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The magnetic properties of the nickel cofactor F430 in the enzyme methyl-coenzyme M reductase of *Methanobacterium thermoautotrophicum*

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Cofactor 430 of methyl-coenzyme M reductase from *Methanobacterium thermoautotrophicum* was studied in both the extracted form in aqueous solution and protein-bound by using low-temperature magnetic-circular-dichroism spectroscopy. In both forms the nickel was present as high-spin paramagnetic nickel(II), spin $S = 1$, subject to almost equal zero-field splitting (cofactor F430, $D = +9.0\,cm^{-1}$, $E/D = 0$; methyl-coenzyme M reductase, $D = +8.5\,cm^{-1}$, $|E/D| = 0.2$). This suggests identical axial co-ordination by oxygen ligand(s) both in aqueous cofactor F430 and in the investigated state of the protein.

INTRODUCTION

Methanogenic bacteria are strict anaerobes that form part of the primary Kingdom of archaebacteria (Jones *et al.*, 1987). Substrates that can provide the carbon moiety for reduction to methane include CO_2, methanol, acetate and CO (Rouvière & Wolfe, 1988).

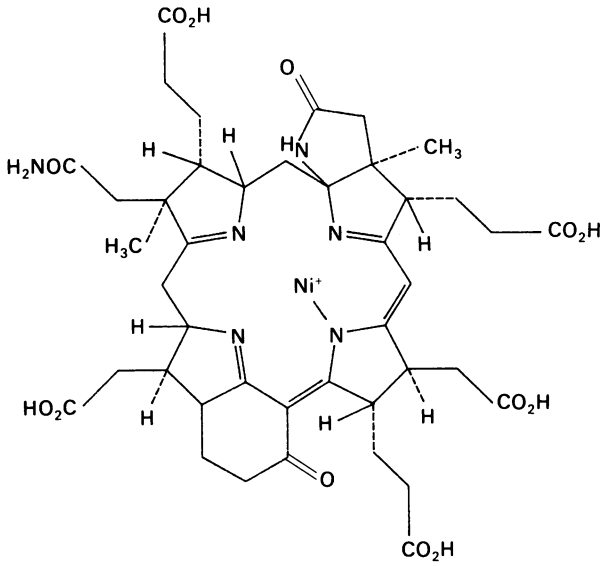
The final step in methane formation is the reduction of methylated coenzyme M [CH_3-S-CoM = 2-(methylthio)-ethanesulphonate] with 7-mercaptoheptanoylthreonine phosphate (H-S-HTP = component B) as electron donor (Bobik *et al.*, 1987; Ellermann *et al.*, 1988):

$$CH_3\text{-S-CoM} + \text{H-S-HTP} \rightarrow \text{CoM-S-S-HTP} + CH_4$$

The enzyme catalysing the reaction is named methyl-CoM reductase (= component C). This is a 300 kDa acidic protein with an $\alpha_2\beta_2\gamma_2$ subunit structure. It contains, at a level of 2 mol/mol of protein hexamer, a tightly bound nickel tetrapyrrole cofactor, cofactor F430 (for references see Ellermann *et al.*, 1988; Rouvière & Wolfe, 1988). The structure of cofactor F430 has been elucidated (see formula) (Pfaltz *et al.*, 1982, 1985; Livingston *et al.*, 1984; Hausinger *et al.*, 1984; Fässler *et al.*, 1985).

Aqueous solutions of coefactor F430 exhibit absorption maxima at 430 nm and 275 nm, which arise from the π–π^* transitions of the unsaturated part of the organic moiety. When bound to the protein, the absorption maximum of the cofactor shifts to 422 nm and a shoulder appears at 445 nm (Ellefson *et al.*, 1982; Ankel-Fuchs *et al.*, 1984). In the present paper we report a comparison between the varied-temperature m.c.d. spectra of an aqueous solution of the extracted cofactor F430 and the native enzyme, methyl-coenzyme M reductase, in order to establish the magnetic parameters of the nickel ion.

Purified methyl-CoM reductase shows only less than 1% of the activity predictable from rates of formation of methane *in vivo* (Ellermann *et al.*, 1988). *In vivo* the enzyme can be in different states, some of which exhibit characteristic e.p.r. spectra that have been assigned to the nickel in cofactor F430 (Albracht *et al.*, 1986, 1988; Jaun & Pfaltz, 1986). A correlation between specific activity and redox state is not yet possible. The results reported in the present paper refer to an e.p.r.-silent state of methyl-CoM reductase with a specific activity of approx. 0.1 μmol of methane/min per mg of protein (Ankel-Fuchs & Thauer, 1986).



