Resonance Raman Spectroscopic Investigation of Axial Coordination in *M. thermoautotrophicum* Methyl Reductase and Its Nickel Tetrapyrrole Cofactor F_{430}

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The *S*-methyl coenzyme M [CH_3-S-CoM, 2-(methylthio)-ethanesulfonic acid] methylreductase enzyme of *Methanobacterium thermoautotrophicum* contains F_{430}, the nickel–tetrapyrrole cofactor which is thought to be the site of reduction of CH_3-S-CoM to methane and HS-CoM.[1,2] Here we report the use of resonance Raman spectroscopy to investigate the well-characterized forms of isolated F_{430} in aqueous solution and the intact methylreductase. The Raman spectra were obtained on pairs of 100–200 μM samples by using a split cell designed for a Raman difference spectrometer described previously.[3] The spectra were excited at 441.6 nm with a 40 mW defocused, unpolarized beam of a helium–cadmium laser (Omnichrome) operating at 4-cm^{-1} resolution.

It has been known for some time that chromatographically and spectroscopically distinct forms of F_{430} can be isolated, depending on the method used to extract the cofactor.[4] Incubation of methylreductase in concentrated salt solutions causes release of F_{430}, presumably with retention of its native conformation, whereas use of high temperatures during isolation or purification of the cofactor causes the epimerization of both side chains (attached to C_{12}, C_{13}) on pyrrolidine ring C.[5] Therefore, the cofactor obtained by salt extraction from the holoenzyme is referred to as F_{430}, and the isomer obtained by heat treatment is referred to as the F_{430} diepimer. F_{430} was obtained by lithium bromide extraction of the chromophore from the holoenzyme, and the diepimer of F_{430} was purified from the protein-free cytosol of lysed cells by column chromatography.[6] The latter form of F_{430} is identical with that obtained from heat treatment of methylreductase, based on UV–vis spectra and reversed-phase FPLC chromatography.

Figure 1 shows the Raman spectra of methylreductase, F_{430}, and the diepimer in the 1280–1680-cm^{-1} frequency range. The Raman spectra are clearly different, especially in the region of the strong lines above 1500 cm^{-1}, and the spectra of the two forms of the isolated chromophore (Figure 1 (parts b and c)) differ from the previously published spectrum of F_{430}.[7] This earlier spectrum is most similar to that of the F_{430} diepimer (Figure 1c) but contains features of the F_{430} spectrum as well. This is not surprising since the sample used in the earlier work was purified from heat-treated cells and would thus contain mainly diepimeric F_{430}.

Resonance Raman spectroscopy has recently provided useful information concerning the coordination state of nickel porphinoids[8-10] as well as some nickel corphinoids[11] having structures

