Kinetics of Binding of O_2 and CO to 'Double-sided' Porphyrinatoiron(II) Complexes†

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The kinetics of binding of O_2 and CO to double-sided porphyrinatoiron(II) complexes having ester pockets on both sides of the porphyrin plane has been studied. The specific environment created by the four ester groups around the central iron(II) ion of 5,10,15,20-tetrakis(2,6-di-*tert*-butylacetoxyphenyl)-porphyrinatoiron(II) **2** results in binding which is not affected by solvation. The lower binding affinity for CO of 5,10,15,20-tetrakis(2,6-dipivaloyloxyphenyl)porphyrinatoiron(II) **1** compared to that of 5,10,15,20-tetra (*o*-pivalamidophenyl)porphyrinatoiron(II) is attributed to the unfavourable steric repulsions between the axial imidazole ligand and the pivaloyloxy groups, and is reflected in decreased association and increased dissociation rates. On the other hand, axial base ligation to **2** is not inhibited by the *tert*-butylacetoxy groups. Therefore, the lower binding affinity for O_2 exhibited by **2** compared to that of an amide fenced porphyrin complex is ascribed to the loss of local polarity in the cavity. The less-polar ester groups of the double-sided porphyrinatoiron complex result in an increased rate of dissociation of O_2. The activation energy for gaseous ligand association to complex **2** was determined.

In haemoglobin (hb) and myoglobin (mb) the haem group is included in a 'haem pocket' constructed from apoprotein. The active site is completely surrounded by hydrophobic residues of the globin chain which enables the formation of a dioxygen adduct stable to irreversible oxidation through a proton-driven process. Further in the haem pocket, very polar groups within van der Waals distances are present around the co-ordination site and form a suitable polar environment for dioxygen binding. X-Ray structural analyses and neutron diffraction studies of oxy-hb and oxy-mb have provided direct evidence that the bound O_2 ligand forms a hydrogen bond with distal histidine (His E7).[1,2] This electrostatic intermolecular interaction contributes to the stabilization of the dioxygen adduct.

Many models of natural dioxygen carriers so far synthesised have bulky cavities on only one side of a porphyrin plane to prevent μ-oxo dimer formation, but the rear side is unprotected.[3–7] So these single-face hindered porphyrin complexes were irreversibly oxidized rapidly by dioxygen under conditions where the axial base concentration is very low.

Some both-faces hindered porphyrinatoirons (capped-strapped, bis-pocket, hanging base, doubly bridged and jelly-fish porphyrins, *etc.*) have very interesting properties of gaseous ligand binding.[8–12] However, the steric hindrances of such highly modified porphyrins are not sufficient to provide two pockets on both sides of the ring planes. It is important for a mimicry of the unique function of haemoprotein to construct a specific environment around the axial co-ordination sites.

Recently, we reported that highly symmetric 'double-sided' porphyrinatoiron(II) complexes, having two pockets on both sides of the macrocycle, form reversible and stable dioxygen adducts in toluene at 25 °C.[13,14] The four bulky ester groups held rigidly on both sides of the porphyrin ring could prevent the unfavourable intermolecular interaction leading to formation of the μ-oxo dimer.

The most typical model among the protected type of complexes having an appropriate pocket is [Fe(tpvp)] [tpvp = 5,10,15,20-tetra(*o*-pivalamidophenyl)porphyrinate] which forms dioxygen complexes very stable to irreversible oxidation.[4] The presence of weak hydrogen bonding between the bound O_2 and the amide residues in the pocket of haems has been discussed extensively. Jameson and Drago[15] estimated the N···O_2 distance to be ≈4 Å, much longer than those found in oxy-mb and oxy-hb. The possibility of hydrogen bonding was denied by IR and ^{17}O NMR spectra of the dioxygen complex of [Fe(tpvp)].[5,16] However, previous investigations of model compounds were consistent with the view that the amide groups increased the local polarity of the pocket and contributed to stabilization of dioxygen binding.[4–6,17]

We now report the kinetics of binding of O_2 and CO to double-sided porphyrinatoiron complexes having two 'ester' pockets without 'amide' groups on both sides of the porphyrin plane. By studying the dynamics of gaseous ligand binding we can discuss the influence of the ester cavity.

