On the Assignment of Nickel Oxidation States of the Ox1, Ox2 Forms of Methyl−Coenzyme M Reductase

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Methyl−coenzyme M reductase (MCR)[1] catalyzes the chemical step of methane formation by methanogenic organisms (eq 1).[2] The reaction involves the two-electron reduction of $CH_3S{-}CoM$ by *N*-7-mercaptoheptanoylthreonine phosphate (CoB−SH). High-resolution structures of two different states of the inactive enzyme have been determined by X-ray diffraction.[3] In these inactive forms, Factor 430 (F_{430}), the nickel tetrahydrocorphinoid cofactor of MCR, is in an "EPR-silent" Ni(II) oxidation state. Other forms of MCR, however, contain F_{430} in EPR−visible states. Among these, the active form, which is denoted red1, is unambiguously assigned to Ni(I), based on the UV−visible and EPR spectral correspondence with isolated Ni(I)F_{430}.[2,4] There are also inactive forms, denoted ox1 and ox2, for which the Ni oxidation state assignment is not so certain; either Ni(I) (d^9) or Ni(III) (d^7) are plausible.[2,5] Addition of the reductant Ti(III) citrate in vitro converts ox1 to red1, and addition of chloroform eliminates the red1 signal, but does not affect ox1.[5,6] Moreover, red1 is formed when the gas phase normally used for cell growth (80% H_2/20% CO_2) is made more reducing (100% H_2) while ox1 is formed when the gas is made less reducing (80% N_2/20% CO_2).[2,7] When H_2-treated cells are incubated in air, an EPR signal similar to ox1 is observed, which is denoted ox2.[2,7] These observations appear to be compatible with a Ni(III) assignment. However, neither the UV−visible nor EPR spectra of ox1 and ox2[5] correspond to those of isolated Ni(III)$F_{430}Me_5$;[8] the EPR spectrum of Ni(III)$F_{430}Me_5$ is typical for a macrocyclic Ni(III) complex.[4,9] Moreover, it has recently been found that ox1 and ox2 states can be generated by treating growing cells with sodium sulfide and dithionite, respectively,[10] which might act as reductants. We have therefore employed 35 GHz EPR and ENDOR[11] spectroscopy to resolve the oxidation state of Ni in the ox1, ox2, and red1 forms of MCR, isolated from *Methanobacterium thermoautotrophicum* strain Marburg and prepared as described previously.[10]

$$CH_3S{-}CoM + CoB{-}SH \rightarrow CH_4 + CoB{-}SS{-}CoM \qquad (1)$$

Table 1. Electronic (***g*** Tensor) Parameters for MCR States and Models Determined by 35 GHz EPR at 2 K

complex	$\mathbf{g} = [g_{1\,(\max)}, g_{2\,(\mathrm{mid})}, g_{3\,(\min)}]$
MCRred1 (this work)	2.2485(5), 2.070(1), 2.060(1)
MCRox1 (this work)	2.229(2), 2.166(2), 2.148(2)
MCRox2 (this work)	2.227(2), 2.140(5), 2.125(5)
Ni^IF_{430} (ref 13)	2.244, 2.063, 2.063
Ni^IOEiBC (ref 13)	2.204, 2.080, 2.063

[a] All samples were in aqueous solution, except for Ni(I)OEiBC, which was in 2-methyltetrahydrofuran solution.

EPR spectra of the holoenzyme states, MCRox1, MCRox2, and MCRred1, as well as those of Ni(I)F_{430} and Ni(I)OEiBC all are roughly axial with $g_{\parallel}$ (∼2.20−2.25) > $g_{\perp}$ > g_e. For MCR red1 and the models, $g_{\perp} \approx 2.06{-}2.07$, while for MCRox1 and ox2, $g_{\perp} \approx 2.14{-}2.15$ (see Table 1; a more extensive compilation is in Table S1 and 35 GHz spectra are shown in Figure S1, Supporting Information). Regardless of the quantitative differences in $g_{\perp}$, this *g* value pattern is characteristic of a transition metal ion with an unpaired electron in the $d_{x^2-y^2}$ orbital, such as the d^9 ions Cu(II) and Ni(I) in tetragonally elongated or square planar geometry.[12] In contrast, as is typical of d^7 ions such as Co(II) or Ni(III) (including Ni(III)$F_{430}Me_5$;[8] see Table S1), an ion with an unpaired electron in the d_{z^2} orbital has $g_{\perp}$ (∼2.2−2.3) > $g_{\parallel}$ ∼ g_e.[12] Thus, the qualitative features of *all* the EPR−visible states of MCR are more characteristic of Ni(I) than of Ni(III) under typical circumstances.

