J. Synchrotron Rad. (1999). **6**, 409–410

The penta-coordinated vanadium formed on binding of ADP-vanadate-Mg(II) to CF_1-ATPase functions as a transition-state inhibitor

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The structure of vanadate, a phosphate analogue which functions in the presence of tightly bound ADP and divalent cations as a transition state inhibitor of CF_1-ATPase, was investigated by X-ray absorption spectroscopy. There was a decrease in the intensity of the pre-edge transition and a change in the shape and energy of the *K*-edge upon binding of ADP-vanadate-Mg(II) to CF_1. The changes were due to alterations in the structure of vanadium from tetrahedral to five-coordinated trigonal bipyramidal geometry. Simulation of the edge shape and energies and EXAFS analysis confirmed the presence of pentacoordinated vanadium bound to the enzyme. This structure was analogous to the proposed transition state of the phosphate during the synthesis and the hydrolysis of ATP by CF_1.

Keywords: ATP synthase, transition state, kinetics, MgADP, EXAFS, k-edge, vanadate.

1. Introduction

The catalytic sector of the chloroplast H^+-ATPase (CF_1) is similar to F_1-ATPase from bovine heart mitochondria, the crystal structure of which was determined at a resolution of 2.8 Å (Abrahams et al. 1994). The structure supports a catalytic mechanism in which the three interacting catalytic sites, at the 3b subunits, are alternately in a different state of the catalytic cycle and in a changed conformation (Boyer, 1993). The resolved structure confirms our earlier results, obtained by X-ray absorption, of the metal at the three cooperative interacting active sites of the enzyme. The analysis revealed the existence of a ternary complex of enzyme, Mn(II) and ATP at these sites in CF_1 (Carmeli et al. 1986). ATP hydrolysis was proposed to proceed through a pentacovalent phosphate intermediate, yet the structure of the transition state intermediate is not fully resolved. We have previously shown (Hochman et al. 1993; Sagi et al. 1998) that vanadate becomes a strong transition state inhibitor of CF_1-ATPase in the presence of divalent metal ion-ADP. Using ^{51}V NMR and X-ray absorption of vanadium we have shown in preliminary studies that ADP-vanadate bound to three cooperative sites in the enzyme forms trigonal bipyramidal pentacovalent geometry (Sagi et al. 1995).

2. Materials and methods

Sample preparation. CF_1 was isolated from lettuce chloroplast, activated and purified (Hochman et al. 1993). CF_1-vanadate complex was prepared by mixing equimolar ratio of 0.85 mM (of 3 sites) activated CF1 with ADP, $MgCl_2$ and sodium vanadate in 40 mM HEPES-NaOH pH 8 at 50 K.

X-ray absorption data collection. X-ray absorption and fluorescence data were collected at National Synchrotron Light Source (NSLS), Brookhaven National Laboratory (BNL), on beam line X-9A using a double flat Si(111) crystal monochromator and Ni-coated harmonic rejection mirror. X-ray fluorescence was collected using a 13 element Germanium detector. X-ray spectra were recorded from 35 eV below the vanadate edge (5485 eV) to 600 eV above the edge. Edge energies were calibrated using the transmission edge energy of vanadium foil (5474 eV) which was collected simultaneously with the fluorescence data. Data processing and analysis included normalization, background subtraction, non-linear curve fitting using vanadyl acetoacetonate as a model compound and error analysis.

3. Results and discussion

X-ray absorption of vanadate bound to CF_1. We have previously shown (Hochman et al. 1993) that vanadate-ADP-divalent metal ion binds very strongly to CF_1 with a *Kd* of 0.5 nM and acts as a transition state inhibitor. For XAFS measurements the CF_1-Mg-ADP-vanadate complex was prepared by mixing of three equivalents of $MgCl_2$, vanadate and the enzyme leaving no more than 2-5% free ligands. The normalized fluorescence spectra of free vanadate, ADP-vanadate uridine-vanadate and CF_1-Mg-ADP-vanadate are shown in Fig. 1. Free vanadate, which has a tetrahedral coordination about the vanadium, has a very intense 1s-3d peak (at 5473 eV). There was a reduction in the intensity of the pre-edge, an increase in the peak transition at 5485 eV and a disappearance of the valley at 5495 eV. in the pentacoordinated vanadate-ADP and vanadate-uridine complexes. A rather small reduction of the pre-edge and a moderate alternation of edge shape of the CF_1-Mg-ADP vanadate complex might reflect the known asymmetry in the structures of the three active sites of the enzyme.

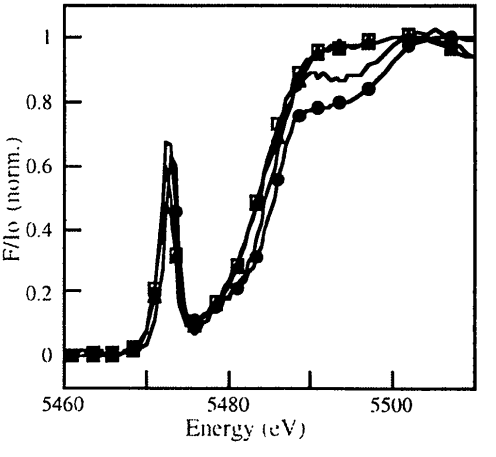

Fig. 1.
The fluorescence X-ray spectra of vanadate *K*-edge. The normalized X-ray fluorescence (F/Io) spectra were measurements in samples of vanadate-ADP-Mg(II) bound to CF_1 (solid line), vanadate-ADP (squares) and vanadate-uridine (triangles) complexes at a 1:50 molar ratio and vanadate in solution (circles).

