Induced Cotton Effects for Complexes of Magnesium Porphyrin with D- and L-proline, L-serine, L-threonine and L-tryptophan

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Circular dichroism and optical rotatory dispersion results are reported for solutions of magnesium protoporphyrin and magnesium mesoporphyrin containing the amino acids, D and L-proline, L-serine, L-threonine and L-tryptophan. The observed induced Cotton effects are attributed to six-coordinate magnesium porphyrin (amino acid)$_2$ species. The Cotton effects are most pronounced for proline and the related pair of amino acids, serine and threonine. In the former case, the observed spectra are inverted with respect to those for other amino acids of the same chirality. It is suggested that this arises from the formation of an additional chiral centre at the ring nitrogen atom due to the coordination of proline to magnesium via this atom. Steric interactions produce an opposite chirality at nitrogen to that existing at the asymmetric carbon atom. For L-serine and L-threonine H-bonding of the -OH groups with the -COO$^-$ porphyrin side chains is proposed as the localising effect that produces the observed induced Cotton rotation. For L-tryptophan, where a weaker CD/ORD effect is observed, stacking of the aromatic rings of the indole and porphyrin groups is considered as a possible localising interaction.

Introduction

The induced Cotton effect of chiral amino acids and peptides on metal ions has been widely studied in order to elucidate the structural and functional relationships of these entities in biological systems [1]. This Cotton effect is also observed when metalloporphyrins are incorporated into the chiral heme pocket of hemoproteins [2]. Recently, the observation of induced Cotton effects of Mg porphyrin in apomyoglobin and with L-histidine has been reported [3]. In this paper, we report the observation of induced Cotton effects of Mg porphyrin with D- and L-proline, the related amino acids, L-serine and L-threonine, and L-tryptophan.

The coordination of proline (an important biological neurotransmitter [4] and catalyst for induction of asymmetry [5]) to a metal ion *via* the nitrogen atom is of particular interest as a new asymmetric centre is formed at this nitrogen, in addition to the asymmetric carbon already present in the five-membered ring [6]. This produces Cotton effects that are the inverse of those observed for other chiral amino acids, as also observed for corresponding Cu^{2+} complexes [7]. However for Mg porphyrins monodentate coordination (in the axial positions) is expected rather than the bidentate type that occurs for Cu^{2+}. Molecular models show that because of steric interactions, the chirality at the donor nitrogen atom must be the opposite to that of the asymmetric carbon atom.

The observation of induced Cotton effects for Mg porphyrin-amino acid complexes is apparently dependent upon some means by which the movement of the amino acid ligands occupying the axial positions is restricted. For L-histidine the restricting effect is possibly the electronic interaction between π electrons of the histidine ring and 3d orbitals, similar to that considered for corresponding iron systems [8]. For chiral proline, there are steric interactions between the saturated proline ring and the porphyrin group. For L-serine and L-threonine, the only other amino acids for which we have observed prominent Cotton effects, molecular models indicate the possibility of hydrogen bonding interactions of the carboxylate sidechains of the porphyrin ring with the OH groups contained in these amino acids. For L-tryptophan, where a weaker Cotton rotation is observed, stacking interactions between the porphyrin and the indole group are considered to be a localising effect.

Experimental

Protoporphyrin dimethyl ester (PPDME) was prepared from whole blood by the procedure of Grinstein [9]. Hydrolysis of PPDME by 1% KOH in methanol gave the desired PP and the purity was checked by the extinction coefficients [10]. Hemin, mesoporphyrin dimethyl ester (MPDME), L-histidine, L-pro-

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line, L-tartaric acid, L-threonine, L-serine, L-cysteine, L-leucine, L-nicotine, L-tryptophan, L-glutamic acid, L-malic acid and D-proline were purchased from Sigma chemical company and British Drug Houses Ltd. D-mandelic acid and S(−)-2-methyl-1-butanol were obtained from Fluka.

MgMPDME was prepared from MPDME and magnesium perchlorate [11]. MgMP was obtained from MgMPDME by hydrolysis using NaOH [12]. A similar procedure was used to obtain MgPP.

