Kinetic Parameters and Linear Free Energy Relationships of O_2 and CO Binding with Closely Related Heme Models of Hemoglobins and Cytochromes P-450

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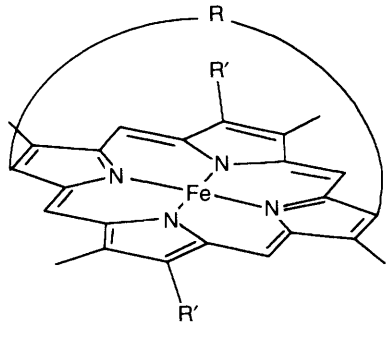
The binding of O_2 and CO with various derivatives of two recently synthesized encumbered porphyrins (**1** and **2**) has been investigated using laser flash photolysis. These compounds bear an aliphatic chain directly anchored on the pyrrole positions of the macrocycle. With either a nitrogenous base or a thiolate group as a fifth axial ligand of the Fe^{II} atom, they model the active site of hemoglobins or cytochromes P-450 respectively and are able to bind O_2 and CO as a sixth ligand. Compared to analogous complexes of slightly encumbered porphyrins, we observed a reduction of O_2 and CO affinities by several orders of magnitude, mainly reflected in decreased association rates. These effects are attributed to the strong central steric hindrance due to the presence of the aliphatic chain. The results also provide evidence for steric discrimination against CO binding. Linear free energy relationships show that all derivatives of **2**, including the thiolate-ligated complex in spite of its very different kinetic and equilibrium parameters, behave as only one homogenous family and reasonably model the reactivity of mammalian hemoproteins for both CO and O_2 binding. On the contrary, compound **1** belongs to the class of severely encumbered porphyrins.

Flash photolysis kinetic studies of liganded Fe^{II} porphyrin models of oxygen-carrying hemoproteins have emphasized the role of distal steric hindrance in discriminating against CO binding.[1-5] The sensitivity towards steric hindrance is different for CO, which preferentially binds to the Fe^{II} atom in a linear fashion,[6] and for O_2, which adopts a bent geometry.[7] In hemoproteins, the consequence of distal steric interactions is an off-axis distortion of the Fe–C–O linkage[8] and a decreased affinity for carbon monoxide. Linear free energy relationships (LFERs) provide a powerful means for comparing the reactivity of heme model compounds to that of hemoproteins.[9] Thus 'pocket'[2] and 'hybrid'[3] TPP (iron^{II}-5,10,15,20-tetraphenylporphyrin) derivatives have been found to reasonably mimic the reactivity of hemoproteins with CO or O_2.[2,3,9] However, crystallographic data indicate that the porphyrin macrocycle is significantly ruffled in these models and that the Fe–CO bond is only slightly distorted, contrary to expectation.[10] It seems therefore that the mechanism by which steric constraints are released is quite different in hemoproteins and in TPP derivatives.

The porphyrin macrocycle of heme model compounds offers two possibilities for anchoring encumbering groups: the *meso*-positions as in the 'picket-fence' and 'hybrid' models considered above or the pyrrole rings, as in the 'strapped', 'cofacial'[1] or 'cyclophane'[11] porphyrins. Since the later positions are probably stiffer than the *meso* positions, it was expected that the energetic balance would favour a distortion of the Fe^{II}–ligand bond rather than macrocycle ruffling. Resonance Raman data[12] indeed suggest that CO is displaced off-axis in 'strapped' porphyrins, whereas the heme geometry remains unaffected; several arguments also indicate that the macrocycle must remain nearly planar in 'cyclophane' models.[11] However, the LFERs obeyed by these compounds are characteristic of a more severe steric encumbrance than in hemoproteins and are consistent with a mechanism of binding involving a substantial conformational change of the strap retained in the liganded complex.[9]

In an attempt to mimic both structural and energetic aspects of hemoproteins reactivity, compounds **1** and **2** have been designed to modulate the amount of central steric hindrance by means of a chain of variable length anchored on the pyrrole positions. Since the chain is purely aliphatic, all other factors which may greatly affect ligand binding such as cage polarity[11] are kept constant. Compounds **1** and **2** differ from the related 'strapped' porphyrins SP-13–SP-15[1] by the chain length, nature and rigidity. In the present work, we have investigated CO and O_2 binding with three nitrogenous base–Fe^{II} complexes of **1** and **2**. We show that compound **1** cannot be distinguished from the 'cofacial', 'strapped' and 'cyclophane' porphyrins and belongs to the class of strongly encumbered compounds. On the contrary, all derivatives of **2** behave as only one homogenous family and follow LFERs which correctly model those of hemoproteins for both CO and O_2 binding.

