Magnetic and Natural Circular Dichroism of Metalloporphyrin Complexes of Human and Rabbit Hemopexin*

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Magnetic circular dichroism (MCD) spectra of several metalloporphyrin complexes of rabbit and human serum hemopexins in the spectral region of 300 to 650 nm and natural circular dichroism (CD) in the 300 to 450 nm region are reported. The MCD spectra of the heme (iron-protoporphyrin IX) complexes of both proteins were essentially identical suggesting similar iron coordination. The Soret region MCD spectrum of ferriheme-hemopexin has a shape and amplitude typical of other completely low spin (S = 1/2) ferric hemeproteins, and the temperature dependence of the MCD intensity indicates that it is composed predominantly of Faraday C-type terms. The visible region MCD spectrum of this complex closely resembles those characteristic of cytochrome b, and other bisimidazole-coordinated heme derivatives. Under aerobic conditions, heme-hemopexin is in the fully oxidized state. The ferroheme-hemopexins also exhibit MCD spectra similar to that of ferrocytochrome b, consistent with a low spin state and histidyl side-chain coordination of the heme iron in the reduced as well as in the oxidized state. The deuteroheme derivatives of rabbit hemopexin exhibit MCD spectra similar to those of the heme complex except for the expected slight differences in wavelength extrema, indicating that the vinyl side chains of protoporphyrin have little influence on the coordination.

In contrast, the natural CD spectra of the heme complexes of rabbit and human hemopexin do not resemble the CD of cytochrome b, reflecting differences in the crevice regions of the different hemoproteins. Furthermore, the CD spectra of the ferroheme complexes of rabbit and human hemopexin point to differences in the local environments of the heme chromophores.

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Hemopexin is a serum β-glycoprotein (1, 2) believed to function in the selective transport of heme† to the liver parenchymal cells (3) where the heme is degraded to bilirubin. The protein possesses a single binding site for heme (4-6) and has an affinity for heme, $K_4 < 10^{-11}$ M (7), considerably greater than that of serum albumin, $K_4$ near $10^{-8}$ M (8). The protein also interacts with a wide variety of naturally occurring and synthetic porphyrins (5, 6, 9-11), but only the binding of iron-porphyrins induces changes in the tertiary conformation of hemopexin (5) which may lead to cellular recognition of the complex. Both the chemistry and physiology of this protein have been recently reviewed (2, 10).

Several experimental approaches have been used to gain insight into the nature of the hemopexin-heme interaction. The absorption and EPR (12, 13) spectra of the protoheme-hemopexin complex are typical of low spin hemoproteins, unlike the high spin heme complex formed with serum albumin (12), and suggest that the heme iron is axially coordinated to two strong field ligands. The absorption spectra of the oxidized and reduced forms of heme-hemopexin resemble those of cytochrome $b_5$ (14) which is known to have 2 histidines bound to the heme iron (15), and chemical (16) and photochemical (17) modification studies have demonstrated that certain histidine residues are essential for heme binding. Changes in the absorption, fluorescence, and optical activity of tryptophan residues (5, 6, 18) upon heme binding suggest...
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that certain indole side chains of hemopexin are also affected by the interaction.

Previous studies have established the utility of the MCD of hemoproteins in determining both the oxidation-reduction and spin states as well as the axial ligation of the heme (see for example, Refs. 19 and 20). The advantages of this technique include the ability to examine ferrous as well as ferric heme at ambient temperature. In contrast to MCD which is determined solely by the electronic state of the porphyrin and metal orbitals, the natural CD properties of these chromophores are strongly influenced by their local environment and symmetry. Free heme in solution has little optical activity but in hemoproteins derives its activity from the disymmetric environment imposed by the polypeptide "solvent" and, hence, is sensitive to the protein structure. In addition, considerable rotatory strength in the Soret region is believed to arise via a coupled oscillator mechanism between the heme moiety and nearby aromatic residues (21). Thus, the use of MCD and CD techniques provides information on both the electronic state and the polypeptide environment of heme.

We report here the MCD and CD spectra of several metalloporphyrin complexes of rabbit and human hemopexin. This information should prove useful for the comparison of the heme coordination sphere of rabbit hemopexin with that of human hemopexin as well as with other heme proteins, such as cytochrome b, for which MCD (20, 22–24) and CD (25–27) spectra have already been reported. The data presented provide additional evidence for bishistidyl-heme coordination in the unmodified proteins and provide a basis for interpretation of the spectroscopic properties of modified derivatives which are currently being investigated.

EXPERIMENTAL PROCEDURES

Details of the experimental procedures are given in the miniprint supplement which follows.3

RESULTS

A detailed description of the results is presented in the miniprint supplement which follows.2

DISCUSSION

The MCD data presented here demonstrate that ferriheme-hemopexin is a fully low spin hemeprotein as indicated by the shape and intensity of the MCD signal associated with the near-ultraviolet Soret band (19). The temperature dependence of the MCD of this derivative-shaped band (Fig. 2) establishes that it is composed predominantly of Faraday C terms as expected for the $S = 1/2$ state. The MCD spectra in Fig. 1 as well as others not shown establish the similarity of ferric human and rabbit hemopexin-heme and deuterohemopexin-hemopexin at pH 7 and pH 9 with regard to spin state. This confirms and extends previous results using EPR in frozen solution (12, 13).

In addition, earlier results indicate that no change in conformation or absorbance of heme-hemopexin ensues between pH 6.5 and 9.1 (5).

The visible MCD spectra of ferriheme complexes of rabbit and human hemopexin (Fig. 3) closely resemble those of bisimidazole type hemoproteins such as cytochrome b (Fig.

