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Characterization of the Metal Clusters in the Nitrogenase Molybdenum–Iron and Vanadium–Iron Proteins of *Azotobacter vinelandii* Using Magnetic Circular Dichroism Spectroscopy†

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ABSTRACT: Low-temperature magnetic circular dichroism (MCD) spectroscopy has been used to investigate the metal clusters in the conventional nitrogenase MoFe protein and alternative VFe protein from *Azotobacter vinelandii*. In the dithionite-reduced state, the MCD spectrum of the MoFe protein is extremely similar to that previously observed for the $S = {}^3/_2$ spin state of the M clusters in the MoFe protein of *Klebsiella pneumoniae*. A paramagnetic cluster with an $S = {}^3/_2$ ground state is also responsible for the temperature-dependent MCD transitions of dithionite-reduced VFe protein. However, the electronic and magnetic properties of this cluster are quite distinct from those of M centers in conventional nitrogenase. When these proteins are oxidized with thionine, the MoFe protein exhibits MCD spectra and magnetization characteristics identical with those observed for the P clusters in *K. pneumoniae*, while those of the VFe protein are only similar. However, the paramagnetism in the thionine-oxidized VFe protein, like the conventional enzyme, probably arises from an $S = {}^5/_2$ spin system with near-axial symmetry and a negative zero-field splitting. Novel clusters with electronic, magnetic, and redox properties similar to those of conventional P clusters are, therefore, present in the VFe protein.

During the past two decades, the nitrogen-fixing enzyme nitrogenase has been isolated from a large number of different bacteria. In all species tested to date, this enzyme consists of two separable proteins, a Fe protein and a MoFe protein (Burgess, 1985). The MoFe protein, or component 1, possesses the active site for substrate reduction. This protein has a molecular weight of about 220 000 in an $\alpha_2\beta_2$ polypeptide pattern and contains 30–33 atoms of iron as well as 2 atoms of molybdenum in clusters of unknown structures. Although it has never been shown specifically, it generally is considered that the Mo atoms are at the active site(s) of the enzyme aiding in both the binding and reduction of substrate.

A major area of the research on nitrogenase in recent years has been the elucidation of the structure and function of the different metal clusters in component 1. The techniques of EPR,[1] ENDOR, EXAFS, MCD, and Mössbauer spectroscopy as well as Fe-S core extrusion have been useful probes of the structure and properties of these clusters. Initial catagorization of these clusters came from Mössbauer spectroscopy (Münck et al., 1975; Zimmermann et al., 1978), which showed that around 94% of the iron existed in two major types of centers in component 1, the M and P clusters, with two and four of each type of cluster, respectively, per enzyme molecule.

The M clusters contain Mo, Fe, and acid-labile S in an approximate ratio of 1:6–8:8–10, respectively (Yang et al., 1982; Nelson et al., 1983). These centers (also called the FeMo cofactor or FeMo-co) are most likely part of the active site of the enzyme (Rawlings et al., 1978). As isolated in the presence of dithionite, the M clusters of component 1 exhibit

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[1] Abbreviations: Av1, Cp1, and Kp1, MoFe protein (component 1) of conventional nitrogenase from *Azotobacter vinelandii*, *Clostridium pasteurianum*, and *Klebsiella pneumoniae*, respectively; Av1′, VFe protein (component 1) of alternative nitrogenase from *A. vinelandii*; CD, circular dichroism; EPR, electron paramagnetic resonance; ENDOR, electron nuclear double resonance; EXAFS, extended X-ray absorption fine structure; ICP, inductively coupled plasma; MCD, magnetic circular dichroism; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; T, tesla.

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an EPR spectrum with inflections at $g = 4.3$, 3.6, and 2.0. This paramagnetism is associated with the ground state of a slightly rhombic $S = {}^3/_2$ spin system having positive zero-field splitting (Münck et al., 1975). The ENDOR spectrum of this center shows at least five different Fe hyperfine coupling constants as well as hyperfine interaction with Mo (Venters et al., 1986). EXAFS studies have further revealed the presence of core sulfur ligands around both Mo and Fe (Cramer et al., 1978a,b; Antonio et al., 1982). Low-temperature MCD spectroscopy of the M clusters of *Klebsiella pneumoniae* nitrogenase corroborates the nature of the spin state of this center (Johnson et al., 1981) and provides an optical fingerprint for identifying this novel center in other enzymes.

