Conformational Control of Intramolecular Hydrogen Bonding in Heme Models: Maximal $Co^{II}-O_2$ Binding in a C-Clamp Porphyrin

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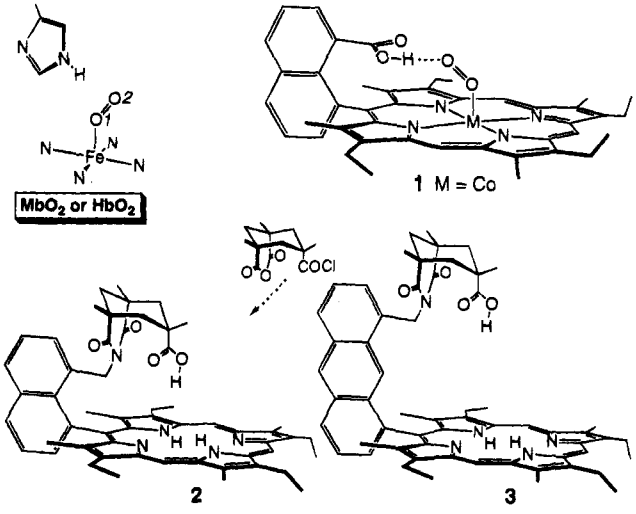
Received January 10, 1995

Hydrogen bonding plays a crucial role in heme protein reactions. In myoglobin (Mb) and hemoglobin (Hb), the interaction between the distal histidyl proton and the heme-bound O_2 is considered the most significant factor controlling O_2 binding.[1] In catalase and peroxidase, a H-bond polarizes the peroxy group to facilitate a heterolytic O–O bond cleavage.[2] Likewise, in cytochrome a_3, the presence of a proton donor is an integral part of O_2 reduction.[3] The study of H-bonding effects, therefore, is always a focal point in heme model chemistry. Earlier, such studies were mainly confined to intermolecular interactions such as solvent effects, but more recently, synthetic models equipped with intramolecular proton donors have emerged.[4–8] While these models clearly established the positive effect of enhancing the O_2 affinity, they have not permitted a closer look at how H-bond geometry and strength would influence the heme–substrate reactions. For example, from X-ray[9] and neutron diffraction[10] data it has been noted that the H-bonding between the distal histidine and Fe–O_2 in Mb and Hb is an oblique one,[7] raising possibilities that the proton could interact with O1 and/or O2. Most model systems available to date, as exemplified by the naphthoic acid model **1**,[5c] attempt a H-bond at O2 in a linear Fe–O1–O2···H. Apart from this "unnatural" conformation, model **1** also suffers from a fatal fault as it undergoes ring degradation giving rise to an oxaporphyrin.[11] In an effort to address the H-bond conformation issue and to sidestep the catabolism of **1**, we relocated the proton donor to an overhanging position. This paper reports the synthesis, structure, and O_2 binding study of this unique model.



Our design begins with **1**, in which the 1,8-naphthalene connector already enforces a U-shaped relationship between the porphyrin plane and the carboxylic acid. The latter can be rotated to a pendent position if another U-turn is inserted, perpendicular to the first one, to achieve an overall 270° C-turn. Thus, Kemp's triacid is incorporated on the premise that the imide connector in this naphthalene Kemp's acid porphyrin (NKAP) **2** should furnish a rigid conformation.[12] Synthetically, we took advantage of Rebek's well-tested coupling between the triacid anhydride–chloride and an amine which in our system is obtained from the naphthalene methanol via mesylate and azide intermediates.[13] Initially, the entire sequence was tested on a homologous anthracene system to give an anthracene Kemp's acid porphyrin (AKAP) **3** before being applied successfully to the more hindered **2**.

The molecular structure of NKAP is confirmed by an X-ray structure (Figure 1).[14] The crystal obtained by slow evaporation of **2** from CH_2Cl_2 contains a water ligand transfixed by the carboxyl group and the ring nitrogens through H-bonds. To accommodate this H_2O, the porphyrin ring undergoes several noticeable deformations at the naphthalene connector. Not only is the C20–C33–C42 angle at 126.54(25)° larger than 123.5-(6)° found in the parent naphthoic acid, but the porphyrin plane also bends outwardly by as much as 9° from the C20–C33 axis (Figure 1C), resulting in a bigger "bite" than otherwise possible. Laterally, the C20–C33 bond deviates from the naphthalene plane by tilting about 6° in the C5 direction and is supplemented by further distortions at C20 (the C19–C20–C33 angle of 117.57(25)° is larger than the C1–C20–C33 angle of 115.06-(25)°), to displace the porphyrin core to the right (Figure 1B), presumably to achieve the best alignment with the water proton. Despite these, it is significant that the imide-to-naphthalene linkage retains its near perfect alignment and C_2 symmetry (with only a slight rotation along the C43–N5 bond). Undoubtedly, much of this rigidity arises from the nonbonding interaction between the C43 methylene and the porphyrin whereby the substituent at C43 obtains a predictable conformation. Thus, the design criteria of the model have been fully met. In solution, the conformation is consistent with the solid state structure as evidenced by ^1H NMR. In particular, the C43 methylene δ in $CDCl_3$ shifts from 3.34 ppm at zero concentration of H_2O to 3.78 ppm (less diamagnetic influence) at saturation support the assertion that, in the absence of the H_2O ligand, the acid group should be even closer to the ring.