Experimental

Materials.—All solvents used were purchased as reagent grade. Toluene was distilled under an argon atmosphere from sodium and benzophenone prior to use. Chlorobenzene and *o*-dichlorobenzene were dried over 4A molecular sieves and vacuum distilled under argon. Dimethylformamide (dmf) was stored over 4A molecular sieves for several days and vacuum distilled under argon before use.

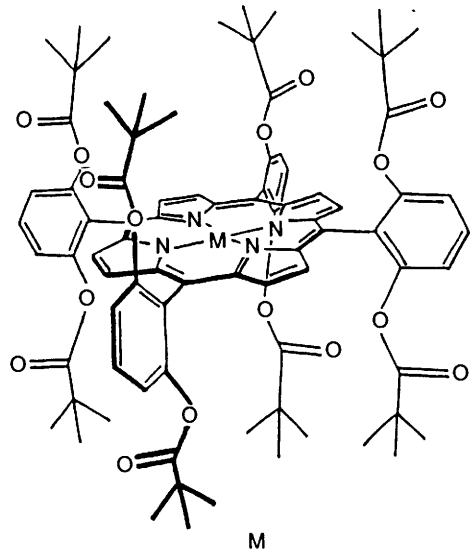
5,10,15,20-Tetrakis(2,6-dipivaloyloxyphenyl)porphyrinatoiron(III) bromide **1′** and 5,10,15,20-tetrakis(2,6-di-*tert*-butylacetoxyphenyl)porphyrinatoiron(III) bromide **2′** were prepared as described previously.[13,14] Reduction to the iron(II) complex was carried out by using $Na_2S_2O_4$ in a heterogeneous two-phase system under anaerobic conditions as previously reported.[13]

Equilibrium Measurements.—Dioxygenation and carbonylation of haems can be expressed by equation (1) where

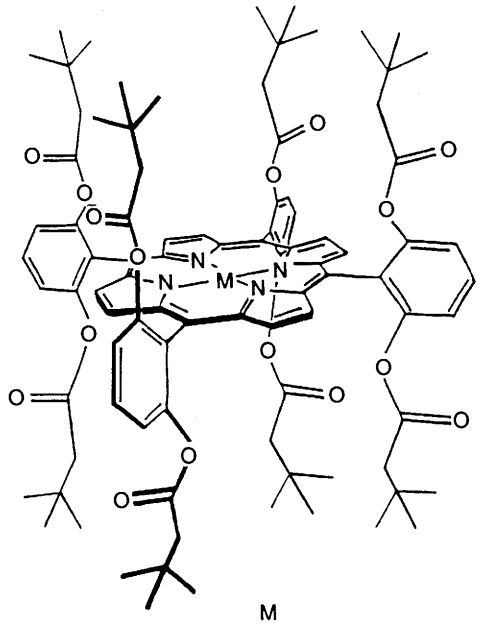
$$\text{Fe(por)B} + \text{L} \overset{K(\text{L})}{\rightleftharpoons} \text{Fe(por)(B)L} \qquad (1)$$

B = axial imidazole base, por = porphyrinate and L = gaseous ligand (O_2 or CO). The affinities for O_2 and CO were determined as described previously.[13,14] Absorption spectra were recorded with a Shimadzu UV-2100 spectrophotometer.

† *Non-SI unit employed:* mmHg ≈ 133 Pa.



M
1′ $Fe^{III}Br$
1 Fe^{II}



M
2′ $Fe^{III}Br$
2 Fe^{II}

Kinetic Measurements.—Kinetic measurements were performed by using laser flash-photolysis techniques. The experiments and data analysis were carried out with a Unisoku USP-500. Rhodamine 590 in absolute methanol was used as the dye and the pulse width was approximately 100 ns. A haem concentration of 1×10^{-5} mol dm^{-3} was used and most experiments were carried out at 25 ± 0.2 °C. In general, porphyrinatoiron(II) can bind one or two axial base ligands following laser flash photolysis of the carbonyl complex in the presence or excess of CO and 1-methylimidazole (mim).[18] The five-co-ordinate adduct of haem is briefly obtained by using a sterically hindered imidazole, 1,2-dimethylimidazole (dmim), incapable of forming a six-co-ordinate complex in reasonable concentration.[4,18] On the contrary, when the dmim concentration is very low, photodissociation of a gaseous ligand may lead to base elimination. This generally results in an increasing apparent rate of rebinding, in particular for CO. When dmim is present in a large excess, no species other than the five-co-ordinate complex FeB formed following flash photolysis and a recombination occurs with k_{obs} given by equation (3). The

