra (---); MCR red1 (green), MCRMe (blue), and MCRMe-NA (gray).

indicating the presence of an axial Ni–O(Gln) bond in MCRMe (Figure 5).

**Geometric Structures of MCR red1 and MCRMe.** The Ni K-edge EXAFS results indicate that the active site in MCR red1–silent is six-coordinate with four Ni–N interactions at 2.08 Å, one Ni–S interaction at 2.41 Å, and one weak Ni–O interaction at 2.27 Å. This is in reasonable agreement with the published crystal structure (PDB entry 1HBO with a resolution of 1.78 Å) (10, 68). The crystal structure reveals that MCR red1–silent is a dimer of two αβγ trimers with nearly identical Ni–porphinoid active sites. The crystal structure also indicates that the Ni cofactor exhibits a large spread in the equatorial Ni–N bond distances, ranging from 1.94 to 2.31 Å. However, fits to the EXAFS data presented here show that the first-shell σ2 value is low, indicating an ordered first shell, which in turn suggests that in solution the equatorial bond distances of the Ni–porphinoid ring of MCR red1–silent are more symmetric than in the crystal.

The fit to the Ni K-edge EXAFS data of MCR red1 indicates a disordered first shell with four Ni–N contributions and a weak axial Ni–O interaction at 2.26 Å. This axial interaction is longer than that reported in a previous EXAFS analysis (2.12 Å); however, the EXAFS data presented in the reported study were limited (k = 2–12 Å−1) (8), and an accurate estimate of a weak axial ligand was not possible. Our study reveals that with the transition from MCR red1–silent to MCR red1, the axial –SR group is lost and the site becomes disordered. One interesting aspect of the EXAFS data is that the average Ni–N bond distance decreases from 2.09 to 2.05 Å with the transition from MCR red1–silent to MCR red1 (69). A similar shortening of the bond distance was observed in the EXAFS data for the isolated Ni2Ni3P3Fe30 cofactor (70, 71). This is counterintuitive since the larger Ni2 in MCR red1 should lead to longer Ni–N bond distances. However, this shortening of the bond can be explained on the basis of back-bonding from the Ni2 center to the low-lying π* orbitals on the corphin ring (72). The charge transfer arising due to back-bonding would be consistent with the low-lying charge transfer transitions observed in the MCD spectrum of MCR red1 and low-energy transitions in the Ni K-rising-edge region (73). This is also consistent with the valence Mulliken populations obtained from the DFT calculations, which show significant valence Ni 3dxy, 3dyz, character in the valence low-lying orbitals for MCR red1 (see Table 3). Thus, a combination of the edge data, the pre-edge energy position, and the EXAFS data reveals a five-coordinate, disordered active site with a weak axial Ni–O bond in MCR red1 (Figure 6).

The Ni K-edge EXAFS data for MCRMe are significantly different from those of MCR red1–silent and MCR red1. In particular, the Ni–S contribution seen in MCR red1–silent is not present and the first shell is composed of five light atom ligands [simulated using five Ni–N components (see Table 2)], in contrast to four in MCR red1 and MCR red1–silent. On the basis of the Ni K-pre-edge data and the TD-DFT calculations, the additional light atom ligand is best described as a methyl group. Since the MCRMe sample had ~33% MCR red1–silent contamination, fits with 4.67 Ni–N components and split first-shell fits with 0.67 Ni–C and 4 Ni–N components were attempted. In the 4.67 Ni–N first-shell case, the fit improved slightly (F = 0.26), with very small changes in the σ2 values of the remaining paths. Since the error in EXAFS coordination number determination is 25%, the fit cannot be differentiated from the best fit presented in Table 2. In the 0.67 Ni–C and 4 Ni–N first-shell case, again, the fit improved slightly (F = 0.26); however, the two first-shell paths (2.02 and 2.08 Å) were within the resolution of the data (~0.10 Å), and the first-shell split was not justifiable. However, both fits are consistent with the best fit presented in Table 2 and support an axial Ni–C(methyl) interaction. It is important to note here that the Ni–C(methyl) interaction cannot be separated from the spread of Ni–N interactions. In addition, the σ2 value of the Ni–N component is on the higher side (~591), indicating that there is a spread in the first-shell bond distances, likely due to the presence of a shorter Ni–C(methyl) component. On the basis of the resolution of the data, the lower limit of the Ni–C bond distance is estimated to be 1.99 Å. The Ni–O bond distance from the weak glutamine ligand is ill-determined from the EXAFS fits. Since the Ni–C bond distance can be modulated by the transaxial ligand, DFT calculations were also performed in the absence of the transaxial ligand. The optimized Ni–C bond distance with and without the lower axial Ni–O ligand is 2.0 and 2.04 Å, respectively, which are both consistent with the EXAFS data and Ni–C bonds in model complexes, which range from 1.95 to 2.04 Å (74). In addition, a more than 3-fold increase in the pre-edge intensity of MCRMe relative to MCR red1–silent suggests that the Ni–C bond is shorter than the average Ni–N distance. Thus, a combination of the Ni K-pre-edge intensity and energy position, DFT calculations, and the EXAFS data reveal a six-coordinate active site with a significantly weak axial Ni–O bond and an axial Ni–C bond with a bond distance of ~2.04 Å (average of EXAFS and DFT bond distances) in MCRMe (Figure 6).

