Poster Session: Porphyrins and Hemoproteins

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Electron Paramagnetic Resonance and Optical Spectroscopic Study of the Heme–Human Serum Albumin Complex

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Heme–human serum albumin (heme–HSA) complex is paramagnetic because of the presence of a Fe^{3+} ion chelated into the protoporphyrin core [1, 2]. At 77 K or lower, the ferric heme in the complex is characterized by a high spin (s = 5/2) electronic configuration. However, the tetragonal symmetry usually displayed by high spin heme iron which is responsible for two EPR lines situated at $g_\perp \cong 6$ and $g_\parallel \cong 2$ (Fig. 1a) appears to be rhombically distorted upon binding by HSA (Fig. 1b). This distortion shows splitting of the $g_\perp \cong 6$ resonance into two resolvable g-values, g_x and g_y (insert of Fig. 1). The EPR spectrum is interpreted in terms of the spin hamiltonian:

$$H = D\{S_z^2 - 1/3S(S+1)\} + E(S_x^2 - S_y^2) + \beta_e\vec{H}\cdot\hat{g}\cdot\vec{S}$$

where $E/D = (g_x - g_y)/48$ is, in the present case, equal to 5.35×10^{-3}, corresponding to a rhombicity [3] of 1.61%.

Departure from tetragonality may be brought about by several reasons, e.g. by mechanical distortion of heme, perturbation of the heme π-electron distribution, or by π-electron binding to the iron at the 5th and 6th ligand positions [3].

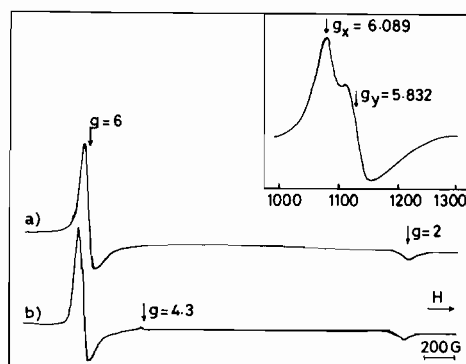

Fig. 1. EPR spectrum of heme (a) and heme–HSA complex (b).

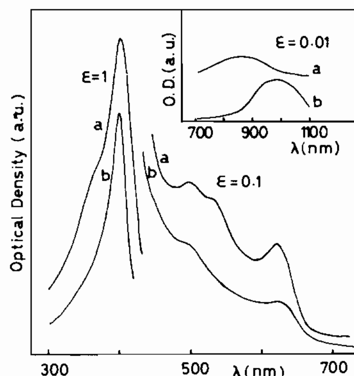

Fig. 2. UV spectrum of heme–HSA complex (a) and heme (b).

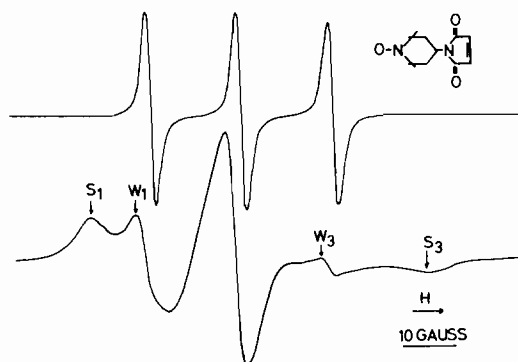

Fig. 3. Spin labeling of sulfhydryl group at position 34.

Very little is known about the molecular structure of the complex and, in particular, about the heme or HSA functional groups involved in the binding. However, engagement of the axial positions of heme iron in the binding has been questioned and the hypothesis that the propionyl side chains are involved in this binding has been put forward [4, 5]. Our study is devoted to gain further information on the molecular aspects of the interaction between heme and HSA, in order to understand why HSA binds so tightly the heme groups (causing distortion of the iron symmetry), although it accomplishes a carrier function.

The following results were obtained:

(i) Absorption optical spectroscopy, in the wavelength range between 300 and 1100 nm, of heme alone (Fig. 2b) and of heme–HSA complex (Fig. 2a) in $DMSO/H_2O$ solvent (in this solvent it has been

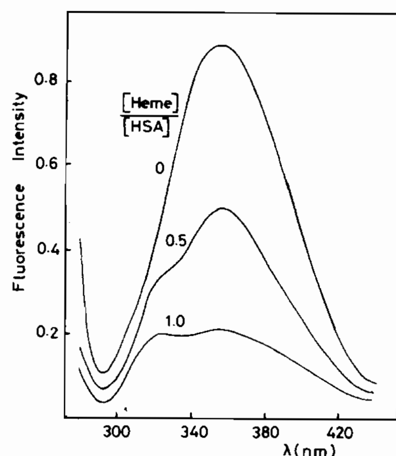

Fig. 4. Emission optical spectrum during formation of heme–HSA complex.

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):

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

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.