[3, 8–10] where, in addition, the influence of variation in the porphyrin ligand on reactivity has been examined [11]. The stoichiometry of formation, spectra and reactivity of intermediates formed with a variety of oxidising substrates (hydroperoxides, chlorite) have also been studied [12, 13].

The results reveal some remarkably simple relationships between the reactivities of enzymic and model species and these afford an approach to assessment of the co-catalytic roles played by protein in the expression of enzymic function.

1 P. Jones and I. Wilson, 'Metal Ions in Biological Systems', Vol. 7, H. Sigel (ed.), Marcel Dekker, New York, p. 185 (1978).
2 P. Jones, 'The Biological Chemistry of Iron', H. B. Dunford *et al.* (eds.) D. Reidel Publishing Co., p. 427 (1982).
3 D. M. Davies, P. Jones and D. Mantle, *Biochem. J., 157,* 247 (1976).
4 D. Job and P. Jones, *Eur. J. Biochem., 86,* 565 (1978).
5 J. E. Frew and P. Jones, *Biochim. Biophys. Acta,* in the press.
6 R. Roman and H. B. Dunford, *Biochemistry, 11,* 2076 (1972).
7 D. Job and H. B. Dunford, *Eur. J. Biochem., 66,* 607 (1976).
8 P. Jones and D. Mantle, *J. Chem. Soc. (Dalton Trans.),* 1849 (1977).
9 P. Jones, D. Mantle and I. Wilson, *Inorg. Biochem.,* in the press.
10 J. E. Frew and P. Jones, *Inorg. Biochem.,* in the press.
11 P. Jones, D. Mantle and I. Wilson, *J. Chem. Soc. (Dalton Trans.),* in the press.
12 P. Jones, D. Mantle, D. M. Davies and H. C. Kelly, *Biochemistry, 16,* 3974 (1977).
13 H. C. Kelly, K. J. Parigi, I. Wilson, D. M. Davies, P. Jones and L. J. Roettger, *Inorg. Chem., 20,* 1086 (1981).

Q14

A Novel Mechanism of Hemoglobin Cooperativity

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Perutz has proposed that the cooperative effect of oxygen binding in tetrameric hemoglobin arises from an equilibration between two quaternary structures: the liganded or relaxed (R) structure with oxygen affinity comparable to the isolated subunit affinity and the unliganded, tensed (T) structure with oxygen affinity lowered by constraining salt bridges [1–3]. Perutz argues that the equilibrium between these two structures is primarily governed by displacement of the iron and proximal histidine from the mean porphyrin plane and that much of the free energy of heme–heme interaction is stored in salt bridges which break upon oxygenation. This theory has received support from other workers [4–6].

On the other hand EXAFS studies have shown that iron–pyrrol nitrogen bond distances do not differ between deoxyhemoglobin A and deoxyhemoglobin Kempsey (β99 asp → asn) which is a high affinity mutant essentially devoid of cooperativity [7]. Thermodynamic studies of the temperature dependence of $\alpha\beta$dimer–tetramer equilibrium point out that the free energy of heme–heme interaction is stored in hydrogen bonds between dimers and not salt bridges [8]. Replacement of iron by cobalt, which remains low spin even in the unliganded tetramer, only slightly diminishes n, the empirical measure of cooperativity [9–11]. Finally, the displacement of iron from the porphyrin plane in deoxyhemoglobin A seems to differ little from iron displacement in the noncooperative monomer deoxymyoglobin [12].

We wish to propose a truly alternative mechanism for cooperative ligand binding by hemoglobin which does not necessitate metal movement or salt bridge energetics. The important molecular movement for an increase in oxygen affinity is porphyrin sliding from the hydrophobic protein interior to a position with increased porphyrin exposure to solvent. In hemoglobin A, this movement is rigidly coupled to breaking the hydrogen bond between β99 asp and α42 tyr as the porphyrin moves towards the protein exterior, and upon oxygenation formation of a new hydrogen bond between β102 asn and α94 asp. Protein crystallographic studies report that the porphyrins of both α and β subunits are more exposed to water in the met-form than for deoxyhemoglobin [2]. The porphyrin is more exposed to solvent in the β chain of the high affinity mutant deoxyhemoglobin Yakima (β99 asp → his) than A [13]. Studies on model cobaltoporphyrins report that oxygen affinity is more dependent upon porphyrin–solvent interactions than upon 2,4-substituents or ligand *trans* to oxygen [14, 15]. Stellwagen has pointed out that the redox potential of hemoproteins is dependent upon the degree of porphyrin exposure to solvent, with the redox potential decreasing as porphyrin exposure increases [16]. That oxygen affinity increases with increasing porphyrin exposure to solvent is consistent with all the above facts and is tied together by the experiments of Basolo and coworkers who have shown that an inverse linear correlation exists between the logK of oxygenation and cobalt(II/III) redox potential for a series of equatorially substituted cobalt complexes [17, 18].

Recent work upon energetics across the $\alpha_1\beta_2$ interface of hemoglobin shows that amino acid mutations in this region drastically alter cooperative energetics while substitutions at other parts of the molecule have little effect upon cooperativity [19]. Substitution of β99 asp by his, gln or gly destroys the lone hydrogen bond which connects the deoxy-dimers and abolishes all cooperative energy,

and the resultant hemoglobins have oxygen affinities close to those of isolated subunits. The NMR resonance position of this hydrogen bond (−14.2 ppm) is in the range of resonance positions of 'strong' hydrogen bonds while the resonance position of the β102 asn–α94 asp proton (−10.3 ppm) appears normal [10, 21]. So energy storage for the deoxy tetramer is primarily localized in this β99 asp–α42 tyr hydrogen bond. Information between subunits is transferred through those amino acids involved in hydrogen bonding to heme pyrrole II and the innate porphyrin rigidity used to modulate porphyrin exposure to solvent which in turn controls oxygen affinity. Porphyrin sliding can also account for Fe–imidazole bond rupture in hemoglobin NO in the presence of IHP [22].

