Biochem. J. (1990) 266, 929–931 (Printed in Great Britain)

Iron *K*-edge X-ray-absorption spectroscopy of the iron–vanadium cofactor of the vanadium nitrogenase from *Azotobacter chroococcum*

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Iron *K*-edge e.x.a.f.s. data for the iron–vanadium cofactor (FeVaco) from *Azotobacter chroococcum* vanadium nitrogenase reported here provide further evidence for the structural similarity between this and the iron–molybdenum nitrogenase cofactor (FeMoco) from *Klebsiella pneumoniae* molybdenum nitrogenase [Arber, Flood, Garner, Gormal, Hasnain & Smith (1988) Biochem. J. **252**, 421–425]. The e.x.a.f.s. data are consistent with the vanadium being present in a V–Fe–S cluster, thus confirming that the *N*-methylformamide extract of the VFe protein component of *A. chroococcum* vanadium nitrogenase does indeed contain a polynuclear metal–sulphur cluster. Additionally, a long Fe–Fe distance is observed as 0.369 nm, demonstrating the presence of a long-range order in the cluster.

INTRODUCTION

Azotobacter is currently thought to possess three genetically distinct nitrogenase systems (Bishop *et al.*, 1982, 1986; Joerger *et al.*, 1986; Robson, 1986). These are the extensively studied molybdenum nitrogenase system, a vanadium-containing system and a system that appears to contain iron and only small amounts of molybdenum and vanadium (Chisnell *et al.*, 1988). Nitrogenase systems containing vanadium have been identified in both *Azotobacter vinelandii* (Hales *et al.*, 1986*a*,*b*) and *Azotobacter chroococcum* (Robson *et al.*, 1986; Eady *et al.*, 1987). E.p.r. and magnetic-c.d. studies Morningstar & Hales, 1987; Morningstar *et al.*, 1987) on the vanadium system indicates that the VFe proteins possess similar redox centres to the MoFe proteins of the molybdenum system. The vanadium *K*-edge e.x.a.f.s. spectrum has been reported for the VFe proteins of both the *A. chroococcum* $Ac1^V$) (Arber *et al.*, 1987, 1989) and *A. vinelandii* ($Av1^V$) (George *et al.*, 1988) nitrogenase systems. These two systems appear to be structurally very similar. The iron–vanadium cofactor (FeVaco), apparently analogous to the iron–molybdenum cofactor (FeMoco) of molybdenum nitrogenase, has been isolated from $Ac1^V$ (Smith *et al.*, 1988). The present study is of the iron *K*-edge e.x.a.f.s. spectrum of this cofactor.

MATERIALS AND METHODS

$Ac1^V$ was purified as described previously (Eady *et al.*, 1987), and FeVaco was isolated and assayed as described by Smith *et al.* (1988). All operations associated with the isolation of FeVaco were conducted anaerobically under N_2 in a glove-box at < 1 p.p.m. O_2. The extracted cofactor was concentrated by evaporation to a final iron concentration of approx. 7.25 mM before being loaded by syringe into the sample cells, which were then sealed. The sample cells were aluminium with rectangular apertures (15 mm × 5 mm) sealed by Mylar windows glued in place. Filled sample cells were subsequently stored in liquid N_2. The ratio of iron to vanadium of the FeVaco sample used was $6.45 \pm 0.05:1$, within the range of previously published values, and had an activity of 33.4 nmol of C_2H_4 produced/min per nmol of V when assayed as described by Smith *et al.* (1988).

E.x.a.f.s. spectra were recorded in the fluorescence mode with a 13-element germanium solid-state detector on the e.x.a.f.s. station 8.1 at the Daresbury Synchrotron Radiation Source operating at 2 GeV. The average current was 160 mA. A slit-less double-crystal Si(111) monochromator and a toroidal mirror were employed in order to maximize the photon intensity on the sample without any degradation of energy resolution of the monochromatic beam (Dobson *et al.*, 1986). During data collection a liquid-N_2 cryostat was used to maintain a sample temperature of approx. 80 K. Nine scans were recorded and averaged. Data analysis was accompanied via the single-scattering curved-wave method for e.x.a.f.s. calculations and phase shifts were derived from calculations *ab initio* as described previously (Lee & Pendry, 1975; Perutz *et al.*, 1982; Gurman *et al.*, 1984; Hasnain, 1988, and references cited therein).

RESULTS AND DISCUSSION

A comparison of the iron *K*-edge e.x.a.f.s. and Fourier transform of FeVaco and FeMoco (Arber *et al.*, 1988) is shown in Fig. 1. The close similarity of the two systems

Abbreviations used: FeMoco, iron–molybdenum cofactor; FeVaco, iron–vanadium cofactor; nitrogenase components are abbreviated as follows: $Ac1^V$ and $Av1^V$ are the VFe proteins of vanadium nitrogenase of *Azotobacter chroococcum* and *Azotobacter vinelandii* respectively.