Cofactor F430

MATERIALS AND METHODS

Cells of *Methanobacterium thermoautotrophicum* (strain Marburg) were grown as previously described

Abbreviation used: m.c.d., magnetic circular dichroism.
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(Schönheit *et al.*, 1979). Preparations of reductase, from 5 g wet wt. of cells, were carried out at 4 °C as described (Ankel-Fuchs & Thauer, 1986).

For low-temperature m.c.d. experiments, ethanediol (50%, v/v) was added to aqueous solutions of cofactor F430, and glycerol (50%, v/v) was added to buffered (50 mM-Pipes, pH 6.7) aqueous solutions of methyl-coenzyme M reductase. These additions left the absorption spectra unchanged. Concentrations of cofactor F430 were calculated by using $\epsilon_{430} = 23000\ M^{-1}\cdot cm^{-1}$ and concentrations of the enzyme were calculated by using $\epsilon_{422} = 46000\ M^{-1}\cdot cm^{-1}$ (Moura *et al.*, 1983). Absorption, e.p.r. and m.c.d. spectra were measured as previously described (Thomson & Johnson, 1980).

RESULTS

Fig. 1 shows the room-temperature absorption spectrum of extracted cofactor F430 in a solution of water/ethanediol (1:1, v/v). The m.c.d. spectra recorded at 5 T and temperatures of 1.7 K, 4.2 K, 10 K and 120 K are also given in Fig. 1. No e.p.r. signals were detected at X-band over a full-field scan and a range of temperatures down to 4 K. The room-temperature absorption spectrum and low-temperature m.c.d. spectra of methyl-coenzyme M reductase are shown in Fig. 2. The e.p.r. spectrum of this sample of enzyme was also devoid of signals. The m.c.d. spectra of cofactor F430 both in extracted form and in the enzyme are similar in form, giving rise to an intense negative and positive peak and trough between 320 nm and 460 nm, the region of the lowest-energy absorption peak. The fine structure partially resolved on the m.c.d. spectra varies slightly between the two. The m.c.d. spectra are temperature-dependent, decreasing in magnitude as the temperature is raised. This shows that cofactor F430 is paramagnetic both in the extracted state and in the enzyme. The m.c.d. magnetization curves have been recorded at the wavelengths of the major positive and negative features for both the extracted cofactor F430 and the enzyme (Fig. 3). The curves are similar in form to one another, showing that the magnetic state of the nickel ion is the same in the two samples. The intensities of the m.c.d. signals are almost linearly dependent upon magnetic-field strength between 0 and 5 T at a temperature of 1.7 K. This shows that the lowest zero-field component of the ground state is non-degenerate. The oxidation state of the metal ion in cofactor F430 is nickel(II) (Livingston *et al.*, 1984) and the spin states accessible are $S = 0$, for a purely square-planar nickel ion, and $S = 1$, for an octahedral, tetrahedral or five-co-ordinate ion. The m.c.d. magnetization curves have been successfully simulated on the assumption of a magnetic ground state with a spin-only moment of $S = 1$ subject to a zero-field splitting represented by two terms, D and E, the axial and rhombic distortions, in the usual spin–Hamiltonian formalism. The transitions that give rise to the m.c.d. signals are taken to be x–y-polarized, that is in the plane of the tetrapyrrole ring. The simulated curves are also shown in Fig. 3 as continuous lines, whereas the experimental data are marked as points. The m.c.d. magnetization curves at 422 nm in the m.c.d. spectrum of the enzyme were fitted with $D = +8.5 \pm 0.3\ cm^{-1}$ and $|E/D| = 0.2 \pm 0.05$, and those of the m.c.d. spectrum of cofactor F430 at 430 nm were fitted with $D = +9.0 \pm 0.3\ cm^{-1}$ and $E/D = 0 \pm 0.05$. Positive D values of this magnitude constrain the majority