The function of the P clusters in component 1 is not understood at present. Core-extrusion (Kurtz et al., 1979) and Mössbauer (Zimmermann et al., 1978) studies suggest them to be [4Fe-4S] clusters, albeit with magnetic properties quite distinct from conventional cubane tetranuclear Fe-S clusters. These P clusters are diamagnetic in the resting state but paramagnetic when oxidized with thionine. However, since both states are EPR silent, EPR and ENDOR spectroscopy cannot be used to study these clusters. Mössbauer (Zimmermann et al., 1978), magnetic susceptibility (Smith et al., 1982), and MCD (Johnson et al., 1981) studies are all consistent with a spin state of ${}^5/_2$ for the oxidized form, and the form of the MCD spectrum is indicative of a novel type of Fe-S center.

Recently, a second or alternative nitrogenase was isolated from the bacteria *Azotobacter chroococcum* (Robson et al., 1986) and *Azotobacter vinelandii* (Hales et al., 1986a), which contains a component 1 different from the conventional enzyme. Not only does this new component 1 have a different polypeptide structure but it also contains vanadium instead of Mo. For both genetic and enzymatic reasons, it obviously is of prime importance to determine whether or not the cluster structure found in the conventional component 1 is conserved in the alternative protein. This paper describes the use of MCD spectroscopy to compare directly the metal clusters of component 1 of conventional and alternative nitrogenase from *A. vinelandii*.

Experimental Procedures

Protein Isolation. The conventional MoFe protein (Av1) was isolated from strain UW of *A. vinelandii* according to the published procedure (Burgess et al., 1980). To obtain alternative component 1 (the VFe protein or Av1′), strain LS15 of *A. vinelandii* (Hales et al., 1986b) was used as previously described (Hales et al., 1986a). The final purity of each protein preparation was judged by specific activity [1500 and 200 nmol of C_2H_2 reduced min^{-1} (mg of protein)$^{-1}$ for Av1 and Av1′, respectively, used in this study] and homogeneity of SDS–PAGE polypeptide patterns (Hales et al., 1986a). With ICP spectroscopy, the Mo and Fe metal content of Av1 was determined to be 1.7 and 28 atoms per protein, respectively, while the V and Fe content of Av1′ was 1.0 and 15 atoms per protein, respectively.

Sample Preparations. All MCD samples contained 50% (v/v) ethylene glycol or glycerol to enable the formation of optical glasses at low temperatures. Protein determinations were made on trichloroacetic acid precipitated material by using the biuret method (Cooper, 1977). Quantitation of MCD spectra was based on protein concentration with 240 000 and 200 000 as the molecular weights of Av1 and Av1′, respectively. Oxidation of MCD samples was accomplished by anaerobic titration with a stock solution of thionine (2 mg/mL in water) previously purged of oxygen. Persistence of a faint blue hue was used as an indication of excess thionine. The excess thionine was not removed, since it was shown not to contribute significantly to the MCD spectrum in the wavelength range 300–1000 nm, even at concentrations 5 times greater than those used in this work.

MCD Spectroscopy. Low-temperature MCD measurements were carried out on the instrumentation previously described (Johnson et al., 1985). MCD spectra are corrected for natural CD and expressed at $\Delta\epsilon$ ($\Delta\epsilon = \epsilon_L - \epsilon_R$), which is the difference between the molar extinction coefficients of left and right circularly polarized light. Any depolarization of the light beam was corrected for by measuring the natural CD of a standard sample of D-tris(ethylenediamine)cobalt(III) chloride placed after the MCD sample. The $\Delta\epsilon$ values are not normalized per unit field, and the magnetic fields used to record the spectra are given in the figure legends.

MCD magnetization plots were constructed by monitoring the MCD intensity at several temperatures as a function of the magnetic field strength. All the magnetization plots shown in this paper correspond exclusively to the paramagnetic centers. The data were corrected for temperature-independent contributions from diamagnetic species by extrapolating plots of MCD intensities vs. inverse temperature to infinite temperature and subtracting a proportional correction at each field. Data are plotted as percent (%) magnetization against $\beta B/2kT$, where % magnetization is the percentage of the MCD intensity relative to saturation, β is the Bohr magneton, B is the magnetic field strength, k is the Boltzmann constant, and T is the absolute temperature. Theoretical magnetization curves were formulated as described previously (Bennett & Johnson, 1987).

