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Functional models for the oxygen binding/activating hemeproteins, myoglobin and cytochrome *c* oxidase

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Abstract

Hemoproteins (hemoglobin and myoglobin) reversibly bind O_2 for transport and storage; other heme proteins (cytochrome *c* oxidase) reduce O_2 to water and couple the energy from that reaction to create a proton gradient for the eventual synthesis of ATP. This paper will describe the design and synthesis of iron porphyrins which are functional models for these oxygen binding and activating heme proteins.

Keywords: Iron porphyrins; Cobalt porphyrins; Oxygen binding; Carbon monoxide binding; Copper–oxygen chemistry

Hemeproteins reversibly bind dioxygen, O_2. Myoglobins, Mb, store O_2 in tissue. Cytochrome *c* oxidase, in the mitochondria, reduces O_2 by $4e^-$; the energy derived from this exergonic reaction is used to translocate protons across a membrane such that ATP can be formed from ADP and phosphate as this proton gradient is relaxed. The present account describes the synthesis of model porphyrins which also bind O_2 reversibly and, in one example, catalyze the reduction of O_2 by an electrocatalytic $4e^-$, $4H^+$ process. The role of CO as a competitive inhibitor of O_2 binding is also presented.

Ferrous porphyrin complexes are thermodynamically unstable in the presence of O_2; simple Fe(II) porphyrins irreversibly react with O_2 according to the following stoichiometry: $4Fe(II)Por + O_2 \rightarrow 2(Fe(III)Por)_2{-}O$. Hemeproteins such as Mb inhibit this reaction, by encapsulating the heme with the protein and preventing bimolecular reactions between two Fe(II) centers and the O_2 molecule. The ternary protein–heme interaction controls the nature and number of axial ligands. In Mb and Cyt_c oxidase one imidazole is coordinated on the 'proximal' face of the porphyrin, affording a high-spin ($S = 2$) Fe(II) porphyrin. The opposite, 'distal' porphyrin face is decorated with residues from the heme protein. These distal residues, especially a valine and another imidazole, help stabilize the polar O_2 adduct, which can be considered an $Fe(III)O_2^-$ group. Note that O_2 binding by any metal involves single electron transfer from the metal to O_2 and simultaneous coordination of the radical anion O_2^- (superoxide) by the oxidized metal center — here Fe(III). The polar $Fe(III)O_2^-$ group is stabilized by a weak

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(~ 2 kcal mol^{-1}) hydrogen bond to the distal histidine.

This $Fe(III)O_2^-$ group is bent, which accounts for its diamagnetism via a weak p-bond formed between the low-spin d_{xz} orbital and the π^* level on O_2^- (both are singly occupied). The bent nature of the O_2 adduct contrasts with the neutral ligand CO, which typically forms linear Fe(II)CO adducts perpendicular to the porphyrin plane. The distal groups play an additional role by destabilizing the CO ligand, seemingly between the distal histidine and valine residues (cf. Fig. 1). The extent and nature of this distortion in the heme proteins is currently very controversial [1].

Carbon monoxide is an intrinsic toxic ligand because CO is produced naturally in the catabolism of heme proteins. Thus aerobic organisms utilizing hemes must tolerate a burden of CO.

Many years ago we prepared Fe(II) porphyrins which are fitted with a polar O_2 binding pocket and a single axial ligand on the opposite porphyrin face. These 'picket fence' and related 'pocket porphyrins' are displayed in Fig. 2; many analogous O_2 binding Fe(II) porphyrins were subsequently prepared. This subject has recently been reviewed [2]. An important experimental measure of reversible O_2 binding should be mentioned; the $Fe(III)O_2^-$ adducts should be diamagnetic as evidenced by sharp ^1H NMR signals at room temperature in the usual chemical shift range exhibited by organic molecules. The $Fe-O_2$ adducts in Fig. 2 and all others described in this account do meet this standard.

Recently we reported a versatile general method for attaching macrocycles over a porphyrin ring in a single high-yielding step. This 'congruent multiple Michael addition' (Fig. 3), does not require high dilution and is facilitated by protic solvents and the presence of metals such as Fe(II), Co(II), or Zn(II) in the porphyrin.

The advent of these new cavity porphyrins led us to reexamine the relative equilibrium affinities of CO versus O_2. Biologists refer to the relative CO/O_2 affinities as M, which is usually measured as $p_{1/2}^{O_2}$ over $p_{1/2}^{CO}$. Recall that $p_{1/2}^{O_2}$ is the partial pressure of O_2 at one half equilibrium binding; it is the reciprocal of the usual equilibrium constant expressed in pressure units.

