dichloride precursor in an unusual reaction in THF at 293 K for 24 h (eq 2). **3Os** can also be prepared from *trans*-OsHCl-*cis*-β-$OsCl_2$(*meso*-tetraphos) + $NaBPh_4$ + $2H_2$ →

trans-[Os(η^2-H_2)H(*meso*-tetraphos)]BPh_4 + NaCl + HCl (2)

(*meso*-tetraphos),[14] $NaBPh_4$, and H_2 in a similar preparation to **2Os**. The distinctive AA'XX' pattern in the ^{31}P NMR spectrum confirms the trans structure. The ^1H NMR spectrum in the high field region at 293 K is consistent with the octahedral structure I in which the η^2-H_2 ligand is trans to the terminal hydride as observed in the crystal structure of **1Fe**. Triplets with ^1J(H,D) couplings of 26.4 Hz grow in the ^1H NMR spectrum with time because of intermolecular H^+/D^+ exchange with acetone-d_6 to give isotopomers containing HD.[21] In contrast to **2Os** where the rate of intramolecular exchange of H atoms is 3000 s^{-1} at room temperature, exchange in **3Os** is slow on the NMR time scale at 293 K.

The T_1 values measured at 200 MHz for the H_2 ligands of the complexes (32 ms for **3Fe**, 49 ms for **3Os**) are shorter than those of comparable complexes **1** and **2** extrapolated to room temperature by use of the known temperature dependences of these T_1 values.[22] For example the H_2 of **1Fe** has a T_1 of ~40 ms and that of **2Os** has a T_1 of ~340 ms at 200 MHz. This information combined with the larger ^1J(H,D) couplings for **4** argues for shorter H-H bonds in these tetraphos complexes relative to bis-diphosphine complexes.

cis-α-OsH_2(*rac*-tetraphos)[14] (structure II, X, Y = H^-) reacts with HBF_4 in ether to give a complex formulated as *cis*-α-[Os(η^2-H_2)H(*rac*-tetraphos)]BF_4, **4Os**.[23] The assignment of cis-α

cis-α-OsH_2(*rac*-tetraphos) + HBF_4·Et_2O →

cis-α-[Os(η^2-H_2)H(*rac*-tetraphos)]BF_4 (3)

geometry of the tetraphos ligand comes from the typical A_2X_2 ^{31}P NMR spectrum[23] and a preliminary X-ray diffraction study.[24] The three hydridic protons at −8.15 ppm remain equivalent to 180 K, and no HD couplings are observed for deuteriated analogues.

The T_1 of the hydridic resonances of **4Os** passes through a minimum value of 160 ms at 252 K, 400 MHz. Crabtree and Hamilton[25] and ourselves[22] have recently described how T_1 measurements can be used to determine the H-H distances of η^2-dihydrogen ligands in transition-metal complexes in solution. We calculate that the H-H distance falls in the range of 1.25–1.6 Å assuming (1) the limits of rapid and no rotation of the H_2 ligand, respectively, (2) dipolar relaxation predominates, (3) the terminal hydride has a T_1 of 600 ms, (4) exchange is of the type $(H_2)(H^*) \rightleftarrows (HH^*)(H)$.[14] Thus some degree of H-H bonding is present although the structure must be close to that of a seven-coordinate trihydride where H-H distances of ≥2 Å are expected. A long, weak H-H bond presumably facilitates H atom exchange,[3a] since $[Ir(H_2)(H)(PPh_3)_2(bq)]^+$ which also has cis H_2 and H ligands and a shorter H-H bond than **4Os** has a higher barrier to exchange.[2] An alternative formulation, *cis*-α-[Os(η^3-H_3)(*rac*-tetraphos)]BF_4, is also possible although for an H_3^- ligand two different types of H atoms and hence two chemical shifts might be expected.[26] Dihydrogen in **4Os** is easily displaced by CH_3CN in boiling CH_3CN/CH_2Cl_2 solution in 2 h to give *cis*-α-[Os(CH_3CN)(H)(*rac*-tetraphos)]BF_4.[14]

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Supplementary Material Available: Characterization of the complexes by FAB MS, C, H analyses, ^1H and ^{31}P NMR as well as calculations based on T_1 values (3 pages). Ordering information is given on any current masthead page.

