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Models for Ferrous Cytochrome b_5: Sign Inversions in the Magnetic Circular Dichroism Spectra of Bis-imidazole Ferrous Porphyrin Systems

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Abstract

The magnetic circular dichroism (MCD) spectrum of bis-imidazole ferrous tetraphenylporphyrin in the Soret region is nearly the mirror image of the spectrum of ferrous cytochrome b_5, a bis-imidazole (histidine)-ligated hemoprotein. Based on previous MCD studies of model and protein heme systems, a sign inversion in the spectra of two heme chromophores having essentially the same coordination structure is unexpected. To investigate whether the nature of the porphyrin itself could account for the observed spectral discrepancy, two additional model complexes, bis-imidazole ferrous protoporphyrin IX dimethylester and bis-imidazole ferrous octaethylporphyrin, whose peripheral porphyrin substituent patterns more closely match that of the protein-bound porphyrin, have been prepared and their MCD spectra measured. In these cases, the band pattern of the ferrous protein in the Soret region is successfully reproduced. It therefore appears that the anomalous MCD spectrum of the tetraphenylporphyrin complex can be attributed to the nature and positioning of the peripheral substituents on the porphyrin ring. Although iron tetraphenylporphyrin complexes are frequently used as models for protoporphyrin-containing hemoproteins, one should be aware that such differences in the peripheral porphyrin substituents may significantly affect the spectral properties of the model complex.

Introduction

One of the central dogmas of bio-inorganic chemistry is that the metal-containing active sites of most, if not all, metalloproteins can be accurately modeled with low molecular weight synthetic analogs [1]. A thorough understanding of the factors that influence the particular spectroscopic properties of a specific type of metal site is therefore of considerable importance in assessing the extent to which an individual metal complex will serve as a good model for a given active site. Thus, studies of models for metalloprotein active sites whose structures have been crystallographically established can still be of importance in that they allow the relationship between structure and spectroscopy to be systematically tested [2]. In this regard, magnetic circular dichroism (MCD)** spectroscopy has been a particularly useful method with which to probe the electronic properties of heme iron model and protein systems and to predict coordination structure from spectroscopic parameters [3]. It was therefore surprising to note that the reported MCD spectrum of bis-imidazole ferrous tetraphenylporphyrin [4] [$(Im)_2Fe(TPP)$] in the Soret region (350–500 nm) was the mirror image of the spectrum of ferrous cytochrome b_5 [5], a bis-Im (histidine) ligated protoporphyrin-containing hemoprotein whose crystal structure has been established [6] (Fig. 1A). This sign inversion was unexpected since the MCD spectral features of other low-spin, six-coordinate ferrous [4, 7] and ferric [8] TPP complexes in the Soret region are generally similar to those of their protoporphyrin-containing hemoprotein [5, 9] and model [10, 11] counterparts. In addition, numerous other studies have indicated that iron TPP complexes can be used as spectral [12] as well as structural [13] models for hemoproteins with similar axial ligation. To address this spectral anomaly, we have measured the MCD spectra of the bis-Im ferrous complexes of protoporphyrin IX dimethylester (PPIXDME) and octaethylporphyrin (OEP) for the first time

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**Abbreviations used: MCD, Magnetic circular dichroism; TPP, the dianion of tetraphenylporphine; PPIXDME, the dianion of protoporphyrin IX dimethyl ester; OEP, the dianion of octaethylporphine; Im, imidazole; 1-MeIm, 1-methylimidazole; Porph, dianion of any porphyrin; EA, electronic absorption.

