MCD spectra of ferrous CPO and P-450-CAM adducts with isocyanides are presented in Fig. 2. While very similar overall, some spectral differences are observed, especially in the more intense visible region MCD features of the CPO adduct. The MCD spectra of the ferrous NO and ferric KCN derivatives of CPO and P-450-CAM are displayed in Fig. 3. As has been observed for native ferric, ferrous and ferrous-CO CPO and P-450-CAM [4], the MCD spectral features of the NO or KCN adducts of both enzymes are similar except for some minor intensity and line shape differences.

In contrast to the above spectral similarities observed between analogous CPO and P-450-CAM derivatives, we have observed significant differences between the two enzymes in the spin state distribution of certain ferric ligand adducts. Although most anionic ligands listed in Table I and some neutral ligands (pyridine, isocyanide, imidazole and NO) form exclusively low spin complexes with ferric CPO, the adducts with other ligands such as neutral sulfur donors are instead a mixture of high and low spin. In addition, formate (low spin) and acetate (mixed spin) behave differently even though both are carboxylate anions at pH 6. Finally, among the heme ligands studied, only fluoride forms a high spin complex with ferric CPO. These diverse spin state properties of the ferric CPO·ligand complexes stand in contrast to the uniformly low spin nature of all ferric P-450-CAM ligand complexes [10].

In conclusion, the hyperporphyrin spectra that are observed for the complex obtained through ligation of a strongly acidic thiol to ferric CPO provide compelling evidence for the presence of an endogenous thiolate ligand to the heme iron of the *ferric* enzyme. The additional spectral similarities observed between analogous ligand complexes of CPO and P-450-CAM add further support to this conclusion for both the ferric and ferrous CPO cases. However, since ligand complexes of P-450-CAM and thiolate-ligated heme models are exclusively low spin, the spin state distribution distinctions seen between some weak field ligand complexes of ferric CPO and P-450 suggests that the heme-iron: thiolate-sulfur interaction in chloroperoxidase is somewhat different from that in P-450 and model complexes. Additional work is in progress to try to identify the cause of these differences.

*Acknowledgements.* This work was supported by NIH Grants GM 26730 (JHD) and GM 07768 (LPH). We thank Laura A. Andersson for her contribution to the bacterial growth and protein purification and Ed Phares and Mary V. Long (Biology Division, Oak Ridge National Laboratories) for assistance in large scale bacterial growth.

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## Q20

## Electron Transfer at Crystallographically Known Long Distances (25 Å) in [$Zn^{II}$, $Fe^{III}$] Hybrid Hemoglobin

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In [Zn, Fe] hybrid hemoglobins, a zinc protoporphyrin (ZnP) and heme are held rigidly at known orientation, with Zn–Fe distance of 25 Å in the functional $\alpha_1$-$\beta_2$ electron transfer entity. Room temperature electron transfer from flash photolytically generated $^3$ZnP to the partner aquoferriheme occurs with rate, $k_t = 60 \pm 25\ s^{-1}$. Oxidation of ferroheme by the partner $(ZnP)^+$ $\pi$-cation radical occurs with rate $k_e = 3.3(\pm 0.7) \times 10^3\ s^{-1}$.

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## Q21

## Gold-induced Spin-state Changes in Haem Proteins

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During the course of our work on anti-arthritic gold drugs, we discovered that $Et_3PAuCl$ converted

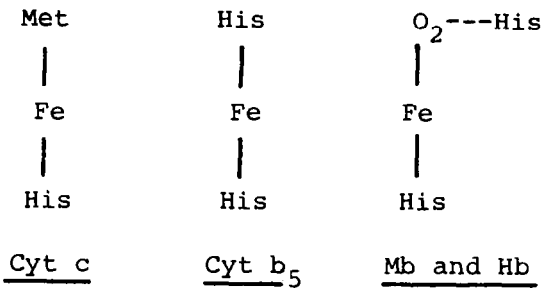


Fig. 1. Axial ligands for iron in haem proteins.

ferric cytochrome into a novel, green high-spin form at pH 7, ambient temperature [1]. The reaction was readily reversed by removal of gold by gel filtration or addition of competing gold-binding ligands. Such a facile spin-state conversion of cytochrome *c* is unusual [2], and we have now extended our studies to other haem proteins with the aim of identifying the critical gold-bonding sites.

