Observation of Induced Cotton Effects for Magnesium Porphyrin–L-Histidine Species

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Complexes of magnesium protoporphyrin and magnesium mesoporphyrin with L-histidine have been prepared in solution and studied by circular dichroism and optical rotatory dispersion spectroscopy. Large induced Cotton effects are observed, in particular for the Soret transitions. These are interpreted in terms of the formation of six-coordinate magnesium porphyrin-(L-histidine)$_2$ species where the asymmetry of the L-histidine groups is coupled to the magnesium porphyrin electronic transitions. The major, five-coordinate components of these solutions do not produce Cotton effects. These results, when compared with data for corresponding protein complexes, lend support for the coupled oscillator description of the origin of optical activity in hemoproteins. The observation of Cotton effects for porphyrin complexes of the type reported here may enable the existence of six-coordinate species to be more clearly established in instances where the major equilibrium product is a five-coordinate species.

Introduction

The induced Cotton effect in hemoproteins is an interesting aspect of the optical activity of heme transitions because metalloporphyrin alone is optically inactive. It has been generally accepted that the nature of the induced Cotton effect in myoglobin and hemoglobin is a result of the coupled oscillator effect [1]. This involves the coupling of heme $\pi-\pi^*$ transitions with $\pi-\pi^*$ transitions of globin aromatic side chains, $\pi-\pi^*$ and $n-\pi^*$ transitions in the polypeptide backbone and the $\sigma-\sigma^*$ transitions in allyl side chains.

We have studied the Circular Dichroism (CD) and Optical Rotatory Dispersion (ORD) spectra of magnesium protoporphyrin–apomyoglobin (MgPP–Mb), magnesium protoporphyrin–apohemoglobin (MgPP–Hb), magnesium mesoporphyrin–apomyoglobin (MgMP–Mb) and magnesium mesoporphyrin–apohemoglobin (MgMP–Hb) [3]. These produce the same kind of Cotton effects as observed for the iron analogues. Thus the presence of d electrons is not a necessary requirement for induced Cotton effects, as already shown by reports that globin containing metal-free porphyrins produces optical rotation of a similar magnitude to that of native hemoglobin [4, 5]. In this paper we report spectral results for the protein-free MgPP and MgMP entities coordinated to L-histidine (the naturally occurring amino acid bound to the iron atom in myoglobin and hemoglobin). Results of this type are important for elucidating the structural and functional relationships between metal ions (*e.g.* Cu [6]) and histidine or histidyl residues in biological systems. Interestingly, marked Cotton effects are observed for aqueous solutions of such mixtures. Comparisons of visible, ORD and CD spectra enable the origin of the Cotton effects to be identified. In addition the results enable a comparison to be made between the Cotton effects of corresponding protein and non-protein systems, because of the availability of data for MgPP and MgMP derivatives of Mb and Hb [3].

Experimental

Protoporphyrin dimethyl ester (PPDME) was prepared from whole blood by the procedure of Grinstein [7]. Hydrolysis of PPDME by 1% KOH in methanol gave the desired PP and the purity was checked by the extinction coefficients [8]. Hemin, Mesoporphyrin dimethyl ester (MPDME) and L-histidine were purchased from Sigma chemical company.

MgMPDME was prepared from MPDME and magnesium perchlorate [9]. MgMP was obtained from MgMPDME by hydrolysis using NaOH [10]. A similar procedure was used to obtain MgPP. Mn-

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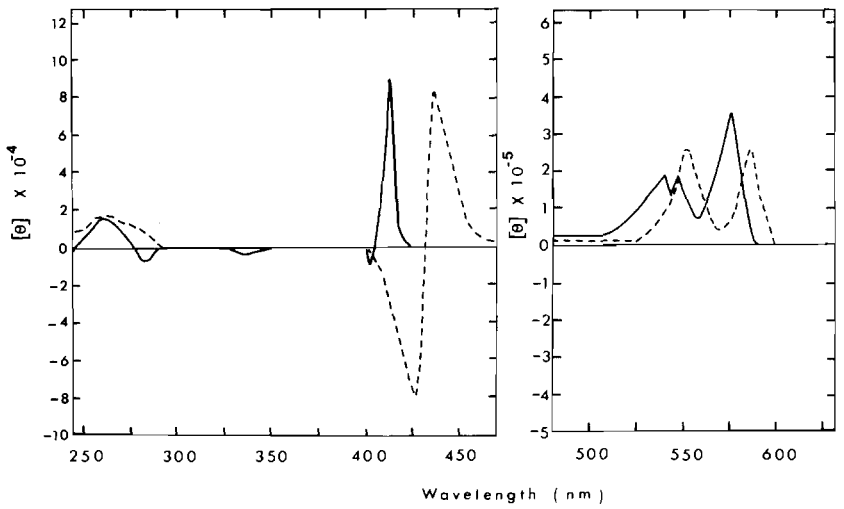