EPR Spectroscopy. EPR spectra were recorded on an IBM ER200D EPR spectrometer interfaced to an Aspect computer for data handling and manipulations. Low temperatures were achieved with an Air Products LTR-3-100E cryostat positioned in a TE_{102} cavity resonating at X-band frequencies.

Results and Discussion

Three distinct preparations of both Av1 and Av1′ were investigated by low-temperature MCD and EPR spectroscopies using either 50% ethylene glycol or glycerol as the glassing agent. The spectra of both thionine-oxidized and dithionite-reduced samples were invariant to the nature of the glassing agent.

Dithionite-Reduced Av1 and Av1′. Figure 1 shows representative MCD spectra between 300 and 1000 nm for dithionite-reduced Av1 and Av1′ measured at 4.5 T at temperatures between 1.64 and 120 K. The spectra for both enzymes consist of positively signed, temperature-dependent MCD transitions throughout the spectral region investigated and are indicative of the presence of paramagnetic Fe-S clusters. For both Av1 and Av1′, the intensity of the bands below 380 nm showed some variability for different samples. Since this variability was apparent in identical samples frozen at different times, it is attributed to variation in the quality of the frozen glass. The effect is only manifest in this spectral region because of the inherently low optical transmission due to protein and dithionite absorption.

The samples of Av1 used for MCD studies all showed the characteristic EPR signal (effective g factors 4.3, 3.6, and 2.0) that is associated with the dithionite-reduced M centers. Moreover, Mössbauer studies reveal that the M center is the only paramagnetic cluster present in this redox state of the enzyme (Huynh et al., 1980; Zimmermann et al., 1978). Therefore, the low-temperature MCD spectrum is attributed to the paramagnetic M centers and provides an optical fin-

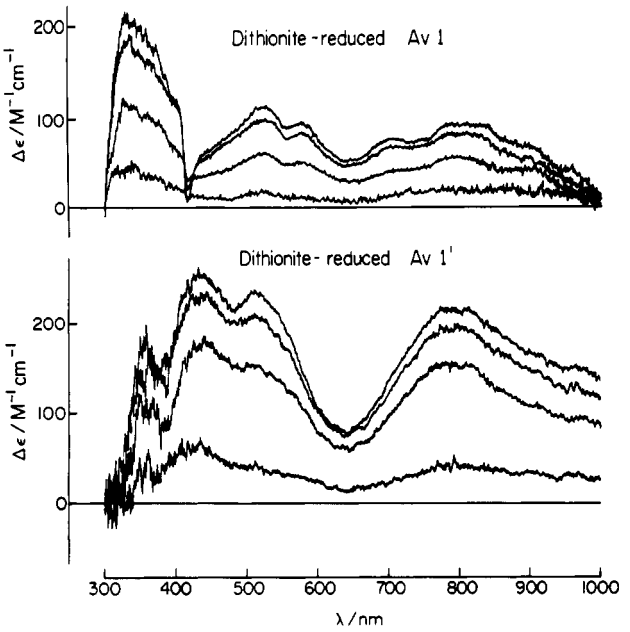

FIGURE 1: Low-temperature MCD spectra of dithionite-reduced Av1 and Av1′. Upper panel: Reduced Av1 (21 mg/mL) in 25 mM Tris-HCl, pH 7.4, 2 mM dithionite, and 0.25 M NaCl, with 50% (v/v) ethylene glycol. Conditions of measurement: temperature, 1.64, 4.22, 15.0, and 120 K; magnetic field, 4.5 T; path length, 0.170 cm. Lower panel: Reduced Av1′ (13 mg/mL) in 25 mM Tris-HCl, pH 7.4, 2 mM dithionite, and 0.25 M NaCl, with 50% (v/v) ethylene glycol. Conditions of measurement: temperature, 1.64, 4.22, 9.5, and 60 K; magnetic field, 4.5 T; path length, 0.175 cm. The intensities of all MCD transitions in both sets of spectra decrease with increasing temperature.