The axial ligands for iron in the proteins studied are shown in Fig. 1. The ferric cytochrome $b_5$ (*Erwinia chrysanthememi*) was predominantly low-spin at pH 7 [3]. Titration with $Et_3PAuCl$ gave rise to an increase in absorption at 610 nm similar to that observed previously with cyt *c* [1], 24 equivalents of gold giving complete conversion to a green high-spin form. The reaction was reversed when gold was removed (gel filtration), but, in contrast to cyt c, too rapid for any kinetic observations by conventional means. From this we concluded that gold attack on either histidine or the haem group itself is involved.

Although $Et_3PAuCl$ rapidly denaturated ferric methaemoglobin, metmyoglobin was fully converted into a green high-spin product by about 30 equivalents of Au at similar haem concentrations (0.07 m*M*). However, in contrast to cytochrome *c*, $Et_3PAuCl$ also reacted with the ferrous proteins. With $MbO_2$, a two-phase reaction was observed leading to the same high-spin green product as from metMb. $HbO_2$ behaved similarly but reacted more rapidly [4]. These gold-induced autoxidations appeared to involve release of $O_2^-$, and that of myoglobin, but not haemoglobin, was reversed by gold removal and $Na_2S_2O_4$ addition.

Direct evidence for structural perturbations around the haem group as a result of gold binding were obtained by a comparison of the paramagnetically-shifted resonances of high-spin ferric myoglobin before and after gold treatment.

Since similar reactions occurred with haemin chloride itself, direct attack of gold on the haem group as well as histidines may be responsible for the observed spin-state changes. Studies on other gold compounds suggest that the Au–P bond remains intact and that N displaces $Cl^-$. A direct attack by gold on pyrrole nitrogens might be enhanced with a bifunctional gold compound, and indeed we find that only a 12-fold molar excess of $[AuCl]_2$[depe], where depe is (1,2-diethylphosphino)ethane, is required for full spin-state conversion of ferric cyt c.

*Acknowledgements.* We acknowledge support from the SERC, MRC, Arthritis and Rheumatism Council, Nuffield Foundation and S K and F Laboratories. Drs. C. Greenwood, A. J. Thompson (UEA) and M. D. Scawen (CAMR) are thanked for valuable discussions.

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## Q22

## The Determination of Binding Sites for Electron Transfer Using Lysine Modified Cytochrome *c* Derivatives

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The heme prosthetic group of cytochrome *c* (MW 12400) is almost completely enfolded by a polypeptide chain of 104 amino acids, leaving the 'front' edge partly exposed to solvent. Although it has been suggested that electron transfer in and out of the protein is via this exposed heme edge [1], the evidence for such a mechanism is often only indirect [2]. At pH 7 cytochrome *c* in the Fe(II) and Fe(III) states carried net charges of +8 and +9 respectively where the distribution of acid residues favours a reaction at the heme edge if the reactant is negatively charged [3]. A positively charged reactant would be guided towards the back of the protein, where the shortest distance to the prosthetic group is 14 Å through the protein. If the exposed heme edge is the only site for electron transfer then positively charged reactants have to overcome a coulombic barrier.

Here we compare the kinetic studies for the oxidation of 4-carboxy-2,6-dinitrophenyl (CDNP) single lysine modified derivatives of horse heart cytochrome *c* [4], with inorganic complexes $Fe(CN)_6^{3-}$ and $Co(phen)_3^{3+}$ [5, 6], and the proteins parsley plastocyanin PCu(II), *Pseudomonas aeruginosa* azurin ACu(II) [7], *Rhus vernicifera* stellacyanin SCu(II), and *Anabaena variabilis* plastocyanin. At pH 7 these proteins have approximate charges of −7, −1, +7 and