Since very little is known about the reactivity of model compounds of cytochromes P-450,[13,14] we also investigated ligand binding to thiolate complexes of **1** and **2**. The carbonylated thiolate derivative of **2** follows the same LFER as model compounds of hemoglobins, though rate and affinity constants are greatly modified. The present results support the



1 R = $-[CH_2]_{12}-$
R′ = $-CH_2-CH_2-CO_2-CH_3$
2 R = $-[CH_2]_{14}-$
R′ = $-CH_2-CH_2-CO_2-CH_3$
Strapped porphyrins (ref. 1):
R = $-[CH_2]_2-CO-NH-(CH_2)_n-NH-CO-[CH_2]_2-$
R′ = pentyl
SP-13: $n = 5$, SP-14: $n = 6$, SP-15: $n = 7$

Table 1 Kinetic rate parameters and equilibrium constants for O_2 and CO binding with derivatives of compounds **1** and **2** and other heme models at 20 °C, in toluene

Compound	k_B^{+CO}/dm^3 mol^{-1} s^{-1}	k_B^{-CO}/s^{-1}	K_B^{CO}/dm^3 mol^{-1}	$k_B^{+O_2}$/dm^3 mol^{-1} s^{-1}	$k_B^{-O_2}$/s^{-1}	$K_B^{O_2}$/dm^3 mol^{-1}	Ref.
1, no base	3.8×10^8	1.9×10^4	2.0×10^4	—	—	—	*c*
1, B = 1-MeIm	2.4×10^2	0.08	3.0×10^3	—	—	—	*c*
2, B = 1-MeIm	7.8×10^4	0.11	7.4×10^5	1.9×10^6	1.1×10^3	1.8×10^3	*c*
Ether–BHP(C_9–Im)(C_{12})[a]	3.0×10^7	0.003	1.0×10^{10}	1.8×10^8	1.3×10^3	1.4×10^5	*c*
2, B = Py	2.4×10^5	0.24	1.0×10^6	3.9×10^6	6.2×10^3	6.3×10^2	*c*
Ether–BHP(C_3PyC_3)(C_{12})[a]	6.8×10^7	0.07	9.9×10^8	3.0×10^8	4.0×10^4	7.5×10^3	9
2, B = 1,2-Me_2Im	2.7×10^4	0.55	4.9×10^4	1.0×10^6	1.0×10^4	1.0×10^2	*c*
2, B = BuS⊖[b]	2.6×10^3	21.9	1.2×10^2	—	—	—	*c*
Protoheme BuS⊖[b]	1.2×10^5	25.0	2.0×10^4	—	—	—	13

[a] BHP = 'Basket-handle' porphyrins, ref. 9. [b] 18-Crown-6-ether complex of potassium butanethiolate in toluene. [c] This work.

Table 2 Absorption maxima and molecular extinction coefficients of some derivatives of compounds **1** and **2** in toluene, at 20 °C

Compound	λ_{max}/nm (ε/dm^3 mmol^{-1} cm^{-1})
1-Fe^{III} (Cl^-)	376 (77), 400 (63), 532 (9.4), 633 (5.5)
1-Fe^{II}	389 (60), 411 (60), 530 (8.3), 566 (10.8)
1-Fe^{II} (1-MeIm)	417 (91), 553 (9.3)
1-Fe^{II} (1-MeIm)(CO)	416 (125), 547 (10.6)
1-Fe^{II} (BuS⊖)	399 (76), 545 (12.6)
2-Fe^{III} (Cl^-)	376 (80), 400 (66), 534 (9.6), 633 (5.9)
2-Fe^{II}	388 (73), 411 (75), 529 (11), 565 (14.9)
2-Fe^{II} (1-MeIm)	415 (104), 538 (11.8)
2-Fe^{II} (1-MeIm)(CO)	412 (162), 544 (11)
2-Fe^{II} (1-MeIm)(O_2)	402 (95), 545 (13.5)
2-Fe^{II} (BuS⊖)	397 (77), 545 (13.0)
2-Fe^{II} (BuS⊖)(CO)	372 (110),[a] 445 (100),[a] 541 (18.6)[a]

[a] Extrapolated to 100% complex formation, using the estimated equilibrium constant (see text).

idea that proximal perturbations can modulate the reactivity without appreciably affecting the 'product' (or 'reactant') character of the transition state.