Aqueous solutions of MgMP and MgPP (which were basic because of the presence of residual NaOH) in the various amino acids and in neat S(−)-2-methyl-1-butanol were freshly prepared and filtered for all the spectral studies. In concentrated solutions of MgPP and MgMP, a red precipitate forms after about 1 hour and in dilute solutions, the pink colour turned to orange and then yellow. Consequently, all spectral measurements must be completed before these changes occur.

Electronic absorption spectra were recorded on a Varian Super Scan 3 UV-visible spectrophotometer. All reagents used were of analytical or chromatographically homogeneous grade. ORD and CD measurements were recorded on a Jasco ORD UV-5 spectrophotometer. Reagents were kept away from strong light and all apparatus was covered by aluminium foil.

Results

Electronic spectra

The electronic spectra of MgMP and MgPP in D-proline, L-proline, L-histidine, L-serine and L-threonine all show a distinct shoulder at 433 nm which is diagnostic of six coordinate species [3] (Fig. 1, Table I). The electronic spectra of MgMP in L-tartaric acid, L-malic acid, D-mandelic acid, L-cysteine, L-leucine and L-glutamic acid show distinct red-shifts in their bands but no band at 433 nm is observed. Furthermore, the relative intensity of the Q_0 band to Q_1 band is reduced significantly. MgMP in neat S(−)-2-methyl-1-butanol and L-nicotine are also red shifted but the relative intensity of Q_0 band to Q_1 band is reduced only marginally. The electronic spectrum of hemin in L-proline shows the characteristic bands of iron in oxidation state 3 [10].

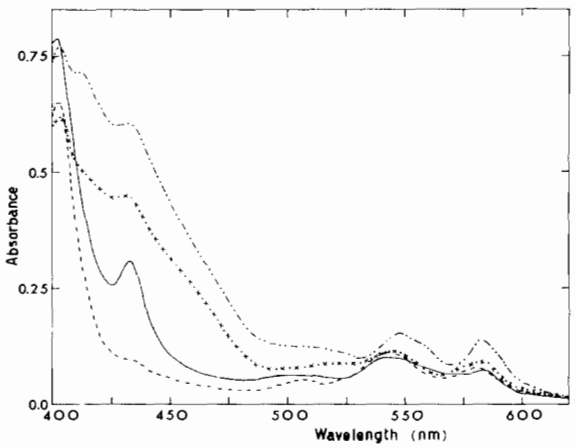

Fig. 1. Electronic spectra of MgMP in L-histidine [——], in L-serine [— · · —], in L-threonine [x·x] and in L-proline [- - -].

TABLE I. Comparison of Electronic Bands of Mg Porphyrin in Chiral Amino Acids.

	MgMP			MgPP		
	Q_0	Q_1	Soret[a]	Q_0	Q_1	Soret[a]
L-proline/ D-proline	581	543	403 433(sh)	594	557	414 433(sh)
L-histidine	584	544	403 433	594	556	416 433
L-serine	583	547	403 413(sh) 433(sh)			
L-threonine	583	546	403 433(sh)			
L-tryptophan	582	545	403 433(sh)			
D-mandelic acid/L-tartaric acid	592	549	401			
S(−)-2-methyl-1-butanol	580	543	406			

[a]sh = shoulder.

When the electronic spectra of MgMP in the chiral amino acids are measured again after one hour, the spectra have the characteristic spectra of MP [10]. This suggests that decomposition of MgMP is a result of Mg^{2+} being removed from the porphyrin ring.