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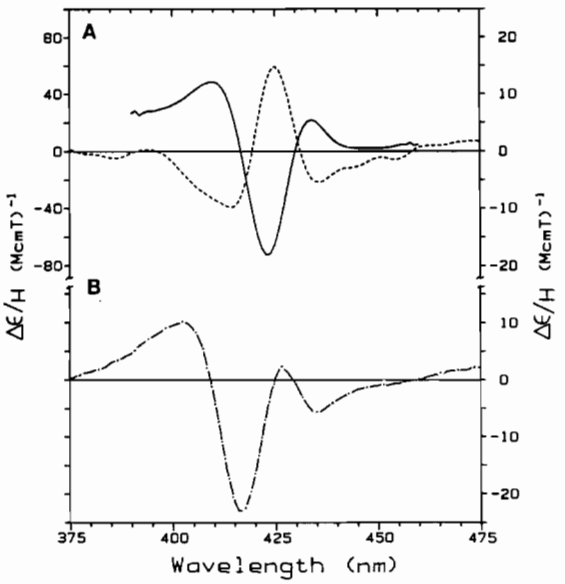

Fig. 1. (A) MCD spectra of bis-Im ferrous TPP (solid line, left scale) and ferrous cytochrome b_5 (dashed line, right scale)*. Note the different scales indicates for each complex. (B) MCD spectrum of 80% ferrous + 20% ferric cytochrome b_5 (dot-dashed line, right scale), produced by a linear combination of the individual spectra.

and have compared them with the previously reported spectra of $(Im)_2Fe(TPP)$ and cytochrome b_5.

Experimental

Ferric TPP chloride [(Cl)Fe(TPP)] was prepared and purified according to literature methods [14a–b]; (Cl)Fe(PPIXDME) was prepared by HCl treatment of the μ-oxo-dimer, which in turn was prepared from hemin chloride (Aldrich) [14c]; (Cl)Fe(OEP) was purchased from Aldrich Chemical Co.; Im (Eastman) was recrystallized from ethanol and 1-methylimidazole (1-MeIm, Aldrich, 99%) was used as received. All solvents were purified according to literature procedures; all other chemicals were of reagent grade and were used as received. Purified cytochrome b_5 (beef liver, tryptic digest, $A_{412.5}/A_{280} = 5.95$ [15]) was the generous gift of Professor A. Grant Mauk.

All three bis-Im ferrous porphyrin complexes were prepared *in situ* in an inert atmosphere glove box (Vacuum Atmospheres) as follows: a solution of $[(Im)_2Fe(Porph)]Cl$** was prepared by dissolving the (Cl)Fe(Porph) (0.05 to 0.50 mM) and either Im or 1-MeIm (5 to 100 mM) in the organic solvent [16]. The complex was then reduced with aqueous $Na_2S_2O_4$ [17], and titrated with additional Im or 1-MeIm until changes in the electronic absorption (EA) spectrum were no longer observed. The cytochrome b_5 sample was prepared in 100 mM potassium phosphate buffer, pH 7.2, and reduced with a slight excess of solid $Na_2S_2O_4$. Iron porphyrin concentrations were determined using published molar extinction coefficients [4, 8, 16], or from the weight of the original (Cl)Fe(Porph) sample. The concentration of cytochrome b_5 was calculated using $\epsilon_{423} = 171\ mM^{-1}\ cm^{-1}$ for the ferrous protein [18]. MCD, EA, and electron paramagnetic resonance (EPR) spectra were obtained as previously described [19] in gas-tight quartz cells to prevent air oxidation. Samples for EPR (0.05 to 0.50 mM) and for EA and MCD (0.05 to 0.10 mM) were analyzed at 77 K and 298 K, respectively. The approximate concentration of contaminating oxidized model complex present in ferrous samples was determined by comparison of the observed EPR intensity to that of the fully oxidized complex under identical conditions.

Results and Discussion

In order to ascertain the origin of the anomalous Soret region MCD spectrum of $(Im)_2Fe(TPP)$ relative to that of ferrous cytochrome b_5 (Fig. 1A), we initially questioned the oxidation state integrity of the sample. Because the MCD spectrum of the ferric cytochrome in the Soret region is much more intense than that of the ferrous protein [5], we reasoned that in the model system, the presence of a small amount of the ferric species could easily cause drastic changes in the observed spectrum of the ferrous complex. To test this, we generated an MCD spectrum of 80% ferrous and 20% ferric cytochrome b_5 by a linear combination of the spectra of the two discrete forms. As seen in Fig. 1B, the computer-derived spectrum is remarkably similar in band pattern to the spectrum of $(Im)_2Fe(TPP)$. However, examination of the ferrous TPP complex by EPR spectroscopy revealed essentially no ($\leqq$5%) ferric complex. Furthermore, a computer-generated spectrum of 95% ferrous and 5% ferric cytochrome b_5 failed to reproduce the inverted bands. The anomalous MCD spectrum of the ferrous TPP complex is, therefore, not due to partial oxidation of the sample but is an inherent feature of the molecule.