**DISCUSSION**

Methyl-coenzyme M reductase catalyzes the reduction of methyl-coenzyme M (methyl-SCoM) with coenzyme B (HSCoB) to methane with the subsequent formation of the heterodisulfide of methyl-SCoM and HSCoB. Several experimental and theoretical studies have been performed in an effort to understand the catalytic mechanism of MCR and the geometric and electronic structures of the intermediates.
in the catalytic cycle, and to date, two mechanisms have been proposed, which differ in the first step: the reaction of MCR_{red1} with methyl-SCoM. The first mechanism involves the formation of an organometallic Ni^{III}--methyl intermediate starting from MCR_{red1} and methyl-SCoM. This is followed by protonolysis to generate methane and the heterodisulfide. The second mechanism involves a direct attack of the Ni^{I} on the S of methyl-SCoM, resulting in a homolytic cleavage of the thioether bond and formation of a Ni^{II}--thiolate species and a CH_{3} radical. The methyl radical reacts with HSCoB to form methane followed by subsequent heterodisulfide formation and reduction of the Ni^{II} species to MCR_{red1}. In the study presented here, the geometric and electronic structures of MCR_{red1} and of a stable Ni^{III}--methyl species (formed from the reaction of MCR_{red1} and MeI) have been elucidated using X-ray absorption spectroscopy.

The formation and stability of this species support the intermediacy of an alkyl--Ni species in the catalytic cycle of MCR, as proposed in mechanism I. The next step in the catalytic cycle (mechanism I) involves the cleavage of the Ni^{III}--methyl bond. This can occur either by homolytic cleavage resulting in Ni^{II} and a CH_{3} radical or by heterolytic cleavage involving two-electron transfer from the methyl group to the Ni center resulting in Ni^{II} and a CH_{3} cation. The results presented in this study indicate that the Ni--C bond in MCR_{Me} is long (74), reminiscent of the long Co^{III}--C bond in adenosylcobalamin (AdoCbl) (∼2.04 Å) (75) and in methylcobalamin (1.96--2.08 Å) (76). In AdoCbl-dependent enzymes, the long weak Co--C bond undergoes a homolytic cleavage forming an Ado^{+} radical, which subsequently initiates radical-based substrate rearrangements (77--80), while in MeCbl, a heterolytic cleavage occurs to leave a methyl cation and Co^{I}. Thus, the long Ni--Me bond in MCR observed by XAS could promote homolysis of the Ni^{III}--methyl bond, which would lead to formation of a methyl radical or enhance the electrophilicity of the methyl group, hence increasing its susceptibility toward nucleophilic attack. In the case of the radical mechanism, the resulting CH_{3} radical can then abstract an H^{+} from HSCoB (either directly or indirectly through another radical intermediate), yielding CH_{4} and SCoB, which subsequently would lead to the formation of CoBS--SCoM and the active MCR_{red1} state of MCR.

