constituent of the MoFe protein of the nitrogenase complex. The absence of Mo has been shown to prevent the synthesis of nitrogenase, but it is not clear how Mo regulates or how MoO_4^{2-} accumulation is regulated.

Nitrogenases from different bacteria have very similar properties. The enzyme consists of the two oxygen-labile proteins — component I (which has two copies each of two different subunits and contains iron and molybdenum atoms) and component II (which has two copies of a single subunit and contains the site of substrate binding and substrate reduction). The active site of nitrogenase resides in an iron-molybdenum cofactor (FeMo-co) that is a part of component I. The role of component II is to reduce component I.

MoFe proteins are complex metalloproteins with MW around 220,000, containing two molybdenum atoms with about 30 iron atoms and a slightly smaller number of acid-labile sulphur atoms per molecule. Mössbauer studies have resulted in identification of three types of metal centre named "P" clusters (16 Fe), the FeMo centres (12-16 Fe) and "S" atoms (2 Fe).

Mo-uptake is energy-dependent and is repressed by NH_4^+ (2 mM) and high concentrations of MoO_4^{2-} (1 mM) in *Clostridium pasteurianum*. In *Azotobacter vinelandii*, Mo-uptake was not inhibited in the presence of NH_4^+, although the rate of uptake of MoO_4^{2-} was slower. During derepression of *Klebsiella pneumoniae* both uptake and nitrogenase activity were maximal at 1 μM MoO_4^{2-} and uptake was inhibited by NH_4^+. *Azospirillum brasilense* showed the maximal nitrogenase activity *in vivo* at a MoO_4^{2-} concentration of 2 μM. *C. pasteurianum* and *A. vinelandii* have Mo-storage proteins which bind MoO_4^{2-} transported into the cell, but *K. pneumoniae* does not. The steps in the transformation of MoO_4^{2-} to the Mo-S environment of FeMo-co may be expected to be very similar in all nitrogen fixing organisms.

ACKNOWLEDGEMENTS

These studies were supported by the Maringá State University, Paraná, Brazil, and by the Ministry of Education of Japan (Monbusho).

* Present address: Department of Agricultural Chemistry, Faculty of Agriculture; Tohoku University — Sendai 980, Japan.

PS1.19 — TH

RICHARD C. REEM
JAMES W. WHITTAKER
EDWARD I. SOLOMON
Department of Chemistry
Stanford University
Stanford, California 94305
U.S.A.

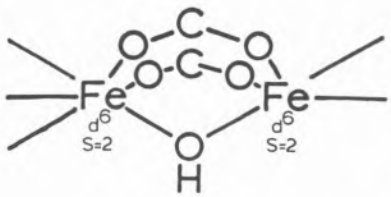
SPECTROSCOPIC STUDIES OF NONHEME IRON ACTIVE SITES

Nonheme iron active sites are involved in the interactions with O_2 in oxygen transport (hemerythrin, Hr), superoxide dismutation (iron superoxide dismutase, FeSD) and lipid peroxidation (soybean lipoxygenase, SBL). We have used a combination of variable temperature absorbance, CD, MCD, and EPR spectroscopies to obtain information on active site electronic structure relating to biological function of these iron sites in both ferric and ferrous oxidation states.

The structure of the binuclear iron active site in Met<Fe(III)Fe(III)>Hr is known to high resolution from X-ray crystallographic studies, which show two irons bridged by an oxo anion as well as aspartate and glutamate carboxylates. One iron is hexacoordinate, bound by three histidyl residues in addition to the bridging ligands; the other iron is pentacoordinate in the Met protein (three bridging ligands plus two histidyl residues) and binds exogenous ligands such as N_3^-, or O_2^{2-} in the oxygenated protein. The results of variable temperature absorbance and CD spectroscopy allow us to distinguish for the first time electronic transitions associated with each iron in the active site and to observe the changes which occur at each iron with temperature and exogenous ligand binding. This, coupled with polarized single crystal absorbance data leads to detailed insight into the electronic structure of the site and perturbation by binding of peroxide.