The bis-Im ferrous complexes of PPIXDME and OEP were also prepared and their MCD spectra compared to that of cytochrome b_5 (Fig. 2). The peripheral porphyrin ring substituents of OEP and

*The MCD spectra of bis-Im ferrous TPP and of ferrous cytochrome b_5 displayed in Fig. 1 are very similar to the previously published spectra [4, 5].

**The MCD and EA spectra of the bis-Im and bis-(1-MeIm) complexes of both PPIXDME and OEP were virtually indistinguinshable.

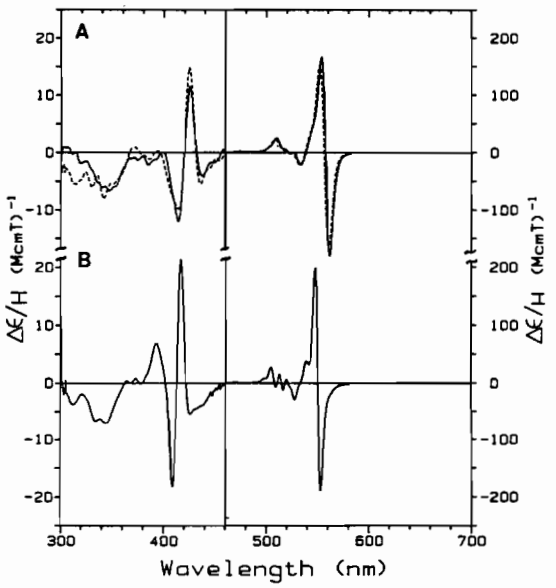

Fig. 2. (A) MCD spectra of ferrous cytochrome b_5 (solid line) and bis-Im ferrous PPIXDME in CH_2Cl_2 (dashed line). Note the different scales: Soret region, left scale; visible region, right scale. (B) MCD spectrum of bis-(1-MeIm) ferrous OEP in toluene. Same conditions as for Fig. 1.

PPIXDME are located on the β-pyrrole carbons, unlike those of TPP which are on the *meso*-carbons. The PPIXDME complex was investigated in order to determine whether the spectral inversion noted in the TPP case was applicable to all bis-Im porphyrin complexes; OEP was chosen since it is the other synthetic porphyrin in addition to TPP that is most frequently used in hemoprotein modeling studies. The MCD spectrum (Fig. 2A) of $(Im)_2Fe(PPIXDME)$ is virtually identical to that of the ferrous protein in both band energies and intensities over the entire 300–700 nm range. Clearly, with the correct porphyrin model, the match-up between the spectra of the model and the protein is excellent. The spectrum of $(1\text{-MeIm})_2Fe(OEP)$ (Fig. 2B) is also very similar to that of the cytochrome although the band energies are slightly blue-shifted (*ca.* 8 nm for the Soret MCD peak)*. Most importantly, the PPIXDME and OEP complexes show the same negative-positive-negative band pattern with decreasing wavelength in their Soret region MCD spectra as does cytochrome b_5.