In this study, Ni K-pre-edge, rising-edge, and EXAFS data analysis have been combined with TD-DFT calculations on the MCR_{red1--silent}, MCR_{red1}, and MCR_{Me} states of MCR to elucidate the geometric and electronic structure differences. It is shown that the Ni K-pre-edge energies (1s → 3d transition) in all three states of MCR are higher than in most Ni complexes (≤8331.5 eV), which provides direct evidence for the strong Ni--F340 cofactor interaction in MCR. For MCR_{red1--silent}, high-k EXAFS data have been used to show that the geometric structure in solution is very similar to that obtained from the crystal structure, a six-coordinate active site with four Ni--N distances of 2.09 Å, one Ni--S distance of 2.41 Å, and one Ni--O distance of 2.26 Å. The data provide high-resolution first-shell bond distances (±0.02 error in bond distance estimation) that have not yet been achieved by X-ray diffraction measurements. For the MCR_{red1} state, a better understanding of the five-coordinate, reduced Ni^{I} active site has been achieved, and it is shown that the first-shell coordination is distorted with a large distribution of the Ni--N distances. The average Ni--N distance is 2.05 Å, and the shorter axial Ni--O distance is 2.25 Å. The first solution structure of an Ni^{III}--alkyl state of MCR, MCR_{Me}, has been determined, which shows that the active site is six-coordinate with four Ni--N distances of 2.08 Å, one Ni--C bond (∼2.04 Å), and a poorly determined lower axial Ni--O interaction of 2.32 Å.

The EXAFS analysis (combined with the Ni K-edge pre-edge data and DFT calculations) presented here unambiguously demonstrates the presence of a long Ni--C organometallic bond (74), which is attributed to an upper axial Ni^{III}--methyl interaction. The EXAFS data reveal that a weak axial Ni--O interaction is present in all three states of MCR, demonstrating that this axial ligand does not participate in strong bonding with the central Ni atom. It is therefore likely that the lower axial glutamine ligand is present to tune the redox potential and/or to provide stability to the active site.

The XAS data for the different forms of MCR show a unique property: the shift in the edge energy positions is relatively small, indicating that the $Q_{Ni}$ on the Ni is similar in all the three states of MCR, consistent with the Ni K-edge EXAFS data. This demonstrates that the Ni--N bond distances have not changed significantly with the transition from MCR_{red1} to MCR_{red1--silent} to MCR_{Me}. The similarity in charge for the three MCR states is a direct consequence of Pauling’s electroneutrality principle which is manifested in this system by two different mechanisms. Upon reduction of MCR_{red1--silent} to MCR_{red1}, the filled 3d orbitals on the Ni center are destabilized, allowing for back-bonding interaction between the Ni center and the F340 cofactor with a partial...
flow of charge from the Ni to the F$_{430}$ orbitals, and hence, the charge on the central Ni atom remains closer to that in MCR$_{\text{red1}}$--silent (closer to Ni$^{\text{II}}$ than Ni$^{\text{I}}$). This is consistent with the similar Löwdin charges on the Ni center in MCR$_{\text{red1}}$ and MCR$_{\text{red1}}$--silent obtained from DFT calculations (Table 3). Calculations also show a dramatic increase in the Ni $3d_{yz}$ and $3d_{xz}$ hole character due to back-bonding interaction with the filled $\pi^*$ orbitals on the F$_{430}$ ring. This increases the total valence Ni character, leading to similar charges on the Ni center in MCR$_{\text{red1}}$ and MCR$_{\text{red1}}$--silent. With the transition from MCR$_{\text{red1}}$--silent to MCR$_{\text{Me}}$, the additional 3d hole created in MCR$_{\text{Me}}$ (Ni$^{\text{II}}$ in $d^8$ configuration) undergoes covalent delocalization over the entire active site, resulting in a species that is best described as [Ni(F$_{430}$Me)$^+$]. Thus, in this case also, the charge on the central Ni atom remains closer to that in MCR$_{\text{red1}}$--silent. Since the total charge on the MCR$_{\text{red1}}$--silent and MCR$_{\text{Me}}$ models chosen for the DFT calculations are different, a direct comparison of the individual fragment charges will be inaccurate; however, the combined valence Ni 3d character in MCR$_{\text{red1}}$ (150%) and MCR$_{\text{red1}}$--silent (141%) are similar, with only a small increase for MCR$_{\text{Me}}$. This, combined with the small edge shift with the transition from MCR$_{\text{red1}}$--silent to MCR$_{\text{Me}}$, indicates similar charges in the two states. This noninnocent role of the F$_{430}$ cofactor in tuning its bonding with the Ni center in different oxidation states is expected to play a direct role in modulating the geometric and electronic structures of the active site and therefore plays an important role in the catalytic pathway. For example, it might be expected that the Ni$^{\text{II}}$ site in MCR$_{\text{Me}}$ would be very unstable due to a high redox potential and might spontaneously reduce to form a Ni$^{\text{I}}$--methyl species. The stability of the MCR$_{\text{Me}}$ can be attributed to the noninnocent participation of the F$_{430}$ cofactor in bonding, which is consistent with the stability observed in reported biochemical studies performed with this state of MCR. In the case of MCR$_{\text{Me}}$, the low charge on a formally Ni$^{\text{III}}$ species would be expected to increase the pK$_a$ of the coordinating anionic nitrogen on the F$_{430}$ ring and destabilize the Ni--N bond toward dissociation and protonation. Here again, the participation of the F$_{430}$ ring in noninnocent bonding results in an increase in Q$_{\text{Ni}}$ and promotes the stability of the MCR$_{\text{red1}}$--silent species. Thus, the Ni K-edge XAS data indicate that the F$_{430}$ cofactor plays a critical role in stabilizing the different forms of MCR and tuning the reactivity of the protein.