Fig. 1. Circular dichroism spectra of MgMP in 0.1 *M* L-histidine [------] and MgMP–Hb [——] redrawn to show main bands.

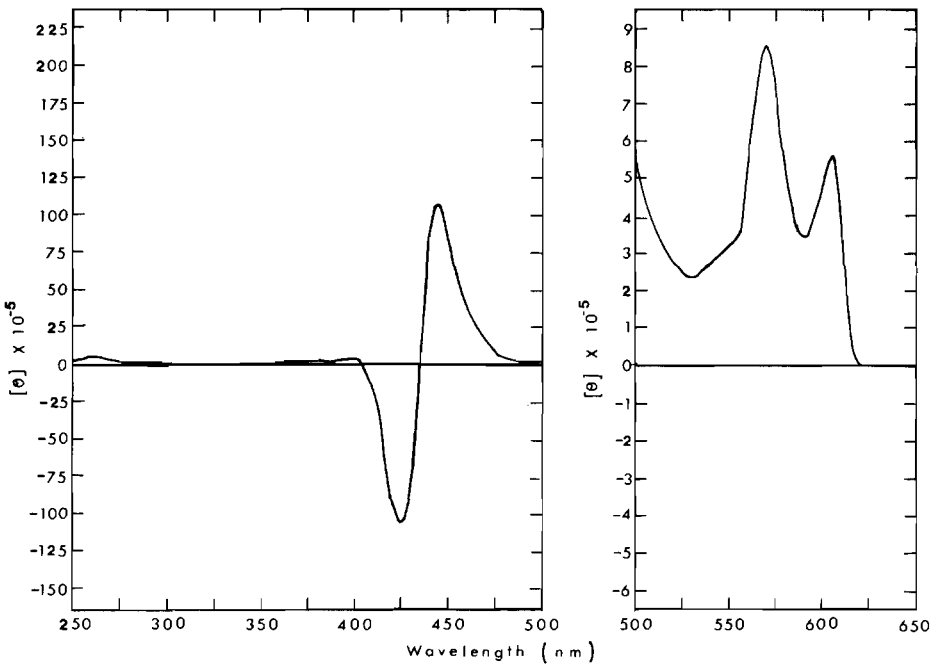

Fig. 2. Circular dichroism spectra of MgPP in 0.1 *M* L-histidine redrawn to show main bands.

MPDME was prepared according to the method by Taylor [11]. Apo–Hb was prepared from sheep Hb according to standard methods [12]. The concentration of apo–Hb was determined by the extinction coefficient of 16.2 mM cm^{-1} at 280 nm [13].

Aqueous solutions of MgMP and MgPP in 0.1 *M* L-histidine were freshly prepared and filtered for all the spectral studies. As a red precipitate forms after about 1 hour spectral measurements must be completed before this occurs.

Electronic absorption spectra were recorded on a Varian Super Scan 3 UV-visible spectrophotometer. Analytical grade pyridine and benzene were purchased from British Drug House Ltd and stored in the dark at 0 °C over 4 Å molecular sieves under nitrogen. Other reagents used were of analytical grade.

