Molybdenum X-Ray Absorption Edge Spectra. The Chemical State of Molybdenum in Nitrogenase

Sir:

The measurement of an atom's K or L x-ray absorption edge energy and line shape can provide useful information about its chemical environment.[1] The "chemical shift" of the absorption edge reflects the net charge on an atom. Furthermore, for transition metal complexes, the fine structure of the metal edge reflects the distribution of empty molecular orbitals and hence the nature of the surrounding ligands. We report here a good correlation between the chemical shift of the Mo K edge and the calculated "coordination charge" for a wide range of Mo compounds. The Mo K absorption edge for the Mo-Fe component of nitrogenase has also been observed, and its position has been used to determine the possible chemical states of Mo in the resting enzyme. These results suggest that absorption edge measurements will be useful in the characterization of the chemical states of metals in metalloenzymes and synthetic catalysts.

The Mo K absorption edge spectra were recorded on the tunable x-ray line of the Stanford Synchrotron Radiation Project.[2] All energies were calibrated relative to a Mo foil standard, whose first absorption inflection point was defined as 20.0039 keV.[3] The raw data from the measurement were essentially reciprocal transmittance vs. monochromator angle. This information was converted to absorbance vs. energy and then numerically differentiated twice by an 11-point finite difference approximation. For the present purposes the edge energy was defined as the major inflection point of the absorbance vs. energy curve. In many of the spectra there are other inflection points due to essentially 1s $\rightarrow$ 5p and 1s $\rightarrow \pi^*$ bound-state transitions, but these are almost always distinguishable from the true edge by their lower energy and sharper features.

The molybdenum oxides and disulfide were run as finely powdered mulls in epoxy matrices on aluminum foil backings. The air-sensitive tri-, tetra-, and pentachloride were run as neat powders, packed between Kapton (polyimide) windows and sealed under nitrogen atmosphere. $(NH_4)_5(Mo_2Cl_8)Cl$,[4] $Na_2(Mo_2O_4Cys_2)$,[5] and molybdenum acetate[6] were synthesized according to literature procedures and run as neat powders; the latter compound was stored at 0 °C under argon until just before the experiment. The molybdate ion was run as a 1 M K_2MoO_4 aqueous solution prepared just prior to taking the spectrum.

The molybdenum-iron component of nitrogenase from *Clostridium Pasteuranium* (molybdoferredoxin) was prepared by the method of Zumft and Mortenson[7] and had activity of about 2.0 μmol of acetylene reduced/(min mg of protein) and a molybdenum content of 1.71 (220 000 daltons). The preparation was dried anaerobically to a powder which was then packed into the measuring cell. The transfer of the dried Mo-Fe protein to the cell was performed in an anaerobic and moisture-free drybox; the latter was absolutely necessary to prevent oxidation and inactivation. In the measuring cell, the dry Mo-Fe protein was protected from moisture by Mylar windows. Samples of the Mo-Fe component prepared by the method of anaerobic drying have been shown to regain up to 90% of their original activity on resolution and assay by standard procedures.

For materials with a high molybdenum concentration, optimum spectra are obtained when the transmittance changes by a factor of $1/e$ over the edge. With a 2 cm × 1 mm x-ray beam, this requires in the range of 50 mg of material for samples 30% in Mo. For more dilute samples, where background absorption from other elements becomes predominant, it is best to work at approximately 10% average transmittance.[8] Good spectra of the nitrogenase Mo-Fe component were obtained with 100 mg of the lyophilized material.

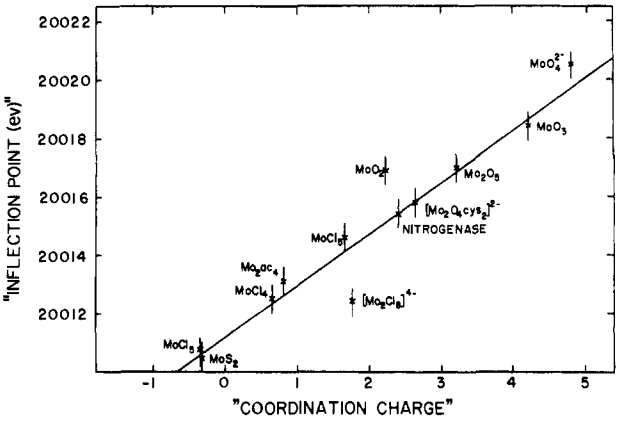

Figure 1. Correlation of absorption edge energy with calculated coordination charge.

The "coordination charge" was calculated from the Allred-Rochow electronegativities[9] according to the method of Batsanov.[10] It is defined as $\eta = m - \Sigma_k n_k c_k$, where m is the formal oxidation state, c_k is the degree of covalence of a bond, and n_k is the number of such bonds. The degree of covalence is in turn defined as $1 - i$, where the ionicity i is obtained from Pauling's formula:[11] $i = 1 - \exp(-1/4(\chi_a - \chi_b)^2)$, using χ_a and χ_b as the Mo and ligand electronegativities. Of course the coordination charge should only be regarded as a rough measure of the net charge on molybdenum, and more sophisticated calculations would give a more precise estimate of the true charge on Mo. Still, the coordination charge calculation serves its purpose—to show the general increase in 1s binding energy with increasing positive charge on Mo.