ACKNOWLEDGMENT

SSRL operations are funded by the Department of Energy, Office of Basic Energy Sciences. The SSRL Structural Molecular Biology program is supported by the National Institutes of Health, National Center for Research Resources, Biomedical Technology Program, and the Department of Energy, Office of Biological and Environmental Research.

SUPPORTING INFORMATION AVAILABLE

Figures and table showing the FEFF fits to the Ni K-edge EXAFS data and their corresponding Fourier transforms of the MCR$_{\text{red1}}$--silent Subtracted MCR$_{\text{red1}}$ and MCR$_{\text{Me}}$ forms and the metrical parameters, respectively, and a shell-by-shell analysis of the EXAFS data for MCR$_{\text{red1}}$, MCR$_{\text{red1}}$--silent, and MCR$_{\text{Me}}$. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

1. Thauer, R. K. (1998) Biochemistry of methanogenesis: A tribute to Marjory Stephenson. Microbiology 144, 2377–2406.
2. DiMarco, A. A., Bobik, T. A., and Wolfe, R. S. (1990) Unusual coenzymes of methanogenesis. Annu. Rev. Biochem. 59, 355–394.
3. Ellefmann, J., Hedderich, R., Böcher, R., and Thauer, R. K. (1988) The first step in methane formation: Investigations with highly purified methyl-coenzyme M reductase (component C) from Methanobacterium thermoautotrophicum (strain Marburg). Eur. J. Biochem. 172, 669–678.
4. Ellefson, W. L., Wolfe, R. S., and Whitman, W. B. (1982) Nickel-containing factor F430: Chromophore of the methyl reductase of Methanobacterium thermoautotrophicum. Proc. Natl. Acad. Sci. U.S.A. 79, 3707–3710.
5. Färber, G., Keller, W., Kratky, C., Jaun, B., Pfaltz, A., Spinner, C., Kobelt, A., and Eschenmoser, A. (1991) Coenzyme F430 from methanogenic bacteria: Complete assignment of configuration based on X-ray analysis of 12,13-Diepi-F430 pentamethyl ester and on NMR spectroscopy. Helv. Chim. Acta 74, 697–716.
6. Shima, S., and Thauer, R. K. (2005) Methyl-coenzyme M reductase and the anaerobic oxidation of methane in methanotrophic archaea. Curr. Opin. Microbiol. 8, 643–648.
7. Thauer, R. K., and Shima, S. (2008) Methane as fuel for anaerobic microorganisms. Ann. N.Y. Acad. Sci. 1125, 158–170.
8. Duin, E. C., Cosser, N. J., Mahlert, F., Thauer, R. K., and Scott, R. (2003) Coordination and geometry of the nickel atom in active methyl-coenzyme M reductase from Methanothermobacter marburgensis as detected by X-ray absorption spectroscopy. J. Biol. Inorg. Chem. 8, 141–148.
9. Signor, L., Knuppe, C., Hug, R., Schweizer, B., Pfaltz, A., and Jaun, B. (2000) Methane formation by reaction of a methyl thioether with a photo-excited nickel thiolate: A process mimicking methanogenesis in microorganisms. Eur. J. Inorg. Chem. 2000, 3508–3516.
10. Graberse, W., Mahlert, F., Duin, E. C., Goebeaud, M., Shima, S., Thauer, R. K., Lamzin, V., and Ermeter, U. (2001) On the mechanism of biological methane formation: Structural evidence for conformational changes in methyl-coenzyme M reductase upon substrate binding. J. Mol. Biol. 309, 315–330.