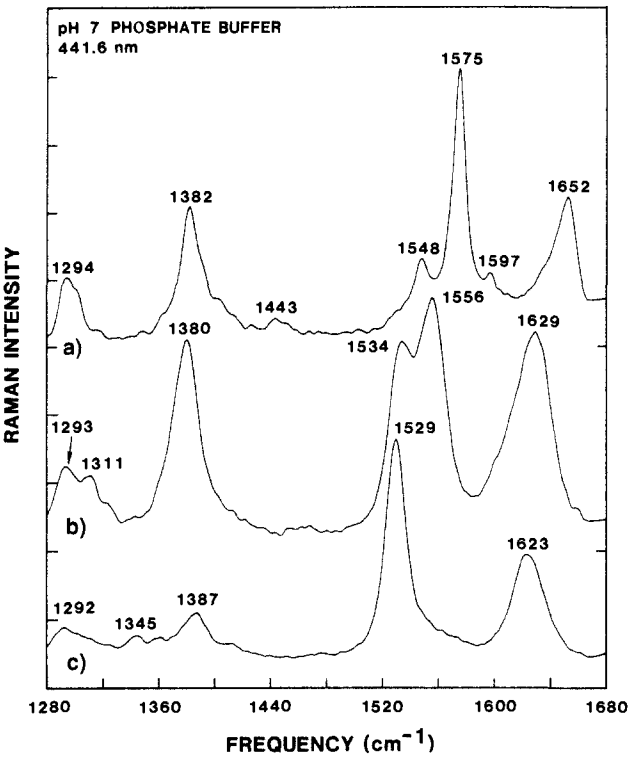

Figure 1. Resonance Raman spectra of methylreductase (a), F_{430} (b), and the diepimer of F_{430} (c) in 10 mM sodium phosphate (pH 7).

Table I. Raman Frequencies and Separation of the Two Strong High-Frequency Lines in Four-, Five-, and Six-Coordinate Complexes of F_{430} Model Compounds[a]

C.N.	ligand	ν_1 (cm^{-1})	ν_2 (cm^{-1})	$\Delta\nu$ (cm^{-1})
4	*b*	(1547)	(1640)	(93)
5	SCN^{-} [b,c]	(1550)	(1631)	(81)
6	MeOH	1556 (1557)	1627 (1628)	71 (71)
6	MeCN	1557	1626	69
6	DMSO	1556	1626	70
6	H_2O	1556	1630	74
6	$Me_2C{=}O$	1556	1628	72
6	Me_2S	1552	1626	74
6	1-methylimidazole	1554	1621	67
6	pyridine	1549	1619	70
6	piperidine	(1555)	(1625)	(70)

[a] Spectra were obtained with 441.6-nm excitation, with the exception of values in parentheses, which are from spectra with 413.1-nm excitation. The model is compound **3** of ref 18; C.N. is the nickel coordination number. Ligand identifies the axial ligands provided by the neat solvents in which the sample was dissolved; $\Delta\nu = \nu_2 - \nu_1$. See ref 11 for experimental details. [b] Spectra obtained in methylene chloride solution. [c] Compound **10** of ref 18.

similar to that proposed[12] for F_{430}. The Raman spectra of the nickel corphins are very similar to F_{430} (in the high-frequency region), and it has been reported that the frequency of the highest energy Raman feature (at ~1630 cm^{-1}) varies inversely with the coordination number of the nickel corphin model.[11] However, upon further study of the corphinoid models with a wide variety of axial

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ligands, we find considerable variation in the frequencies of both high-frequency Raman lines (at ~1540 and ~1630 cm^{-1}, Table I). The *separation* of these lines seems to be a more accurate indication of coordination number. The resonance Raman band frequencies for the nickel corphinoid models shown in Table I demonstrate that this separation is 93 cm^{-1} in the spectrum of the four-coordinate model.[11] However, in this particular case, the separation depends somewhat on the excitation energy, for reasons that are not well understood at this time. Much smaller separations are observed for five- and six-coordinate models (80 and ~71 cm^{-1}, respectively). The 94-cm^{-1} separation of the high frequency lines in the spectrum of the F_{430} diepimer (Figure 1c) indicates that the Raman data are in agreement with nickel X-ray absorption and EXAFS results which show that the F_{430} diepimer is four-coordinate, square planar (with nickel–nitrogen distances of 1.9 Å).[6,13-15]

The simplest interpretation of the F_{430} spectrum (Figure 1b) invokes an equilibrium mixture of two species. The major species has lines at 1556 and 1629 cm^{-1}, whereas the minor species has a peak at 1534 cm^{-1} and a second unresolved feature between ~1622 and ~1632 cm^{-1} (the latter peak is evident from the asymmetry of the 1629-cm^{-1} feature). We note that the separations of these lines are 73 cm^{-1} for the major species and at least 88 cm^{-1} for the minor species. The correlation between peak separation and coordination number (vide supra) would appear to indicate that the major species is six-coordinate, whereas the minor form is four-coordinate.

