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The Cooperative Effect Revealed for a New Hemoglobin Model Complex

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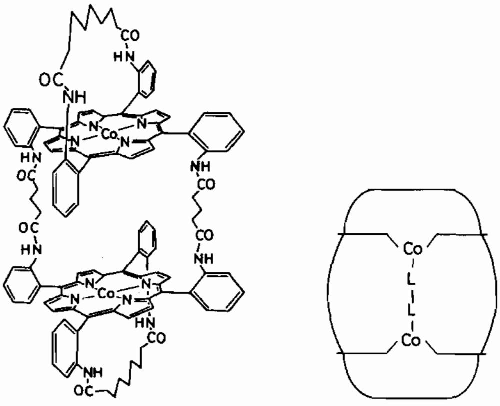
Much interest has been shown recently in cooperative dioxygen binding to hemoglobin (Hb) [1]. According to Perutz's trigger mechanism [2], the shift of the central metal atom in heme relative to the porphyrin plane upon dioxygen binding is one of the key factors in the cooperative effect in Hb and its cobalt analogue (CoHb) [3]. To demonstrate experimentally the logic behind the trigger mechanism, we synthesized a new dimeric model complex in which cooperative dioxygen binding might be expected as a result of structural changes, followed by transmission to other active sites.

The model complex was derived from *meso*-tetra(*o*-aminophenyl)porphyrin [4] and is shown in Fig. 1a. Oxygenation equilibria were determined by spectrophotometric O_2 titration in N,N-dimethylformamide (DMF) at −15.0 °C [5].



(a) (b)

Fig. 1. (a) Synthesized new Hb model complex. (b) Schematic representation of bidentate ligand adducts; 'sandwich geometry', where L–L represents bidentate ligands.

To prevent the binding of pyridine derivatives to one side of the porphyrinato complex, and aliphatic chain was designed to bridge over each porphyrin plane and consequently the bindings of the bidentate ligands were forced between the two porphyrin planes. As seen in Table I, the large binding constants for bidentate ligand addition also suggest that the structures of these adducts have 'sandwich geometry' in Fig. 1b.

As seen in Table I, dioxygen binding to the model complex is not cooperative in the case of the 4,4′-bipyridine (BIPY) adduct or the 1,3-bis(4-pyridyl)-propane (BIPYPr) adduct, while it is cooperative in the case of the 1,2-bis(4-pyridyl)ethane (BIPYEt) adduct. The Hill coefficient (n = 1.3) for dioxygen binding for the BIPYEt adduct was not changed in the concentration range 2.5 mM–10 mM of BIPYEt where concentration of the complex was 0.04 mM. This is in remarkable contrast to Tabushi's model complex [6], for which the Hill coefficient for the dioxygen binding varies with concentration of base, thus their proposed mechanism for cooperativity is intermolecular.

TABLE I. Pyridines and Dioxygen Bindings to Model Complexes.

	K_B^{a} (M^{-1})	$P_{1/2}O_2$^{b} (Torr)	n^c
PY	1.7×10^3	3.2×10^2	1.0
BIPY	2.3×10^5	4.5×10^2	1.0
BIPYEt	9.2×10^4	4.9×10^1	1.3
BIPYPr	6.0×10^4	1.6×10^2	1.0

In DMF at −15.0 °C.

^{a}Binding constants to pyridines. ^{b}Half-saturation pressures for dioxygen binding. ^{c}Hill's coefficient for dioxygen binding reaction.

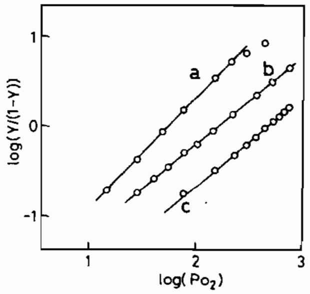


Fig. 2. Hill plot for dioxygen binding reaction to the model complex in DMF at −15.0 °C. (a) axial base = BIPYEt, slope = 1.3; (b) axial base = BIPYPr, slope = 1.0; (c) axial base = BIPY, slope = 1.0.

On the other hand, it is confirmed that some changes in dioxygen binding to the first active site will be transmitted to the second through the sandwiched BIPYEt molecule intramolecularly. The distance between two cobalt atoms in the model complex is about 12 Å, and the molecular sizes of BIPY, BIPYEt and BIPYPr are 7 Å, 9 Å and 10 Å, respectively, according to the Dreiding stereomodel. Assuming that the Co–N (pyridyl nitrogen) distance

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is about 2.4 Å [7], the distance between two cobalt atoms might not be long enough to accommodate BIPYEt without some constraint. Constraint might therefore be imposed upon the BIPYEt molecule bound to two cobalt atoms. While a certain electronic interaction between two active sites through BIPYEt could be responsible for the observed cooperativity, its contribution would be very small in our model system. This is because cooperative dioxygen binding is not found in the BIPY adduct which is considered to have a stronger electronic interaction than does the BIPYEt adduct with respect to the separation between two porphyrin planes.

We propose a mechanism for cooperative dioxygen binding behavior for the BIPYEt adduct as follows. With the first dioxygen binding, the shift of the cobalt atom into the porphyrin plane would release the constraint in BIPYEt and make the second dioxygen binding more favorable than the first. The absence of cooperativity in the BIPYPr adduct would be responsible for the flexibility of the propane chain connecting the two pyridyl groups in BIPYPr and such constraint would not be accumulated in the BIPYPr adduct. Furthermore, this kind of structural constraint might not be accumulated in the BIPY adduct because BIPY is a small molecule and can plunge between two cobalt atoms without constraint.

Our results represent dynamic evidence for the basic mechanism of the cooperative effect on Hb where four binding sites exist. In such cases, the small shift of the central metal atom at the first dioxygen binding site in the heme initiates the changes in tertiary structure. These changes can be spread into the quaternary structure mechanically and consequently regulate cooperatively the dioxygen affinities. Details of our model system will be published elsewhere.

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