ORD and CD measurements were recorded on a Jasco ORD UV-5 spectrophotometer. Reagents were kept away from strong light and all apparatus

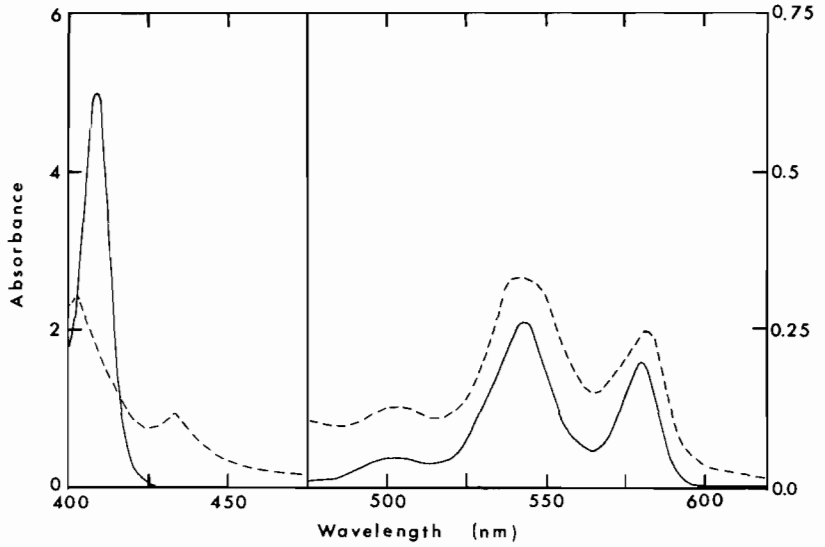

Fig. 3. Electronic spectra of MgMP in 0.1 *M* L-histidine [------] and MgMP–Hb [———].

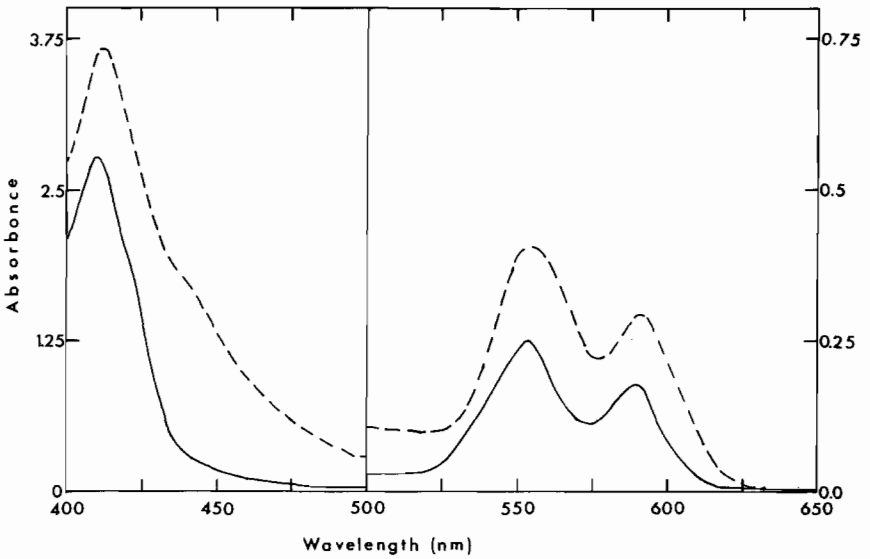

Fig. 4. Electronic spectra of MgPP in 0.1 *M* L-histidine [------] and in 0.1 *M* pyridine [———].

was covered by aluminium foil. All the protein preparative work was carried out at temperatures between 0° and 4 °C.

Results

CD spectra

MgMP and MgPP do not produce optical rotation in solvents like ether, benzene and pyridine. 0.1 *M* L-histidine, by itself, has no significant CD spectrum in the visible region. However, MgMP in 0.1 *M* L-histidine shows two well-formed bands between 500 nm and 600 nm (Fig. 1). It is likely that these two bands correspond to the Q_0 and Q_1 bands observed in the electronic spectrum (Fig. 3). As also observed for MgPP in L-histidine, the relative positions of these two bands do not appear to vary significantly as the concentrations of L-histidine are increased from 0.05 *M* to 0.2 *M*. The Q_0 and Q_1 bands of MgMP in L-histidine are at 589 nm and 556 nm respectively, while the Q_0 and Q_1 bands of MgPP in L-histidine are at 604 nm and 568 nm (Fig. 2). The Soret band is split into two equal and opposite bands. The positive band is at 445 nm and the negative band is at 425 nm. In both MgMP and MgPP, the positions of

