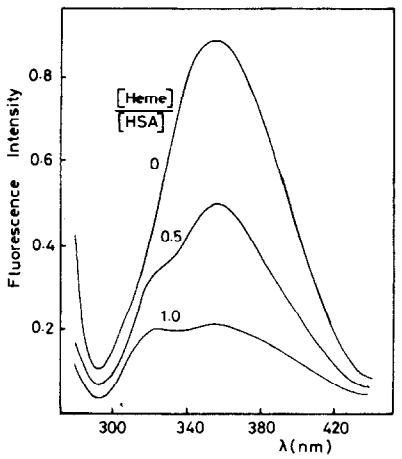

Fig. 4. Emission optical spectrum during formation of heme–HSA complex.

shown that hemes are monomeric and HSA is not denaturated [4]) permitted us to ascertain definitively that at least one axial position of iron is involved in the binding;

(ii) Spin labeling of the lone sulfhydryl group at position 34 of the protein, by a maleimide nitroxide (Fig. 3), led us to estimate using Leigh's theory a distance of about 10 Å between the paramagnetic ferric heme ion and the bound spin label;

(iii) The use of Fe^{3+}–protoporphyrin, spin labelled at its propionyl carboxyl groups, provided strong evidence that these groups are directly involved in the formation of the complex; probably via hydrogen bonds.

(iv) Emission optical spectroscopy showed that progressive quenching of the lone tryptophan (at position 214) fluorescence occurred during the formation of the complex (Fig. 4). According to the expression (6):

$$E = \frac{8.8 \times 10^{-25}\, Q n^{-4} K^2 \int_0^\infty F_D(\nu)\epsilon_A(\nu)\nu^{-4}\, d\nu}{8.8 \times 10^{-25} Q n^{-4} K^2 \int_0^\infty F_D(\nu)\epsilon_A(\nu)\nu^{-4}\, d\nu + r^6}$$

the distance r between the heme group and tryptophan was estimated to be very close to 1.5 nm.

Taking into account these results, and the fact that KCN converted the complex to a low spin form only at high concentrations, we can imagine that the heme group is bound in a rather hydrophobic pocket to HSA, which provides a ligand to the 5th axial position of iron and further hydrogen bonds to the propionyl side chains. The pocket is situated very close to the SH group and the close-lying aromatic tryptophan is probably responsible for the rhombic distortion, via a perturbation of the heme π-electron distribution [7]. Further thermodynamic results suggest the occurrence of an entropy-controlled internalization process of the bound heme, to provide steric protection against diffusion off the carrier protein surface, thus allowing for efficient release only at the target.

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Q2

Magnesium Porphyrin-Globin and -Amino Acid Complexes

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Complexes of magnesium mesoporphyrin (MgMP) and magnesium protoporphyrin (MgPP) with apomyoglobin and apohemoglobin, and amino acid species of the type MgMP/PP (amino acid)$_2$ have been studied by electronic, circular dichroism (CD) and optical rotatory dispersion (ORD) spectroscopy [1–3].

In the case of the protein complexes, specific spectral differences were observed for the myoglobin (Mb) and hemoglobin (Hb) products. These have been interpreted in terms of the formation of six-coordinate magnesium aquo species, Mg(porphyrin)(-histidine)(H_2O), for MgMPMb and MgPPMb and five-coordinate, Mg(porphyrin)(-histidine), species for MgMPHb and MgPPHb [1]. These results highlight the importance of relatively small differences in the protein environment of the heme group in myoglobin and hemoglobin, on binding at the sixth coordination site. It is possible that a favourable hydrogen bonding interaction with the distal imidazole group (of the type recently described for oxy Mb [4] and CO heme proteins [5]) may stabilise the binding of a water molecule to Mg in the Mb species.

