An extended-X-ray-absorption-fine-structure (e.x.a.f.s.) study of coenzyme F430 from *Methanobacterium thermoautotrophicum*

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Coenzyme F430 is a nickel porphinoid found in all methanogenic bacteria. Extended-X-ray-absorption-fine-structure (e.x.a.f.s.) spectra have been recorded above the nickel K-edge of coenzyme F430 and two model compounds, (5,10,15,20-tetramethylporphinato)nickel(II) and (5,10,15,20-tetramethylchlorinato)nickel(II). The results show that the four nickel–nitrogen distances in F430 are split, with two nitrogen atoms at 0.192 nm and two at 0.210 nm.

INTRODUCTION

Coenzyme F430 is a yellow nickel porphinoid found in all methanogenic bacteria (Gunsalus & Wolfe, 1978; Diekert *et al.*, 1981). It is the chromophore of the enzyme methyl-CoM reductase (Ellefson *et al.*, 1982), to which the coenzyme is tightly bound. F430 dissociates from the enzyme on denaturation at low pH, at high salt concentrations in 80% (v/v) ethanol, or at high temperature (Ankel-Fuchs *et al.*, 1984; Hausinger *et al.*, 1984). A structure of the protein-free factor has been proposed on the basis of non-crystallographic methods (Fig. 1) (Pfaltz *et al.*, 1982, 1985; Livingston *et al.*, 1984; Huster *et al.*, 1985).

Protein-free F430 has an absorption maximum at 430 nm; methyl-CoM reductase with bound F430 shows an absorption maximum at 422 nm and a shoulder at 445 nm (Ellefson *et al.*, 1982; Ankel-Fuchs *et al.*, 1984). This finding indicates that F430 is altered on binding. The mode of binding is not known. As a first step in the elucidation of this problem, the e.x.a.f.s. of the protein-free F430 was measured. To assist in the assignment of the correct phase shift for nickel, the e.x.a.f.s. of Ni(tmp) and of Ni(tmc), for which X-ray-crystallographic data exists (Gallucci *et al.*, 1982), were recorded.

MATERIALS AND METHODS

Cells (1700 g wet wt.) of *Methanobacterium thermoautotrophicum* (strain Marburg) were extracted five times with 3.7 litres of aq. 50% (v/v) acetone at 0 °C. The extracted cells, which contained F430 bound to methyl-CoM reductase, were suspended in 1.4 litres of water and the pH was adjusted to 2 with HClO₄. After separation by centrifugation (30 min, 16000 g), the pellet was extracted three times with 1.4 litres of 10 mM-HClO₄. The combined supernatants were adjusted to pH 7, diluted to four times the original volume with water, and applied to a QAE-Sephadex column (6 cm × 12 cm) previously equilibrated with 50 mM-Tris/HCl, pH 7. The column was washed with 5 litres of the same buffer containing 70 mM-NaCl. F430 was eluted in 1.7 litres after increase of the NaCl concentration to 300 mM. The fractions

Fig. 1. Proposed structure of coenzyme F430

Abbreviations used: e.x.a.f.s., extended X-ray absorption fine structure; Ni(tmp), (5,10,15,20-tetramethylporphinato)nickel(II); Ni(tmc), (5,10,15,20-tetramethylchlorinato)nickel(II); QAE-, quaternary ammonium ethyl-; F430, coenzyme F430.

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containing F430 were combined, diluted to four times the original volume with water and reapplied to a QAE-Sephadex column (6 cm $\times$ 12 cm), previously equilibrated with 50 mM-Tris/HCl, pH 7. After washing the column with 5 litres of 1 mM-HCl, F430 was eluted with 5 mM-HCl. Chromatography on QAE-Sephadex in HCl was repeated once. After freeze-drying, 100 mg of a yellow material was obtained containing 97 μ mol of F430 as determined photometrically ($\epsilon_{430} = 23\,000 \text{ M}^{-1} \cdot \text{cm}^{-1}$). The M_r of F430 as its chloride is 940. The preparation is therefore more than 94% pure. The release of F430 from methyl-CoM reductase appears to be an irreversible process (Livingston *et al.*, 1984; Hausinger *et al.*, 1984).

