MAGNETIC CIRCULAR DICHROISM OF NON-HEME IRON PROTEINS*

D. D. Ulmer†, B. Holmquist and B. L. Vallee+

The Biophysics Research Laboratory, Department of Biological Chemistry, Harvard Medical School, Division of Medical Biology, Peter Bent Brigham Hospital, Boston, Massachusetts

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The magnetic circular dichroism (MCD) at 45 kgauss has been determined for a group of non-heme iron proteins. Both transferrin and conalbumin exhibit a single, positive ellipticity band at 330 nm ($[\theta]_M$ = 560). Oxy- and methemerythrin, spinach and clostridial ferredoxins and rubredoxin all display distinctive multibanded spectra which may reflect such factors as coordination of the metal, its ligands, metal bridging by other atoms, and varying degrees of metal-metal coupling. The MCD spectra of both ferredoxins and rubredoxin undergo dramatic change upon oxidoreduction providing a potential means for relating the electronic structure of the iron to protein function. In contrast to the plant ferredoxins, the magnetic field does not significantly affect the CD spectra of adrenodoxin and putidaredoxin.

Studies of the extrinsic Cotton effects of metalloproteins by circular dichroism (CD) have contributed importantly to the understanding of their conformations and metal-binding sites (1-3). Whereas conventional CD signals arise only from asymmetric chromophores, imposition of a properly oriented magnetic field, MCD, can induce optical activity -- the Faraday Effect -- in virtually all electronic transitions. Since the physical origin of the Faraday Effect and that of the Cotton Effect are quite distinct (4,5) the former would be expected to provide further insight into the electronic structure of the metal sites of metalloproteins.

To assess the potential usefulness of magnetic circular dichroism (MCD), we have examined a group of non-heme iron proteins which have been characterized previously by conventional CD and resonance spectral methods.

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†Present address: Martin Luther King, Jr. Hospital, Department of Medicine, Los Angeles, California.

+To whom inquiries should be addressed.

A preliminary report of this work and related studies of Co(II) substituted proteins has been given (6,7).

METHODS: Metal complexes of human serum transferrin (Hoechst Chemical Co.) and conalbumin (Sigma Chemical Co.) were prepared as described previously (1) and measured in 0.1 M Tris-Cl buffer, pH 8.5. Spinach ferredoxin, type III, was diluted to 1 mg/ml in 0.15 M Tris-0.8 M NaCl, pH 7.3, prior to measurement. Clostridial ferredoxin, kindly provided by Dr. Walter Lovenberg, was dissolved on 0.1 M Tris-Cl, pH 7.28 and Clostridial rubredoxin, also a gift of Dr. Lovenberg, was dissolved in 0.15 M Tris-Cl, pH 7.3. The ratio of absorption at 280/490 nm of this material was 2.55. Hemerythrin, a gift of Dr. Irving Klotz, was dialyzed against 0.1 M Tris-cacodylate, pH 8.0, to remove ethanol and excess salt, while adrenodoxin, a gift of Dr. T. Kimura, was dissolved in 0.01 M sodium phosphate, pH 7.49, just prior to spectral measurements. Putidaredoxin, generously prepared by Drs. J. L. Lipscomb and I. C. Gunsalus, was stored under liquid N_2 prior to measurement in 0.05 M Tris-Cl, pH 7.4. Xanthine oxidase, from butterfat (Sigma Chemical Co.) was dialyzed versus 0.01 M Tris-Cl, pH 8.5, for several days to remove ammonium sulfate.

MCD spectra were measured on a Cary Model 61 CD instrument equipped with a Varian Model V-4145 superconducting solenoid at field strengths of 40 to 45 kgauss. The slit width was adjusted to permit spectral dispersion of 2 nm or less. CD spectra were measured under identical experimental conditions in the absence of a magnetic field. MCD is expressed as $[\theta]_M$ in degrees cm^2 $decimole^{-1}$ $kilogauss^{-1}$, where $[\theta]_M$ is calculated as for conventional CD. All samples were corrected for appropriate solvent blanks.

RESULTS:

Figure 1 compares the CD and MCD spectra of iron transferrin. A broad negative CD ellipticity band centered at 465 nm coincides precisely

with the λmax of the absorption spectrum; there is also a sharper, positive band at 330 nm. A magnetic field of up to 47 kilogauss markedly enhances the 330 nm band but not that at 465 nm (Figure 1). The MCD spectrum, the