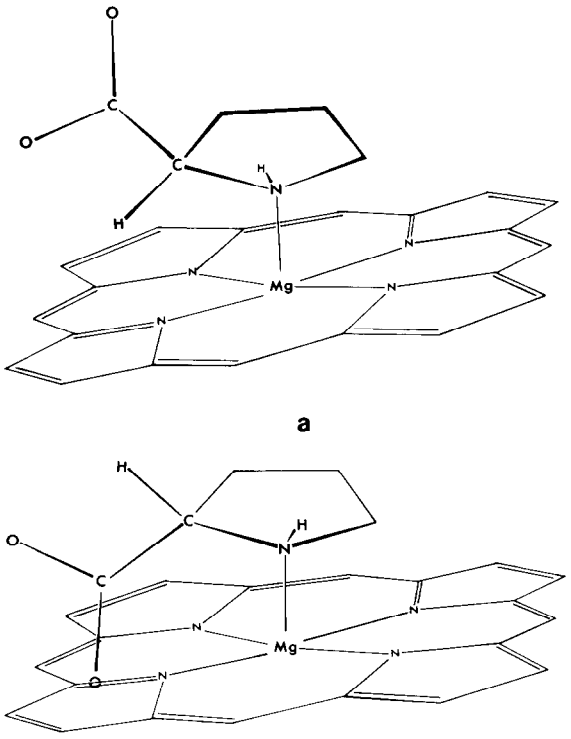

Fig. 1. Diagrams illustrating the formation of an opposite chirality at N to that existing at C for the binding of L-proline to Mg(porphyrin) (as for the optically active Mg(porphyrin)(L-proline)$_2$ complexes). (a) Sterically acceptable opposite-chirality situation. (b) Sterically unacceptable same-chirality arrangement.

Aqueous solutions of the magnesium porphyrins containing the chiral amino acids, L-histidine, L-serine, L-threonine and L-proline, produce prominent induced Cotton effects [1, 2]. However the CD/ORD spectra of these species differ from those of the magnesium porphyrin Mb and Hb protein complexes. Electronic band positions indicate the complexes producing the Cotton effects to be six-coordinate Mg(porphyrin)(amino acid)$_2$ entities, containing two amino acids bound to the metal rather than only one amino acid residue as for the protein species. The electronic spectra also indicate the presence of five-coordinate Mg(porphyrin)(amino acid) species in the solutions but these do not produce Cotton effects.

Induced Cotton effects of the magnitude observed for the Mg(porphyrin)(amino acid)$_2$ complexes presumably require reasonable firm stereochemical location of the coordinated chiral amino acids. For L-histidine we suggest the ligands are localised primarily by π–π bonding interactions between the ligand aromatic π system and the d_π orbitals of Mg [2]. For the similar amino acids, L-serine and L-threonine, molecular models indicate that hydrogen bonding between the ligand –OH groups and the porphyrin COO^- side chains fix these ligands in well-defined positions.

L-proline is a special case in that binding through the ring nitrogen atom produces a new chiral centre at that atom. Interestingly, steric interactions between proline and the porphyrin ring determine the chirality of nitrogen to be opposite to that at the asymmetric carbon atom (as illustrated in Fig. 1). Inversion of this type occurs for a similar reason in the case of proline complexes of Cu^{2+} [6].

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Q3

Electron Transfer Mechanism Studies of Cytochrome c_3: pH Dependence of the Redox Equilibria

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Cytochromes c_3 are tetrahaem electron transfer proteins isolated from *Desulfovibrio* spp. which play an important role in the metabolism of sulfur compounds and hydrogen [1]. They have a molecular weight of approx. 13,000 Daltons [1, 2]. The four haems, mesoporphyrins, are covalently bound to the polypeptide chain through thioether linkages provided by cysteinyl residues. The axial ligands are two hystidinyl residues [3, 4]and all the haems have negative, although different mid-point redox potentials [5–8].

Several physico-chemical techniques, mainly Mössbauer [9], EPR [4–7], NMR [3, 10–16], cyclic voltammetry, and differential pulse polarography [6, 7], have been applied to elucidate the mechanism of electron transfer in cytochrome c_3. However, the mechanism by which electrons are transferred between cytochrome c_3 and other electron carriers is only poorly understood. NMR has been shown to be a suitable technique to elucidate the electron exchange mechanism of cytochrome