Figure 1 shows that a good correlation exists between the edge inflection point energy and the calculated charge on Mo. This approximately linear relationship has been established previously for manganese[10] K absorption edges. Such correlations also exist in x-ray photoelectron,[12] x-ray fluorescence,[13] and Mossbauer[14] spectroscopy. The common theme in all these studies is that inner electronic or nuclear energy levels are measurably affected by chemical changes in the valence electron distribution.

The most interesting result from the present work is the location of the Mo K edge for the Mo-Fe component of nitrogenase. Within experimental error, (±0.5 eV), Mo in resting nitrogenase and Mo in the oxo-bridged Mo(V) cysteine complex have the same chemical shift, as shown in Figure 2. From the present results, one reasonable oxidation state for the Mo in the resting enzyme is Mo(V) coordinated to one sulfur ligand. Such an environment has been suggested by chemical studies with Mo thiol model systems.[15] However, given the range of error in the edge measurement and the edge shift correlation, several other possible Mo environments are clearly possible. With a value of 20 015.5 ± 0.5 eV for the nitrogenase Mo edge, and using the parameters of a least-squares fit straight line through the known data points, a value of 2.3 ± 0.3 was calculated for the coordination charge. Assuming hexacoordinate Mo, the possible environments shown in Table I were calculated.

Further work with edge fine structure analysis of more biochemically relevant model compounds should help resolve the present ambiguity. By studying Mo(V) with various amino acid ligands, one could construct a finer scale with which to characterize the possible environments. More precise absorption edge measurements will also be possible with planned instrumental improvements.[16]

Table I

Oxidation state	No. of nitrogens	No. of oxygens	No. of sulfurs	Calcd coord charge
IV	1	5	0	2.05
	0	6	0	2.21
V	2	2	2	2.04
	4	1	1	2.15
	1	3	2	2.20
	6	0	0	2.26
	3	2	1	2.31
	0	4	2	2.36
	5	1	0	2.42
	2	3	1	2.47
	4	2	0	2.58
VI	0	1	5	2.09
	2	0	4	2.19
	1	1	4	2.35
	3	0	3	2.46
	0	2	4	2.51

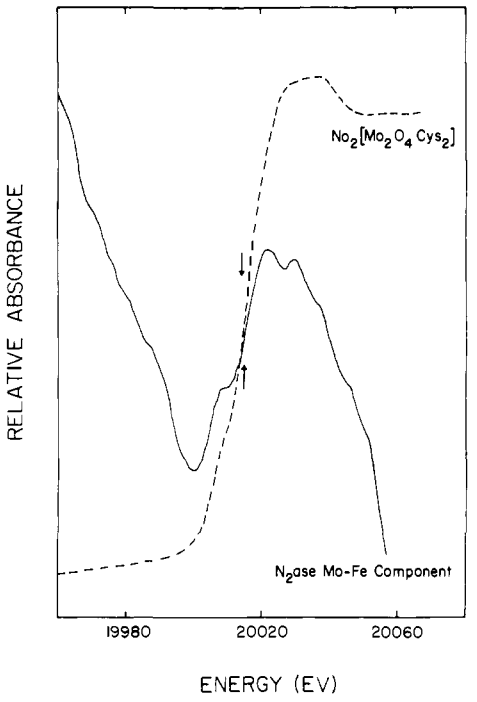

Figure 2. A comparison of the Mo K absorption edges for the nitrogenase Mo-Fe component and the oxo-bridged Mo(V)-cysteine complex. Note sloping background of protein spectrum due to residual absorption and scattering by other elements.

The observed correlation over a wide range of formal oxidation states between edge energy and coordination charge indicates the sensitivity of the Mo K absorption edge to the chemical state of Mo. This x-ray absorption edge method is especially useful because edge measurements can be made on any phase of matter, without the vacuum system required by photoelectron spectroscopy. The measurements are element specific and nondestructive.[17] Using fluorescence detection techniques, relatively small quantities of material are needed.[18] The x-ray absorption method is thus extremely useful for cases where other kinds of spectra are obscured or unavailable.

The present work indicates that Mo in the resting nitrogenase Mo-Fe component could well be Mo(V) with at least one cysteine sulfur ligand. Together with the lack of an observable ESR signal (as expected for Mo(V)) and the presence of two Mo atoms per protein complex,[7] this is evidence for an antiferromagnetically coupled Mo dimer in the resting enzyme. Binuclear metal complexes have been observed in iron proteins such as hemerythrin[19] and 2 Fe-S* ferredoxins,[20] while they have been invoked as general components for copper proteins[21] and molybdenum proteins.[22] Further x-ray absorption studies on nitrogenase model compounds and on the actively functioning enzyme will help to elucidate the mechanism of nitrogen fixation, while analysis of the extended x-ray absorption fine structure of nitrogenase will further test the existence of a binuclear metal active site.[23]

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