gerprint for identifying this type of novel Fe-Mo-S center. Of all the nitrogenase component 1 proteins that have been purified to date, the only one for which the low-temperature MCD spectra have been reported is Kp1 (Johnson et al., 1981). Comparison of the MCD data for dithionite-reduced Kp1 and Av1 reveals a close similarity in both form and intensity of the spectra. The only significant difference lies in the appearance of a sharp negatively signed band at approximately 420 nm in the spectrum of Av1. This feature appeared with varying intensity for all three samples of dithionite-reduced Av1 investigated and is attributed to a trace impurity of cytochrome. *A. vinelandii* grows by respiration and, as such, produces a high concentration of intracellular soluble cytochromes. Low-temperature MCD spectroscopy is particularly sensitive for detecting paramagnetic cytochromes, even when they are present in nanomolar concentrations (Thomson & Johnson, 1980). Therefore, even though cytochromes are not detected in our protein samples by SDS–PAGE or absorption spectroscopy, their presence can be observed by MCD spectroscopy. Apart from this minor band at 420 nm, the marked similarity in the electronic properties of dithionite-reduced M centers, as revealed by MCD spectroscopy, indicates that the structure of this cluster is highly conserved in Mo-containing nitrogenases from different nitrogen-fixing organisms. A similar conclusion was reached on the basis of a comparison of the Mössbauer and EPR properties of the M centers in Av1, Cp1, and Kp1 (Huynh et al., 1980; Zimmermann et al., 1978; Smith et al., 1980).

The samples of dithionite-reduced Av1′ used for MCD studies showed EPR spectra analogous to those reported previously (Hales et al., 1986a), with signals indicative of paramagnetic clusters with $S = {}^1/_2$ and ${}^3/_2$ ground states. Spin quantitation of the $S = {}^1/_2$ resonance ($g = 2.04$ and 1.93) reveals it to be a minor paramagnetic component, accounting

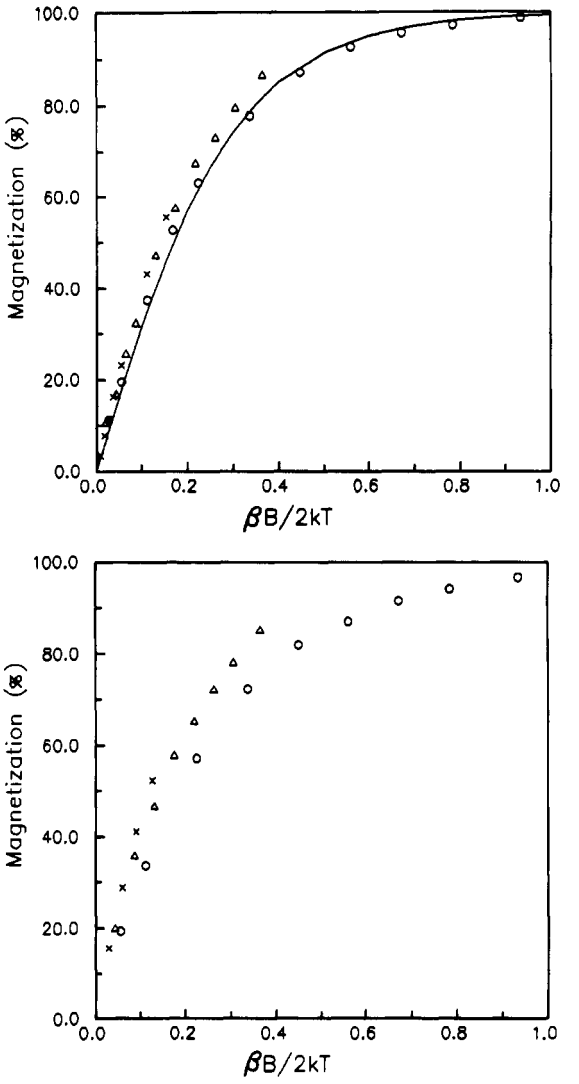

FIGURE 2: MCD magnetization plots for dithionite-reduced Av1 and Av1′. Upper panel: Reduced Av1 at 520 nm. Sample conditions are given in the legend to Figure 1. Temperatures: (○) 1.64 K; (△) 4.22 K; (×) 10.0 K. Magnetic fields are between 0 and 4.5 T. The solid line corresponds to the theoretical magnetization curve for $g_\perp = 4.0$, $g_\parallel = 2.0$, and $m_z/m_{x,y} = -0.3$. Lower panel: Reduced Av1′ at 520 nm. Sample conditions are given in the legend to Figure 1. Temperatures: (○) 1.64 K; (△) 4.22 K; (×) 12.2 K. Magnetic fields are between 0 and 4.5 T.