$$\mathrm{Fe(por)(B)L} \xrightarrow{h\nu} \mathrm{Fe(por)B} \underset{k_{off}(L)}{\overset{k_{on}(L)}{\rightleftharpoons}} \mathrm{Fe(por)(B)L} \quad (2)$$

$$k_{obs} = k_{on}(L)[L] + k_{off}(L) \quad (3)$$

gaseous ligand was always in large excess over the haem so that the pseudo-first-order approximation could be applied throughout.

Arrhenius activation parameters for ligand association were determined by measuring ligand rebinding rates at 10–35 °C.

Results and Discussion

Effect of Steric Repulsion at the Rear Side on O_2 *and* CO *Binding.*—In order to study gaseous ligand-binding equilibria, conditions must be chosen to ensure dominance of the five-co-ordinate complex in solution. Therefore, spectrophotomeric O_2 and CO titrations of double-sided porphyrinatoiron complexes were carried out under conditions of excess of sterically hindered imidazole ([dmim] = 0.3 mol dm^{-3} for complex **2**). The value of $P_{50}(O_2)$ was determined from the spectral changes at various partial pressures of dioxygen using an equation employed by Collman *et al.*,[19] because binding of O_2 to double-sided porphyrinatoiron complexes at 25 °C was incomplete even at 760 mmHg O_2. Repeated experiments yielded $P_{50}(O_2)$ consistent to within ± 10%. On the other hand, the CO binding affinities of these complexes are large enough to apply the Hill plot. Values of P_{50} for dioxygenation or carbonylation are shown in Table 1.

The O_2 binding affinity of the **1**–dmim complex was significantly lower than that of the [Fe(tpvp)]–dmim.[13] Our previous work on **1** showed that the unfavourable steric repulsion between the bulky pivaloyloxy groups and an imidazole ligand must play a major role in the reduced binding affinity for O_2. We further clarified this by use of two newly synthesized models in which the steric bulk at the rear side of the porphyrin plane was reduced.[14] That is, the $P_{50}(O_2)$ values of double-sided porphyrinatoiron complexes could be controlled by the pocket structure for axial base binding. As described earlier, the equilibrium constant for the binding of dmim to **2** having flexible *tert*-butylacetoxy groups was 1.3×10^3 dm^3 mol^{-1}, which is nearly the same as those for the single-face hindered porphyrinatoirons and flat open 5,10,15,20-tetraphenylporphyrinate (tpp). However, the O_2 binding affinity of the **2**–dmim complex was still lower than that of [Fe(tpvp)]. Therefore, the reduced affinity for O_2 cannot be entirely attributed to steric hindrance of the rear pocket. It may be concluded that the change in local polarity around the binding site due to replacement of amide by ester groups depresses the O_2 binding affinity.

The $P_{50}(CO)$ value of the **1**–dmim complex was larger than that of **2**–dmim and [Fe(tpvp)]–dmim complexes. This is mainly the result of the weak axial base ligation, in the same manner as for the binding of O_2. The values of $P_{50}(CO)$ for the **2**–dmim complex having ester pockets constructed by *tert*-butylacetoxy groups is 0.017 mmHg, which indicates that the steric repulsion at the rear side of the porphyrin plane is appreciably relieved.