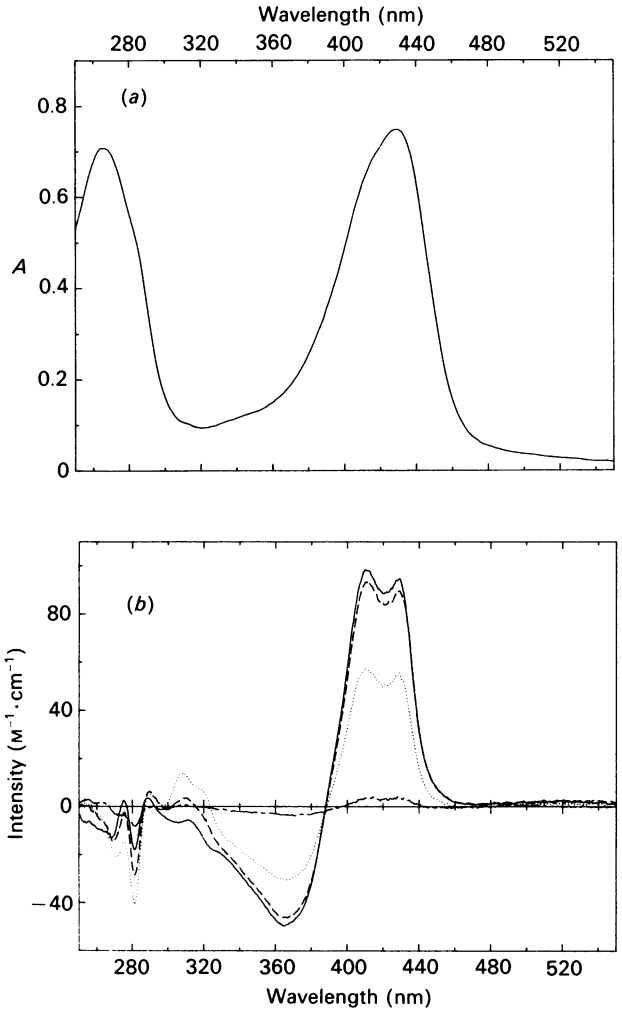

Fig. 1. Room-temperature electronic absorption and low-temperature m.c.d. spectra of cofactor F430

The sample concentration was 325 μM in water/ethanediol (1:1, v/v). The pathlength was 1 mm. (*a*) Room-temperature absorption spectrum. (*b*) M.c.d. spectra recorded with a magnetic field of 5 T. Temperatures: ——, 1.7 K; – – – –, 4.2 K; · · · · ·, 10.0 K; —·—, 120 K.

of the population to the lowest, $m_s = 0$, component of the $S = 1$ ground state when the sample is at 1.7 K. Consequently the m.c.d. signal is linearly dependent on the magnetic field until second-order Zeeman mixing with the $m_s = \pm 1$ components becomes appreciable. The onset of this is apparent by the departure of the 1.7 K line from linearity at high field.

A second method was used to determine D in the case of the enzyme. A plot of the m.c.d. intensity at 422 nm against 1/(absolute temperature, T) was made with the use of magnetic fields at which the m.c.d. is linearly dependent upon field, that is 2.5 T. This plot (Fig. 4) can be fitted to an expression for the m.c.d. intensity, I, of the form:

$$I = (1-\alpha_2)b_1 + \alpha_2 b_2 + \alpha_2(c_2/T)$$

where α_2, the fraction population of the $m_s = \pm 1$ doublet, is given by:

$$\alpha_2 = 2\exp(-DkT)/[1+2\exp(-D/kT)]$$

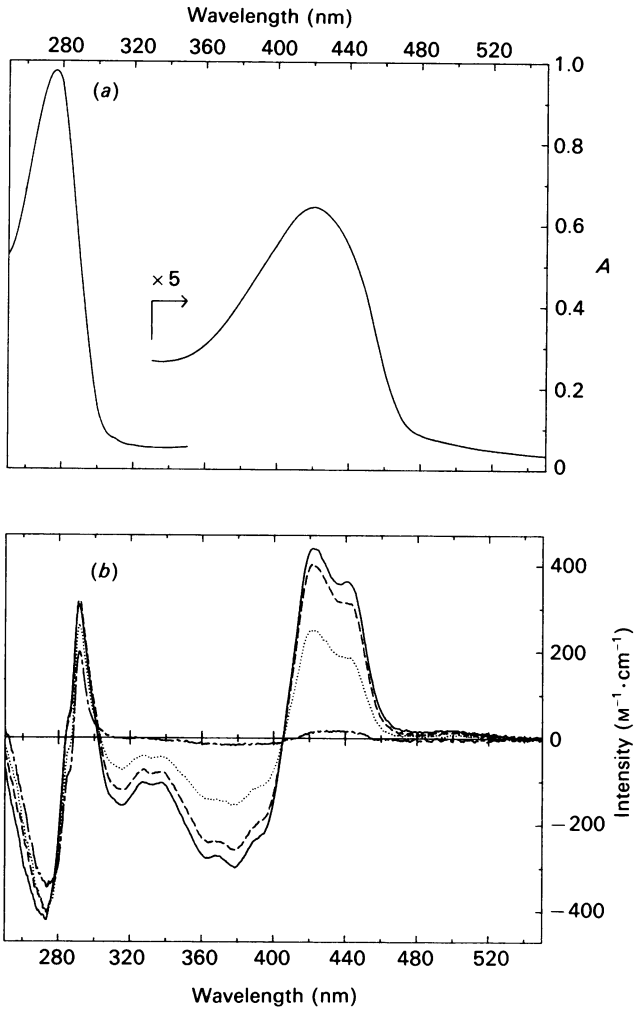

Fig. 2. Room-temperature electronic absorption and low-temperature m.c.d. spectra of methyl-coenzyme M reductase

The sample concentration was 28 μM in 50 mM-Pipes buffer, pH 6.7, containing glycerol (50%, v/v). The pathlength was 1 mm. (*a*) Room-temperature absorption spectrum. (*b*) M.c.d. spectra recorded with a magnetic field of 5 T. Temperatures: ——, 1.6 K; – – – –, 4.2 K; · · · · ·, 10.0 K; — · — · —, 116 K.

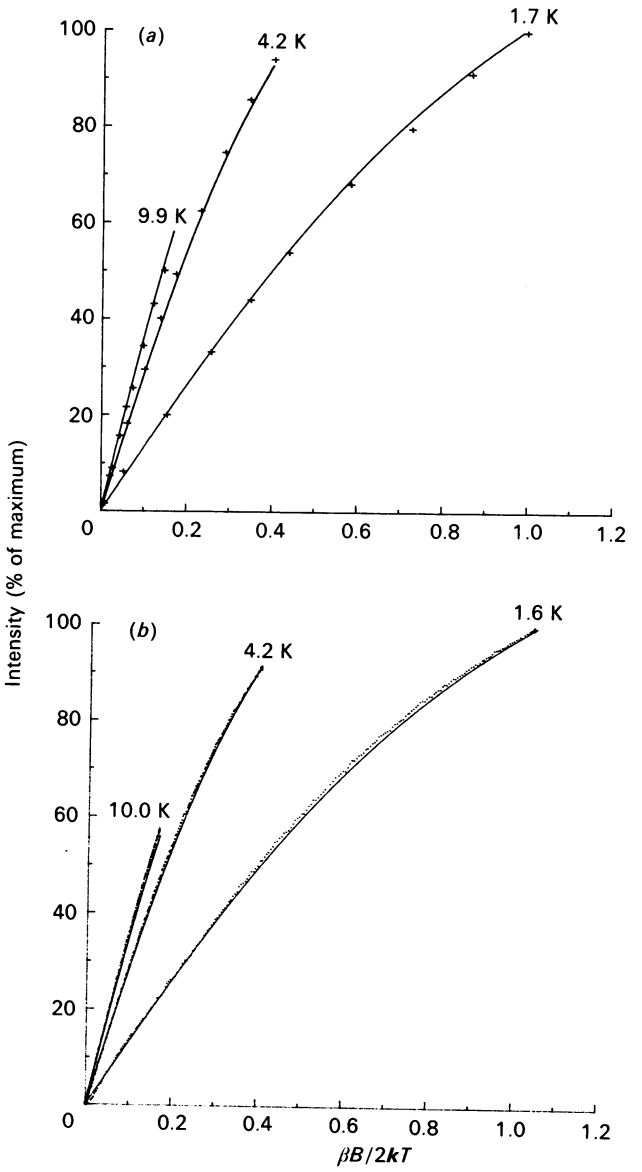

Fig. 3. M.c.d. magnetization data for cofactor F430 and methyl-coenzyme M reductase

(*a*) +, Data recorded for cofactor F430 at a wavelength of 430 nm and at magnetic fields of between 0 and 5 T. The sample was as for Fig. 1 and temperatures were as indicated. Continuous lines indicate the theoretical fits to the data as described in the text. (*b*) · · · · ·, Data recorded for methyl-coenzyme M reductase at a wavelength of 422 nm and at magnetic fields of between 0 and 5 T. The sample was as for Fig. 2 and temperatures were as indicated. Continuous lines indicate the theoretical fits to the data as described in the text.