Results and Discussion

Compounds **1** and **2** have been synthesized according to the procedure described in ref. 15. The bimolecular association rate constants k^{+CO}, k_B^{+CO} and $k_B^{+O_2}$ and the first order dissociation rates k_B^{-CO} and $k_B^{-O_2}$ were obtained either directly from the relaxation kinetics following laser photodissociation of the respective five-coordinated CO–Fe^{II}, six-coordinated B–Fe^{II}–CO and six-coordinated B–Fe^{II}–O_2 complexes, or by using the technique of competitive rebinding.[16] Carbon monoxide affinities K^{CO} and K_B^{CO} were obtained by spectrophotometric titration. Values of k^{-CO} and k_B^{-CO} were calculated as the ratio k^+/K. For 1-MeIm–**1**, K_B^{CO} was derived from the kinetic measurements of k_B^{+CO} and k_B^{-CO}. The notation of rate and equilibrium constants follows the definitions of refs. 17 and 18, with B denoting either a nitrogenous base or a thiolate ligand.

Photodissociation of the Five-coordinated CO–Fe^{II} *Complexes.*—Addition of CO to tetra-coordinated TPP derivatives usually leads to a mixture of the five- and six-coordinated adducts CO–Fe^{II} and CO–Fe^{II}–CO.[19] This was found to be also the case with porphyrin **2**. Static absorption spectra and rebinding kinetics following laser photodissociation in presence of pure or diluted CO revealed the presence of more than one CO-complex. On the contrary, spectrophotometric titration of **1** with CO up to saturation gave a series of spectra with well-defined isosbestic points, and the rebinding kinetics were monoexponential. The absence of bis ligation upon CO addition was the first evidence of a severe central steric hindrance in compound **1**. The equilibrium and kinetic rate constants given in Table 1 are in good agreement with those previously reported for deuteroheme dimethyl ester in benzene.[17,18] CO reacts very quickly with the unprotected face of a tetra-coordinated porphyrin. The values of k^{-CO} and k_B^{-CO} given in Table 1 show that the five-coordinated complex CO–Fe^{II} is far less stable than the six-coordinated carboxyhemochrome B–Fe^{II}–CO.

Photodissociation of the Hexa-coordinated (B)–Fe^{II}–CO *and* (B)–Fe^{II}–O_2 *Complexes.—Nitrogenous base–heme complexes.* The absorption spectrum of porphyrins **1** and **2** was modified upon addition of organic nitrogenous bases B = 1-methylimidazole (1-MeIm), 1,2-dimethylimidazole (1,2-Me_2Im) or pyridine (Py) (Table 2). Spectrophotometric titrations with **2** led to affinity constants K_B of the order of 10^4 dm^3 mol^{-1}. Even in the presence of a large excess of base (up to 0.1 mol dm^{-3}), the B–Fe^{II}–B hemochrome was not formed, which indicates that the steric hindrance provided by the chain prevents base ligation within the cage. Thus, binding of nitrogenous ligands preferentially occurs on the unencumbered face while the protected face remains vacant for subsequent binding of gaseous ligands.

Auto-oxidation is often a problem with model compounds, rendering spectrophotometric titrations of the various Fe^{II} complexes inaccurate and in some cases impossible. Partial auto-oxidation occurred to some extent with derivatives of **1** and **2** even in presence of a large excess of base, due to an unavoidable residual amount of oxygen contained in CO or argon. As a consequence, only an order of magnitude could be estimated for K_B. This sensitivity to oxygen was particularly pronounced with compound **1** and practically prevented a direct determination of K_B^{CO}.

However, because Fe^{III} complexes do not photodissociate, kinetic rate parameters obtained in flash photolysis measurements are not affected by auto-oxidation, except for a decrease of the signal intensity due to the slow conversion of Fe^{II} into Fe^{III}-complexes. All investigated six-coordinated complexes of porphyrins **1** and **2** were found to be photolabile. When carbonylated complexes of **1** and **2** at various concentrations of CO and axial base were photolyzed, biphasic recombination kinetics were usually observed. The proportion of the initial fast phase decreased upon increasing the proximal base concentration and vanished at base concentrations as high as 0.5–4 mol dm^{-3} (see Experimental section for more details). The fast rebinding phase is likely to correspond to CO reacting with the four-coordinated species generated by rapid base elimination from the intermediate five-coordinated complex.[18] At high base concentration, clean monophasic kinetics corresponding to CO rebinding with the five-coordinated species were observed, and the values of $k_{obs}/[CO]$ became independent of both base and CO concentrations. For the complexes of **1** with pyridine and 1,2-Me_2Im no regime could be found where these conditions could be satisfied and no values of k_B^{+CO} were obtained.