(20) **3Os**: only characterized in solution since it is extremely difficult to crystallize; FAB MS calcd for $C_{42}H_{45}{}^{192}OsP_4$ 865.4, obsd 861 ($M^+ - 4$ H); δ (^1H, 293 K, acetone-d_6, 200 MHz) −6.36 (br s, H_2, T_1 = 49 ms) −11.21 (quintet, J(H,P) = 18.9 Hz, T_1 = 650 ms); δ (^{31}P versus 85% H_3PO_4, THF) 80.1 (m), 32.5 (m) (AA'XX', J_{AX} = 185 Hz).

(21) [Os(HD)H(*meso*-tetraphos)]$^+$: δ (^1H, 293 K, acetone-d_6) −6.36 (^1J(H,D) = 26.4 Hz), −11.25 (^2J(H,P) = 18 Hz); [Os(HD)(D)(*meso*-tetraphos)]$^+$: −6.28 (^1J(H,D) = 26.4 Hz).

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(23) **4Os**: white powder, 42% yield; FAB MS, calcd for $C_{42}H_{45}{}^{192}OsP_4$ 865.4, obsd 861 ($M^+ - 4$ H); Ir (Nujol) 2043 (vw), 2016 (vw), 1983 (vw) cm^{-1} (Os-H); δ (^1H, 252 K, CD_2Cl_2, 400 MHz) −8.20 (quintet, J(H,P) = 12.1 Hz, T_1 = 160 ms which is the minimum value; δ (^{31}P versus 85% H_3PO_4, acetone) 81.0 (s), 40.2 (s). Anal. Calcd for $C_{42}H_{45}BF_4OsP_4$·CH_2Cl_2: C, 49.87; H, 4.57. Found: C, 49.43; H, 4.23.

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X-ray Absorption of *Azotobacter vinelandii* Vanadium Nitrogenase

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Evidence for the existence of a vanadium-containing nitrogenase has existed for more than half a century,[1] but progress in understanding this enzyme has only come recently.[2] In 1980, Bishop and co-workers proposed that an alternative nitrogen-fixing enzyme exists in *Azotobacter vinelandii*[3] and subsequently proposed that vanadium was involved.[4] In 1986, Robson et al. demonstrated clearly that the alternate nitrogenase from *Azotobacter chroococcum*, Acl*, contained vanadium[5] instead of molybdenum. Hales et al. have shown that vanadium is also found in the *Azotobacter vinelandii* alternative component I, Avl'.[6]

The molybdenum and vanadium nitrogenase proteins are similar in many respects. Like the molybdenum enzyme, both Acl* and Avl' exhibit an EPR spectrum characteristic of a species with an $S = {}^3/_2$ ground state;[7,8] Avl' also contains the so-called P-clusters.[9] Additionally Acl* has recently been shown to possess

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Table I. Comparison of Vanadium and Molybdenum Nitrogenase Curve-Fitting Results

sample	M–S			M–O			M–Fe		
	N^a	$R,^b$ Å	$\sigma,^c$ Å	N^a	$R,^b$ Å	$\sigma,^c$ Å	N^a	$R,^b$ Å	$\sigma,^c$ Å
Azotobacter vinelandii vanadium nitrogenase[d] 4 K	3 3–4	2.337 2.34	0.032	3 2–3	2.136 2.15	0.047	3 2–4	2.759 2.76	0.054
Azotobacter chroococcum vanadium nitrogenase[e] 80 K	2 1–3	2.32 2.32	0.122	4 3–5	2.14 2.14	0.022	3 2–3	2.74 2.74	0.083
Clostridium pasteurianum molybdenum nitrogenase[f] 273 K	4–6	2.35		1–2	2.2		2–4	2.68	
Klebsiella pneumoniae molybdenum nitrogenase[g] 4 K	4 4–5	2.370 2.37	0.056	 0–2	 2.15		3 2–4	2.691 2.69	0.023

[a] Coordination number; values reported as integers were fixed during refinement and represent an individual fit; values reported as a range represent the scope of acceptable fits combined with systematic error. [b] M–X distance; systematic error ca. 0.02 Å. [c] Root-mean-square deviation of R; error in this parameter can be very large if the wrong coordination number is used. [d] This work; although other coordination numbers can fit they data, they require physically unreasonable σ values. [e] Reference 11. [f] Reference 16. [g] Reference 17.