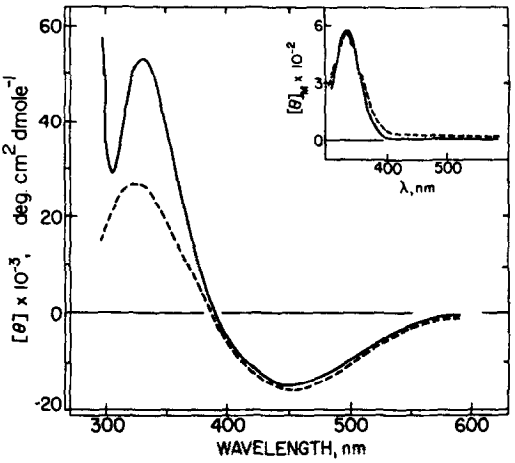

Fig. 1. CD spectra of human serum iron transferrin in the absence (····) and presence (——) of a 47 kgauss magnetic field. INSERT: MCD spectra of transferrin (——) and conalbumin (- - -). For conditions see the text.

difference between measurements in the presence and absence of a magnetic field (Figure 1, insert), manifests as a single, sharp signal at 330 nm ($[\theta]_M$ = 560). This transition likely represents a B-term since its intensity is not altered by variations in temperature from -30 and +30° C (See below), though definitive elimination of a C-term requires the examination of a wider temperature range. The MCD spectrum of conalbumin is virtually identical to that of transferrin (Figure 1, insert).

Figure 2 a-c shows the MCD spectra of hemerythrin, from sipunculid worms, and of a bacterial and a plant ferredoxin. Hemerythrin contains two iron atoms per mole thought to have nearly identical electronic structures in both the oxy- and met-forms of the protein (8). The CD spectra of the two forms are very similar, but their MCD spectra differ significantly: oxyhemerythrin exhibits three distinct positive MCD bands, at 330, 380 and 503 nm, superimposed

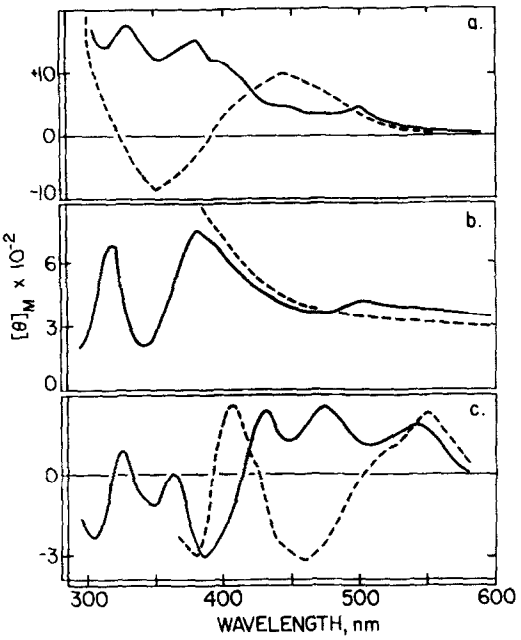

Fig. 2. MCD spectra of a) oxyhemerythrin (——) and methemerythrin (- - -); of b) oxidized (——) and reduced (- - -) clostridial ferredoxin; and c) of oxidized (——) and reduced (- - -) spinach ferredoxin. For conditions see text.

upon a broad envelope of positive ellipticity increasing with decreasing wavelengt (Figure 2a). In contrast, methemerythrin generates a broad biphasic band with a positive peak at 438 nm, and a negative trough at 350 nm. MCD appears to be a highly discriminative sensor of the electronic properties of the oxy- and met-forms.

A high magnetic field also resolves details not observed in the absorption or conventional CD spectra of oxidized Clostridial (8 g at Fe/mole) (Figure 2 b), and spinach ferredoxin (2 g at Fe/mole) (Figure 2c) as exemplified by the oxidized spinach protein which exhibits seven distinct new ellipticity bands over and above those seen in the CD spectrum. The ferredoxins function in electron transfer and their reduction with sodium dithionite resolves yet additional transitions, (Figures 2b,c) suggesting that MCD may serve to correlate their structural with pertinent functional properties. Both the MCD and CD spectra of xanthine oxidase (9) closely

resemble those of spinach ferredoxin. The implication that this reflects analogous electronic structures of both these non-heme iron proteins requires further study.

Above 300 nm the MCD of oxidized rubredoxin manifests as an envelope of overlapping, intense bands. A biphasic component, centered between 380-390 nm, may represent an A-term (Figure 3) (see discussion), and could be particularly helpful in defining the environment of the single iron atom. Reduction markedly enhances the negative and positive ellipticity bands at 312 and 336 nm (Figure 3) identified previously in the CD spectrum of rubredoxin.

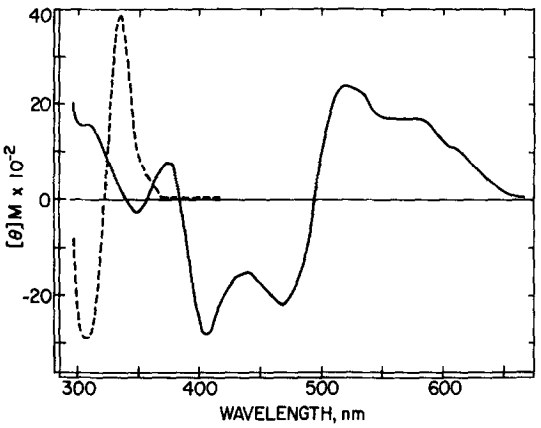

Fig. 3. MCD spectra of oxidized (——) and reduced (- - -) rubredoxin. For conditions see the text.