In Fig. 4 are shown values of $p_{1/2}^{O_2}$ and $p_{1/2}^{CO}$ for hemoglobin in the low affinity 'T state', Hb(T), and various of our model complexes. It should be noted that the T state of Hb can be mimicked by using as axial ligands 2-methyl or 1,2-dimethyl imidazole (2-MeIm and 1,2-Me_2Im). Although the picket fence T-state model exhibits an O_2 affinity similar to Hb(T), the former binds CO irreversibly so that $M >$ 4000. Two new porphyrins fitted with a 1,4,7-triazacyclononane cap manifest an even higher affinity for O_2 but these show a much reduced

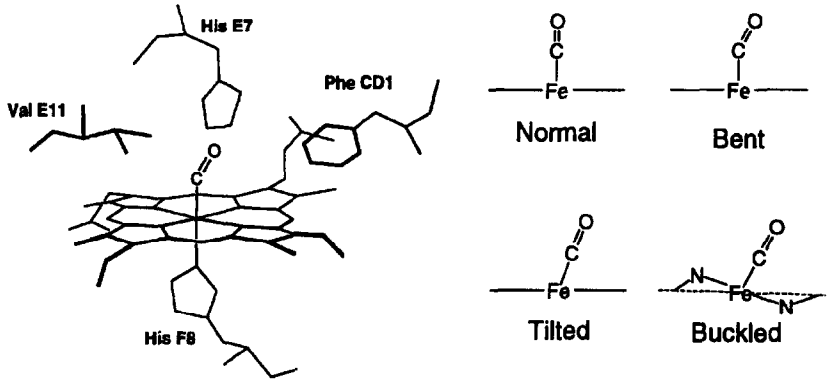


Fig. 1. Hb and Mb active site destabilizes CO.

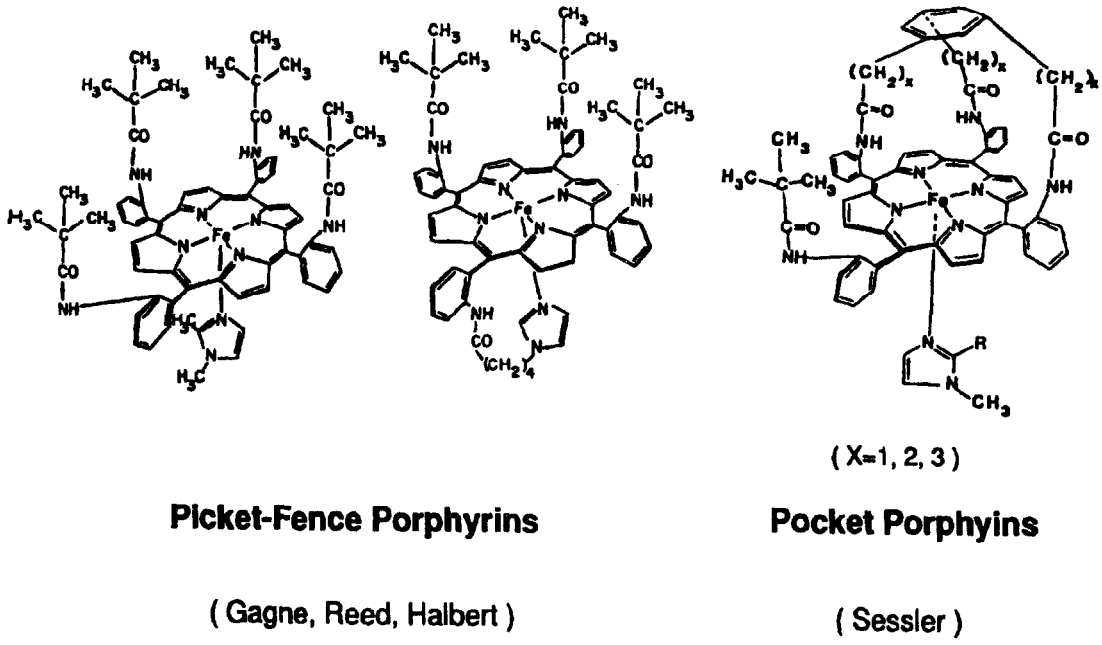


Fig. 2. Oxygen binding Mb analogues.