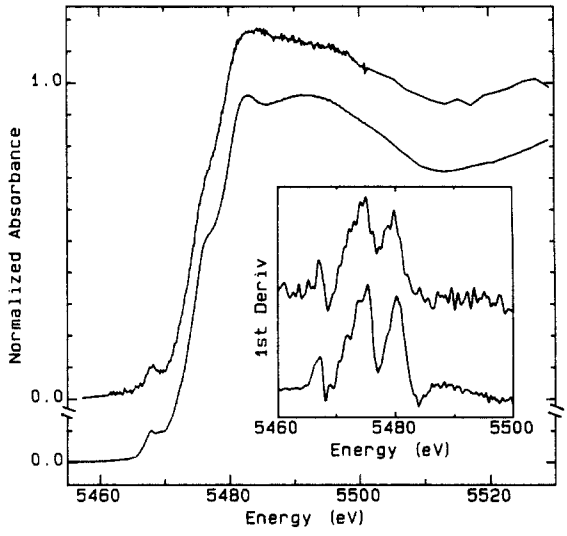

Figure 1. Vanadium K X-ray absorption edge for Av1′ (top), compared with the spectrum of $[Me_4N][VFe_3S_4Cl_3(DMF)_3]\cdot 2DMF$. Inset: first derivative spectra.

an *N*-methylformamide soluble cofactor, FeVco, analogous to the well-known iron–molybdenum cofactor FeMoco.[10] Arber et al. have reported X-ray absorption spectra for the Ac1* enzyme and interpreted the EXAFS as evidence for a V–Fe–S cluster.[11] The local vanadium structure is proposed to resemble a recently synthesized cubane-like VFe_3S_4 cluster,[12] and analogies are drawn with the EXAFS-derived structure reported for the molybdenum nitrogenases.[13–17] We report herein an X-ray absorption spectroscopic study of *A. vinelandii* vanadium nitrogenase, Av1′,[18]

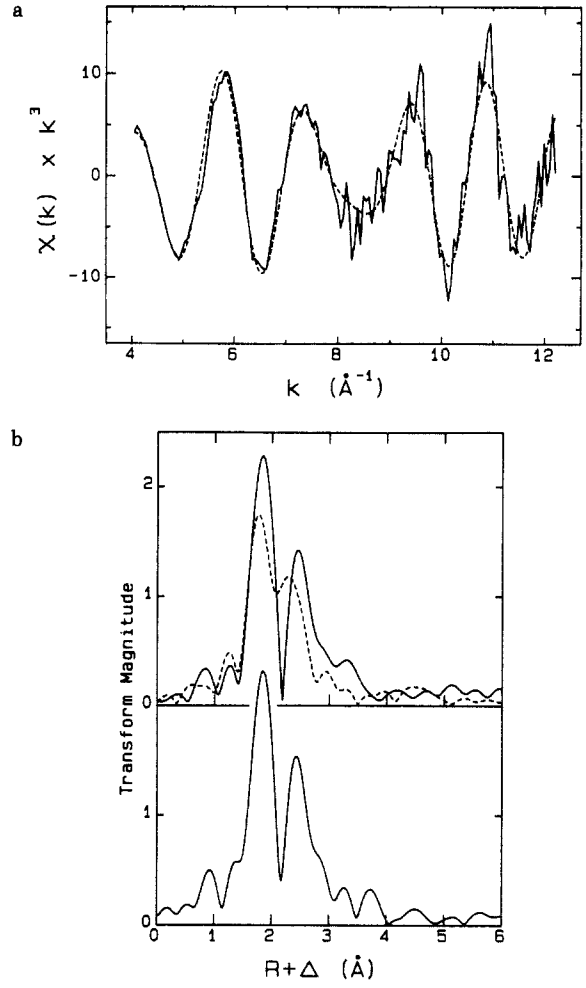

Figure 2. (a) EXAFS of Av1′ (—) and best fit (---). (b) k^3EXAFS Fourier transforms (3–12.2 Å^{-1}) for (top) Av1′ (—) versus Mo Kp1 (---) and (bottom) $[Me_4N][VFe_3S_4Cl_3(DMF)_3]\cdot 2DMF$.

which supports and extends the work of Arber et al.