b_1 and b_2 are the m.c.d. *b* terms arising from the $m_s = 0$ and $m_s = \pm 1$ levels respectively and c_2 is the m.c.d. *c* term of the $m_s = \pm 1$ doublet. This expression ignores splitting of the $m_s = \pm 1$ doublet by rhombic distortions. The best fit to this expression (Fig. 4) gave $D = +8.5 \pm 0.2\ cm^{-1}$ (and $b_1 = 100$, $b_2 = -55$, $c_2 = 20$), showing good agreement with the D value obtained from the simulation of the magnetization curves.

Although the general form of the m.c.d. spectrum and the magnetization properties of cofactor F430 show no marked changes upon co-ordination to the protein, the measured m.c.d. intensities are different. The intensity of $\Delta\epsilon \approx 400\ M^{-1}\cdot cm^{-1}$ at 1.7 K and 5 T and 425 nm for the enzyme is approximately twice that expected for 2 molar equivalents of cofactor F430, which has a $\Delta\epsilon$ value of $\approx 100\ M^{-1}\cdot cm^{-1}$ under the same conditions. The evidence is clear from structural determinations that 2 mol of cofactor F430 is present per mol of protein. An explanation may be that, as released from the protein, cofactor F430 samples include various amounts of a diepimer form (Pfaltz *et al.*, 1985; Shiemke *et al.*, 1988) that is restricted to a square-planar co-ordination of nickel(II), and this remains low-spin diamagnetic. In this case the contribution to the m.c.d. spectrum of this form of the extracted cofactor F430 could be negligible, and we may be underestimating the intensity of the $S = 1$ form of cofactor F430.

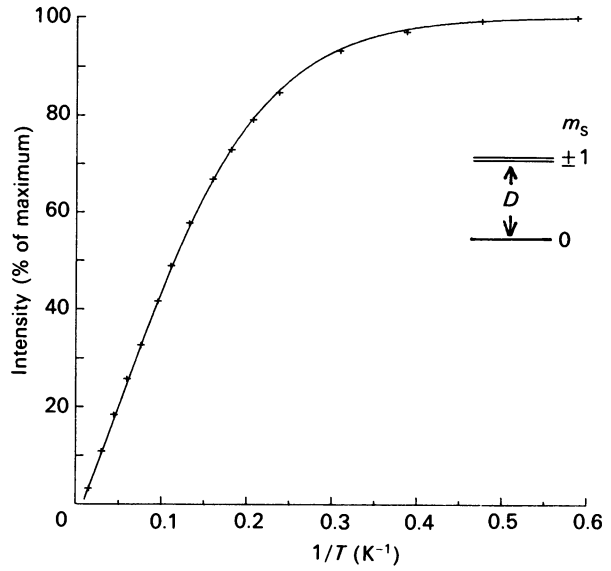

Fig. 4. Temperature-dependence of the intensity of the m.c.d. spectrum of methyl-coenzyme M reductase at 422 nm

+, Experimental data. The continuous line indicates the theoretical fit to the data as described in the text.

CONCLUSIONS

The m.c.d. spectrum of methyl-coenzyme M reductase arising from the cofactor F430 paramagnetic centre is very similar to that of the protein-free aqueous cofactor in the wavelength region 320–520 nm. The m.c.d. magnetization behaviour indicates that the nickel is present in the high-spin nickel(II), $S = 1$, state, which has undergone an axial zero-field splitting, D, of 9.0 cm^{-1} in the case of protein-free cofactor F430 and 8.5 cm^{-1} in the enzyme. This value of D is an indication of the binding strength of the axial ligation to nickel(II) as compared with that of the equatorial tetrapyrrole ligand. It was concluded from studies on the free aqueous cofactor F430 and model chromophores that the high-spin nickel(II) is bound axially by water (Cheesman, 1984). Without axial ligands, the square-planar environment leaves nickel(II) in the $S = 0$ diamagnetic state. The close similarity of the D values in the protein-free and bound forms of cofactor F430 indicates that the nickel environment is similar in both in terms of the basicity of the axial ligands. We therefore conclude that protein-bound cofactor F430, in the e.p.r.-silent form of methyl-CoM reductase, either has water co-ordination or is axially ligated by residues that generate a base strength similar to that of water.

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