for approximately 0.2–0.3 spin/molecule. Similar spin quantitations were found in the absence of ethylene glycol or glycerol. The shape of the $S = {}^3/_2$ resonance is broad and temperature-dependent, with poorly resolved maxima at $g =$ 5.3 and 4.3, and is quite distinct from that observed from the M centers of Av1. Comparison of the form of the low-temperature MCD spectra for dithionite-reduced Av1 and Av1′ (Figure 1) also indicates marked differences in the nature of the constituent paramagnetic chromophores. The observed spectra differ in terms of both the intensity and frequency of all temperature-dependent MCD transitions.

In an attempt to establish the nature of the paramagnetic species that contribute to the MCD spectrum, magnetization curves (Thomson & Johnson, 1980) were constructed for Av1 and Av1′ at 800 and 520 nm. The plots were qualitatively similar at both wavelengths, and the data corrected for diamagnetic contributions at 520 nm are shown in Figure 2. For both proteins, the magnetization plots consist of a nested set of curves, in that the data points recorded at each temperature lie along separate curves. This nesting results from the presence of low-lying states that become populated over the

temperature range of the experiment and is indicative of ground states with $S > {}^1/_2$ (Thomson & Johnson, 1980). The MCD magnetization plot for dithionite-reduced Av1 is very similar to those reported for dithionite-reduced Kp1[2] and the extracted FeMo cofactor of Kp1 (Johnson et al., 1981; Robinson et al., 1984), which have been rationalized qualitatively in terms of the ground-state parameters of the paramagnetic M center, i.e., slightly rhombic $S = {}^3/_2$ ground state with $2D$ between +10 and + 12 cm^{-1} (Münck et al., 1975; Venters et al., 1986). Complete theoretical simulation of MCD magnetization curves for ground states with $S > {}^1/_2$ is a complex task involving a number of parameters and has yet to be accomplished. Here we restrict our analysis to comparing the magnetization data at the lowest temperature (1.64 K) with theoretical curves based on the effective g factors of the lowest zero-field doublet (i.e., $g_\perp = 4.0$, $g_\parallel = 2.0$). Such an analysis neglects effects resulting from field-induced mixing of zero-field components. Nevertheless, with judicious choice of the transition polarization ratio ($m_z/m_{x,y}$), a good fit is readily obtained (see Figure 2), showing that the experimental data are consistent with the available ground-state parameters of the $S = {}^3/_2$ M center.

The pronounced nesting of the MCD magnetization data for dithionite-reduced Av1′ indicates that the transition originates, for the most part, from the EPR-detectable $S = {}^3/_2$ species, with only minor contributions from the paramagnetic component with an $S = {}^1/_2$ ground state. In accord with the marked differences in the form of the $S = {}^3/_2$ EPR resonance (Hales et al., 1986a), the MCD magnetization data for dithionite-reduced Av1′ are quite distinct from Av1, showing more pronounced nesting and more gradual saturation at the lowest temperature (1.64 K). On the basis of the available EPR data, it seems probable that these differences are interpretable in terms of changes in the zero-field splitting parameters. Further analysis of the magnetization data must await elucidation of the ground-state parameters of the $S = {}^3/_2$ cluster in dithionite-reduced Av1′ via a detailed study of the temperature dependence of the EPP resonances. It is tempting to speculate, by analogy with the conventional nitrogenase, that this $S = {}^3/_2$ cluster is a V-Fe-S (or FeV-co) cluster and that the spectroscopic differences between this center and the M center relate to the substitution of V for Mo. Experiments designed to test this hypothesis, involving the extraction and characterization of a low molecular weight cofactor from Av1′, are currently in progress in this laboratory.

Thionine-Oxidized Av1 and Av1′. No EPR signals were observed for thionine-oxidized Av1 or Av1′ over the temperature range 4.2–30 K. In contrast, both samples exhibit intense temperature-dependent MCD spectra in the wavelength range 300–1000 nm. MCD spectra over this wavelength at temperatures from 1.72 to 30 K and at a magnetic field of 4.5 T are shown in Figure 3. All MCD bands are attributed to paramagnetic Fe-S clusters with the exception of the sharp negative feature at approximately 420 nm that appears with varying intensity for different samples and arises from a trace impurity of cytochrome.