CD Spectra

MgMP and MgPP are optically inactive, while the chiral amino-acids and alcohol used in these experiments have no significant CD spectra in the visible region. However, freshly prepared solutions of magnesium porphyrin and L-proline, L-serine, L-threonine, D-proline and L-histidine show distinct CD bands in the visible region (Table II). Magnesium porphyrin in L-tryptophan shows very weak CD bands. The shapes and signs of the curves of MgMP in L-serine and L-threonine (Fig. 4) are similar to those of MgMP in L-histidine [3]. However for MgMP and MgPP in L-proline, the signs of the bands are opposite to those of MgMP and MgPP in L-histidine respectively. Increasing the concentration of L-proline from 0.05 *M* to 1 *M* does not change the positions and signs of the bands of MgMP in L-proline. The only difference is that at higher concentrations of L-proline, these CD bands are more intense. Nonetheless, decomposition of MgMP still persists after one hour. The CD spec-

TABLE II. Comparison of Circular Dichroism Bands of Mg Porphyrin in Chiral Amino Acids.

	MgMP			MgPP		
	Q_0	Q_1	Soret	Q_0	Q_1	Soret
L-proline	586	552	435 (negative) 425 (positive) 430 (cross-over point)	603	564	446 (negative) 429 (positive) 438 (cross-over point)
D-proline	586	552	425 (negative) 435 (positive) 430 (cross-over point)	603	564	429 (negative) 446 (positive) 438 (cross-over point)
L-histidine	589	556	425 (negative) 445 (positive) 435 (cross-over point)	604	568	425 (negative) 445 (positive) 435 (cross-over point)
L-serine	589	559	420 (negative) 448 (positive) 434 (cross-over point)			
L-threonine	586	558	421 (negative) 435 (positive) 428 (cross-over point)			
L-tryptophan[a]	589	554	421 (negative) 435 (positive) 438 (cross-over point)			

[a]Very weak signal.

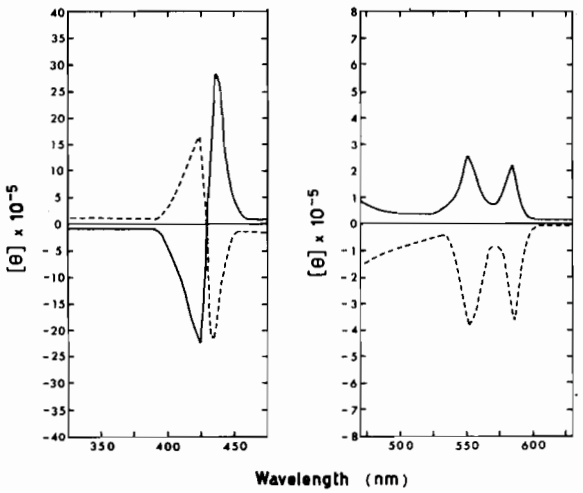

Fig. 2. Circular dichroism spectra of MgMP in 0.1 *M* L-proline [- - -] and in 0.1 *M* D-proline [——] redrawn to show main bands.

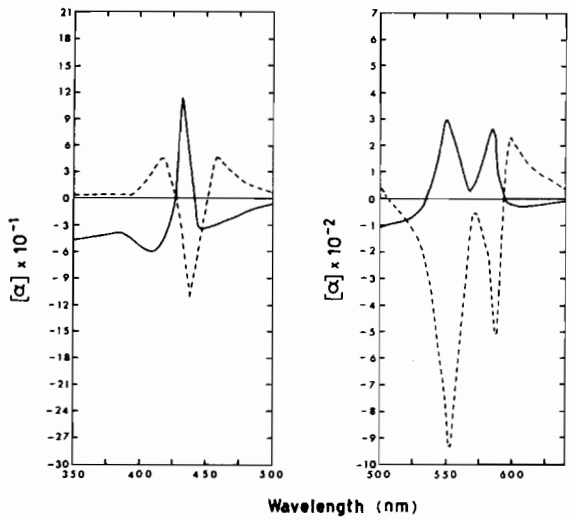

Fig. 3. Optical rotatory dispersion of MgMP in 0.1 *M* L-histidine [- - -] and in 0.1 *M* L-proline [——] redrawn to show main bands.

trum of MgMP–D-proline is the opposite to that of the corresponding L-proline sample, as expected (Fig. 2).