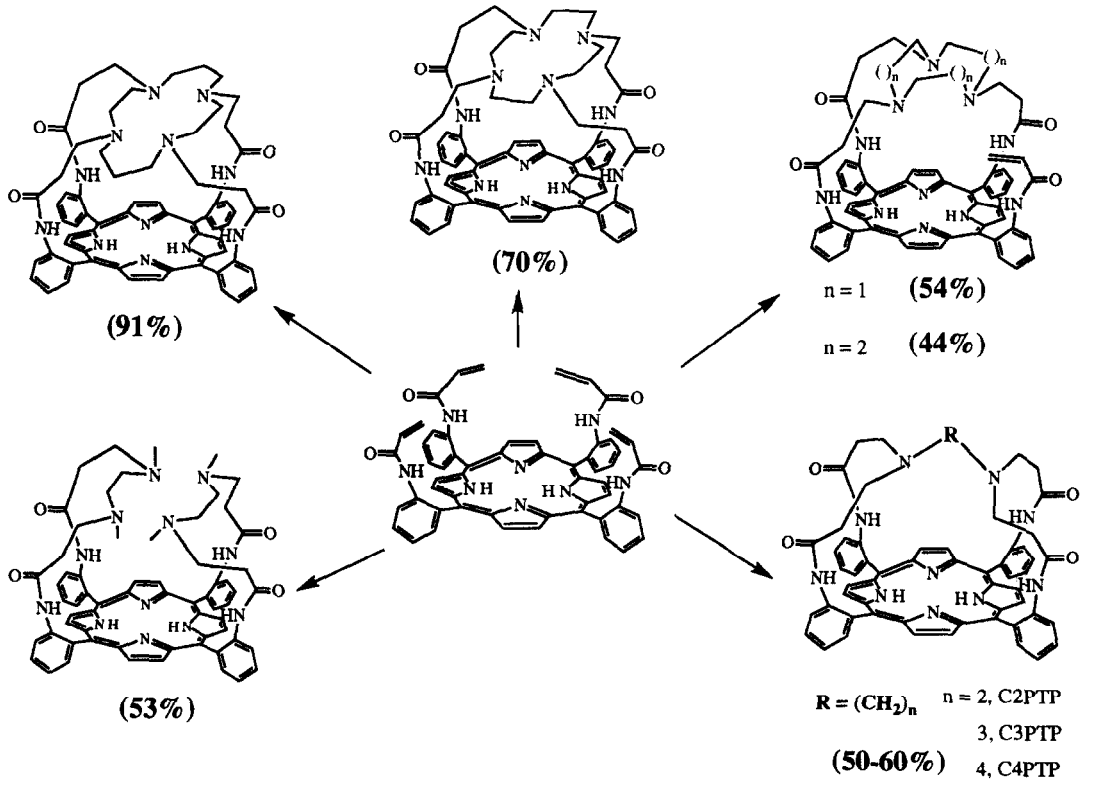


Fig. 3. Congruent multiple Michael addition.

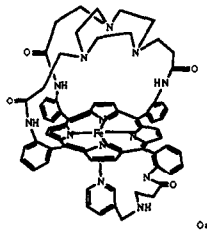
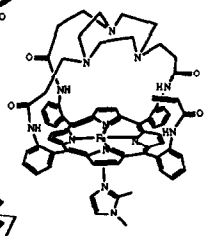
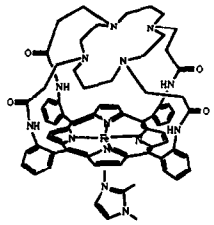
Porphyrin	$P_{1/2}(O_2)$ (torr)	$P_{1/2}(CO)$ (torr)	$M = \frac{P_{1/2}(O_2)\ (torr)}{P_{1/2}(CO)\ (torr)}$
Hb (T)	40 140	0.30	135 460
Fe(Picket Fence) (1, 2-Me_2Im)	38	0.0089	4280
	3.7	1.5	2.47
	2.3	2.9	0.79
	25	>3500	<0.007

Fig. 4. Summary of gas binding thermodynamics.

CO affinity, such that M is close to 1. The Fe(II) complex of the 'cyclam' capped porphyrin is even more remarkable; whereas it exhibits a 'normal' O_2 affinity, it does not bind CO at all (at 1 atm); the M value is < 0.007! The probable origin of this phenomenon can be

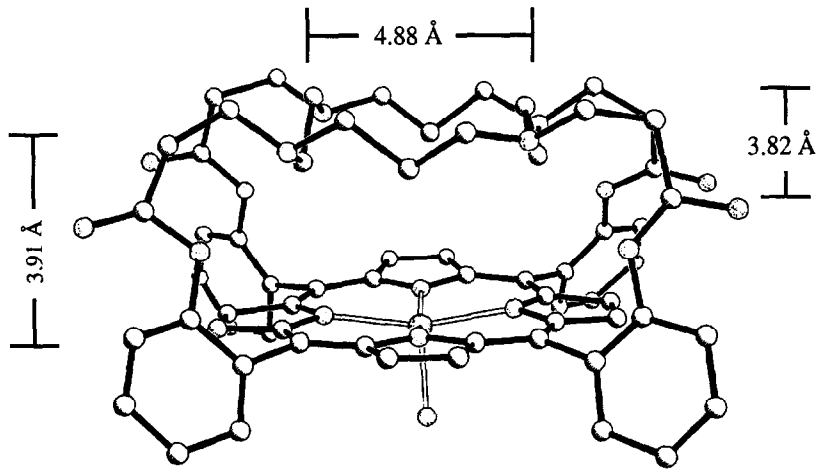


Fig. 5. Cyclam capped Zn porphyrin.