In contrast to the well defined geometric structure of the Met Hr iron site which has emerged, the

deoxy Hr active site is known at a lower resolution. In addition, previous spectroscopic studies have been impaired by the absence of absorbance features clearly associated with the iron in the accessible spectral range. The application of variable temperature CD and MCD have allowed us to detect the Fe(II) ligand field transitions of deoxy Hr near 1000 nm and use them as probes of the iron site in this oxidation state. The MCD spectrum of deoxy Hr is shown in Fig. 1A. At 5 K, the zero field CD spectrum is coincident with the 5 T MCD spectrum. As the temperatue is raised, while keeping the field constant, an MCD signal is observed, reaching a maximum intensity near 65 K and declining at higher temperatures. Assuming a largest reasonable value for the zero field splitting to be $D = 12$ cm^{-1} this MCD data requires an antiferromagnetic exchange interaction $-J > 13 \pm 5$ cm^{-1} within the binuclear center. Model studies have shown that the carboxylate bridges cannot account for an exchange of this magnitude. A hydroxo or oxo bridge would provide the required coupling, and, although the possibility of a protein conformational change which introduces a new bridging ligand cannot be ruled out, a hydroxo bridge is most likely present in deoxy hemerythrin. This data, combined with CD data which indicates the presence of one 5- and one 6-coordinate iron, allows us to present the following model for the deoxy Hr active site:



CD studies of the Fe(II) ligand field bands in deoxy Hr show that N_3^- and OCN^- bind at the pentacoordinate iron in deoxy Hr, each with a single binding constant of about 70 M^{-1}. F^- also binds with $K_B \sim 7$ M^{-1}, but no other anions have been found to bind with $K_B > 0.5$ M^{-1}. Binding of anions generates large changes in the CD and MCD spectra of deoxy Hr, as illustrated in Fig. 1. The MCD spectrum of deoxy N_3^- Hr (Fig. 1B) indicates a groundstate paramagnetism which saturates at low temperature with $g > 8$. We also observe an unusual Fe(II) EPR signal (Fig. 1C)

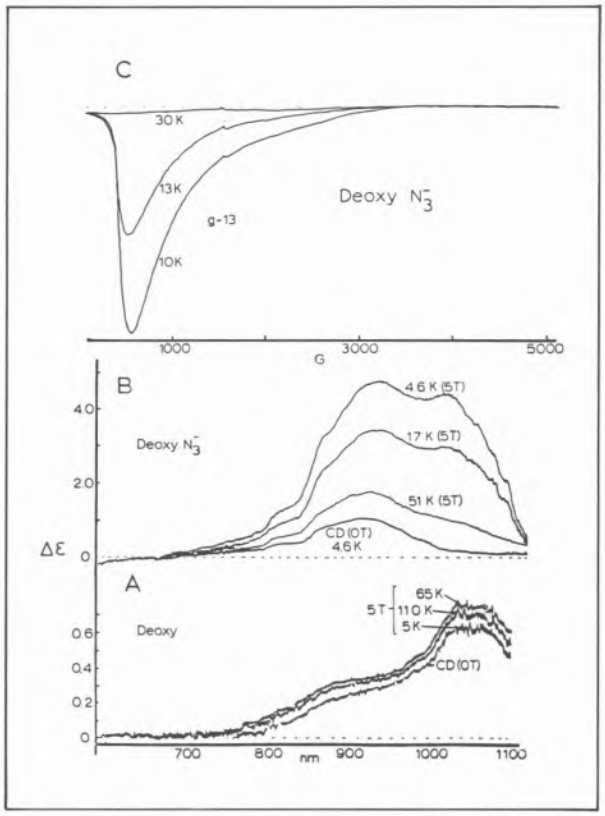


Fig. 1
EPR and MCD Spectra of Deoxy and Deoxy-N_3^- Hemerythrin

with $g_{eff} \sim 13$. The observation of paramagnetism indicates that the groundstate magnetic properties of the binuclear ferrous site are no longer dominated by antiferromagnetic coupling. The most probable explanation of this effect is the breaking of, or perturbation of the hydroxo bridge. This could result in labilization of the oxo bridge which would be consistent with the observed rapid exchange of the bridge oxygen with solvent water in the presence of N_3^- or OCN^-.