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(14) $C_{55}H_{61}N_5O_4 \cdot H_2O$, monoclinic, space group $C2/c$, a = 19.557(4) Å, b = 11.9541(17) Å, c = 42.602(8) Å, β = 105.201(17)°. The structure was solved by direct methods. All non-hydrogen atoms were refined anisotropically. The protons on O5, O1, and N1 to N4 were located from a difference Fourier map and positionally refined; final R_F = 0.047, R_w = 0.044. See supplementary material.

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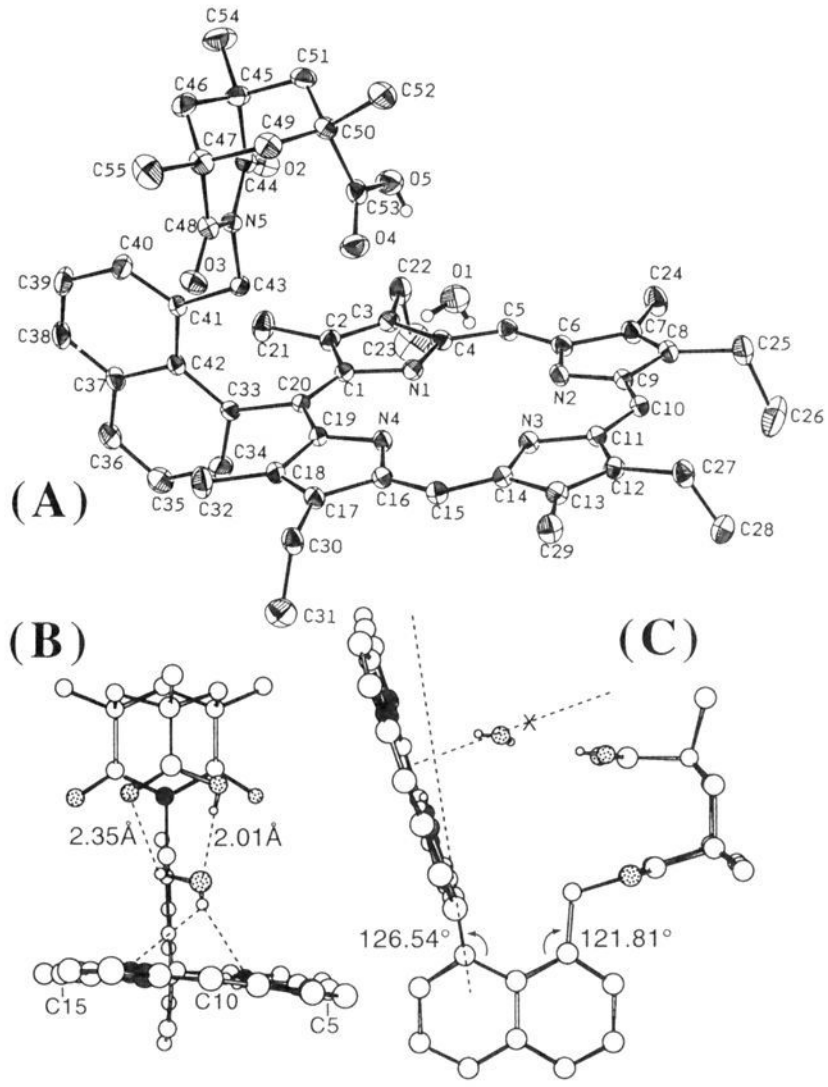

Figure 1. (A) ORTEP plot and labeling scheme for **2**, complexed with H_2O. (B) Side view of the X-ray structure showing H-bonds. The distance between the water oxygen and the porphyrin center is 2.31(4) Å. (C) Another view showing distortions around the C20−C33 connector.

Table 1. O_2 Binding Constants in DMF

Co^{II} complex	T (°C)	$P_{1/2}$ (Torr)	ΔH (kcal mol^{-1})	ΔS (cal mol^{-1} K^{-1})[a]
2	−42	0.009 ± 0.001		
	−30	0.028 ± 0.005		
	−17	0.11 ± 0.02	−11.4 ± 1.2	−40 ± 5
	0	0.38 ± 0.10		
	(25	2.3)[b]		
3	−42	2.4 ± 0.3		
2/3 Me ester	−42	≥200		
CoMb[c]	25	57	−11.3	−46

[a] Standard state, 1 Torr. [b] Extrapolated from the van't Hoff plot. [c] Equine Mb, from the following: Spilburg, C. A.; Hoffman, B. M.; Petering, D. H. *J. Biol. Chem.* **1972**, *247*, 4219.