11. Duin, E. C., and McKee, M. L. (2008) A new mechanism for methane production from methyl-coenzyme M reductase derived from density functional calculations. J. Phys. Chem. B 112, 2466–2482.
12. Pelmschenkow, V., and Siegbahn, P. E. (2003) Catalysis by methyl-coenzyme M reductase: A theoretical study for heterodisulfide product formation. J. Biol. Inorg. Chem. 8, 653–662.
13. Pelmschenkow, V., Blomberg, M. R., Siegbahn, P. E., and Crabtree, R. H. (2002) A mechanism from quantum chemical studies for methane formation in methanogenesis. J. Am. Chem. Soc. 124, 4039–4049.
14. Grabarse, W. G., Mahlert, F., Shima, S., Thauer, R. K., and Ermeter, U. (2000) Comparison of three methyl-coenzyme M reductases from phylogenetically distant organisms: Unusual amino acid modification, conservation and adaptation. J. Mol. Biol. 303, 329–344.
15. Tang, Q., Carrington, P. E., Horng, Y. C., Maroney, M. J., Ragsdale, S. W., and Bocian, D. F. (2002) X-ray absorption and resonance Raman studies of methyl-coenzyme M reductase indicating that ligand exchange and macrocycle reduction accompany reductive activation. J. Am. Chem. Soc. 124, 13242–13256.
16. Yang, N., Reider, M., Wang, M., Harmer, J., and Duin, E. (2007) Formation of a nickel-methyl species in methyl-coenzyme M reductase, an enzyme catalyzing methane formation. J. Am. Chem. Soc. 129, 11028–11029.
17. Dey, M., Telser, J., Kunz, R. C., Lees, N. S., Ragsdale, S. W., and Hoffman, B. M. (2007) Biochemical and spectroscopic studies of the electronic structure and reactivity of a methyl-Ni species formed on methyl-coenzyme M reductase. J. Am. Chem. Soc. 129, 11030–11032.
18. Kunz, R. C., Horng, Y. C., and Ragsdale, S. W. (2006) Spectroscopic and kinetic studies of the reaction of bromopropanesulfonate with methyl-coenzyme M reductase. J. Biol. Chem. 281, 34663–34676.
19. Kunz, R. C., Dey, M., and Ragsdale, S. W. (2008) Characterization of the thioether product formed from the thiolytic cleavage of the methyl thioether.
alkyl-nickel bond in methyl-coenzyme M reductase. *Biochemistry* 47, 2661–2667.

Dey, M., Kunz, R. C., Lyons, D. M., and Ragsdale, S. W. (2007) Characterization of alkyl-nickel adducts generated by reaction of methyl-coenzyme M reductase with brominated amino acids. *Biochemistry* 46, 11969–11978.

Hinderberger, D., Piskorski, R. P., Goenrich, M., Thauer, R. K., Schweiger, A., Harmer, J., and Jaun, B. (2006) A nickel-alkyl bond in an inactivated state of the enzyme catalyzing methane formation. *Angew. Chem., Int. Ed.* 45, 3602–3607.

Tenderholt, A. P., Pysplin, and QMForge.

Rehr, J., and Albers, R. (2000) Theoretical approaches to X-ray absorption fine structure. *Rev. Mod. Phys.* 72, 621–654.

Westre, T. E., Kennepolph, P., DeWitt, J. G., Hedman, B., Hodgson, K. O., and Solomon, E. I. (1982) Observation of an electric quadrupole transition in the X-ray absorption spectrum of the Ni(II) complex. *Chem. Phys.* 64, 4146–4156.