The vanadium K X-ray absorption edge for Av1′,[19] Figure 1, is similar to the Ac1* spectrum reported by Arber et al.[11] but with slightly improved resolution. There is a small 1s → 3d transition

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(18) Component I of the alternate nitrogenase Av1′ was prepared as described by Hales et al.[6] The final V concentration was 1.1 mM. After concentration, the sample was loaded into a 1 × 2 × 20 mm Lucite cuvette with a 6.3 μ polypropylene window on a long face. Model compounds were prepared as finely powdered solids and diluted as necessary with boron nitride. $[Me_4N][VFe_3S_4Cl_3(DMF)_3]\cdot 2DMF$ was prepared as described by Kovacs and Holm.[12]

(19) X-ray absorption spectra were recorded at the Stanford Synchrotron Radiation Laboratory on Beam Line IV-2 by using Si(220) or Si(400) crystal monochromators. Fluorescence detection was employed for the enzyme spectra, by using a 13-element intrinsic Ge detector.[20] The reported spectrum represents the average of 10 20-min scans. During data collection the sample was maintained at 4 K with an Oxford Instruments CF 204 cryostat. The spectra were calibrated with respect to the first inflection point of a V foil, defined as 5465.2 eV.[24,25]

at 5469 eV, a shoulder at 5477 eV, and an overall maximum at 5484 eV. The relatively weak 1s → 3d transition precludes the presence of terminal V=O bonds or tetrahedral symmetry at the V site.[21] Although the 1s → 3d transition for many vanadium compounds often has resolved structure due to splitting of the 3d levels, such features are not evident in the enzyme data. Lorentzian deconvolution of the data (not shown) indicates that the ligand field splitting is less than 2 eV.

The Av1′ edge is quite similar to that of the $[Me_4N][VFe_3S_4Cl_3(DMF)_3]\cdot 2DMF$ model compound, although some subtle differences remain. The model compound spectrum has some structure in the 1s → 3d region, which is especially evident in the derivative curve. The peak near 5484 eV, presumably a 1s → 4p transition, appears sharper in the model compound spectrum. Furthermore, the broad maximum near 5492 eV is stronger and sharper in the model data; in this region there is better correspondence with the V_2S_3 spectrum reported by Wong et al.[21]

The nitrogenase EXAFS spectrum, Figure 2, can be simulated by a fit with three components, corresponding to V-(O,N), V-(S,Cl), and V-Fe shells.[22] Using the $[VFe_3S_4Cl_3(DMF)_3]^-$ cluster as a model, a value of 3 ± 1 V-Fe interactions at an average distance of 2.76 ± 0.03 Å is calculated. The fits also indicate 3–4 V-S interactions at 2.33 ± 0.03 Å and 2–3 O and/or N neighbors at 2.15 ± 0.03 Å. The predicted number of sulfur ligands is higher than the value of 2 ± 1 reported by Arber et al. Additionally, the new estimates of the Debye–Waller factors fall into a more chemically reasonable range than those determined by Arber et al., and the V-Fe distance does not require the addition of any correction factor (Arber et al. used a +0.05 Å correction). In part, the increased k-range of the new data allows more accurate determination of these quantities.