To obtain a more detailed picture of the electronic structure of the ox1, ox2, and red1 states of MCR, we collected CW 35 GHz ENDOR spectra from them, as described earlier for Ni(I)F_{430} and Ni(I)OEiBC.[13] Figure 1 displays the ^{14}N ENDOR spectra[14] for MCRox1, ox2, red1, and Ni(I)OEiBC taken at the magnetic field corresponding to each sample's maximum *g* value ($g_{\max} \equiv g_1 = 2.20{-}2.24$). The spectra are strikingly similar in that in each case the hyperfine couplings to the pyrrole-like ^{14}N at $g_{\max}$ have the value $A(^{14}N) \approx 26$ MHz.[15] Moreover, the "2-D" field-frequency ^{14}N patterns show that the hyperfine interactions all are dominated by an isotropic ^{14}N coupling in the range 26−31 MHz (see Telser et al.[13] and Figures S3, S4, and S5, Supporting Information). The 2-D patterns further show that the pyrrole-like nitrogen ligands of these asymmetric macrocycles are of at least two slightly different types.[16,17] The patterns were analyzed to obtain the ^{14}N hyperfine and quadrupole tensor parameters presented in Table S2 (Supporting Information); the table also includes data for other relevant complexes.

The large, roughly isotropic ^{14}N couplings for the equatorially coordinated pyrrole-like ligands in MCRox1 and ox2 (Table S2)

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(1) Abbreviations used: bpy, 2,2′-bipyridine; CoM, coenzyme M, 2-(methylthio)ethanesulfonic acid; DAPA, 2,6-bis[(1-phenylimino)ethyl]pyridine; F_{430}, nickel tetrapyrrole pentacarboxylic acid cofactor; $F_{430}Me_5$, nickel tetrapyrrole pentamethyl ester cofactor; hfc, hyperfine coupling; OEiBC, octaethylisobacteriochlorin; py, pyridine; tbp, trigonal bipyramidal; TPP, tetraphenylporphyrin.

(2) Thauer, R. K. *Microbiology* **1998**, *144*, 2377−2406.

(3) Ermler, U.; Grabarse, W.; Shima, S.; Goubeaud, M.; Thauer, R. K. *Science* **1997**, *278*, 1457−1462.

(4) Telser, J. In *Structure and Bonding*; Williams, R. J. P., Ed.; Springer-Verlag: Heidelberg, 1998; Vol. 91, pp 31−63.

(5) Goubeaud, M.; Schreiner, G.; Thauer, R. *Eur. J. Biochem.* **1997**, *243*, 110−114.

(6) Rospert, S.; Böcher, R.; Albracht, S. P. J.; Thauer, R. K. *FEBS Lett.* **1991**, *291*, 371−375.

(7) Albracht, S. P. J.; Ankel-Fuchs, D.; Böcher, R.; Ellermann, J.; Moll, J.; van der Zwaan, J. W.; Thauer, R. K. *Biochim. Biophys. Acta* **1988**, *941*, 86−102.

(8) Jaun, B. *Helv. Chim. Acta* **1990**, *73*, 2209−2216.

(9) Lovecchio, F. V.; Gore, E. S.; Busch, D. H. *J. Am. Chem. Soc.* **1974**, *96*, 3109−3118.

(10) Becker, D. F.; Ragsdale, S. W. *Biochemistry* **1998**, *37*, 2369−2647.

(11) Hoffman, B. M.; DeRose, V. J.; Doan, P. E.; Gurbiel, R. J.; Houseman, A. L. P.; Telser, J. In *Biological Magnetic Resonance*; Berliner, L. J., Reuben, J., Eds.; Plenum Press: New York, 1993; Vol. 13, pp 151−218.

(12) Maki, A. H.; Edelstein, N.; Davison, A.; Holm, R. H. *J. Am. Chem. Soc.* **1964**, *86*, 4580−4587.

(13) Telser, J.; Fann, Y.-C.; Renner, M. W.; Fajer, J.; Wang, S.; Zhang, H.; Scott, R. A.; Hoffman, B. M. *J. Am. Chem. Soc.* **1997**, *119*, 733−743.