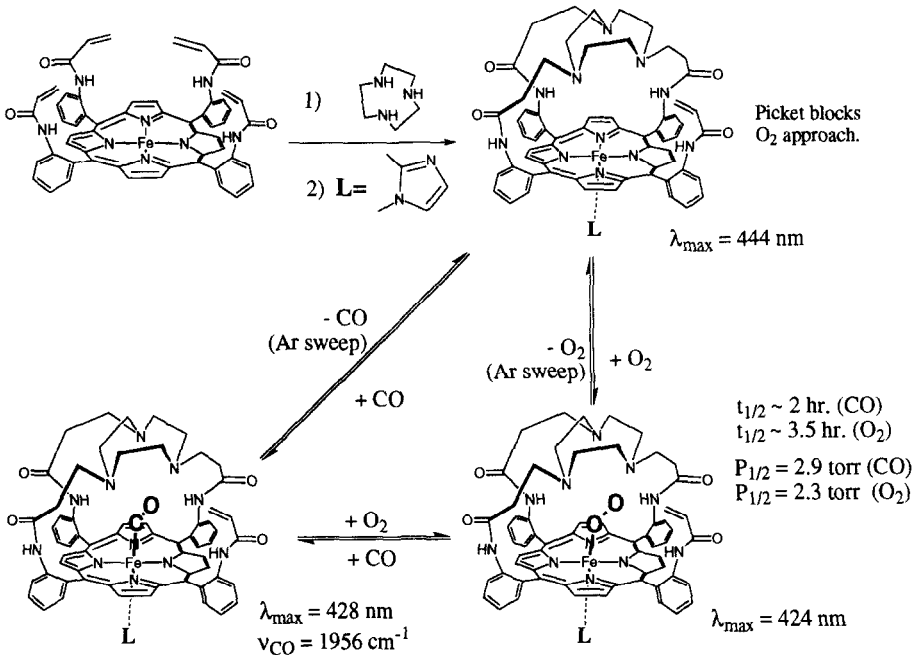


Fig. 6. Synthesis of an Fe capped porphyrin; slow CO/O_2 binding.

ascertained by examining the X-ray structure of the corresponding Zn(II) complex. An ortep drawing is displayed in Fig. 5. The CH_2 groups just over the metal core have H-atoms projected directly at an axis perpendicular to the porphyrin and emanating from the metal porphyrin

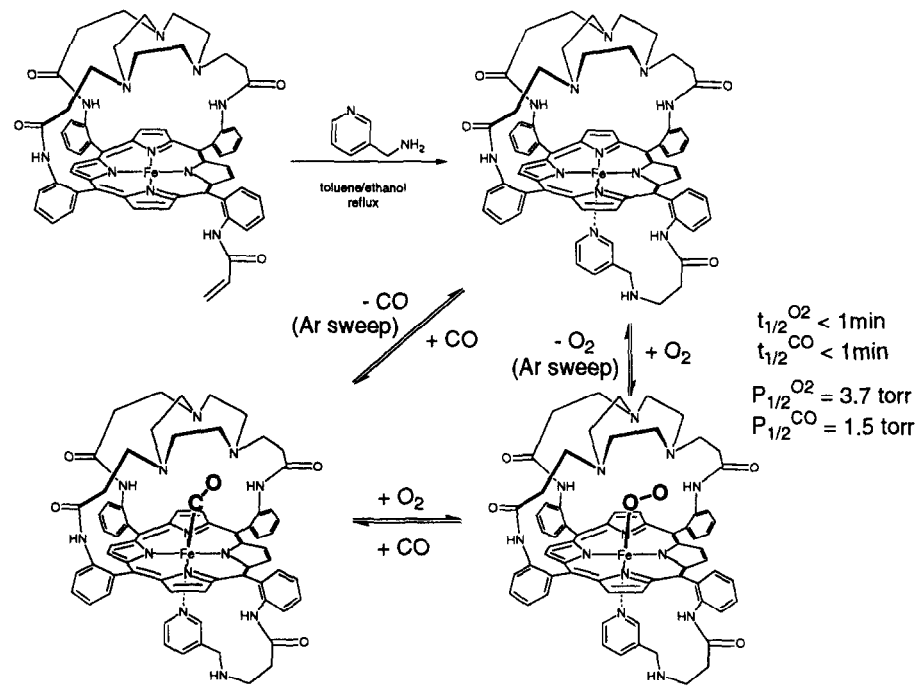


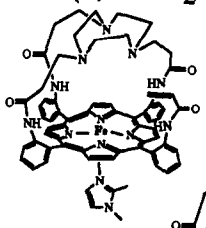
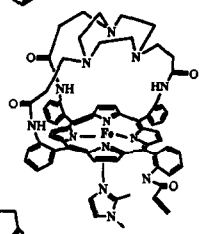
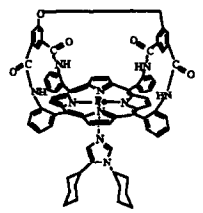
Fig. 7. Synthesis and CO/O_2 binding of a pyridine tailed Fe porphyrin.

center. The intrinsically bent O_2 ligand would not suffer this non-bonded interaction, but the CO ligand would be strongly distorted and is, consequently, destabilized.

We have also discovered some unusual kinetic phenomena by studying the on-rates for O_2 and CO binding. In Fig. 6 the synthesis of an iron(II) triazacyclononane capped porphyrin is shown. Note that this complex has a remaining 'unconsummated' acrylamide substituent which partially blocks the molecular window through which O_2 or CO must pass to bind to the Fe(II) center. The half lives for O_2 and CO binding are very long; these are very slow reactions even though the equilibrium binding constants are 'normal'. The steric origin of this kinetic effect was confirmed by preparing the pyridine tailed complex as shown in Fig. 7. The blocking acrylamide 'picket' has been rotated to the other face of the porphyrin ring and used to deliver a pyridine ligand intramolecularly to Fe(II) — by another Michael addition. The resulting complex binds both CO and O_2 at a rate $> 10^2$ that of the related complex having an acrylamide group blocking access to the inner cavity CO/O_2 binding site.