Solvation Effects.—In the case of 'flat' haems (tpp or chelated haem) the solvation effect is the dominant factor responsible for the lower gaseous ligand affinities compared to those of the 'protected' type, [Fe(tpvp)], *etc.*[4] It is generally accepted that increased solvent polarity enhances binding of O_2 due to stabilization of the expected charge separation upon Fe–O_2 bonding in unprotected porphyrin complexes.[3,4,9,20,21] In contrast, the situation for binding of CO is confusing.[4,6,9,20,21] Perhaps change in polarity has little effect on $P_{50}(CO)$ since the

Table 1 Solvent effect on equilibria for binding of O_2 and CO to iron(II) porphyrin complexes at 25 °C[a]

Complex	Solvent	ε	$P_{50}(O_2)$/mmHg	$10^2P_{50}(CO)$/mmHg	Ref.
1–dmim	Toluene	2.38	870	16	12, This work
2–dmim	Toluene	2.38	230	1.7	13, This work
	Chlorobenzene	5.67	250	1.3	This work
	o-Dichlorobenzene	9.93	250	1.7	This work
	dmf	36.71	240	2.4	This work
[FeL^1]–dmim[b]	Toluene	2.38	508	0.91	8
	Chlorobenzene	5.67	299	1.2	8
	o-Dichlorobenzene	9.93	277	1.6	8
[FeL^2]–mim[c]	Toluene	2.38	4.5[d]	0.08	3
	CH_2Cl_2	8.9	6.5[d]	—	3
	dmf	36.71	3.3[d]	0.03	3

[a] Estimated errors < 10%. [b] L^1 = Bis-pocket porphyrinate. [c] L^2 = Capped porphyrinate. [d] At 0 °C.

Table 2 Parameters for binding of O_2 and CO to iron(II) porphyrin complexes in toluene at 25 °C*

Complex	$k_{on}(O_2)$/dm^3 mol^{-1} s^{-1}	$k_{off}(O_2)$/s^{-1}	$P_{50}(O_2)$/mmHg	$k_{on}(CO)$/dm^3 mol^{-1} s^{-1}	$k_{off}(CO)$/s^{-1}	$P_{50}(CO)$/mmHg	Ref.
1–dmim	—	—	870	3.0×10^5	0.49	0.16	This work
2–dmim	4.5×10^7	7.9×10^4	230	9.0×10^5	0.15	0.017	This work
[Fe(tpvp)]–dmim	1.2×10^8	3.4×10^4	37	1.6×10^6	0.15	0.009	This work
	1.1×10^8	4.6×10^4	38	1.4×10^6	0.14	0.009	4
tpp–dmim				1.6×10^5	0.24	0.15	4

* Estimated errors < 10%.

resulting complexes demonstrate little charge separation upon Fe–CO bonding.

The affinity for O_2 of a bis-pocket porphyrinatoiron having non-polar and wide pockets on both sides of the porphyrin plane increased with solvent polarity due to stabilization of the charge separation for bound dioxygen.[9] The result indicated that its cavity could not impede the insertion of solvent molecules.

The $P_{50}(O_2)$ and $P_{50}(CO)$ values of the complex **2**–dmim in several solvents are shown in Table 1. The binding affinities for O_2 and CO of the double-sided porphyrinatoiron complexes were independent of the relative permittivities of the solvents. This indicates that the cavities of complex **2** inhibit access of solvent molecules to the ligand binding site, hence weakening the solvent effect on the gaseous ligand affinities. Therefore, the presence of four ester groups on both sides of the macrocycle can result in a specific environment around a central iron ion such that binding is not affected by solvation. Therefore, we suggest that the systematically lower binding affinity for O_2 of complex **2** be mainly attributed to the less-polar ester pockets in comparison to amide pockets.

Kinetics of O_2 *and* CO *Binding.*—In order to clarify the lowered ligand binding affinities of double-sided porphyrins, the dynamics of gaseous ligand binding was explored by laser flash photolysis.

When a toluene solution of the complex **2**–dmim–CO ([haem] = 1×10^{-5}, [dmim] = 0.3 mol dm^{-3}) was photolysed linear decay plots of log ΔA *vs.* t were obtained. This indicates clean monophasic rebinding. The value of $k_{on}(CO)$ was obtained using equation (3). The dissociation rate constant $k_{off}(CO)$ was calculated from $k_{on}(CO)/K(CO)$. Under the same conditions, flash photolyses of the stable dioxygen complex **2**–dmim–O_2 were carried out over a range of dioxygen concentration. Values of $k_{on}(O_2)$ and $k_{off}(O_2)$ were obtained using equation (3). The $K(O_2)$ derived from $k_{on}(O_2)$ and $k_{off}(O_2)$ matches that obtained directly under equilibrium conditions. The binding affinity for O_2 of the **1**–dmim complex was too low to determine the rate parameters for dioxygenation at 25 °C. Table 2 contains P_{50} and kinetic parameters for the gaseous ligation of double-sided porphyrinatoiron complexes.