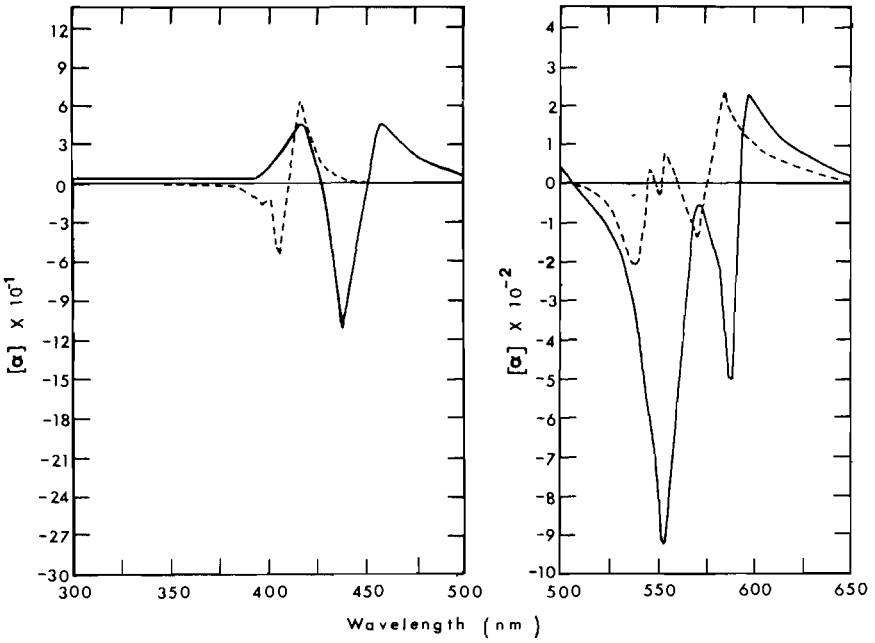

Fig. 5. Optical rotatory dispersion of MgMP in 0.1 *M* L-histidine [———] and MgMP–Hb [------] redrawn to show main bands.

these two bands appeared to be the same and the crossover point is at 435 nm.

MgMP–Hb also shows the Q_0 and Q_1 bands (Fig. 1). But the relative intensity of the Q_0 and Q_1 band is different from that in L-histidine and the ratio of Q_0 and Q_1 is greater than unity. The Q_0 band is at 575 nm and the Q_1 band is split into two positive bands at 548 nm and 542 nm. The Soret band shows a dominant positive band at 412 nm and a small negative band at 402 nm.

ORD Spectra

MgMP and MgPP do not show any optical rotation while pure L-histidine shows a rotational angle of −59.8 [14]. MgMP in 0.1 *M* L-histidine has a complex curve with peaks at 597, 570, 445 and 425 nm and troughs at 588, 553 and 435 nm (Fig. 5). MgPP in L-histidine has a similar complex curve with peaks at 607, 578, 445 and 425 nm and troughs at 598, 562 and 435 nm (Fig. 6). MgMP–Hb has peaks at 584, 553, 546, 417 and 401 nm and troughs at 570, 549, 538, 407 and 398 nm (Fig. 5).

The ORD spectra of MgMP in L-histidine and in apo–Hb are similar in shape in the visible region and the significant difference is in the Soret region. The crossover point between the positive and negative band in the Soret region of MgMP–Hb corresponds closely to the maximum absorption of its CD spectrum. However, in the Soret region of MgMP in L-histidine, the maximum negative value at 435 nm corresponds to the crossover point of the CD spectrum.