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1 M. F. Perutz, *Nature, 228,* 726 (1970).
2 M. F. Perutz, *Brit. Med. Bull., 32,* 195 (1976).
3 J. V. Kilmartin, *Brit. Med. Bull., 32,* 209 (1976).
4 J. J. Hopfield, *J. Mol. Biol., 77,* 207 (1973).
5 J. L. Hoard and W. R. Scheidt, *Proc. Nat. Acad. Sci. U.S.A., 70,* 3919 (1973).
6 A. Warshel, *Proc. Nat. Acad. Sci. U.S.A., 74,* 1789 (1977).
7 P. Eisenberger, R. G. Shulman, R. M. Kincaid, G. A. Brown and S. Ogawa, *Nature, 274,* 30 (1978).
8 G. K. Ackers, *Biophys. J., 32,* 331 (1980).
9 B. M. Hoffman and P. H. Petering, *Proc. Nat. Acad. Sci., U.S.A., 67,* 637 (1970).
10 L. C. Dickinson and J. C. W. Chien, *J. Biol. Chem., 248,* 5005 (1973).
11 K. Imai, M. Ikeda-Saito, H. Yamamoto and T. Yonetani, *J. Mol. Biol., 138,* 635 (1980).
12 M. F. Perutz, S. S. Hasnain, P. J. Duke, J. L. Sessler and J. E. Hahn, *Nature, 295,* 535 (1982).
13 P. D. Pullsinelli, *J. Mol. Biol., 74,* 57 (1973).
14 H. C. Stynes and J. A. Ibers, *J. Am. Chem. Soc., 94,* 5125 (1972).
15 H. Yamamoto, T. Takayanagi, T. Kwaan and T. Yonetani, *Bioinorg. Chem., 7,* 189 (1977).
16 E. Stellwagen, *Nature, 275,* 73 (1978).
17 M. J. Carter, L. M. Engelhardt, D. P. Rillema and F. Basolo, *J. Chem. Soc., Chem. Commun.,* 810 (1973).
18 M. J. Carter, D. P. Rillema and F. Basolo, *J. Am. Chem. Soc., 96,* 392 (1974).
19 P. W. Pettigrew, P. H. Romeo, A. Tsapis, J. Thillet, M. L. Smith, B. W. Turner and G. K. Ackers, *Proc. Nat. Acad. Sci., U.S.A., 79,* 1849 (1982).
20 K. J. Wiechelman, *U. F. Fairbanks and C. Ho, Biochemistry, 15,* 1414 (1976).
21 J. Emsley, *Chem. Soc. Rev., 9,* 91 (1980).
22 J. C. Maxwell and W. S.Caughey, *Biochemistry, 15,* 388 (1976).

Q15

^{57}Fe NMR of Iron(II) Low Spin Hemes

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Electronic structures of iron in hemoproteins have close correlation with their reactivity. Many physicochemical techniques have been applied to hemoproteins to elucidate electronic structures of heme irons. However, conclusive experimental determination of the electronic structures of low spin iron(II) heme, especially oxygenated hemes, waits for further examinations. In order to determine the electronic structures of the hemes directly from the view point of their ground state, we have started ^{57}Fe NMR studies of heme complexes in succession to our MCD [1] and *ab initio* calculation [2] on the heme complexes. ^{57}Fe NMR spectra were obtained by using steady-state free precession and the quadriga Fourier transform technique [3–5]. First, T_1 values of ^{57}Fe nuclei have been estimated for $Fe(CO)_5$ and ferrocene with natural abundance of ^{57}Fe. ^{15}N-enriched, α,β,γ,δ-*meso*-tetra-phenylporphine (TPP) and α,β,γ,δ-*meso*-*p*-tolylporphine (TTP) were prepared from ^{15}N-labeled pyrrole (CEA Saclay, 95% enrichment), and benzaldehyde or *p*-tolylaldehyde by the usual method [6]. Incorporation of ^{57}Fe (Spire, 95.45% enrichment) was accomplished by Rothemund's method [7]. Bis-pyridine and bis-pyrrolidine complexes of [^{15}N,^{57}Fe]-TPP and [^{15}N,^{57}Fe]-TTP with Fe(II) low spin state were prepared by a conventional method [8]. The ^{57}Fe NMR spectra recorded on a Bruker CXP-300 NMR spectrometer incorporated with a Bruker ^{57}Fe probe (using 15 mm sample tube), can be tuned between 10.2 and 9.2 MHz. The chemical shift values of the bis-pyridine complex of [^{15}N,^{57}Fe]-TPP, of the bis-pyrrolidine complex of [^{15}N,^{57}Fe]-TTP, of $K_4[Fe(CN)_6]$ and of ferrocene are 7341, 7258, 2495, and 1531 ppm low field from that of $Fe(CO)_5$ as a reference. The variation of chemical shifts of various iron(II) complexes will be discussed in terms of the d–d energy splitting in a similar way to that of ^{59}Co-complexes [9].