Table 1 displays both thermodynamic ($p_{1/2}^{O_2}$) and kinetic second-order on-rates for binding O_2 to Hb(R) and a series of model hemes. The last entry is one of the 'picnic basket' porphyrins

Table 1
Dioxygen binding kinetics

Porphyrin	$P_{1/2}O_2$ (torr)	k_{on}/(Msec)
Hb (T)	40 140	10^6 - 10^7
Fe(Picket Fence) (1, 2-Me_2Im)	38	10^8 Sessler
	3.7	10^{-2}
	~ 4	1
	0.28	10^9

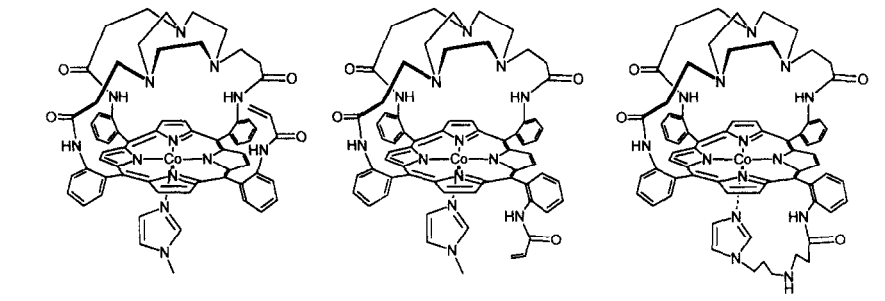


Fig. 8. Impact of steric conformation on dioxygen binding rate constants.

(as an 'R-state' model). The kinetic effect of sterically inhibiting the entrance to the O_2 binding site is self evident.

This kinetic phenomenon is not restricted to iron. Fig. 8 contrasts the second-order O_2 on-rates for a series of three closely related cobalt substituted cavity porphyrins, each having an axial ligand bound to the proximal face. The 10^2 rate difference between the complex on the left (which has a blocked window to the O_2 binding site) and the two on the right is consistent with results described above on structurally related iron porphyrins.

These Co(II) cavity porphyrins were prepared (Fig. 9) by the congruent Michael reaction as described earlier for the Fe(II) complexes. Again, an axial ligand has been used to bind an axial ligand covalently on the proximal face. These complexes bind O_2 reversibly; their $p_{1/2}^{O_2}$ values are compared with cobalt substituted myoglobin, CoMb, and the cobalt picket fence in Table 2.

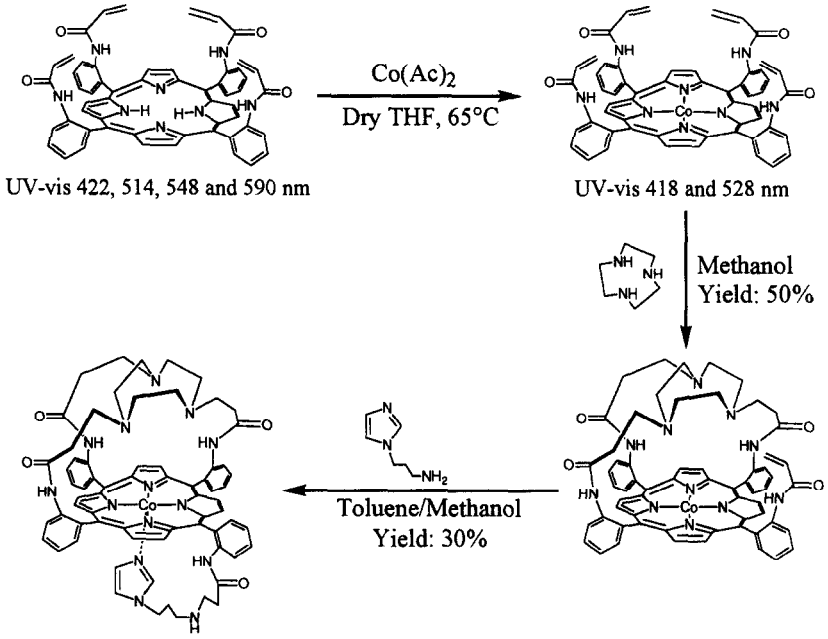


Fig. 9. Synthesis of ECN–Co(II)–Im-tailed porphyrin.

Cytochrome *c* oxidase is at the terminus of oxidative metabolism. As shown schematically in Fig. 10, this terminal oxidase is responsible for reducing O_2 and using the energy of this exergonic reduction to convert ADP and phosphate into ATP for energy storage ('oxidative phosphorylation'). The electrons are stripped from foodstuffs (fats, carbohydrates, and proteins) through a multitude of enzymatic reactions. The electrons are transported to cytochrome *c* oxidase by cytochrome *c*, a coordinatively saturated hemoprotein. Fig. 11 shows a more intimate view of the membrane bound multi-metallic cytochrome oxidase system. There are four redox-active metal centers: Cu_A (a mixed valence Cu(II)/Cu(I) dimer), heme-a (a coordinatively saturated heme having two histidine derived imidazole ligands), and a 'reaction center' which has a Cu(I) and heme-a_3 (both are coordinatively unsaturated). Both mammalian (beef heart) and bacterial cytochrome oxidase systems have recently been structurally characterized; confirming the structural nature of the oxygen binding/activating site [3,4].