No significant circular dichroism was observed for MgMP dissolved in L-tartaric acid, L-glutamic acid, L-cysteine, D-mandelic acid, L-malic acid, L-leucine, L-nicotine solutions and also in S(−)-2-methyl-1-butanol. As for hemin in L-histidine, no significant CD spectrum was obtained for hemin dissolved in a solution of L-proline.

ORD Results

MgMP and MgPP show no optical activity while all the amino acids and the chiral alcohol show rotational properties [12]. However, extra features are observed in the visible region when Mg porphyrin is dissolved in solutions of L-serine, L-threonine, D-proline, L-proline and L-histidine (Fig. 3, Table III). Some variation in the features of the ORD spectra of the proline solutions was observed in the visible region. No significant extra ORD features were obtained for MgMP

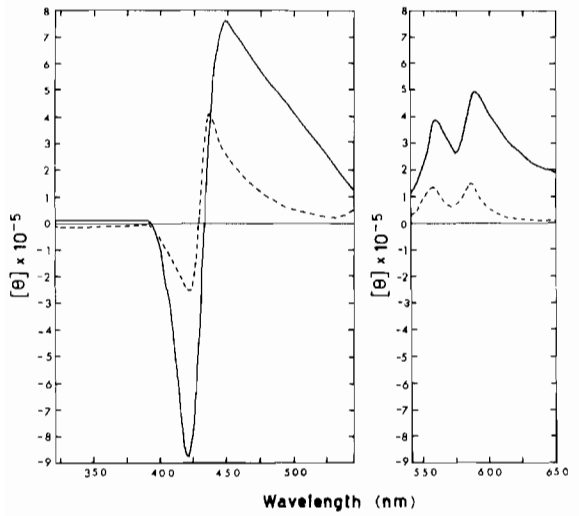

Fig. 4. Circular dichroism spectra of MgMP in 0.1 *M* L-serine [——] and in 0.1 *M* L-threonine [- - -] redrawn to show main bands. (The concentrations of the MgMP species producing these spectra are not known, see text).

TABLE III. Comparison of Optical Rotatory Dispersion of Mg Porphyrin in Chiral Amino Acids.

	MgMP	MgPP
L-proline (peaks)	584, 548, 431	601, 568, 438
(troughs)	598, 568, 447, 412	626, 588, 445, 431
D-proline (peaks)	598, 568, 447, 412	
(troughs)	584, 548, 431	
L-histidine (peaks)	597, 570, 447, 412	607, 578, 445
(troughs)	588, 553, 435	598, 562, 435
L-serine (peaks)	599, 566, 450, 410	
(troughs)	586, 550, 434	
L-threonine (peaks)	604, 564, 448, 412	
(troughs)	580, 544, 428	

dissolved in solutions of L-tartaric acid, L-glutamic acid, L-cysteine, D-mandelic acid, L-malic acid, L-leucine, L-nicotine and in S(−)-2-methyl-1-butanol. Similarly, no ORD was obtained for hemin dissolved in a solution of L-proline.

Discussion

The interaction of amino acids with MgPP and MgMP produces a variety of molecular species. This is indicated by multiple features of the electronic spectra in the Soret region where two or three absorption bands are generally observed (Fig. 1). The high energy bands at ~403 nm and ~415 nm are probably diagnostic of five-coordinate MgPP/MP(L) species while those at ~433 nm arise from six-coordinate MgPP/MP(L)$_2$ entities [3]. A third band at ~413 nm for L-serine with MgMP may indicate the formation of a six coordinate MgMP(L)(H_2O) species. All of the amino acid–magnesium porphyrin complexes decompose quite readily to give the free porphyrin (which is readily identified from its electronic spectrum). Presumably axial coordination facilitates the removal of magnesium from the porphyrin rings.

The equilibrium constants for MgPP/MP(L)$_2$ complexes are generally small [3, 14]. However for certain chiral amino acids these complexes produce Cotton effects of significant magnitude. This enables quite specific structural information to be deduced for these entities even when they are present in only small proportions. The observed Cotton effects, as judged by the Soret region in particular (where the ORD/CD features are centered on the ~433 nm electronic band alone) arise exclusively from the six-coordinate MgPP/MP(L)$_2$ complexes.