X-ray-absorption spectra were obtained at the nickel K-edge in standard transmission mode, on powdered samples, at the Daresbury Synchrotron Radiation Source (Hasnain, 1981). The storage ring was operating at 1.8 GeV and an average current of 100 mA. Harmonic contamination of the incident beam was minimized by using an Si111 monochromator and by choosing a sample thickness, t , such that μt was less than 1, where μ is the attenuation coefficient of the sample. For the factor, three scans were used to produce the averaged spectrum shown in Fig. 2; no evidence of radiation damage was observed when the spectra were compared. Single scans only were collected for the model compounds (Fig. 3). All data were collected at room temperature. Data analysis utilized the single-scattering spherical-wave method for calculating e.x.a.f.s. with phase-shifts derived from 'ab initio' calculations (Lee & Pendry, 1975; Perutz *et al.*, 1982; Alagna *et al.*, 1984). Quality of fit and refinement was examined as described previously (Blackburn *et al.*, 1983).

Fig. 2. Nickel K-edge e.x.a.f.s. of coenzyme F430

E.x.a.f.s. [$\chi(k)$] is presented as $k^3\chi(k)$ plotted versus k , where k is in $\text{\AA}^{-1}$ and is equivalent to 10 nm^{-1} . Continuous lines represent the experimental spectra, broken lines the simulated spectra. (a) 4:0 split; (b) 3:1 split; (c) 2:2 split.

Fig. 3. Nickel K-edge e.x.a.f.s. of (a) Ni(tmp) and (b) Ni(tmc)

E.x.a.f.s. [$\chi(k)$] is presented as $k^3\chi(k)$ plotted versus k , where k is in $\text{\AA}^{-1}$ and is equivalent to 10 nm^{-1} . Continuous lines represent the experimental spectra, broken lines the simulated spectra.

RESULTS

E.x.a.f.s. spectra and theoretical fits of Ni(tmp) and Ni(tmc) are shown in Fig. 3. The theoretical reproducibility of all the features present in the experimental e.x.a.f.s. was achieved only by inclusion of all atoms out to about 0.49 nm.

Table 1 includes the results of a detailed analysis of the e.x.a.f.s. data and also the averaged crystallographic distances of the model compounds. Comparison between the e.x.a.f.s. and crystallographic distances are very good, and in view of this we felt justified in using the phase shifts calculated for these model compounds to analyse the data of coenzyme F430. Fig. 2 shows the experimental data and theoretical fits for the coenzyme F430. These three theoretical spectra are the best fits achieved for a variation of the four Ni-N distances such that: (a) all four Ni-N distances are equivalent at 0.196 nm (we refer to this as a '4:0 split'), (b) three Ni-N distances are equivalent at 0.196 nm and one at 0.214 nm (3:1 split), and (c) two Ni-N distances are equivalent at 0.192 nm and two at 0.210 nm (2:2 split). Table 2 contains the

Table 1. Parameters used to simulate the e.x.a.f.s. associated with the nickel K-edge of Ni(tmp) and Ni(tmc)

R denotes the e.x.a.f.s. distances of atoms from the central nickel ion; R' are average values of non-crystallographically equivalent distances; σ^2 is a Debye–Waller type factor and is equivalent to ΔR^2 r.m.s. (root mean square).

Shell no.	Atom type	Ni(tmp)				Ni(tmc)			
		No.	σ^2 (nm ²)	R (nm)	R' (nm)	No.	σ^2 (nm ²)	R (nm)	R' (nm)
1	N	4	0.00007	0.193	0.195	4	0.00006	0.192	0.193
2	C	8	0.00017	0.297	0.301	8	0.00016	0.293	0.297
3	C	4	0.00007	0.328	0.342	4	0.00008	0.326	0.338
4	C	8	0.00023	0.426	0.423	4	0.00037	0.419	0.418
5	C	—	—	—	—	4	0.00016	0.429	0.423
6	C	4	0.00015	0.486	0.494	4	0.00010	0.485	0.488

Table 2. Parameters used to simulate the e.x.a.f.s. associated with the nickel K-edge of coenzyme F430

R denotes the e.x.a.f.s. distances of atoms from the central nickel ion; σ^2 is a Debye–Waller type factor and is equivalent to ΔR^2 r.m.s. (root mean square).