Comparison of the spectra in Figure 1 (parts b and c) shows that the minor form of F_{430} is not due to contamination of the sample with diepimer: the major peak in the diepimer spectrum occurs at 1529 cm^{-1}, whereas the analogous feature occurs at 1534 cm^{-1} for the minor component in the F_{430} spectrum. One possible explanation for the spectral difference between the diepimer and the minor four-coordinate form of F_{430} is the altered configuration of the pyrrolidine ring C side chains in the diepimer relative to their "native" configuration in both F_{430} species; i.e., the equilibrium between the two species evident in Figure 1b involves changes in axial ligation but not isomerization of the macrocycle. X-ray absorption edge and EXAFS data indicate that in aqueous solution F_{430} is six-coordinate with an expanded 2.1 Å Ni–N core;[6,13,14] the X-ray results contain no evidence for the presence of a four-coordinate form. This apparent conflict may be explained by the difference in sample temperature for the X-ray and Raman experiments (10 and 298 K, respectively), with only the more stable six-coordinate form being present at the lower temperature. Consistent with this proposal is the absence of the 1534-cm^{-1} feature in preliminary low-temperature (77 K) Raman spectra of F_{430}. The nature of the axial ligands in the six-coordinate form of aqueous F_{430} is unknown at present. Further comparison to Raman spectra of model compounds and ligated derivatives of F_{430} should resolve this question. We are also pursuing an X-ray absorption study to determine the nature of the axial ligands.

These additional studies may also help to explain the anomalous nature of the methylreductase spectrum (Figure 1a). Since only F_{430} can be reconstituted into the apoenzyme to give active methylreductase,[16] one would expect the methylreductase spectrum to be more similar to that of F_{430}. Actually, the Raman spectrum of methylreductase is considerably different from that of either F_{430} or the diepimer. The frequencies of the two strong lines in the methylreductase spectrum (1575 and 1652 cm^{-1}) are much higher than the analogous features in the spectra of the isolated cofactor (Figure 1 (parts b and c)). The methylreductase peak separation (77 cm^{-1}) is between that found for the five-coordinate nickel corphinoid model complex (81 cm^{-1}) and the 71 ± 2 cm^{-1} separation observed for the six-coordinate models with a variety of axial ligands (Table I). However, more variability in the separation of the two high-frequency lines is noted for six-coordinate complexes of isolated F_{430}, and separations approaching that of the holoenzyme are observed with bis-pyridine ligation of the isolated cofactor.[17] Although no six-coordinate F_{430} complexes thus far examined reproduce the relatively high frequencies of these lines in methylreductase, it is possible that a six-coordinate cofactor with novel ligation is responsible for the anomalous holoenzyme spectrum. Since the X-ray absorption edge spectrum of methylreductase is apparently inconsistent with a 5-coordinate structure,[13,14] the latter possibility bears consideration.

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Synthesis of $[Mo_6S_8(PEt_3)_6]$ by Reductive Dimerization of a Trinuclear Molybdenum Chloro Sulfido Cluster Complex Coordinated with Triethylphosphine and Methanol: A Molecular Model for Superconducting Chevrel Phases

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The cluster core of the superconducting Chevrel phases is an octahedron of six molybdenum atoms with eight face-bridging chalcogens.[1] The preparation of the soluble molecular complexes with the cluster units found in the nonmolecular inorganic solids has been an attractive synthetic objective.[2] The relationships between the geometry of the cluster core and the cluster valence electron concentration[3] and the energy bands[4] are among the more important problems related to the Chevrel phases. The electronic states of the hypothetical molecular $Mo_6(\mu_3\text{-}S)_8$ compounds have been computed and compared with those of the solid-state Chevrel phases,[5] but the synthesis of a molecular cluster complex with this unit has not been achieved.[6,7]

We now report the first synthesis of a molecular analogue of the Chevrel phases by reductive dimerization of a trinuclear molybdenum sulfido cluster, which itself is a new class of cluster condensation.[8] The trinuclear cluster has been prepared by the

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