6) and complexes such as imidazole-myoglobin (19). The spectra of the diagnostic charge-transfer bands of other heme-ligand systems, such as the methionyl-histidyl of cytochrome c (20) or the proposed thiolate-histidyl coordination of low spin forms of cytochromes P-450 (24, 43, 44), are distinctly different. This assignment is also supported by previous work showing that chemical modification (16) of histidine residues of rabbit hemopexin prevents formation of a hemichrome complex, but does allow heme to associate with the protein forming a complex with no apparent strong field iron axial ligands from the protein. The properties of the bromoacetate-modified hemopexin-heme complex are being examined in more detail using MCD techniques.4

The Soret and visible region MCD spectra of reduced heme-hemopexin resemble those of the low spin ferrocytochrome b, (Fig. 6) but are unlike the low spin oxy- and carbonyl-derivatives of myoglobin or c-type cytochromes (19, 20). In the Soret region, the hemopexin complexes exhibit spectra very similar to the weak inverse derivative-shaped $A$ terms seen in the spectra of cytochrome b, with a zero crossing near the absorption band maximum (Figs. 4 and 6). In the visible region, the hemopexin MCD spectra display an intense $A$ term associated with the $Q_y$ transition, typical of low spin ferrohemoproteins (Figs. 5 and 6). This strongly implies that the reduced heme-hemopexin complex is low spin ($S = 0$) and is supported by the absorption spectrum of reduced heme-hemopexin which is similar to other low spin hemoproteins.

In the visible region, ferrodeuterohemepexin-hemopexin displays MCD generally similar to the MCD of the protoheme complexes. However, there are several well resolved MCD bands in the $Q_y$ region and a distinct secondary MCD band in the $Q_x$ at 335 nm (Fig. 5). This complex MCD spectrum is reflected in the unusual absorption spectrum of ferrodeuterohemepexin-hemopexin in which the $b_{-1}$ band shows a distinct shoulder and the $a$-band presents a double maximum with an intensity near that of the $b$-band. This is not found in other reduced low spin deuterohemepexin complexes, e.g. ferrodeuterohemepexin-cytochrome b, (14) in which the $a$-band has a single maximum of greater intensity than the $b$-band. However, "split" $a$-bands in heme proteins at ambient temperature have been reported previously, e.g. ferromesohemepexin-cytochrome b, (14), Pseudomonas cytochrome c peroxidase (45), and cytochrome oxidase (46). The splitting of the $Q_y$ band could arise from an internal inequivalency of heme $x - y$ axes in the protein-bound state, but other causes can be envisioned and the basis of this effect is not clear at this time.

The MCD spectra of cobalt- and nickel-deuteroporphyrin IX bound to hemopexin differ from those seen with iron-porphyrins (Figs. 7 and 8). The Soret and visible MCD spectra observed arise predominantly from porphyrin $\pi-\pi^*$ bands, and no $C$ terms are expected with diamagnetic porphyrins. In the visible region MCD spectra, $A$ terms associated with the $Q_y$ bands are observed, but unlike iron-porphyrins, no evidence is found for additional transitions in the 440 to 490 nm region.

While the MCD and absorption spectra of the iron porphyrin-hemopexin complexes and cytochrome b, show a high degree of similarity, do their EPR (12, 13, 47) and oxidation-reduction potentials (48, 49), their CD spectra in the Soret region contain some interesting differences. Both the proto- and deuteroheme complexes of rabbit hemopexin in the ferric and ferrous oxidation-reduction states have a single positive

3 Some of the data are presented as a miniprint supplement immediately following this paper. Figs. 1 through 5, 7 and 8, and Tables I through III are found on pp. 2944–2945. Miniprint can be easily read with a large (3' x 3') standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document No. 71M-1505, cite author(s), and include a check or money order for $2.50 per set of photocopies.

4 Unpublished results.
ellipticity in this spectral region, with maxima near the absorption band maxima. Human ferriheme-hemopexin has a significantly weaker ellipticity in the Soret region than its rabbit counterpart (Figs. 1 and 4) and human and rabbit ferroheme-hemopexin have Soret CD of opposite sign (Fig 4). Since the rotatory strength of the heme chromophore derives predominantly from interactions with close lying tryptophan and tyrosine residues (21), these differences in CD reflect differences in the location or orientation, or both, of aromatic amino acid side chains near the heme-binding site of the two hemopexin species. Further differences are seen in comparing the CD spectra of the heme-hemopexins with cytochrome b₅ (Figs. 1, 4, and 6) and point to dissimilarities in their respective heme environments. These differences in CD are also reflected by the large accessibility of the heme chromophore to solvent in the rabbit heme-hemopexin complex (18), whereas the heme of cytochrome b₅ lies in a restricted crevice (15).

In summary, the work presented here shows that human and rabbit heme-hemopexin, like cytochrome b₅, are low spin, bisimidazole heme complexes in both the oxidized and reduced oxidation states. However, the similarity does not extend to the local environments of their heme chromophores. The difference between human and rabbit hemopexin may indicate that the mechanism whereby hemopexin delivers heme to the liver for degradation may show at least slight species differences.
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Hemopexin is known to bind a variety of negatively and positively charged ligands, including its ligands. The CD spectra of hemopexin and its complexes have been measured in the near-ultraviolet and visible regions. The CD spectra of hemopexin in the presence of different ligands have been measured in the near-ultraviolet and visible regions. The CD spectra of hemopexin in the presence of different ligands have been measured in the near-ultraviolet and visible regions. The CD spectra of hemopexin in the presence of different ligands have been measured in the near-ultraviolet and visible regions. The CD spectra of hemopexin in the presence of different ligands have been measured in the near-ultraviolet and visible regions. The CD spectra of hemopexin in the presence of different ligands have been measured in the near-ultraviolet and visible regions. The CD spectra of hemopexin in the presence of different ligands have been measured in the near-ultraviolet and visible regions. 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