Adrenodoxin, in accord with earlier observations at lower field strengths (Kimura, personal communication), and putidaredoxin, kindly supplied by Dr. I. Gunsalus, do not exhibit appreciable MCD at fields up to 45 kilogauss, indicating that in contrast to the more negative potential plant ferredoxins, both these proteins lack significant degeneracy in the visible and near ultraviolet iron transitions.

DISCUSSION: Zeeman splitting of degenerate ground or excited states of an atom generates characteristic MCD signals categorized in accord with

their origins and differentiated by means of their patterns and temperature dependencies. C terms are monophasic and A terms are biphasic; the former are temperature dependent, the latter are not. B terms, which are monophasic and temperature-independent result from magnetic field induced mixing of non-degenerate ground and excited states of different electric dipole polarization (5).

The MCD spectra of simple chromophoric molecules reflect magnetic and symmetry properties of their electronic states and of polarization characteristics of their transitions. These can lead to spectroscopic assignments which provide a quantitative basis for theoretical calculations. The degree to which MCD can assist in the analysis of more complex molecules, e.g. metalloproteins, is as yet uncertain. However, it should prove valuable in delineating transitions concealed in absorption and CD spectra, either because of low transition probabilities or because of overlaps. The present studies support such expectations, though compared to simpler systems, spectral interpretation remains much more empirical.

The rich diversity of the non-heme iron MCD spectra doubtless is influenced by a variety of factors including the valence of the metal, varying degrees of metal-metal interaction, metal-bridging by other atoms, as well as the ligands of the iron atoms and their coordination geometries. As concerns the latter, we have found the MCD spectra of cobalt II complexes to be characteristic of particular geometries suggesting those of some Co(II) substituted metalloenzymes (6).

Transferrin and conalbumin contain two high spin Fe (III) atoms (10) octahedrally coordinated to nitrogen and phenolic oxygen ligands (11). The metals are not thought to interact (12). Based on the stability of their complexes, binding of the two iron atoms seems identical though differences in their resonance signals have been interpreted to suggest that at any given time each of the two irons may exist in a different conformation (13,14). The virtually identical, single transition in the MCD spectra of the two

proteins (Figure 1) may be characteristic of high spin Fe (III), coordinated to oxygen and nitrogen.

The MCD spectra of hemerythrin and the bacterial and plant ferredoxins are significantly more complex (Fig. 2, a-c). However, the electronic features of all of these proteins are believed to reflect varying degrees of iron-iron coupling (8, 15, 16) which, taken together with the differences of their metal ligands, may likely account for their distinctive spectra. In this regard the three positive ellipticity bands in nearly the same spectral positions of both oxyhemerythrin and oxidized Clostridial ferredoxin indicate that analogous transitions of the iron in both proteins may respond to the magnetic field. Oxygen is thought to form a bridge between closely adjacent metal atoms of hemerythrin (17) and sulfur might likely play an analogous role in some ferredoxins, accounting perhaps for those features of their MCD which are similar.

The sulfur bound iron atoms of oxidized spinach ferredoxin, also exhibit three positive MCD maxima at or near the position of the bands noted above (Fig. 2 c). However, yet additional ultraviolet and visible maxima distinguish the MCD spectrum of the plant protein, perhaps due to unusually strong anti-ferromagnetic exchange coupling between metal centers, as suggested by NMR studies (15). The reduced forms of plant and bacterial ferredoxins differ strikingly both from their oxidized equivalents and from each other, suggesting that the iron atom has a unique environment in each. It may be anticipated that investigation of a series of these proteins from different sources may clarify the basis of these variations.

The single Fe atom of oxidized and reduced rubredoxin, coordinated with sulfhydryl groups of cysteine in a distorted tetrahedron, is in the high spin Fe(III) and high spin Fe (II) states, respectively (18,19). The MCD spectrum of the oxidized protein is unusually intense compared to those of the other non-heme iron proteins here examined, and its bands are split with an apparent A-term centered between 380-390 nm (Fig. 3), in marked contrast with

the high spin iron in transferrin and conalbumin (Fig. 1). The intense, biphasic signal of reduced rubredoxin at shorter wavelengths is not observed in the reduced forms of any of the other non-heme iron proteins examined. These distinctive MCD signals may prove characteristic for high spin tetrahedral iron-sulfur coordination. However, such conjectures remain to be explored in more detailed investigations now underway.

Systematic studies of synthetic analogues with structural and electronic properties akin to those of the iron-sulfur proteins, such as reported recently by Holm and his collaborators (20), and of other complexes of iron with nitrogen and oxygen ligands are needed to discern the precise basis of the spectra observed so far.

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