The induced Cotton effects reported here for L-serine, L-threonine and L-proline and earlier for L-histidine [3] are of the primary type [15] rather than the weaker secondary effect observed for the interaction of a non chiral solute with a chiral solvent [16]. As reported above, no significant Cotton effects were observed for MgMP dissolved in S(−)-2-methyl-1-butanol. This means that those amino acids which produce significant Cotton effects must be bound to the MgPP/MP entities in a stereospecific manner. For L-histidine, as mentioned in the Introduction, electronic effects may produce the required specificity of interaction. For L-serine and L-threonine, it will be argued that hydrogen bonding of the –OH groups to carboxylate sidechains of MgPP and MgMP is the crucial stereochemical factor. By contrast an indirect stereochemical effect appears to be decisive for L-proline [17, 18].

L-proline is unique in that it is the only amino acid for which an additional chiral centre may be formed on coordination to a metal ion. This is for coordination through the nitrogen atom and in the case of MgPP/MgMP species molecular models show that the opposite chirality is produced to that existing at the asymmetric carbon centre. In Figs. 5 and 6, stereoscopic views of MgMP(L-proline)$_2$ (which were derived from 1 Å = 2 cm models based on unrefined bond angles and distances) show that steric interactions of the $-COO^-$ group with the porphyrin ring prevent the formation of the same chirality at nitrogen as at carbon. This analysis is substantiated by the observation of inversed Cotton effects for L-proline compared with those produced by other L-amino acids. In the latter cases the effects are determined by the chirality of the carbon centre located within the ligand group whereas for L-proline it is the chirality of the ligating nitrogen atom which predominates. When the chirality of the carbon atom is altered the ORD and CD spectra invert as expected (Fig. 2). It is of interest that the induced Cotton effect for proline coordinated to Cu^{2+} is also inverted with respect to that observed for other amino acids of the same chira-

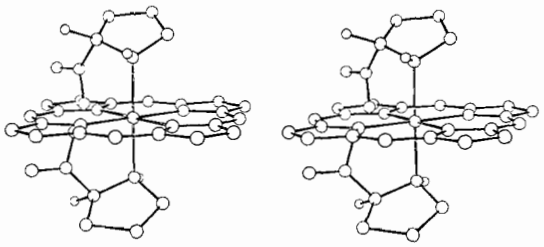

Fig. 5. Stereoscopic view of a model of MgMP (L-proline)$_2$ to show that steric interactions prevent the formation of nitrogen coordination with L-chirality at that atom.

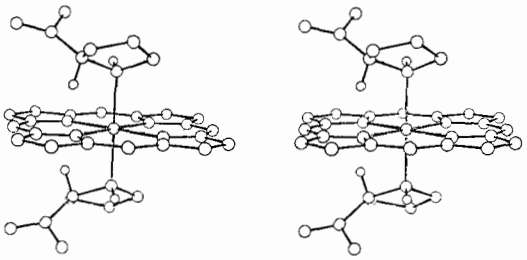

Fig. 6. Stereoscopic view of a model of MgMP (L-proline)$_2$ to show formation of D-chirality at the coordinated nitrogen atom for a stereochemically acceptable structure.

lity [7, 19]. An analogous but different (because proline is a bidentate ligand in Cu^{2+} complexes) stereochemical explanation has been proposed for the Cu^{2+} compounds [17, 20].