Discussion

The spectra for the MgPP and MgMP–pyridine and L-histidine mixtures (Figs. 3 and 4) show broad electronic bands with some indication of multiple bands in the Soret region. Magnesium porphyrin has a high affinity for nitrogenous bases like pyridine to form five-coordinate species (*e.g.* MgPP + py $\rightleftharpoons$ MgPP(py) [15–18]). The degree of binding of a second axial ligand is measurable and Storm *et al.* [17] obtained a value of 0.242 for the equilibrium constant for MgPP(py) + py $\rightleftharpoons$ MgPP(py)$_2$ in 2,6-lutidine, using relative intensities of distinguishable Soret bands for the five- and six-coordinate species. From our studies of this equilibrium in benzene we obtained a value of 1.45, using the visible Q_0 band (Fig. 7). This increase in the equilibrium constant by about 6 times agrees well with the ratio value of 5.02 found for the equilibrium constants of MgTPP(DME) with pyridine in benzene and 2,6-lutidine. Thus either the Soret or the Q_0 band may be used for equilibrium studies of this type.

For the nitrogenous base L-histidine it was not possible to study the five-coordinate/six-coordinate equilibrium because of the inability to dissolve sufficient L-histidine in benzene. However the spectra of MgPP and MgMP in the presence of this base do have a Soret absorption at about 433 nm (Figs. 3 and 4). From the equilibrium studies it is apparent that this band is diagnostic of six-coordinate bis axial ligand magnesium porphyrin complexes. Thus,

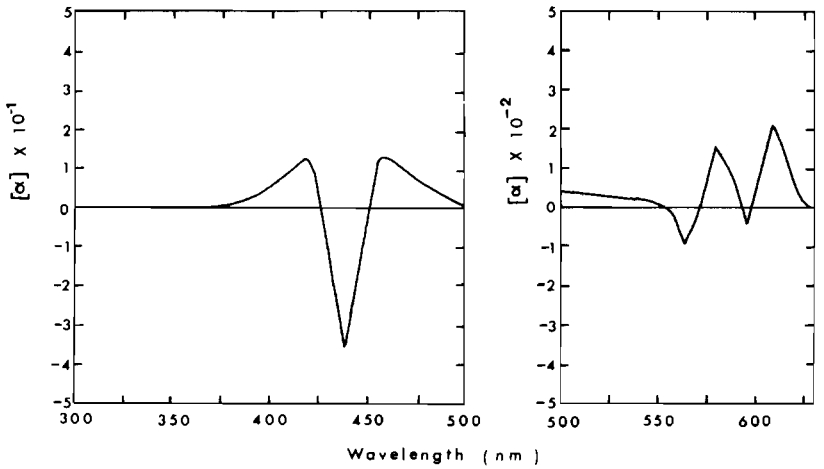

Fig. 6. Optical rotatory dispersion of MgPP in 0.1 *M* L-histidine redrawn to show main bands.

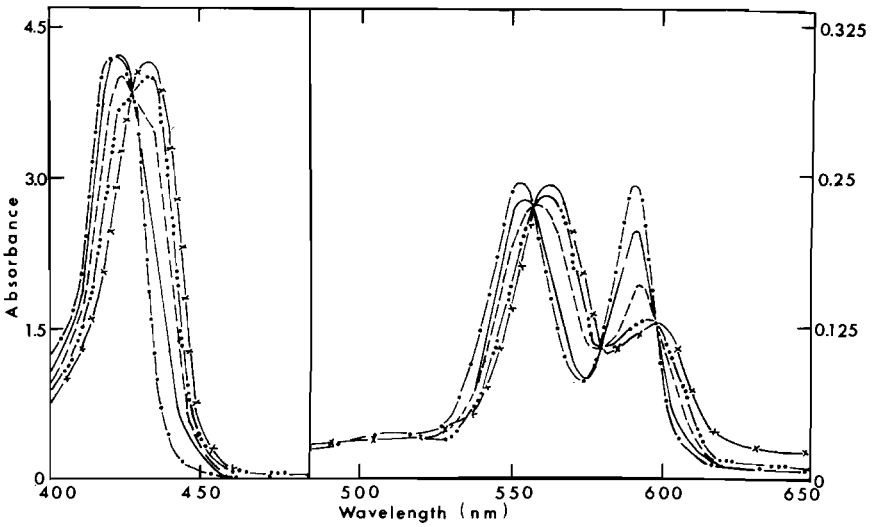

Fig. 7. Electronic absorption spectra of MgPP under various pyridine concentrations at 20.9 °C to determine the equilibrium constant MgPP(pyridine) + pyridine ⇌ MgPP(pyridine)$_2$.

although the visible bands are not resolved, the Soret peaks show the formation of a five-coordinate/six-coordinate equilibrium mixture for both MgPP and MgMP with L-histidine.