Similar studies on the ligand field spectra of the ferrous mononuclear iron active sites in FeSD and SBL are providing information on the environment and accessibility of the iron in this oxidation state. Transitions observed near 1000 nm in CD and MCD spectra reflect the coordination environment of the iron centers and have been used to probe ligand binding to the ferrous sites. In addition, we have used low temperature MCD spectroscopy to probe the mononuclear iron sites in the ferric enzymes and their interactions with exogenous ligands.

PS1.20 — MO

B. BARATA
I. MOURA
A.V. XAVIER
J.J.G. MOURA
Centro de Química Estrutural
FCL, UNL, Complexo I, IST
Av. Rovisco Pais, 1000 Lisboa
Portugal
J. LIANG
B.H. HUYNH
Department of Physics
Emory University
Atlanta, Georgia 30322
U.S.A.
J. LEGALL
Department of Biochemistry
University of Georgia
Athens, Georgia 30602
U.S.A.

EPR AND MÖSSBAUER STUDIES ON *DESULFOVIBRIO GIGAS* Mo(Fe-S) PROTEIN

The Mo(Fe-S) protein from *Desulfovibrio gigas*, a sulfate reducing organism, was shown to contain one Mo and approx. 12 Fe per molecule and a molecular weight of 120 KDa. No evidence was found for the presence of subunits. Its physiological role has not yet been determined. Optical, EPR and CD data strongly suggest the presence of [2Fe-2S] clusters. At ~70 K the EPR spectrum of the dithionite reduced sample exhibits a Mo signal centered around $g = 1.97$ and signals at $g = 2.02$, 1.94 and 1.93, corresponding to one type of [2Fe-2S] centers, named (Fe-S I). At lower temperature ($T < 40$ K) an additional signal appears at $g = 2.06$ and 1.90, indicating the presence of a second [2Fe-2S] center (Fe-S II). Redox titration studies revealed yet another Fe-S center with type I EPR signal. The two type I centers are termed (Fe-S I A) and (Fe-S I B).

When observed at temperatures lower than 40 K, the type I Fe-S EPR features at $g = 2.02$ split into two peaks separated by approx. 15 G. Such splitting can be explained either by coupling of the paramagnetic site to a nearby $I = 1/2$ nucleus, such as a proton, or a slight difference in the resonances of Fe-S I A and I B centers. The EPR signals of the Fe-S centers and molybdenum of the reduced protein are compared in H_2O and D_2O.

Recently, the protein was purified from ^{57}Fe grown cells. The quality of the Mössbauer data obtained in the native and partially reduced Mo(Fe-S) protein enabled us to pursue the characterization of the [Fe-S] centers in correlation with the previously reported EPR data.

In the native state, the Mössbauer parameters of the only quadrupole doublet observed at 4.2 K with an external field of 500 G applied parallel to the gama beam ($\Delta E_Q = (0.63 \pm 0.02)$ mm/s, and $\delta = (0.27 \pm 0.02)$ mm/s), are typical of high-spin ferric ions with tetrahedral sulfur coordination.

Partially reduced states of the protein show two types of doublets, at 150 K. The central quadrupole doublet is similar to that of the oxidized Mo(Fe-S) protein. The outer doublet represents the ferrous site in the reduced [2Fe-2S] clusters. The shape of the ferrous doublet indicates that it consists of at least two unresolved doublets. This observation is consistent with the EPR finding that the Mo(Fe-S) protein contains more than one type of [2Fe-2S] cluster. The Mössbauer parameters for the two ferrous sites are $\Delta E_Q = (3.27 \pm 0.02)$ mm/s, $\delta = (0.57 \pm 0.02)$ mm/s and $\Delta E_Q = (2.79 \pm 0.02)$ mm/s, $\delta = (0.59 \pm 0.02)$ mm/s.

Low temperature studies are being carried out in order to compare the above data with [2Fe-2S] clusters of reduced ferredoxin from spinach and Rieske centers.

ACKNOWLEDGEMENTS

This work was supported by INIC, JNICT and NATO.

REFERENCES

J.J.G. MOURA, A.V. XAVIER, R. CAMMACK, D.O. HALL, M. BRUSCHI, J. LEGALL, *Biochem. J.*, **173**, 419-425 (1978).