The results for L-proline appear to be interpretable only in terms of binding through the nitrogen atom. For L-serine and L-threonine coordination could have occurred via the $-COO^-$ group, rather than NH_2, for the basic conditions used. In order to check this possibility studies were made of the interaction of D-mandelic acid and L-malic acid. No significant Cotton effects were observed, although this did not conclusively eliminate the carboxylate coordination possibility as an additional interaction, fixing the positions of these ligands, would also be required. Again it was the construction of molecular models that effectively enabled nitrogen coordination to be established and the observed Cotton effects for these amino acids to be rationalised. For both L-serine and L-threonine the $-OH$ and $-NH_2$ groups have the appropriate stereochemical locations for simultaneous binding of $-NH_2$ to the metal and hydrogen bonding of the $-OH$ to the carboxylate ($-COO^-$) sidechains of the magnesium porphyrin entity (as shown in the Fig. 7 stereoscopic diagrams). Coordination via $-NH_2$ is favoured because it is a stronger donor than $-OH$ to metal ions. L-threonine has an additional chiral carbon centre (arising from the substitution of CH_3 for H when compared with L-serine). The effect of this centre, being more distant from the metal atom, would be expected to be smaller. However, as can be

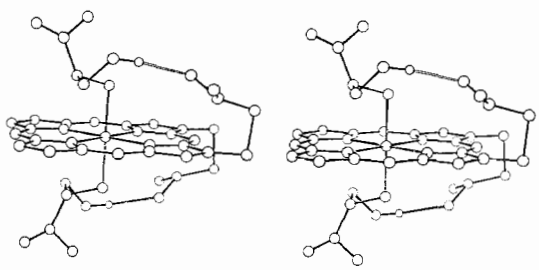

Fig. 7. MgMP (L-serine)$_2$ to show the effect of hydrogen bonding of the amino acid -OH group to the $-COO^-$ porphyrin side chain in locating the coordinated amino acid groups.

seen from the CD spectrum of the L-threonine complex (Fig. 4 – note differences with respect to L-serine spectrum), this carbon centre does influence the optical rotation to some extent.

It appears that induced Cotton effects are not produced by other possible complexes of L-proline, L-serine and L-threonine, having one rather than two axial ligands bound in the manner shown in Figs. 6 and 7 (*i.e.* for MgPP/MP(L) or MgPP/MP(L)(H_2O) species). No ORD or CD bands appear at electronic positions other than that assigned to MgPP/MP(L)$_2$. It is difficult to completely eliminate the possibility that the ~433 nm band might be due to six-coordinate MgPP/MP(L)(H_2O) species rather than MgPP/MP(L)$_2$ ones. However studies reported in connection with the L-histidine study [3] strongly support the MgPP/MP(L)$_2$ formulation. Thus two asymmetric centres are apparently required in order for induced Cotton effects to be produced for planar entities like MgPP/MP.

Although electronic spectra show that the other amino acids studied bind to MgPP/MP, no significant Cotton effects were observed, presumably because of the absence of additional localising effects as exist for L-serine and L-threonine. L-proline groups may be localised to some extent by steric interactions but this would not be as critical as for the other amino acids because of the direct binding of a chiral centre to the metal atom. L-tryptophan produces weak Cotton effects, possibly due to the presence of some localising effects (a molecular model study showed that stacking of the indole and porphyrin groups can occur for the favoured 3.4 Å distance, for NH_2 binding to the metal atom).

The studies reported here are for species of limited stability and also of undetermined concentration (because of the experimental procedure used, see Experimental Section). The ease with which Mg is removed from the porphyrin ring may be related to the photosensitive nature of the solutions. In addition it has been observed that the intensities of the ORD/CD transitions are markedly reduced at elevated temperatures. Another related aspect is the observation

that comparable iron porphyrin complexes do not produce similar ORD/CD effects. The difference in electron occupancy of the d orbitals may be important in relation to the electronic coupling processes [3]. While the observed induced Cotton effects may be rationalised in terms of plausible models, there are additional features requiring further elucidation.

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Note in Proof (June 3, 1983)

In further studies with another preparation of MgPP, (using PP from Sigma) reversed CD bands were observed for L-serine and L-histidine compared with those reported above and earlier [3] (although for D- and L-proline the same CD spectra as reported here were obtained). No ORD/CD effect has been observed for Mg deuteroporphyrin with chiral amino acids. Particular preparative conditions may be important in determining what ORD/CD effect is observed. Our main procedure has been to add solid Mg porphyrin to solutions of the chiral amino acids and to filter before recording spectra.