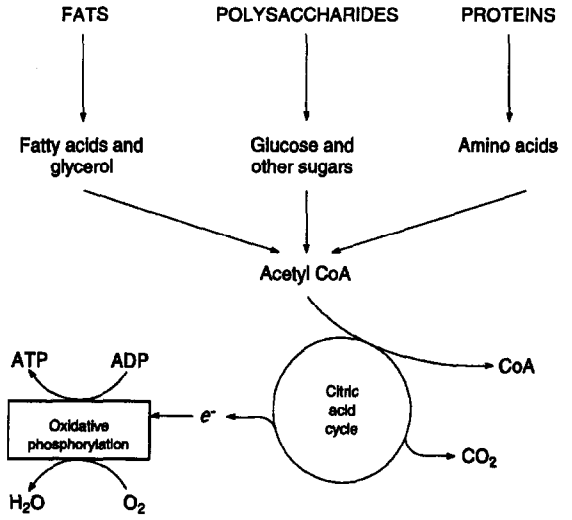
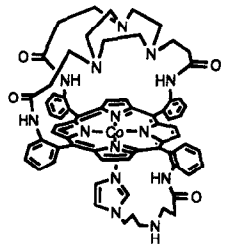


Fig. 10. Role of cytochrome *c* oxidase in oxidative phosphorylation.

A plausible mechanism by which the oxygen binding site in cytochrome oxidase binds and reduces dioxygen to water is illustrated in Fig.

Table 2
Cobalt $p^{O_2}_{1/2}$ values

Systems	$P_{1/2}{}^{O2}$, torr	Conditions
CoMb	57	25°C, pH 7.4
Co(TpivPP)/N-MeIm	140	25°C, Toluene
	35	25°C, Toluene

$p^{O_2}_{1/2}$: dioxygen partial pressure at half oxygenated state.

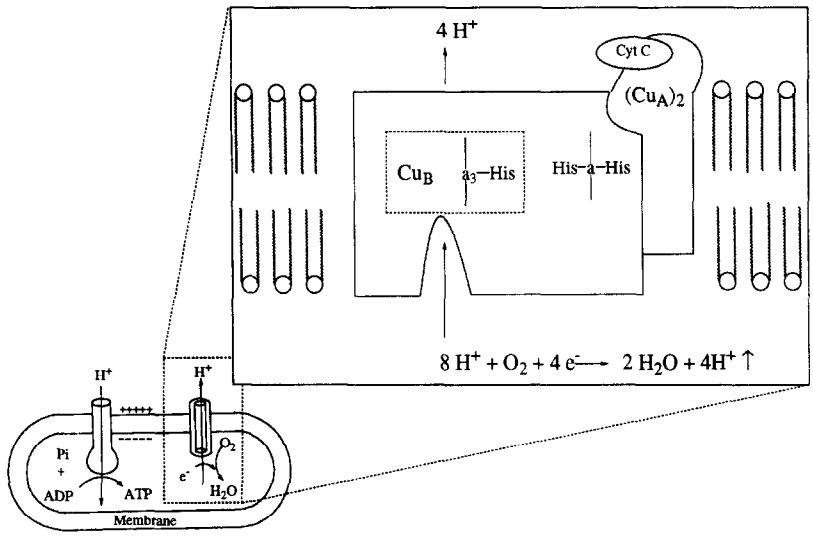


Fig. 11. Schematic view of cytochrome *c* oxidase in the mitochondrial inner membrane.

12. It is proposed that O_2 binds through the fully reduced, Cu(I)/Fe(II) center, first forming a Mb-like O_2 adduct and subsequently forming a bridging $Fe(III)O_2^{2-}Cu(II)$ complex. Subsequently, addition of $1e^-$ and $2H^+$ yields a $Fe^{IV}=O$ ferryl complex, which is further reduced to the resting Fe(III)/Cu(II) stage of the enzyme. These states have been observed by time resolved resonance Raman spectroscopy using $^{16}O/^{18}O$ isotopes. There is disagreement