The spectral inversion in the TPP complex thus appears to be a function of the porphyrin peripheral substituents. Changes in the porphyrin ring substituents have been found to affect the spin-state of the central iron as well as the coordination structure, most notably in the work of Scheidt on the bis-3-chloropyridine [20] and isothiocyanate-pyridine [21] complexes of ferric TPP and OEP. He ascribed the structural differences between the OEP and TPP complexes to '*cis*-effects' arising from the influence of the porphyrin and its peripheral substituents on the characteristics of the complex. This is in contrast to '*trans*-effects', which reflect the influence of the axial ligands on the iron. Other studies have shown that the MCD spectra of metal-free and zinc-bound chlorins (dihydroporphyrins) are highly influenced by the position of the reduced double bonds as well as the peripheral substituents on the chlorin ring [22]. The reported MCD spectrum of bis-(1-MeIm) ferrous heme *a* [23]** in the Soret region displays a simple derivative-shaped band pattern distinct from that shown by either the TPP or PPIXDME models. Curiously, the spectra of the bis-pyridine [11] and bis-butylamine† complexes of ferrous PPIXDME are similar in shape as those of the corresponding ferrous TPP complexes [4]. Thus, the MCD spectra of bis-Im ferrous porphyrins seem to be more sensitive to *cis*-effects than those of other six-coordinate, low spin ferrous porphyrin complexes.

In summary, MCD spectroscopy has been used to systematically analyze and compare the bis-Im ferrous complexes of the three most commonly used porphyrins: TPP, OEP, and PPIXDME. The sign-inverted Soret region MCD spectrum of $(Im)_2$-Fe(TPP) has been found to be an inherent property of the TPP complex. In contrast, the MCD spectrum of $(Im)_2Fe(PPIXDME)$ is virtually superimposable upon that of protoporphyrin-containing bis-histidine-ligated cytochrome b_5. The MCD spectrum of $(1\text{-MeIm})_2Fe(OEP)$ was found to also exhibit a non-inverted band pattern in the Soret region. The spectral inversion of the TPP complex, relative to the spectra of the PPIXDME and OEP complexes, has been attributed to the position and type of peripheral porphyrin substituents. Such '*cis*-effects' have been found to cause iron spin-state and coordination structural changes in other iron porphyrin complexes as well as spectral changes in metal-free and zinc-bound chlorine complexes. Therefore, one must scrutinize spectroscopic studies in which

*The absorption maxima of the ferrous OEP complex (320, 413, 521, and 549 nm) are blue-shifted relative to those of the PPIXDME complex (330, 423, 528, and 558 nm) due to the difference in substituents on the β-pyrrole carbons (alkyl groups on OEP *vs.* vinyl and alkyl groups on PPIXDME); a similar blue-shift occurs when mesoheme (alkyl substituents) is substituted for protoheme in cytochrome b_5 [18].

**Heme *a* has a similar structure to protoporphyrin IX except that one of the vinyl groups is replaced by a formyl group.

†The bis-(1-butylamine) complexes of both ferrous PPIXDME and TPP exhibit similar positive-negative-positive MCD patterns (data not shown).

iron TPP complexes are used to model protoheme-containing hemoproteins; changes in the porphyrin periphery may in some cases drastically alter the spectral characteristics of the model complex.