Since Mössbauer studies of thionine-oxidized Av1 indicate that oxidized P clusters are the only paramagnetic centers

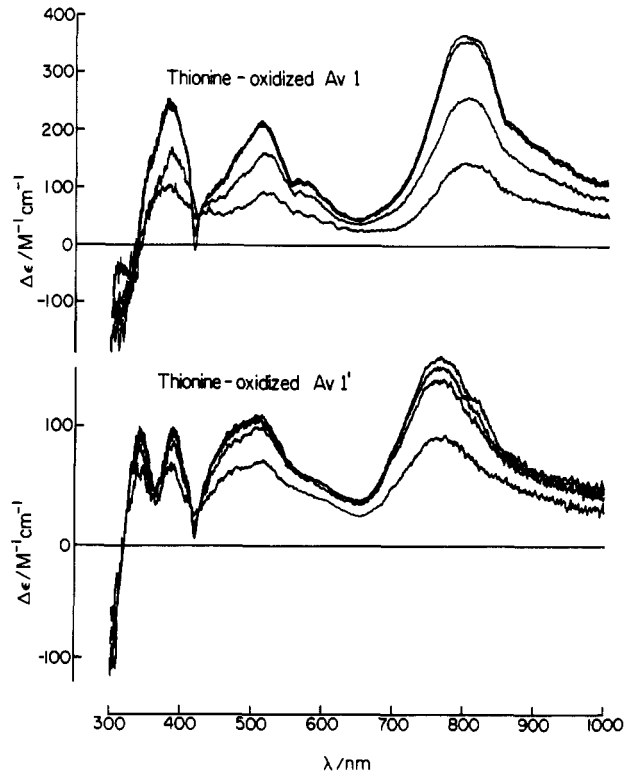

FIGURE 3: Low-temperature MCD spectra of thionine-oxidized Av1 and Av1′. Upper panel: Thionine-oxidized Av1 (20 mg/mL) in 25 mM Tris-HCl, pH 7.4, and 0.25 M NaCl, with 50% (v/v) ethylene glycol. Conditions of measurement: temperature, 1.57, 4.22, 18.0, and 30.0 K; magnetic field, 4.5 T; path length, 0.178 cm. Lower panel: Thionine-oxidized Av1′ (17 mg/mL) in 25 mM Tris-HCl, pH 7.4, and 0.25 M NaCl, with 50% (v/v) ethylene glycol. Conditions of measurement: temperature, 1.58, 4.22, 7.6, and 20.0 K; magnetic field, 4.5 T; path length, 0.175 cm. The intensities of all MCD transitions in both sets of spectra decrease with increasing temperature.

(Zimmermann et al., 1978), the temperature-dependent transitions observed in the low-temperature MCD spectrum can be similarly assigned. Moreover, the spectra are identical in both form and intensity with that observed under comparable conditions for thionine-oxidized Kp1 (Johnson et al., 1981). In this case the assignment of the spectrum to P clusters was confirmed by studies of component 1 from the *nifB*⁻ mutant, which is deficient in M clusters (Robinson et al., 1984). The results reported here attest to the homogeneity of P clusters in Mo-containing conventional nitrogenases from diverse nitrogen-fixing organisms. In contrast, thionine-oxidized Av1′ exhibits a low-temperature MCD spectrum that is similar in form to, but not identical with, that of Av1. Notably, the band centered at around 800 nm in Av1 has shifted to 760 nm in Av1′, and the band centered at 380 nm in Av1 is clearly resolved into two components at 390 and 340 nm in Av1′. Also, the intensity of the MCD transitions is decreased by a factor of between 2 and 3.

Since the unique feature of the MCD characteristics of oxidized P clusters that distinguishes them from all other known Fe-S clusters is the steepness of their magnetization curves (Johnson et al., 1981), magnetization plots at 800 and 760 nm for thionine-oxidized Av1 and Av1′, respectively, are shown in Figure 4. To facilitate comparison of the data, theoretical MCD magnetization data for an isolated ground-state doublet with effective g factors of $g_\parallel = 10$ and $g_\perp = 0$ are shown as a solid line in both sets of data. These are the g factors anticipated for the $M_s = \pm {}^5/_2$ doublet ground state of an $S = {}^5/_2$ spin system which would arise from negative axial zero-field splitting, corresponding to the best description

[2] The published MCD magnetization data for dithionine-reduced Kp1 (Johnson et al., 1981) were not corrected for contributions from diamagnetic P clusters. The diamagnetic contribution manifests itself as a greater degree of nesting and incomplete magnetic saturation of the lowest temperature data. However, comparison of the uncorrected magnetization data for dithionine-reduced Av1 and Kp1 shows a close similarity.