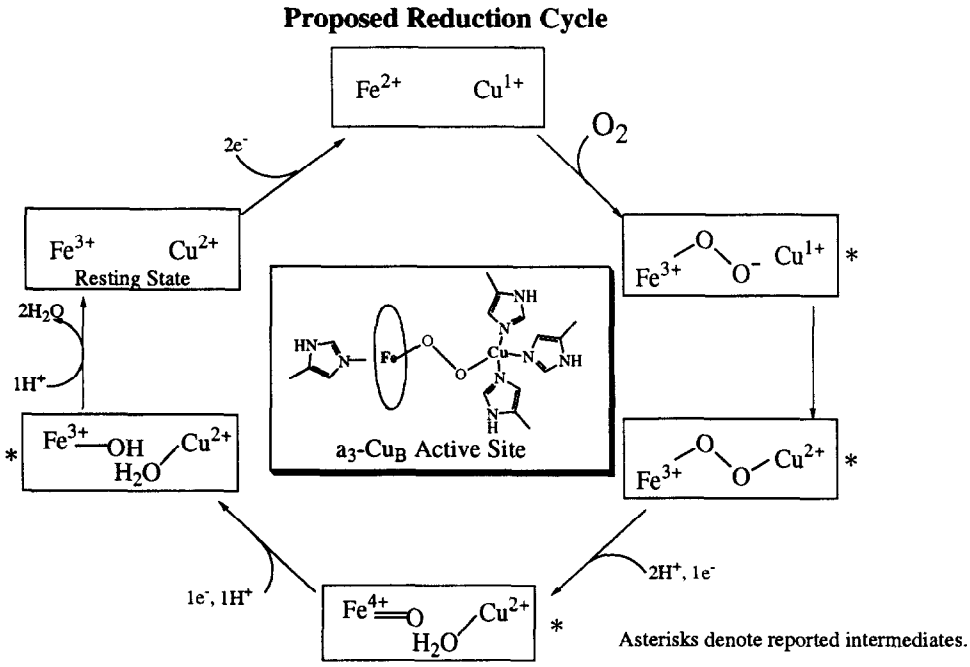


Fig. 12. Four-electron reduction of O_2 by cytochrome *c* oxidase proposed bridged μ-peroxo intermediate.

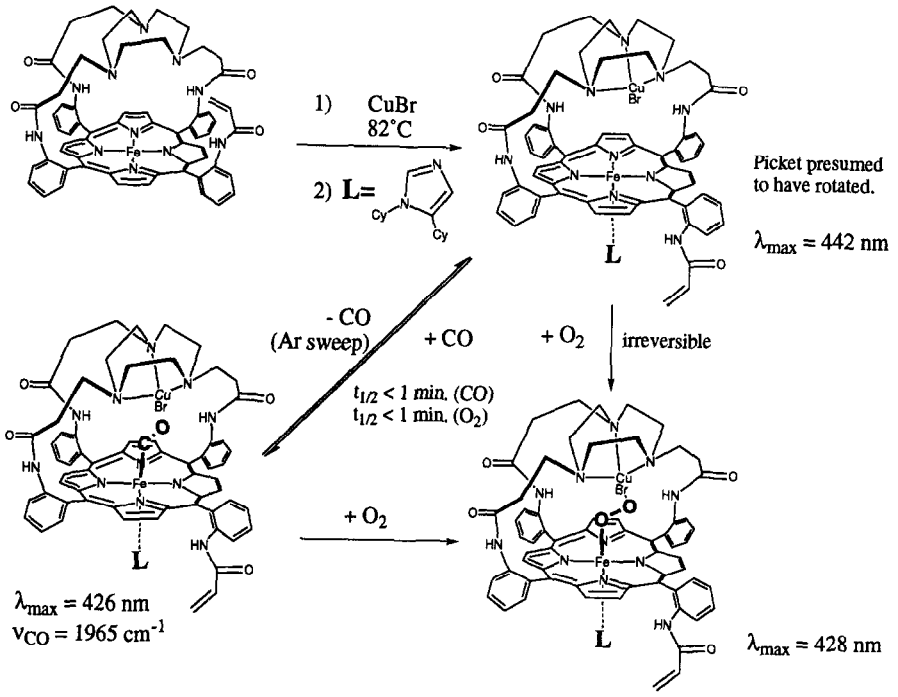


Fig. 13. Synthesis of a model for the O_2 binding site in cytochrome c oxidase.

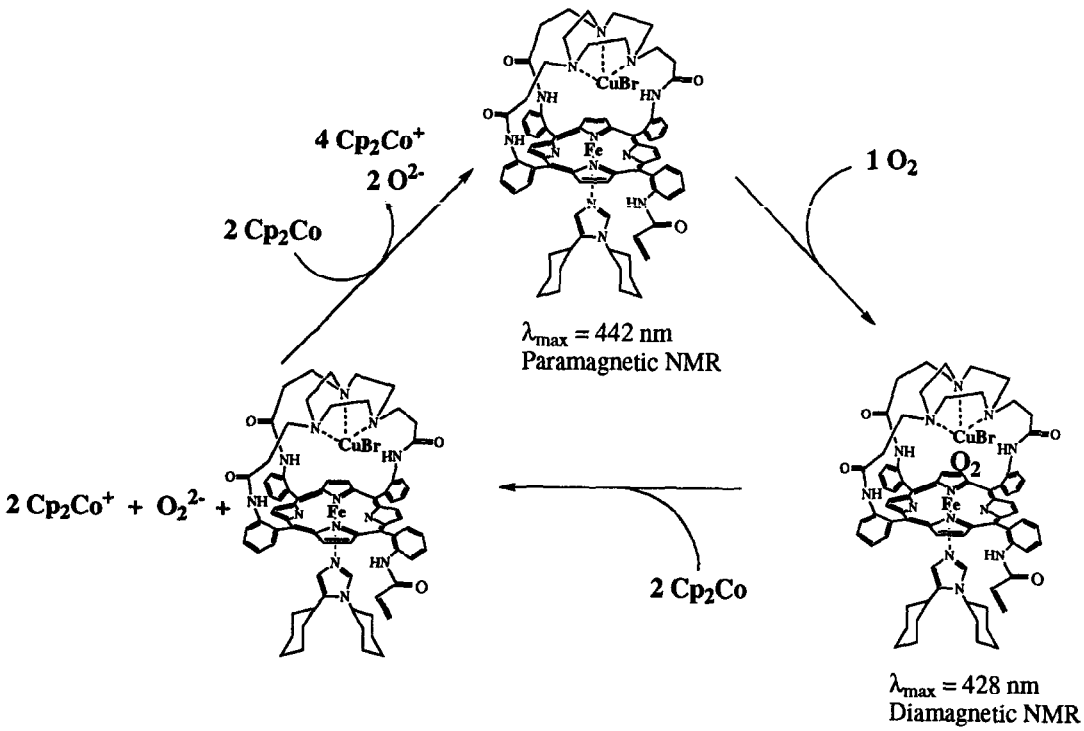


Fig. 14. Redox titration of the bridging peroxide adduct.