Several earlier works have clarified that the gaseous ligand affinities of deoxy haems utilizing dmim are about two orders of magnitude lower than those of complexes derived from mim.[4,6,18] The kinetic data for these T-state models were reflected in decreased ligand association and increased dissociation rates for binding of CO and in increasing dissociation rate for binding of O_2.

(*a*) CO. Our kinetic data for the complex **1**–dmim–CO demonstrated that the lower CO affinity predominantly arose from decreased $k_{on}(CO)$ and increased $k_{off}(CO)$ in comparison to [Fe(tpvp)]. As described above, the contribution from the solvation effect can be ruled out. So we can conclude that the low affinity for CO of complex **1** is attributed to the steric repulsion between the axial base and the rear pivaloyloxy fences.

In contrast, the CO binding parameters of the **2**–dmim complex were similar to those of [Fe(tpvp)]. This result indicated that the influence of the small steric repulsion at the rear side of the complex **2** is negligible.

(*b*) O_2. The lower binding affinity for O_2 of the **2**–dmim complex compared to [Fe(tpvp)] was attributed to the slightly decreased association rate and the increased dissociation rate.

In the case of the pocket porphyrins and anthracene haem cyclophane, the rate constants for dioxygen association were decreased only by distal steric hindrance of the protecting pocket.[4,6] The shape of the cavity of complex **2** could be different owing to the presence of the more flexible *tert*-butylacetoxy rather than pivalamide groups. This may result in slightly different association rate constants for dioxygen binding between **2** and [Fe(tpvp)]. If the slightly low $k_{on}(O_2)$ of complex **2** were caused by a steric effect of the *tert*-butylacetoxy groups, $k_{off}(O_2)$ should be negligibly decreased or unchanged.[4,6] However, the rate of dissociation of O_2 from complex **2** was increased. That is, changing the attached fences from amide to ester caused a decrease in the local polarity of the cavity and destabilized the dioxygen species. This agrees with results for ether 'hanging-base' porphyrins. Momenteau and co-workers[17] considered that the large difference in binding affinities for O_2 of ether and amide 'hanging-base' porphyrins resulted from a difference in the dissociation rate of O_2. They indicated that the presence of NH groups strongly increased the intrinsic stability of the dioxygen derivatives.

The activation energy for gaseous ligand association was determined from Arrhenius plots (Table 3). The thermodynamic values for complex **2** are similar to those for [Fe(tpvp)].

In this paper, we have described the kinetic properties of

Table 3 Arrhenius parameters for the association of O_2 and CO to iron(II) porphyrin complexes in toluene

Complex	Ligand	E_a/kJ mol^{-1}	log A	ΔG_{eq}/kJ mol^{-1}	$\Delta G^{\ddagger}$/kJ mol^{-1}	Ref.
2–dmim	O_2	20	11	16	30	This work
	CO	33	12	44	39	This work
[Fe(tpvp)]–dmim*	O_2	13	10	20	27	4

* Values calculated from $\Delta H^{\ddagger}$ and $k_{on}(O_2)$ in ref. 4.

double-sided porphyrinatoiron complexes which have ester cavities on both sides of the ring plane as a haem pocket. Four *tert*-butylacetoxy groups formed 'less-polar' pockets around the binding sites. The ester pocket of the complex **2** reduced the binding affinity for O_2 reflected by the increased dissociation rate compared to that of [Fe(tpvp)].

Although the co-ordination structure of double-sided porphyrin complexes has not been examined, their electronic nature and axial bonding characters have been investigated by using physicochemical measurements (ESR, IR and Mössbauer spectroscopy, *etc.*) as detailed in the following paper.

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