and 1-decanol have similar effects on the behavior of the system when present in the supporting electrolyte. The influence of the aliphatic alcohols is reversible and persists only during their presence in the bulk of the electrolyte solution.

It seems reasonable to explain these observations by postulating that the alcohol molecules associate with the octadecylviologen layer, likely through intercalation of their hydrocarbon tails into the $C_{18}MV^{2+}$ layer. This then leads to a drastic change of solvation environment around the bipyridyl groups and prevents the formation of electroinactive dimers and/or aggregates. The dimerization of methylviologen cation radical has been known to occur in aqueous solutions and does not take place in media of low polarity.[10] Recently the equilibrium constant for the dimerization of $MV^{\bullet+}$ was observed by Kaifer and Bard to decrease in the presence of anionic micelles and was interpreted to indicate that $MV^{\bullet+}$ species reside in a hydrophobic environment of the micelles' core.[11] The increase of the apparent diffusion coefficient reported above is related to an increase of the fluidity of the $C_{18}MV^{2+}$ monolayer intercalated with alcohol molecules.

The results of the electrochemical experiments reported here constitute, to our best knowledge, the first direct measurements of the lateral electron transport in organized monolayers. These measurements were possible primarily because of the unique geometry of the pore structure of the aluminum oxide films used as substrates.[3a,b] Many intriguing questions related to the dynamics of the electron propagation in self-assembled layers as a function of their composition and the type of intercalating agents are currently under investigation.

Acknowledgment. We gratefully acknowledge the support of the National Science Foundation (CHE-8504368).

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Structural Diversity of F_{430} from *Methanobacterium thermoautotrophicum*. A Nickel X-ray Absorption Spectroscopic Study

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Received December 23, 1985

Nickel metalloenzymes have been found in all methane-producing bacteria examined.[2] Two hydrogenases from *Methanobacterium thermoautotrophicum* contain nickel,[3-5] which has been shown to be coordinated by S-containing ligands in one form.[5]

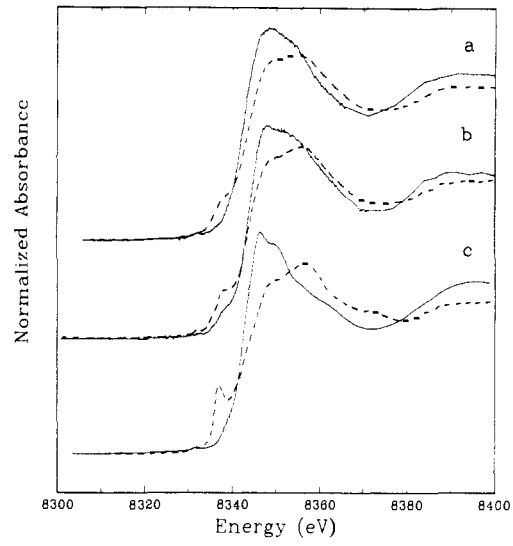

Figure 1. Nickel K absorption edge spectra for various forms of F_{430} from *M. thermoautotrophicum* compared with model compounds: (a) intact component C (solid line) and free F_{430} (dashed line), (b) salt-extracted F_{430} (solid line) and heat-extracted F_{430} (dashed line), and (c) six-coordinate $[Ni(en)_3]^{2+}$ (solid line) and four-coordinate square-planar Ni(ibc) (dashed line). The preedge feature at 8336 eV is a signature for Ni four-coordinate square-planar geometry (see text).

A very similar Ni-containing hydrogenase occurs in *Desulfovibrio gigas*.[6] A compound called F_{430} has been isolated from *M. thermoautotrophicum*, strain ΔH,[7] and has been identified as the Ni-containing prosthetic group in protein component C of the *S*-methyl coenzyme-M reductase system.[8,9] This enzyme complex catalyzes the final step in methanogenesis, the two-electron reduction of the methyl group in *S*-methyl coenzyme M [2-(methylthio)ethanesulfonate] to methane:

$$CH_3SCH_2CH_2SO_3^- + 2e^- + 2H^+ \rightarrow CH_4 + HSCH_2CH_2SO_3^-$$

F_{430} has been established to have a Ni(II)-tetrapyrrole structure,[10] constituting the only known biological example of nickel in a tetrahydro reduced corphine. The detailed mechanistic role of F_{430} in methyl group reduction has not been identified.