Neese, F. (2002) Prediction and interpretation of the Fe-57 isomer shift in Fe(II) systems. *Chem. Phys. Lett.* 34, 595–598.

Rehr, J., Mustre de Leon, J., Zabinsky, S., and Albers, R. (1991) Theoretical X-ray absorption fine structure standards. *J. Am. Chem. Soc.* 113, 5135–5140.

Ermier, U., Grubacek, W., Shima, S., Goubeaud, M., and Thauer, R. K. (1997) Crystal structure of methyl-coenzyme M reductase: The key enzyme of biological methane formation. *Science* 278, 1457–1462.

Stern, E. A. (1993) Number of relevant independent points in X-ray absorption fine structure spectra. *Phys. Rev. B: Condens. Matter Mater. Phys.* 48, 9825–9827.

Pople, J. (2004) *Gaussian 03*, revision C.02.

Lee, C., Yang, W., and Parr, R. (1988) Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Phys. Rev. B: Condens. Matter Mater. Phys.* 37, 785–789.

Mihelich, B., Savin, A., Stoll, H., and Preuss, H. (1989) Results for benzene obtained with the correlation energy density functionals of Becke, Lee, Yang and Parr. *Chem. Phys. Lett.* 157, 200–206.

Becke, A. (1993) Density functional thermochemistry. 3. The role of exact exchange. *J. Chem. Phys.* 98, 5648–5652.

Krishnan, R., Binkley, J., Seeger, R., and Pople, J. (1980) Self consistent molecular-orbital methods. 20. Basis set for correlated wave functions. *J. Chem. Phys.* 72, 650–654.

McGrath, M., and Radom, L. (1991) Extension of Gaussian-1 (G1) theory to bromine-containing molecules. *J. Chem. Phys.* 94, 511–516.

Curtiss, L., McGrath, M., Blaued, J., Davis, N., Blinning, R., and Radom, L. (1995) Extension of Gaussian-2 theory to molecules containing 3rd row atoms Ga-Kr. *J. Chem. Phys.* 103, 6104–6113.

Rassolov, V. A., Pople, J. A., Ratner, M. A., and Windus, T. L. (1998) 6-31G* basis set for atoms K through Zn. *J. Chem. Phys.* 109, 1223–1229.

Harirahan, P., and Pople, J. (1973) The influence of polarization functions on molecular orbital hydrogenation energies. *Theor. Chim. Acta* 25, 209–222.

Francal, M., Pietro, W., Bocian, D., Pigitt, J., Gordon, M., DeFrees, D., and Pople, J. (1982) Self consistent molecular-orbital methods. 23. A polarization type basis set for 2nd-row elements. *J. Chem. Phys.* 77, 3654–3665.

Neesa, F. (2004) ORCA: An ab initio, DFT and semiempirical Electronic Structure Package.

Neesa, F., and Olbrich, G. (2002) Efficient use of the resolution of the identity approximation in time-dependent density functional calculations with hybrid density functionals. *Chem. Phys. Lett.* 362, 170–178.

Neesa, F. (2002) Prediction and interpretation of the Fe-57 isomer shift in M. *Chin. Acta* 337, 181–189.

Szymanski, S., Strobel, S., and Neese, F. (2005) Performance of nonrelativistic and quasi-relativistic hybrid DFT for the prediction of electric and magnetic hyperfine parameters in Fe-57 M spectra. *Inorg. Chem.* 44, 2244–2254.

Schaefer, A., Horn, H., and Ahrlich, R. (1992) Fully optimized contracted Gaussian basis sets for atoms Li to Kr. *J. Chem. Phys.* 97, 2571–2577.

Schaefer, A., Huber, C., and Ahrlich, R. (1994) Fully optimized contracted Gaussian basis sets of triple-ζ valence quality for atoms Li to Kr. *J. Chem. Phys.* 100, 5829–5835.

Krause, M., and Oliver, J. (1979) Natural widths of the atomic K-levels and L-levels, K-alpha X-ray lines and several KLL Auger lines. *J. Phys. Chem. Ref. Data* 8, 329–338.