Theoretical computations of XANES . Calculations were performed using FEFF6 (Rehr et al. 1991). For the dependence of the edge structure on bond lengths, only trigonal bipyramidal structures were considered and the *3d* state was omitted. Both the equatorial oxygen atoms which were positioned at bond length a1 and the axial oxygens which were assigned distance a2, were ranged between 1.6 to 2.2 Å in 0.1 Å steps. In all, more than 100 calculations were done including tetrahedral symmetry. Multiple scattering paths up to 6 legs and 10 total path lengths were calculated, and the scattering potentials were generated individually for each structure because they depend slightly on the interatomic distances. Experimental spectra were determined only by *module* a scale factor and a smooth background, and the comparison of theory with experiment accounted for this uncertainty. The best match of each theoretical spectrum to the experimental data while floating a multiplicative scale factor and additive background was accomplished by solving a linear system of algebraic equations, which yields a unique solution. The background was approximated over the edge region as a quadratic function of energy: a +b(E-E0) + c $(E-E0)^2$. The mean-square residual obtained in this matching procedure provides a measure of the discrepancy between theory and experiment for various hypothetical structures. A scatter-plot of the residual error vs average bond length in the calculations gave an approximately parabolic function (data not shown), with a minimum at the best fit distance. The position, width, and shape of the absorption edge depend strongly on bond length because they are dominated by single scattering; the fine structure in addition is modulated by multiple scattering (Bunker & Stern, 1984). The longer the average bond length, the lower the edge position, and the faster the rise (Fig. 2). As a consequence, the average bond length can be estimated from the shape of the edge. An average bond length of 1.89 Å gave the best fit for trigonal bipyramidal geometry.

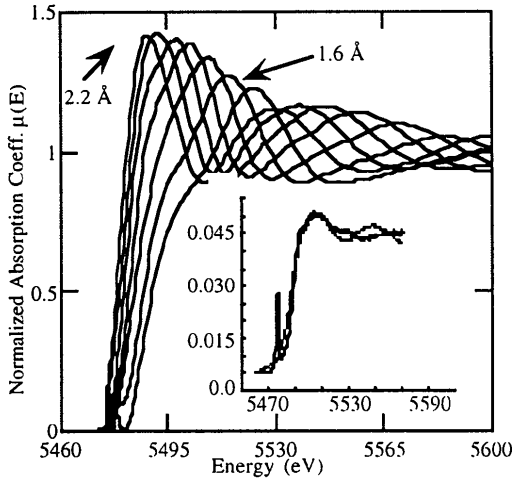

Fig. 2
Effect of average bond length on edge position. In the theoretical calculations shown, the equatorial and axial bond lengths are taken to be equal and are varied between 1.6 and 2.2 Å in 0.1 Å steps. The 3d pre-edge is not included. The best fit between the experimental (solid lines) bound vanadate spectra and the simulated spectra (dashed line) is shown in the insert.

EXAFS analysis. The trigonal bipyramidal and square planar models used in the simulation are clearly oversimplified. The relatively high symmetry assumed results in large EXAFS-type oscillations above the edge, larger than seen experimentally. This indicates that the real five coordinated cluster in the enzyme is more highly distorted than assumed in the trigonal bipyramidal models. Indeed the EXAFS analysis indicated that the two atoms type fit gives the best results. EXAFS analysis for only for two atoms fit is summarized in Table 1. The best fit indicates a trigonal bipyramidal geometry about the vanadium atom with three equatorial and two axial V-O bonds of 1.63 Å and 1.92 Å, respectively. The negative Debye-Waller factors arise, in large part, from correlation between the parameters used to interpret the back scattering from the inner shell and due to the uncertainty in the coordination number parameter N. Other combinations of the two atoms fit resulted in either a higher chi-square or high Debye-Waller factor. All attempts to obtain a valid single atom fit failed. The relatively short equatorial bond distance at 1.63 Å reflects the existence of a mixture structures of vanadate bound to the three asymmetric active sites as observed in our edge studies.

Table 1
Curve-fitting results for V K-edge EXAFS of CF_1-ATPase-ADP-vanadate complex. Vanadate is bound to three active sites of the enzyme. Pentacoordinated trigonal bypiramidal geometry gave the best fit (Fit # 1). N is the number of scatterers per metal, R is the metal-scatterer distance. σ is Debye-Waller values for R. Eo is the shift in Eo in the fit. The error values on R are calculated from normalized error (σ). Shell k ranges between 1.5-10.5/Å.

Fit	Shell	N	R(Å)	σ^2 (Å)	ΔEo(eV)	X^2
1	V-O	3	1.63±0.02	-0.001	6	3.2
	V-O	2	1.92±0.03	-0.002	8	
2	V-O	4	1.615±0.04	0.005	2	10.1
	V-O	1	1.95±0.05	0.010	8	
3	V-O	4	1.62±0.002	0.002	6	5.4
	V-O	2	1.93±0.005	-0.005		

Conclusions. The XANES data clearly show that there is some heterogeneity in the structure of the enzyme bound vanadium ions. A rather small reduction of the pre-edge and a moderate alternation of the edge shape of the CF_1-Mg-ADP vanadate complex might reflect either shorter bond or a distortion in the symmetry of pentacoordinated trigonal bipyramid sphere. The EXAFS results support a pontacoordination environment around the vanadium but also suggest that the structure of the complex is not identical in all the sites.

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(Received 10 August 1998; accepted 5 November 1998)