The Fourier transform of the EXAFS, Figure 2, shows a well-resolved splitting between the V-Fe shell and the first coordination sphere interactions. The transforms reported by Arber et al. do not show this splitting, presumably because the data were processed over a narrower spectral range ($k_{max} \cong 10$ Å^{-1}). In fact, the splitting is greater than that observed for the Mo nitrogenase over the same spectral range. This reflects the larger spread between V-S and V-Fe distances, $\Delta R = 0.42$ Å, compared to the Mo-S versus Mo-Fe differential in the Mo enzyme, $\Delta R = 0.32$ Å, and suggests a slight but significant difference in the cluster geometry in the vicinity of the V site.

The modestly different V-S and V-Fe distances for the V nitrogenase compared to the Mo enzyme require a slight change in the MFeS cluster geometry. It is known that the vanadium enzyme has different reactivity with respect to substrates, for example, reducing acetylene in part to ethane.[26] If at some point in the reaction mechanism substrates bridge across a mixed-metal pair, V-Fe versus Mo-Fe, then the slightly longer distance could be involved in changing the reaction energetics and pathways.

Acknowledgment. The spectra were recorded at the Stanford Synchrotron Radiation Laboratory, which is supported by the Department of Energy, Office of Basic Energy Sciences, and the National Institutes of Health, Biotechnology Resource Program, Division of Research Resources. This work was supported in part by the National Institutes of Health under Grant GM 33965 (B.J.H.). We thank Dr. T. Haibert for the sample of $(NH_4)_3VS_4$ and Dr. E. I. Stiefel for helpful discussions.

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Effect of Ion Pairing on Bond Order and Charge Localization in Alkyl Phosphorothioates

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Recent papers dealing with physicochemical properties of phosphorothioate anions support the claim that in aqueous solutions the P–S bond order is close to one, the P–O bond orders are higher than one, and approximately one negative charge is localized on sulfur. In doubly or triply charged anions the additional charges are delocalized over two or three oxygens.[1-3] Calculations for unsolvated species are consistent with conclusions based on experimental data, with particular reference to P–S bond order.[4] We show here that ion pairing in solution induces a moderate perturbation on the P–O bond order.

Phosphorothioate anions bound at sites on enzymes are often ion paired with the guanidinium or ammonium functions of arginine or lysine so that structures in aqueous solutions may not model phosphorothioates bound at enzymic active sites. Bonding in enzyme-bound species may be more similar to that in crystalline salts of phosphorothioates. In crystals the counter cations are closely coordinated with the P–O bonds of phosphorothioates and draw electron density toward oxygen, with consequent delocalization of negative charge away from sulfur and lengthening of P–O bonds.[1,5]

To examine this question, we have prepared the cyclohexylammonium, tetramethylammonium, and tetrabutylammonium salts of *O,O*-diethyl [^{18}O]phosphorothioate.[6] We compared the ^{18}O-induced isotope shifts of the ^{31}P NMR signals for these salts in organic solvents, in which they should be ion paired, with those in D_2O, in which they should be solvated, and obtained the results set forth in Table I. We obtained 15 independent determinations of the isotope shifts for each sample, in all of which the lines were base line resolved and well determined. The average isotope shifts and standard deviations were calculated for each sample. Isotope shifts have been linearly correlated with bond order.[7]

The isotope shift for cyclohexylammonium and tetramethylammonium diethyl [^{18}O]phosphorothioate in D_2O is 0.039 ppm, slightly less than the 0.041 ppm reported for cyclic thio[^{18}O]-phosphodiesters.[8] The value for a single bond is 0.021 ppm,[1] so that 0.039 ppm corresponds to a P–O bond order of 1.9. In 1,4-dioxane-d_6 the isotope shift is 0.036 pm, and in acetonitrile-d_3 it is 0.037 ppm, corresponding to a bond order of 1.7–1.8. The bond order is clearly reduced in the organic solvents, presumably due to ion pairing. Furthermore, the effect is the same in the H-bonding cyclohexylammonium salt as in the non-H-bonding tetramethylammonium salt, so H-bonding does not contribute to the effect.

The isotope shift for the tetrabutylammonium salt is 0.036 ppm in D_2O as well as in 1,4-dioxane-d_6. The shift in D_2O relaxes to 0.039 ppm over a period of 1 h upon addition of ammonium acetate (to 0.1 M) to the NMR tube. It appears that the highly

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