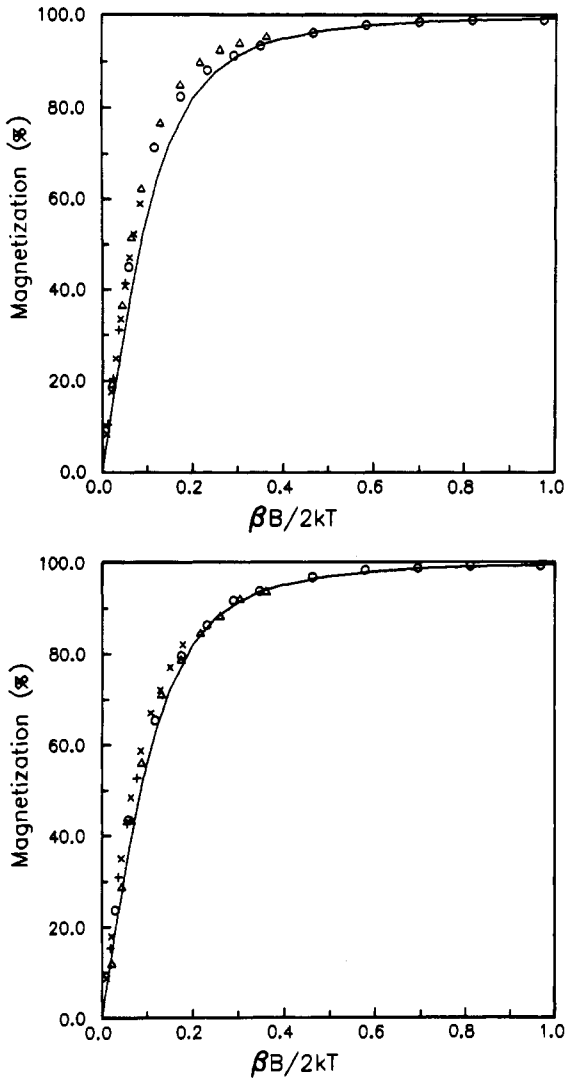

FIGURE 4: MCD magnetization plots for thionine-oxidized Av1 and Av1′. Upper panel: Thionine-oxidized Av1 at 800 nm. Sample conditions are given in the legend to Figure 3. Temperatures: (○) 1.57 K; (△) 4.22 K; (×) 18.0 K; (+) 30.0 K. Magnetic fields are between 0 and 4.5 T. Lower panel: Thionine-oxidized Av1′ at 760 nm. Sample conditions are given in the legend to Figure 3. Temperatures: (○) 1.58 K; (△) 4.22 K; (×) 8.6 K; (+) 20.0 K. Magnetic fields are between 0 and 4.5 T. The solid line in both plots corresponds to the theoretical magnetization curve for an isolated doublet ground state with effective g factors of $g_{\parallel} = 10$ and $g_{\perp} = 0$.