UV–vis absorption measurements on the MCRred and MCRMe samples showed 36 and 35% MCRred and MCRMe contributions, respectively. The MCRred−silent spectrum has been quantitatively subtracted from the MCRred and MCMa spectra, and the data have been renormalized.
66. Sarangi, R., DeBeer George, S., Rudd, D. J., Szilagyi, R. K., Ribas, X., Rovira, C., Almeida, M., Hodgson, K. O., Hedman, B., and Solomon, E. I. (2007) Sulfur K-edge X-ray absorption spectroscopy as a probe of ligand-metal bond covalency: Metal vs ligand oxidation in copper and nickel dithiolene complexes. J. Am. Chem. Soc. 129, 2316–2326.

67. Craft, J. L., Horng, Y.-C., Ragsdale, S. W., and Brunold, T. C. (2004) Spectroscopic and computational characterization of the nickel-containing F430 cofactor of methyl-coenzyme M reductase. J. Biol. Inorg. Chem. 9, 77–89.

68. The reported average standard deviation in bond distances in 1HBO (Cruickshank’s DPI) is approximately ±0.14 Å (although the standard deviation in the Ni—N bond distances is expected to be better), while that for the Ni—N bond distances obtained from the EXAFS data is ±0.02 Å.

69. Although the resolution of \(k^2 \approx 17\) Å EXAFS data is ~0.1 Å, the standard deviation of the first shell obtained from the EXAFS data presented here is ±0.02 Å.

70. Furenlid, L. R., Renner, M. W., and Fajer, J. (1990) EXAFS studies of nickel(II) and nickel(I) factor 430M. Conformational flexibility of the F430 skeleton. J. Am. Chem. Soc. 112, 8987–8989.

71. Shiemke, A. K., Shelnutt, J. A., and Scott, R. A. (1989) Coordination chemistry of F430: Axial ligation equilibrium between square-planar and bis-aquo species in aqueous solution. J. Biol. Chem. 264, 11236–11245.

72. Dey, M., Kunz, R. C., Van Heuvelen, K. M., Craft, J. L., Horng, Y.-C., Tang, Q., Bocian, D. F., George, S. J., Brunold, T. C., and Ragsdale, S. W. (2006) Spectroscopic and computational studies of reduction of the metal versus the tetrapyrole ring of coenzyme F430 from methyl-coenzyme M reductase. Biochemistry 45, 11915–11933.

73. Duin, E. C., Signor, L., Piskorski, R., Mahlert, F., Clay, M. D., Goenrich, M., Thauer, R. K., Jaun, B., and Johnson, M. K. (2004) Spectroscopic investigation of the nickel-containing porphinoid cofactor F430. Comparison of the free cofactor in the (+)1, (+)2 and (+)3 oxidation states with the cofactor bound to methyl-coenzyme M reductase in the silent, red and ox forms. J. Biol. Inorg. Chem. 9, 563–576.

74. On the basis of ~160 structures submitted to the Cambridge Structure Database (CSD), the average Ni—C(alkyl) bond distance in NiII- and NiI-containing complexes is ~1.98 Å.

75. Ouyang, L., Rulis, P., Ching, W. Y., Nardin, G., and Randaccio, L. (2004) Accurate redetermination of the X-ray structure and electronic bonding in adenosylcobalamin. Inorg. Chem. 43, 1235–1241.

76. Drennan, C. L., Huang, S., Drummond, J. T., Matthews, R. G., and Ludwig, M. L. (1994) How a protein binds B12: A 3.0 Å X-ray structure of B12-binding domains of methionine synthase. Science 266, 1669–1674.

77. Marsh, E. N., and Drennan, C. L. (2001) Adenosylcobalamin-dependent isomerases: New insights into structure and mechanism. Curr. Opin. Chem. Biol. 5, 499–505.

78. Banerjee, R. (2001) Radical peregrinations catalyzed by coenzyme B12-dependent enzymes. Biochemistry 40, 6191–6198.

79. Ludwig, M. L., and Matthews, R. G. (1997) Structure-based perspectives on B12-dependent enzymes. Annu. Rev. Biochem. 66, 269–313.

80. Warncke, K., Schmidt, J. C., and Ke, S. C. (1999) Identification of a rearranged-substrate, product radical intermediate and the contribution of a product radical trap in vitamin B-12 coenzyme-dependent ethanolamine deaminase catalysis. J. Am. Chem. Soc. 121, 10522–10528.