Shell no.	Atom type	4:0			3:1			2:2		
		No.	σ^2 (nm ²)	R (nm)	No.	σ^2 (nm ²)	R (nm)	No.	σ^2 (nm ²)	R (nm)
1	N	4	0.00026	0.196	3	0.00013	0.195	2	0.00005	0.192
2	N	0	—	—	1	−0.00001	0.213	2	0.00005	0.210
3	C	8	0.00044	0.287	6	0.00030	0.290	4	0.00022	0.286
4	C	0	—	—	2	0.00034	0.310	4	0.00022	0.302
5	C	4	0.00044	0.324	4	0.00037	0.326	4	0.00024	0.327
6	C	8	0.00025	0.434	8	0.00024	0.436	8	0.00027	0.437
7	C	4	0.00053	0.482	4	0.00035	0.455	4	0.00062	0.453
Fit index...		1.0139			0.4311			0.3851		

parameters used to simulate the e.x.a.f.s. associated with the nickel K-edge of coenzyme F430. The fit index improved as we varied the four Ni–N distances as described above. The fit index is defined as:

$$\sum_k [\chi_{\text{exp.}}(k) - \chi_{\text{theor.}}(k)]^2$$

$k = \sqrt{[0.263(E - E_0)]}$, where E = energy of the beam and E_0 = energy zero of the photoelectron wave. $\chi_{\text{exp.}}(k)$ and $\chi_{\text{theor.}}(k)$ are the experimental and theoretical e.x.a.f.s. in k -space.

The nearest eight carbon atoms are split in an analogous way to the four nitrogen atoms, i.e. four carbon atoms at 0.286 nm and four at 0.302 nm. The four carbon atoms at the 5,10,15,20 positions are not split, although their Debye–Waller factor is greater than for the model compounds, suggesting disorder for these atoms.

DISCUSSION

On examination of the 4:0 theoretical fit (Fig. 2a), it is noticed that the peak, occurring at approx. 0.6 nm^{−1}, is larger than the experimental peak; also, theory produces a beat region at approx. 0.825–0.925 nm^{−1}, which is not present in the experimental spectra. In addition, the Debye–Waller factor for the first nitrogen shell is considerably higher than the value obtained from the model compounds. This suggests non-equivalence of the four nearest nitrogen atoms. A high Debye–Waller factor is also found for the eight nearest carbon atoms

at 0.28 nm. In view of this it was decided to split the first shell 3:1.

By using a 3:1 split (Fig. 2b), the fit improved markedly, as seen in the value of the fit index. However, it is to be noted that the Debye–Waller factor for the three nitrogen atoms at 0.195 nm is twice that of the model compounds. The single nitrogen atom at 0.213 nm has a negative value for the Debye–Waller factor, indicating that a higher occupation number is required at this distance. The unsatisfactory values for the Debye–Waller factors led us to investigate a 2:2 split (Fig. 2c). It can be seen that there is a marginal improvement in fit index in going from a 3:1 to a 2:2 split, but a dramatic improvement in Debye–Waller factors to a value almost identical with that of the model compounds. A similar variation is also seen in the first eight carbon atoms, i.e. the Debye–Waller factors tend to the value observed for the eight carbon atoms in the model compounds in going from an 8:0 to a 6:2 to a 4:4 grouping of these atoms.

On the basis of these observations we suggest that the four nitrogen atoms in coenzyme F430 are not equivalent, but two are at 0.192 nm and two at 0.210 nm; also, this splitting is relayed to the first eight carbon atoms and presumably to other atoms in the ring system.

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