(14) ^{14}N ($I = 1$) gives first-order ENDOR signals with $\nu\pm(m) = \nu_N \pm A_N/2 + 3P_N(2m - 1)/2$, where P_N is the quadrupole coupling constant with $m = 0, 1$.[11]

(15) The ν-partners in ENDOR spectra of the MCR samples have unpredictable intensity/phase behavior; such effects are commonly seen at 35 GHz.[11]

(16) For MCRox1, it was possible to distinguish four magnetically distinct sets of N ligand. The detailed analysis of these magnetic parameters for all MCRox and red species is in progress.[17]

(17) Telser, J.; Horng, Y. C.; Becker, D. F.; Ragsdale, S. W.; Hoffman, B. M. Manuscript in preparation.

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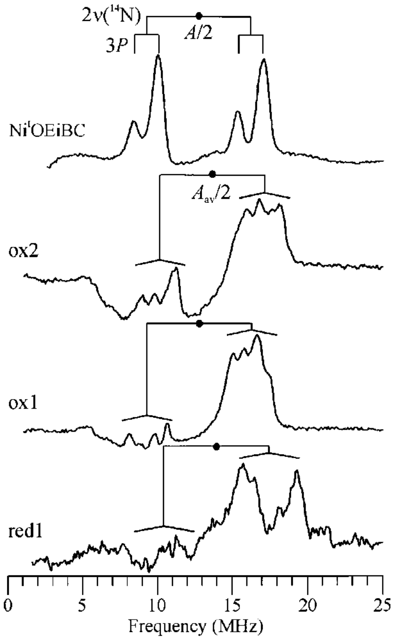

Figure 1. 35 GHz CW ^{14}N ENDOR spectra (2 °K) at g_1 ($g_\parallel$) of the following (top to bottom): Ni(I)OEiBC,[13] MCRox2, MCRox1, and MCRred1. Ni(I)OEiBC shows a single type of ^{14}N at this g value; the closed circle indicates $A/2$, the line passing through the circle indicates twice the ^{14}N Larmor frequency ($2\nu(^{14}N) \approx 7$ MHz), and the "goalposts" indicate the quadrupole splitting ($3P$). For the MCR spectra, for which the line shapes are grossly distorted by a variety of relaxation effects, closed circles indicate half the average hyperfine coupling for ^{14}N ($A_{av}/2$), while the braces above the ν_+ and ν_- branches indicate the quadrupole-split peaks from at least two different types of ^{14}N. Experimental conditions: see Figures S3, S4, and S5 for ox1, ox2, and red1, respectively.

are characteristic of a $d_{x^2-y^2}$ odd-electron ground state, and confirm that these EPR−visible forms of MCR share this state with such d^9 complexes as Ni(I)F_{430} and Cu(II)TPP.[18] In contrast, the d_{z^2} ground state, which is typical of low-spin d^7 complexes, exhibits very weak couplings to the equatorial ligands[19] (e.g., in Co(II)-TPP(py): $a_{iso} = 3.1$ MHz for the pyrrole nitrogens),[20] and instead has extremely strong couplings to axial ligands.[9,21−24]

Could MCRox1 or ox2 nonetheless be a Ni(III) species? Electrochemical data disfavor this assignment. Formation of Ni(III) in macrocyclic complexes occurs at very positive potentials,[8,25−27] requiring strong oxidants such as peroxydisulfate.[24] In contrast, "ox" states appear without the use of strong oxidants. Moreover, we find that "ox1" and "red1" states can coexist in the presence of excess Ti(III) citrate reductant (see Figure S6). Regardless, could the $d_{x^2-y^2}$ ground state exhibited by ox1 and ox2 be consistent with Ni(III) species? A $d_{x^2-y^2}$ ground state could occur in a trigonal bipyramidal (tbp) geometry, and molecular mechanics calculations suggest that such a distortion is possible for the *free* Ni(II)F_{430} ligand.[28] The crystal structures of MCR show F_{430} to be planar with numerous H-bonds to peptide amide nitrogens;[3] thus, the "rigid binding to protein"[3] makes distortion of *bound* F_{430} to tbp geometry less likely. Indeed, there are no examples of tbp geometry with tetraazamacrocyclic ligands.[29] Tripodal ligands are required to obtain isolable tbp complexes of, e.g., Cu(II) and Ni(I),[30,31] and even such a tbp complex of Ni(III), $[NiDAPA(SPh)_2]^+$, gives an EPR spectrum indicating a d_{z^2} ground state (see Table S1).[32]