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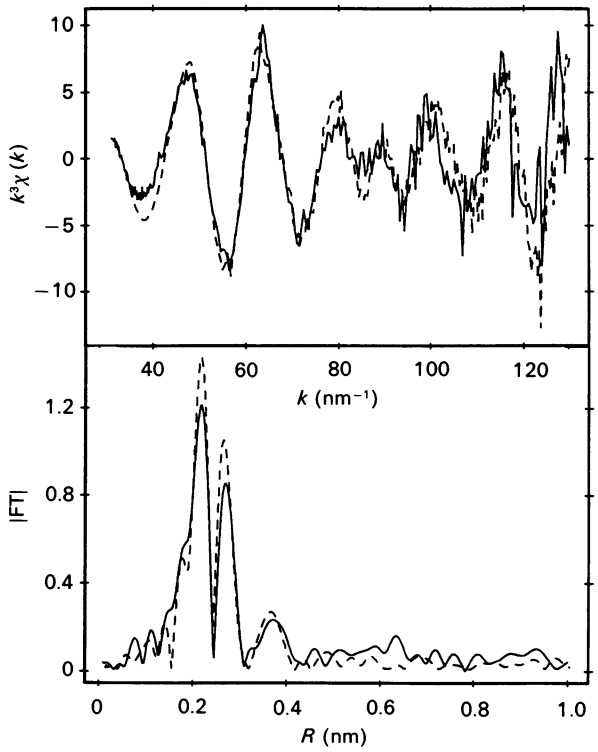

Fig. 1. Comparison of the e.x.a.f.s. and Fourier transforms of FeVaco and FeMoco

——, FeVaco; – – – –, FeMoco.

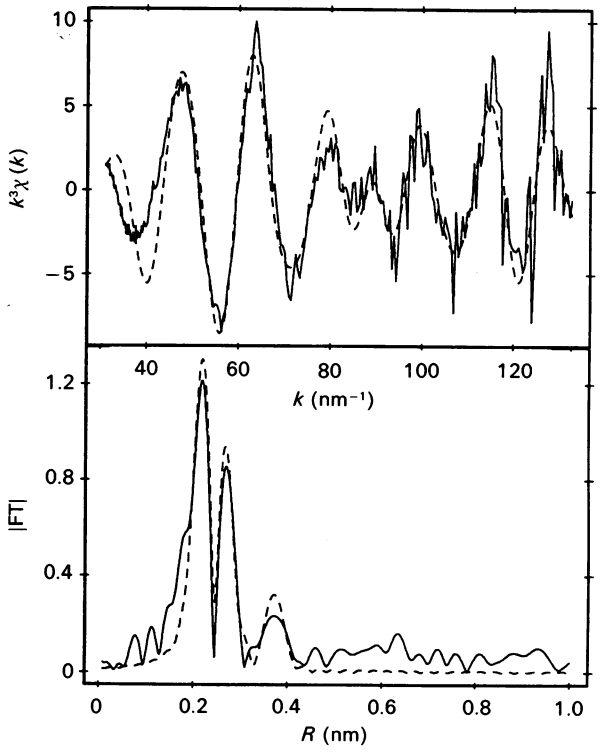

Fig. 2. E.x.a.f.s. and Fourier transform together with the theoretical fit for FeVaco using the parameters of model a given in Table 1

——, FeVaco; – – – –, model a.

is apparent, both in the e.x.a.f.s. data and in their Fourier transforms. The Fourier transforms show that there are three major distances in the co-ordination sphere of iron, and that the back-scatterers are of similar nature and at similar positions for both cofactors.

Because of the overall similarity of the two cofactors, the starting model chosen for the refinement for the FeVaco e.x.a.f.s. data was based on the parameters calculated for FeMoco (Arber *et al.*, 1988), that is a sulphur shell at approx. 0.22 nm, an iron shell at 0.27 nm and a longer iron shell at 0.36 nm. In addition to this, a V–Fe distance of 0.275 nm was included, as this has been clearly defined from the analysis of the vanadium *K*-edge e.x.a.f.s. of Acl^V and Avl^V (Arber *et al.*, 1989; George *et al.*, 1988). Least-squares refinement of this model resulted in an excellent simulation of both the e.x.a.f.s. data and the Fourier transform over the whole data range, as shown in Fig. 2. The difference (observed minus simulated; see Fig. 3) spectrum shows no other back-scattering contribution. The shoulder at approx. 0.19 nm in the Fourier transform is therefore an artifact of background subtraction and/or is due to the atomic e.x.a.f.s. effects. Thus, as in the analysis of the e.x.a.f.s. data for FeMoco, no evidence for a light-element back-scattering approx. 0.18 nm is obtained. The parameters used for this simulation are compared with the iron environment derived from the e.x.a.f.s. data for FeMoco in Table 1. The three major back-scattering distances for the two cofactors are very similar, giving an Fe–S distance of 0.224 nm, an Fe–Fe distance of 0.265 nm and the long Fe–Fe distance of 0.369 nm for the FeVaco as compared with 0.220 nm, 0.262 nm and 0.365 nm. However, the Fe–V distance was found to increase to 0.291 nm on refinement for the FeVaco as compared with the refined Fe–Mo distance of 0.269 nm for FeMoco. This is clearly at variance with the value obtained from the vanadium *K*-edge for the V–Fe distance. The vanadium *K*-edge data provide unique information about this distance because of the absence of interfering back-scattering processes in that case. Therefore the refined value for the Fe–V distance obtained from the iron *K*-edge data cannot be regarded as correct. Refinement of the model with Fe–V distance constrained to the value deduced from the vanadium *K*-edge data gives essentially the same structural parameters for all of the other shells. However, the quality of simulation is significantly inferior both visually and as measured by the fit index (see Table 1). The reason for this apparent discrepancy for the Fe–V distance in the presence of a similar Fe–Fe distance is not clear at this stage, and systematic work on chemical systems with mixed second shells containing iron and vanadium back-scattering atoms may prove helpful.