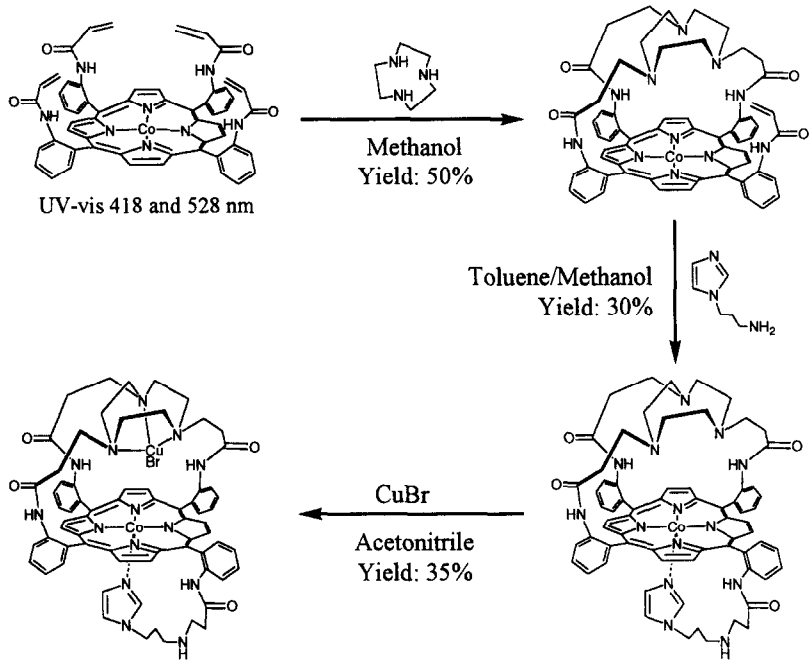


Fig. 15. Synthesis of Cu(I)–Co(II)–Im-tailed porphyrin.

as to whether the bridging peroxide stage has been observed spectroscopically or whether it reacts rapidly, forming the ferryl stage. Proton pumping is thought to take place during the last two intramolecular electron transfer states.

As illustrated in Fig. 13 we have made a synthetic analogue of this O_2 binding activating center [5]. Again the multiple Michael addition was used; in this case to fashion a Cu(I) complex over the distal side of an iron porphyrin. The proximal side is ligated with 1,5-dicyclohexylimidazole which is used in excess and is incapable of penetrating the inner cavity so that the Fe(II) porphyrin remains 5-coordinate. As shown in Fig. 13, this system binds CO reversibly; however O_2 binds irreversibly. The resulting 1:1 O_2 adduct is diamagnetic as judged by ^{1}H NMR, and preliminary Mössbauer and magnetic measurements. Apparently there is strong antiferromagnetic exchange between the low spin Fe(III) and the Cu(II) centers via a peroxide bridge. The character of the peroxide ligand is further bolstered by a resonance Raman O–O frequency present at 758 cm^{-1} and, as illustrated in Fig. 14, a $4e^-$ redox titration with the $1e^-$ reducing agent cobaltocene Cp_2Co.

A closely related Co(II)/Cu(I) complex has been prepared using the scheme illustrated in Fig. 15. This model is fitted with a covalently attached axial imidazole. This complex irreversibly forms a 1:1 complex with O_2, affording an analogous Co(III)/Cu(II) bridging peroxide. The latter has been characterized by titration with 4 equivalents of Cp_2Co. More importantly, when this Co(II)/Cu(I) macrocycle is adsorbed on a graphite electrode in a rotating ring–disc device, it shows clean electrocatalytic $4e^-$ reduction of O_2 in aqueous solution at physiological pH (7.3). The iron derivatives have not yet been studied.

Acknowledgements

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References

[1] G.B. Jameson, in: Metal-Containing Polymeric Materials, C. Carraher, M. Zeldin, J. Sheats, B. Culbertson and C.U. Pittman, Jr. (Eds.) (Plenum, New York, 1996) pp. 421–468.
[2] M. Momenteau and C. Reed, Chem. Rev. 94 (1994) 659.
[3] T. Tsukihara, H. Aoyama, E. Yamashita, T. Tomizaki, H. yamaguchi, K. Shinzawa-Itoh, R. Nakashima, R. Yaono and S. Yoshikawa, Science 269 (1995) 1069.
[4] S. Iwata, C. Ostermier, B. Ludwig and H. Michel, Nature 376 (1995) 660.
[5] J.P. Collman, P.C. Herrmann, B. Boitrel, X. Zhang, T.A. Eberspacher and L. Fu, J. Am. Chem. Soc. 116 (1994) 9783.