Another means of obtaining a $d_{x^2-y^2}$ Ni(III)F_{430} is via strong tetragonal compression (see Figure S2), as observed for Ni(III) with two *trans*-cyano ligands in $[Ni(CN)_4(bpy)]^-$,[33] and in $[Ni(TPP)(CN)_2]^-$.[34] The strongest possible ligands for ox1 and ox2 are S-donors,[10] and the structure of $MCR_{ox1-silent}$ indeed shows that CoM coordinates to Ni as CoM−S(H)−Ni(II).[3] However, this ligand is weak and only the upper face is available for an exogneous ligand,[3] and model chemistry indicates that it is unlikely that a single such ligand could invert the d_{z^2} and $d_{x^2-y^2}$ levels in a Ni(III)F_{430} species.[9,22,23,35] Indeed, MCD spectroscopy shows that even the axial coordination of two cyanides to Ni(II)F_{430} gives a tetragonally elongated geometry with the energy of the d_{z^2} orbital well below that of $d_{x^2-y^2}$.[36]

The fact that both MCRox1 and ox2 have roughly the same ^{14}N isotropic hfc as the Ni(I) in red1, $26 \leq a_{iso} \leq 31$ MHz (see Figure 1 and Table S2), invalidates an alternative description for $MCR_{ox1,2}$, i.e., that the ox1,2 EPR signals arise from a radical and a high-spin Ni(II), spin coupled to give total spin, $S = 1/2$ ($RS^\bullet$; $S = 1/2$)−(Ni(II); $S = 1$).

We conclude that neither ox1 nor ox2 are Ni(III) states. The combined spectroscopic and chemical evidence further strongly suggests that not only the red1 but the ox1 and ox2 states as well all contain Ni(I)F_{430}.

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Supporting Information Available: EPR spectra of MCR species and model complexes, tables of electronic and nuclear parameters, and MO diagram for $d^{7,9}$ systems (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Brown, T. G.; Hoffman, B. M. *Mol. Phys.* **1980**, *39*, 1073−1109.

(19) Wirt, M. D.; Bender, C. J.; Peisach, J. *Inorg. Chem.* **1995**, *34*, 1663−1667.

(20) van Doorslaer, S.; Bachmann, R.; Schweiger, A. *J. Phys. Chem., A* **1999**, *103*, 5446−5455.

(21) Ragsdale, S. W.; Lindahl, P. A.; Münck, E. *J. Biol. Chem.* **1987**, *262*, 14289−14297.

(22) Lappin, A. G.; Murray, C. K.; Margerum, D. W. *Inorg. Chem.***1978**, *17*, 1630−1634.

(23) Haines, R. I.; McAuley, A. *Inorg. Chem.* **1980**, *19*, 719−723.

(24) Gore, E. S.; Busch, D. H. *Inorg. Chem.* **1973**, *12*, 1−3.

(25) For example, the following electrochemical potentials (vs NHE) have been reported: for isolated aqueous F_{430}, $E(Ni^{2+/+}) = -0.65$ V;[26] while for isolated $F_{430}Me_5$ in acetonitrile, $E(Ni^{3+/2+}) = +1.45$ V,[8] and analogous complexes in aqueous solution have, e.g., $E(Ni^{3+/2+}) = +1.34$ V.[27]

(26) Holliger, C.; Pierik, A. J.; Reijerse, E. J.; Hagen, W. R. *J. Am. Chem. Soc.* **1993**, *115*, 5651−5656.

(27) Jaacobi, M.; Meyerstein, D.; Lilie, J. *Inorg. Chem.* **1979**, *18*, 429−433.

(28) Zimmer, M.; Crabtree, R. H. *J. Am. Chem. Soc.* **1990**, *112*, 1062−1066.

(29) See Donnelly and Zimmer for an analysis of all structurally characterized tetraazamacrocyclic complexes of Ni: Donnelly, M. A.; Zimmer, M. *Inorg. Chem.* **1999**, *38*, 1650−1658.

(30) Jiang, F.; Karlin, K. D.; Peisach, J. *Inorg. Chem.* **1993**, *32*, 2576−2582.

(31) Barbucci, R.; Bencini, A.; Gatteschi, D. *Inorg. Chem.* **1977**, *16*, 2117−2120.

(32) Baidya, N.; Olmstead, M. M.; Mascharak, P. K. *J. Am. Chem. Soc.* **1992**, *114*, 9666−9668.

(33) Wang, Y. L.; Beach, M. W.; Pappenhagen, T. L.; Margerum, D. W. *Inorg. Chem.* **1988**, *27*, 4474−4472.

(34) Seth, J.; Palaniappan, V.; Bocian, D. F. *Inorg. Chem.* **1995**, *34*, 2201−2206.

(35) Martin, L. Y.; Sperati, C. R.; Busch, D. H. *J. Am. Chem. Soc.* **1977**, *99*, 2968−2981.

(36) Ma, L. Ph.D. Thesis, University of Georgia, 1993.