The identity of the back-scatterer making up the third shell (0.369 nm) is open to some debate. The back-scattering contributions from a vanadium or iron atom located at approx. 0.37 nm are rather similar, and thus an unambiguous assignment is not feasible. However, we note that the quality of the fit is slightly better for this shell if iron is taken as the back-scatterer. Additionally, this assignment is supported by the analysis of the equivalent shell in FeMoco (Arber *et al.*, 1988).

In conclusion, the iron *K*-edge e.x.a.f.s. data presented here add further support to the view that FeVaco and

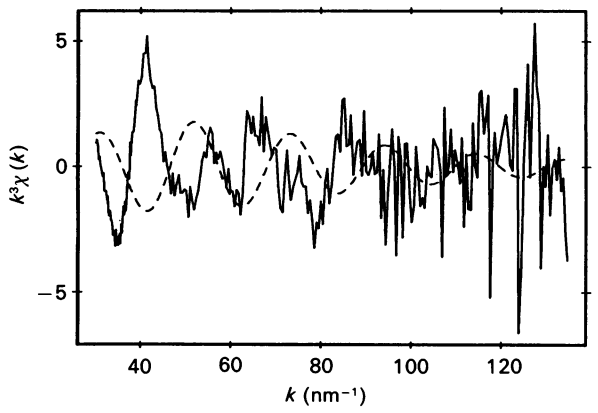

Fig. 3. E.x.a.f.s. difference spectrum for FeVaco using the parameters of model a given in Table 1

——, Theoretical spectrum for FeVaco; – – – –, theoretical spectrum for an O atom at 0.19 nm.

Table 1. Comparison of the iron *K*-edge e.x.a.f.s. parameters for FeVaco (present work) and for FeMoco (Arber *et al.*, 1988)

For FeVaco: $\Delta E_0 = 9.15$ eV; energy range, 14–753 eV. Errors in bond distances are considered to be $\leqslant \pm 0.003$ nm.

Model	Cofactor	Atom	*N*	*R* (nm)	$2\sigma^2$ (nm^2)	Fit index
a	FeVaco	S	3	0.224	0.00011	6.79
		Fe	2	0.265	0.00010	
		V	1	0.290	0.00011	
		Fe/V*	1	0.369	0.00008	
b	FeVaco	S	3	0.224	0.00011	9.38
		Fe	2	0.263	0.00011	
		V	1	0.275	0.00019†	
		Fe/V*	1	0.369	0.00008	
	FeMoco‡	S	3	0.220	0.00009	2.84
		Fe	2	0.262	0.00011	
		Mo	1	0.269	0.00008	
		Fe	1	0.365	0.00009	

* Parameters are for iron as the back-scatterer.

† Values constrained, and taken from the vanadium *K*-edge e.x.a.f.s. of Acl^V (Arber *et al.*, 1989).

‡ For FeMoco, parameters are slightly different from those published and have been obtained by using the same phase shifts etc. as used for the FeVaco. $\Delta E_0 = 12.8$ eV; energy range, 14–842 eV.

FeMoco are structurally very similar and confirm that the *N*-methylformamide extract of Acl^V contains a polynuclear metallosulphur cluster. The cluster possesses long-range order and a mixed iron/vanadium second shell. Although the synthetic cluster $\{VFe_3S_4Cl_3[HCON(CH_3)_2]_3\}^-$ (Kovacs & Holm, 1986) acts as a reasonable model for both the vanadium and the iron *K*-edge e.x.a.f.s. of FeVaco, no cluster has yet been synthesized that mimics both the mixed second shell and long-range structure now shown to be present in both FeVaco and FeMoco.

Received 27 November 1989/19 January 1990; accepted 25 January 1990

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