of the nature of the ground state in light of the available MCD, Mössbauer, magnetic susceptibility, and EPR data (Johnson et al., 1981; Zimmermann et al., 1978; Smith et al., 1982). The observed magnetization data for thionine-oxidized Av1 are in excellent agreement with those reported for Kp1 (Johnson et al., 1981). The intercept value of the initial slope and the saturation limit has been used as a convenient measure of the steepness of MCD magnetization curves (Thomson & Johnson, 1980). For thionine-oxidized Av1, the intercept value, $I = 0.125$, compares well with values of $I = 0.12$ and $I = 0.127$ for thionine-oxidized Kp1 (Johnson et al., 1981) and the *nifB*⁻ strain of Kp1 (Robinson et al., 1984), respectively. Comparison of the magnetization data for thionine-oxidized Av1 and Av1′ (Figure 4) also reveals a close correspondence in the magnetic properties of the paramagnetic chromophores in these proteins. The plots differ slightly in terms of the steepness of the magnetization curves, $I = 0.135$ for Av1′, suggesting minor differences in the ground-state zero-field splitting parameters. In both cases, the data at the lowest temperature (only the lowest doublet signficantly populated) magnetize more steeply than the theoretical plot. We attribute this deviation to rhombic distortion of the ground state and/or field-induced mixing of zero-field components. More complete analysis of the magnetization data obtained for all the major bands in the MCD spectrum, involving experimental estimation of zero-field splitting parameters and including second-order Zeeman effects, is currently in progress. However, on the basis of the preliminary analysis presented here, we conclude that the minor differences in the MCD magnetization behavior of thionine-oxidized Av1 and Av1′ can be explained in terms of slight differences in zero-field splitting parameters and that novel clusters with ground state $S = {}^5/_2$ spin systems ($D < 0$) are present in both enzymes.

On the basis of the form of the low-temperature MCD spectrum, the MCD magnetization behavior, and the absence of EPR resonances, we conclude that clusters with similar electronic and magnetic properties to P clusters are present in the alternative enzyme. These centers will be termed P′ clusters, so as to distinguish them from conventional P clusters. The intensity of the MCD spectrum for thionine-oxidized Av1′ compared to Av1 suggests that the alternative enzyme contains approximately two P′ clusters, as opposed to four P clusters in the conventional enzyme (Zimmermann et al., 1978). However, this conclusion should be viewed as tentative at present, since the intensity of the low-temperature MCD spectrum can only be rigorously used for quantitative of Fe-S centers when a sample of known concentration containing clusters with identical MCD spectra is available.

Finally, these results also have important genetic and enzymatic implications. In constructing strain LS15, a deletion was transformed in the *nifHDK* genes of *A. vinelandii* that encode for the structural proteins of both conventional components 1 and 2 (Hales et al., 1986b). The presence of this deletion proves that the alternative nitrogenase is transcribed from structural genes different from those required for the conventional enzyme. This difference has been corroborated by the facts that Av1′ is antigenically different from Av1 (Hales et al., 1986b) and Av1′ is composed of polypeptides of different molecular weights from Av1 (Hales et al., 1986a). On the other hand, since these genes are contiguous on the genome (Brigle et al., 1985), no other nitrogen-fixing genes were altered by this transformation. This means that, although the structural genes are different, the alternative enzyme may utilize some or all of the remaining nitrogen-fixing genes that are needed in the processing of the conventional enzyme. In *K. pneumoniae*, where the genetics of nitrogen fixation are best understood, the products of the *nifE*, *-N*, *-V*, *-B*, and *-Q* genes have been associated with the synthesis of M clusters, while the genes associated with P clusters are not known (Cannon et al., 1985). Unfortunately, for *A. vinelandii*, the genomic structure used for the processing of nitrogenase is only partially understood (Kennedy et al., 1985). However, it was recently implied (Joerger et al., 1986) that *nifB* is needed by both enzyme systems in *A. vinelandii* while *nifN* is not. The MCD results presented here are the first to provide spectroscopic evidence for similarities between the two nitrogenase component 1 proteins. Specifically, these results imply that *A. vinelandii* may use similar or identical genes for the processing of the P clusters and P′ clusters. Furthermore, the conservation of this type of cluster in the two proteins lends support to their importance in the enzymology of nitrogen fixation.

CONCLUSION

The low-temperature MCD data presented here confirm that the constituent clusters in component 1 of conventional nitrogenase are highly conserved in enzymes isolated from

diverse organisms. Moreover, they attest to differences in the nature of the clusters in the recently isolated VFe protein from the alternative nitrogenase from *A. vinelandii*. The new types of clusters have been identified in Avl′. One is paramagnetic with an $S = {}^3/_2$ ground state in the dithionite-reduced enzyme and becomes diamagnetic upon oxidation with thionine. By analogy with the conventional MoFe protein, this center probably corresponds to a novel V-Fe-S cluster. The other type of cluster is diamagnetic in the dithionite-reduced enzyme but becomes paramagnetic with an $S = {}^5/_2$ ground state after thionine oxidation. The electronic, magnetic, and redox properties of this cluster are similar to but not identical with those of the P clusters in the conventional MoFe protein.

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