We report here a Ni X-ray absorption spectroscopic (XAS) study of several forms of F_{430} and component C isolated from *M. thermoautotrophicum*, strain ΔH. These preliminary results indicate that "free" F_{430}[11] is structurally distinct from the F_{430}-containing protein. Also, depending upon the conditions used, extraction of F_{430} from the protein yields at least two structurally distinct forms, one similar to that in the intact protein and one similar to free F_{430}. Analysis of the extended X-ray absorption fine structure (EXAFS) data and comparison with a number of structurally characterized Ni(II) complexes provide evidence that the flexibility of the tetrapyrrole is responsible for the different structural forms.

M. thermoautotrophicum cell extract was prepared as described previously[12] and component C was prepared from the cell extract following published procedures[13] except that phenyl-Sepharose

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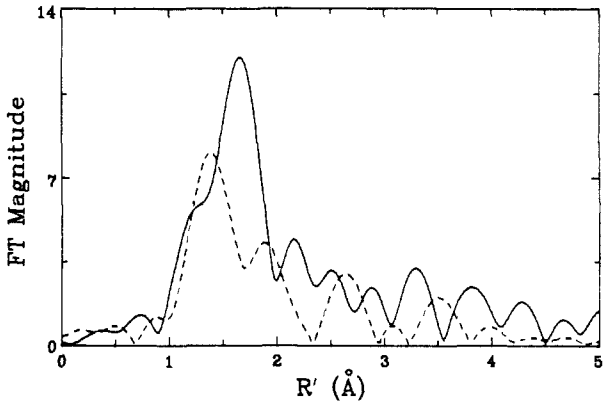

Figure 2. Fourier transforms (k = 3.5–12.5 Å^{-1}, k^3 weighting) of Ni EXAFS of *M. thermoautotrophicum* component C (solid line) and heat-extracted F_{430} (dashed line). The major FT peak of component C represents a single shell of N scatterers at a long Ni–ligand distance, whereas the major FT peak of F_{430} is split, representing two Ni–ligand interactions, the major component being at a shorter Ni–ligand distance.

was used in the final purification step. The specific activity of this preparation was 5800 nmol of CH_4 mg^{-1} h^{-1}. Free F_{430}[11] was purified from the cell-free extract, whereas the other F_{430} samples were derived from component C by heat extraction or salt extraction.[14]

XAS data were collected at the Stanford Synchrotron Radiation Laboratory (SSRL) on wiggler side station beam line VII-3 under dedicated conditions (3.0 GeV) using Si[220] monochromator crystals. All F_{430} and component C data were collected at 4 K by fluorescence excitation monitored by scintillation detectors[15] with Co filters. XAS data of model compounds were collected at 4 K by standard transmission techniques on SSRL beam line II-3. The model compounds used in this study were $[Ni(en)_3]Cl_2 \cdot 2H_2O$ (en = ethylenediamine),[16] Ni(ibc) (ibc = isobacteriochlorin),[17] $[Ni(phen)_3]Cl_2 \cdot 7H_2O$ (phen = 1,10-phenanthroline),[18] and $[Ni([9]aneN_3)_2](ClO_4)_2$ ([9]aneN$_3$ = 1,4,7-triazacyclononane).[19] All data were internally calibrated by using a nickel foil reference.[20]

The Ni K absorption edge spectra of various forms of F_{430} are compared with the model compounds $[Ni(en)_3]^{2+}$ and Ni(ibc) in Figure 1. A preedge feature at ca. 8336 eV can be seen in the free F_{430}, heat-extracted F_{430}, and Ni(ibc) spectra. We characterize this peak as a signature for Ni four-coordinate square-planar geometry. Recently, Smith et al. observed a similar preedge feature in some Cu(II)(d^9) square-planar complexes and assigned it to a $4p_z \leftarrow 1s$ transition.[21] By analogy, we tentatively assign the 8336 eV peak to a $4p_z \leftarrow 1s$ transition in Ni(II)(d^8) square-planar complexes. In agreement with other model compounds, the sharp, somewhat featureless edge rise observed for $[Ni(en)_3]^{2+}$ is indicative of Ni six-coordinate geometry. The resemblance of the edges of free F_{430} and component C (Figure 1a) to the edges of Ni(ibc) and $[Ni(en)_3]^{2+}$ (Figure 1c), respectively, implies that the Ni in free F_{430} is four-coordinate square planar and upon incorporation into the protein the Ni coordination number increases beyond 4. A comparison of the salt- and heat-extracted forms of F_{430} (Figure 1b) shows that the salt extraction apparently results predominantly in a form of F_{430} similar to component C (in terms of the inner Ni coordination sphere), whereas heat extraction produces predominantly the four-coordinate square-planar form similar to free F_{430}.