To determine O_2 binding, both Co^{II} and Fe^{II} of **2** have been studied but Fe^{II}NKAP proves to be too labile even at −42 °C. The O_2 adduct of Co^{II}NKAP in DMF is more stable; appreciable autoxidation does not occur below −20 °C ($t_{1/2}$ at 0 °C is about 4 min).[15] As shown in Table 1, the $P_{1/2}$ obtained at −42 °C from CoNKAP is an all-time record for Co^{II} porphyrins, being three times better than **1** studied previously,[5c] and the more than 10^4-fold enhancement from the ester to the acid is truly dramatic. In the anthracene case, the enhancement is less impressive. This may be due to a mismatch of the geometry and/or increased motion of the pendent group. With Co^{II}**2**, perhaps the surprising result is its relatively large affinities observed at higher temperatures, a deviation from **1** which exhibits a greater sensitivity to temperature. Consequently, ΔH and ΔS for O_2 binding in CoNKAP are smaller. The difference between **1** and Co^{II}**2** at first seems hard to reconcile. Even if the previous $P_{1/2}$ of **1** at 0 °C is given a 10-fold reduction,[16] the adjusted ΔH and ΔS of **1** are still much more negative than those of Co^{II}**2**. We believe that the disparity is caused by the mode of H-bonding. In **1**, the coplanar and inflexible Fe−O−O···H has the highest gain in ΔH but suffers the highest loss in ΔS. In **2**, because the C-clamp-shaped host is ideal for biting a monoatomic ligand, the H-bond would aim at O1, which may not achieve the best gain in ΔH but is conformationally less restrictive for the chelated O_2.[17] Thus, the smaller loss in ΔS is apparently more than enough to compensate for the enthalpic loss to afford large binding constants throughout the temperature range.[4a]

To lend further support to our conjecture that **2** disfavors a H-bond at the open end of diatomic ligands, ^{15}N-NMR was used to probe that ligand environment.[18] H-bonding is known to cause a large upfield shift of the ^{15}N signal for $(C^{15}N^-)_2Fe^{III}$ porphyrins, due to reduced spin transfer from iron to cyanide.[19] Typical biscyanomet hemes of etioporphyrin and octaethylporphyrin, in DMSO containing 10-fold excess $KC^{15}N$, exhibit one single peak around δ 720 (referenced from free $KC^{15}N$ at δ −100). With the anthracene **3**, which should clamp down a linear C≡N strongly if the acid group is in place, biscyano-FeAKAP has two peaks at δ 622 and 490. When the CO_2H is reduced to CH_2OH, the corresponding peaks become δ 701 and 657. The more upfield ^{15}N signal belongs to the H-bonded CN which weakens the axial ligand field and simultaneously shifts the other trans CN ligand signal. Notice that the spread between the two signals becomes smaller as the H-bond becomes weaker. With **2**, the $(C^{15}N^-)_2$ FeNKAP shows two peaks at δ 715 and 670, suggesting a quite ineffective H-bond to this cyano nitrogen. Using Figure 1C as a model, an iron-bound CN would place the N at X, a very awkward position to align with the carboxylic proton. It can be further estimated that replacing the linear CN by a bent O=O would not give a better O2···H−O_2C alignment without first requiring twisting the acid group off center (estimated C43−N5 rotation of at least 40° for an optimum H-bond). While such rotation may still happen, the more likely H-bond occuring in this model would be one that is energetically less steep or a multiwelled interaction toward the π-bond or O1, if the X-ray structure is any indication.

This study demonstrates the intricacies of distal H-bonding effects in heme-O_2 reaction; it highlights the fact that, due to the entropic factor, a high O_2 binding constant does not necessarily come from a maximum Fe−O−O···H interaction. Rather, the binding is always enhanced with less restrictive H-bond(s) at the physiological temperature.[20]

Acknowledgment. This work was supported in part by the NIH. C.K.C. dedicates this paper to the memory of the late Professor Teddy G. Traylor.

Supplementary Material Available: Synthesis, O_2 titrations, and crystal structural data (13 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

JA9500872

(15) O_2 titrations of the Co^{II} porphyrin−DMF solution in a vacuum tonometer were followed by UV−vis. The $P_{1/2}$ reported are the average of 3−5 runs, with precision indicated in Table 1. The rapid oxidation at 0 °C allows only one or two data points; the $P_{1/2}$ is estimated by combining data from five separate runs and confirmed by several independent samples.

(16) The O_2 affinity of **1** at 0 °C is subject to a high degree of uncertainty due to additional complications brought about by the catabolic reaction.[11] During re-examination, the $P_{1/2}$ (0 °C) could be as low as 5−10 Torr. (At 5 Torr, $\Delta H = -14.3$ kcal mol^{-1} and $\Delta S = -56$ cal K^{-1} mol^{-1}.)

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