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References

1 J. A. Ibers and R. H. Holm, *Science, 209,* 223 (1980).
2 F. A. Walker, D. Reis and V. L. Blake, *J. Am. Chem. Soc., 106,* 6888 (1984).
3 (a) M. Hatano and T. Nozawa, *Adv. Biophys., 11,* 95 (1978); (b) B. Holmquist, in D. Dolphin (ed.), 'The Porphyrins', Vol. 3, Academic Press, New York, 1978, p. 249; (c) J. H. Dawson and D. M. Dooley, in A. B. P. Lever and H. B. Gray (eds.), 'Iron Porphyrins – Part 3', Academic Press, New York, in press.
4 J. P. Collman, F. Basolo, E. Bunnenberg, T. J. Collins, J. H. Dawson, P. E. Ellis, Jr., M. L. Marrocco, A. Moscowitz, J. L. Sessler and T. Szymanski, *J. Am. Chem. Soc., 103,* 5636 (1981).
5 (a) L. Vickery, A. Salmon and K. Sauer, *Biochem. Biophys. Acta, 286,* 87 (1975); (b) L. Vickery, T. Nozawa and K. Sauer, *J. Am. Chem. Soc., 98,* 351 (1976).
6 F. S. Mathews, P. Argos and M. Levine, *Cold Springs Harbor Symp., Quant. Biol., 36,* 387 (1972).
7 J. P. Collman, J. I. Brauman, K. M. Doxsee, T. R. Halbert, E. Bunnenberg, R. E. Linder, G. N. LaMar, J. Del Gaudio, G. Lang and K. Spartalian, *J. Am. Chem. Soc., 102,* 4182 (1980).
8 H. Kobayashi, T. Higuchi and K. Eguchi, *Bull. Chem. Soc. Jpn., 49,* 457 (1976).
9 L. Vickery, T. Nozawa and K. Sauer, *J. Am. Chem. Soc., 98,* 343 (1976).
10 J. P. Collman, T. N. Sorrell, J. H. Dawson, J. R. Trudell, E. Bunnenberg and C. Djerassi, *Proc. Nat. Acad. Sci., U.S.A., 73,* 6 (1976).
11 T. Shimizu, T. Nozawa and M. Hatano, *Bioinorg. Chem., 6,* 119 (1976).
12 (a) M. Schappacher, L. Ricard, R. Weiss, R. Montiel-Montoya, E. Bill, U. Gonser and A. Trautwein, *J. Am. Chem. Soc., 103,* 7646 (1981); (b) J. P. Collman and S. E. Groh, *J. Am. Chem. Soc., 104,* 1391 (1982); (c) F. A. Walker, J. Buehler, J. T. West and J. L. Hinds, *J. Am. Chem. Soc., 105,* 6923 (1983).
13 (a) T. Mashiko, C. A. Reed, K. J. Haller, M. E. Kastner and W. R. Scheidt, *J. Am. Chem. Soc., 103,* 5758 (1981); (b) P. D. Smith, B. R. James and D. H. Dolphin, *Coord. Chem. Rev., 39,* 31 (1981).
14 (a) A. D. Adler, F. R. Longo, J. D. Finarelli, J. Goldmacher, J. Assour and L. Korsakoff, *J. Org. Chem., 32,* 476 (1967); (b) E. B. Fleischer, J. M. Palmer, T. S. Srivastava and A. Chattergee, *J. Am. Chem. Soc., 93,* 3162 (1971); (c) T. N. Sorrell, *Ph.D. Thesis,* Stanford Univ., 1977, p. 139.
15 L. S. Reid and A. G. Mauk, *J. Am. Chem. Soc., 104,* 841 (1982).
16 T. Yoshimura and T. Ozaki, *Bull. Chem. Soc. Jpn., 52,* 2268 (1979).
17 D. Brault and M. Rougee, *Biochemistry, 13,* 4591 (1974).
18 J. Ozols and P. Strittmatter, *J. Biol. Chem., 239,* 1018 (1964).
19 J. H. Dawson, L. A. Andersson and M. Sono, *J. Biol. Chem., 257,* 3606 (1982).
20 (a) W. R. Scheidt, D. K. Geiger and K. J. Haller, *J. Am. Chem. Soc., 104,* 495 (1982); (b) W. R. Scheidt, D. K. Geiger, R. G. Hayes and G. Lang, *J. Am. Chem. Soc., 105,* 2625 (1983).
21 W. R. Scheidt, Y. J. Lee, D. K. Geiger, K. Taylor and K. Hatano, *J. Am. Chem. Soc., 104,* 3367 (1982).
22 (a) J. D. Keegan, A. M. Stolzenberg, Y.-C. Lu, R. E. Linder, G. Barth, A. Moscowitz, E. Bunnenberg and C. Djerassi, *J. Am. Chem. Soc., 104,* 4305 (1982); (b) J. D. Keegan, A. M. Stolzenberg, Y.-C. Lu, R. E. Linder, G. Barth, A. Moscowitz, E. Bunnenberg and C. Djerassi, *J. Am. Chem. Soc., 104,* 4317 (1982).
23 K. Carter and G. Palmer, *J. Biol. Chem., 257,* 13507 (1982).