Figure 2 compares the Fourier transforms (FTs) of the Ni EXAFS data for heat-extracted F_{430} and component C. The main Ni–ligand distance is longer in component C as seen by the shift in position of the major peak in the FT. The obvious split in the largest peak in the F_{430} FT indicates that two Ni–ligand distances are present in the average first-coordination sphere. The outer-shell contributions are due to the carbon atoms in the corphine structure. Curve fitting was performed[22] on filtered first-shell contributions utilizing phase and amplitude functions derived from complex backtransformations[20] of the Ni–N shell of $[Ni(en)_3]^{2+}$. For component C, best fits were obtained for a single Ni–N distance of 2.09 Å and a coordination number of 5 or 6. Studies by Eschenmoser and co-workers have shown that axially coordinated Ni(II) tetrapyrroles adopt a planar conformation with Ni–N distances of about 2.1 Å.[10] In contrast, curve-fitting results for F_{430} data indicate that there are N scatterers at two distinct Ni–N distances, 1.91 and 2.14 Å, with an overall coordination number between 4 and 6.[22] Other workers have observed similar disorder in the F_{430} Ni–ligand distances.[23] As Eschenmoser and co-workers have shown, shorter Ni–N distances occur in four-coordinate nickel corphinates due to an S_4 ruffling of the corphinate framework which compresses the nickel first-coordination sphere.[10] The presence of two different Ni–N distances may be due to an asymmetric corphine ligand that is incompletely ruffled resulting in two or three short and two or one longer Ni–N bonds. Alternatively, sample heterogeneity could explain two Ni–N distances. Heat-extracted F_{430} may be a mixture of the four-coordinate square-planar ruffled structure (short Ni–N bonds) and the six-coordinate structure with a planar corphine and axial ligation (longer Ni–N bonds).

These EXAFS results support the edge spectral assignments for the Ni coordination environments in F_{430} and component C. In component C, F_{430} exists as a planar Ni corphinate with axial ligation (to protein residues?), whereas outside of the protein, F_{430} may adopt at least two different structural forms: a four-coordinate, square-planar form with short Ni–N distances and probably a ruffled corphinate ligand (as in free and heat-extracted F_{430}) or a five- or six-coordinate form with long Ni–N distances and probably a planar corphinate ligand (as in salt-extracted F_{430}). It is attractive to speculate that the substrate-binding and/or methyl-reduction steps of the methyl reductase activity require a planar nickel corphinate with an axial coordination site that would not be available in the ruffled structure.

Acknowledgment. The X-ray absorption experiments were carried out at the Stanford Synchrotron Radiation Laboratory (SSRL) which is supported by the Department of Energy, Office of Basic Energy Sciences, and the National Institutes of Health, Biotechnology Resource Program, Division of Research Resources. XAS work at Illinois under R.A.S. is supported by NSF (DMB 85-02707) and R.S.W. is supported by NSF (DMB 81-18178) and USPHS (AI 12277). R.A.S. is a Presidential Young Investigator and an Alfred P. Sloan Research Fellow.

Supplementary Material Available: Tables of raw Ni XAS data for the following samples: $[Ni(en)_3]Cl_2 \cdot 2H_2O$; [Ni(ibc)]; $[Ni(phen)_3]Cl_2 \cdot 7H_2O$; $[Ni([9]aneN_3)_2](ClO_4)_2$; component C; free F_{430}; salt-extracted F_{430}; heat-extracted F_{430} (50 pages). Ordering information is given on any current masthead page.

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