Surprisingly, we have found that these products produce significant Cotton effects. Furthermore it can be seen from the CD spectra that the crossover positions of the Soret peaks at 435 nm correspond closely to the 433 nm peak position in the electronic spectrum. Thus the ORD/CD results may be interpreted in terms of Cotton effects arising from the six-coordinate bis complexes, MgPP(L-histidine)$_2$ and MgMP(L-histidine)$_2$, on the assumption that the five-coordinate species in the mixture do not produce Cotton effects. This is consistent with the results from theoretical calculations for hemoproteins which suggest that the fifth ligand, histidine (93, 8F) alone does not play a major role in producing the induced Cotton effect [1, 2]. This group is coordinated in a symmetrical manner with respect to the porphyrin ring in hemoproteins and the imidazole group in the five-coordinate MgPP and MgPP complexes is likely to coordinate in the same manner (as also observed for comparable zinc and cobalt complexes [19]).

If this interpretation of the spectral results is correct it is of interest that it is the coupling mechanism of a second L-histidine ligand which gives rise to the observed Cotton effects in the non-protein Mg porphyrin complexes. For the naturally occurring

proteins the presence of comparable asymmetric entities located *trans* to a bound asymmetric ligand (such as the distal histidine in myoglobin and hemoglobin) may be crucial for the production of coupled rotational effects, as indicated by the calculations of Hsu and Woody [1, 2]. It is of interest that the magnitude of the rotation in the Soret region is at least three times greater for the bis L-histidine porphyrin complexes (where the second asymmetric entity is significantly closer to the metal centre) than for the corresponding hemoproteins. Thus, overall our results substantiate the coupled oscillator interpretations of the observed Cotton effects for hemoproteins [1, 2].

The protein and non-protein species differ from each other with respect to the details of molecular asymmetry. In the former case the groups, whose transitions are likely to be coupled with the heme $\pi-\pi^*$ transitions, are those that are asymmetrically oriented with respect to the heme group. For the non-protein six-coordinate complexes the two histidine ligands, while being asymmetric, will be bound in equivalent positions *trans* to each other. This may be the reason for the more symmetrical nature of CD and ORD Soret bands observed for these complexes, by comparison with corresponding bands of the magnesium hemoproteins species. (Figs. 1–4).

The symmetrical form of the ORD spectrum in the Soret region, for the MgPP(L-histidine)$_2$ complex in particular, is characteristic of entities having degenerate or near degenerate electronic transitions [22]. Neglecting the porphyrin side chains, the Soret band is doubly degenerate [1, 2]. The extent to which this degeneracy is resolved would probably be greater for the protein species where the local metal site symmetry is likely to be lower than for the non-protein complexes. This is substantiated by the ORD results where the protein species give more complex spectra [3].

We were unable to obtain comparable Cotton effects for corresponding solutions of ferric and manganic porphyrin containing L-histidine. This may be due to some advantage that a magnesium(II) d^0 configuration has over an ion having d electrons, so far as the mechanism of the electronic coupling is concerned. Alternatively the orientation of the L-histidine ligands may be crucial for the production of Cotton effects. Presumably such ligands must be coordinated in a well-defined manner (with no free rotation about the metal–nitrogen bond) for Cotton effects to be observed. Theoretical considerations of induced circular dichroism (ICD) have identified two possible situations – one where there is a fixed mutual orientation of the chiral and achiral molecules [20] and another where the molecules are randomly orientated [21]. The magnitude of the Cotton effects observed here suggests the former case for the Mg porphyrin-(L-histidine)$_2$ complexes. It may also be necessary for the two ligands *trans* to each other to be bound with a particular mutual orientation for optical rotation to occur.

The existence of marked Cotton effects for this type of porphyrin complex could be extended to other systems involving the study of the binding of asymmetric ligands to axial positions where equilibrium mixtures of five- and six-coordinate complexes are formed. As five-coordinate species do not apparently produce Cotton effects the CD and ORD spectra enable details of the six-coordinate species to be selectively studied.

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