A good estimate of K_B^{CO} was obtained for 1-MeIm-**1** as the ratio k_B^{+CO}/k_B^{-CO} due to the fact that k_B^{-CO} was large enough compared to k_B^{+CO}[CO] to be determined as the intercept of the linear plot of the equilibrium relaxation rate *versus* the CO concentration.

The lifetime of the oxy-hemochromes of compound **2** was long enough to allow their direct kinetic study by flash photolysis within a few minutes after their preparation. At the same base concentrations as used for the analogous CO adducts, clean exponential kinetics were observed. The values of the association and dissociation rate constants obtained in this way matched within errors those obtained by using the alternative technique of photocompetitive rebinding. In contrast, oxyhemochromes of compound **1** oxidized too rapidly to allow a direct determination of the kinetic rate parameters. Attempts to use the alternative technique of competitive rebinding were also unsuccessful. This technique implies that the carboxyhemochrome must be the dominant species at equilibrium and requires experimental conditions under which oxygen rebinds faster than CO after flash-off. These conditions could not be fulfilled within the available range of O_2 and CO concentrations, probably because of unfavourable relative values of the rate constants of **1**.

The kinetic rate and equilibrium constants listed in Table 1 are consistent with the values previously reported for 'strapped' porphyrins.[1] Values for two 'basket-handle' porphyrins chelated with the same proximal bases (imidazole or pyridine) but bearing a distal chain long enough to be representative of a small 'peripheral' steric hindrance[9] are also given for comparison. The most striking fact is a reduction of the CO affinities by several orders of magnitude in **1** and **2** mainly manifested in the association rate constants. This decrease of K_B^{CO} and k_B^{+CO} may be attributed to the strong central steric hindrance provided by the chain anchored on the pyrrole positions. The effect is more pronounced with **1** where the reduction reaches six and five orders of magnitude for K_B^{CO} and k_B^{+CO} respectively. Actually, 1-MeIm-**1** has the smallest association rate constant k_B^{+CO} reported to date. These observations appear consistent with the fact that the distal chain is shorter in **1** than in any 'strapped' or 'cyclophane' porphyrins. Equilibrium and association rates are decreased for both CO and O_2, but the effect is much more important for CO. Since the distal chain does not contain any polar group which could stabilize the oxygenated complex leading to a partial compensation of steric effects, the present results may be interpreted in terms of a strong steric discrimination against CO.

Thiolate–heme complexes. When the crown ether complex of potassium butanethiolate (BuS⊖) was added to Fe^{II} porphyrins **1** or **2**, the Soret bands shifted to 399 and 397 nm respectively (Table 2) and the α bands to about 545 nm, indicating the formation of a five-coordinated thiolate complex. Thiolate ions do not form bis-adducts with Fe^{II} porphyrins, even when the latter are not protected on their distal side;[13,14] in the present case, binding undoubtedly must occur on the free face. Addition of a large excess of butanethiolate was required in order to avoid auto-oxidation of the porphyrin. Again a direct titration of the thiolate complex was precluded. Upon CO addition, no spectral change was detected with the thiolate complex of porphyrin **1**, whereas a second species developed with **2**; it exhibited a 'hyper' Soret spectrum with two absorption maxima at 445 and 372 nm, together with a band centred at 541 nm. This spectrum is typical of the six-coordinated BuS^-–Fe^{II}–CO complex; the small band observed at 403 nm may be attributed to a contribution of the five-coordinated species, which indicates that even with 1 atm CO, the porphyrin is not entirely hexa-coordinated. The affinity constant K_B^{CO} was estimated to be about 200 ± 100 $dm^3\ mol^{-1}$ by spectrophotometric titration. Although this determination lacked accuracy because of some unavoidable porphyrin auto-oxidation, the order of magnitude is in agreement with the value K_B^{CO} = 100 $dm^3\ mol^{-1}$ obtained from the kinetic measurements. The absence of CO binding to BuS^-–**1** as well as the decrease by about two orders of magnitude of the affinity of BuS^-–**2** compared to protoheme[13] or thiolate chelated hemes[14] (see Table 1) is a further evidence of the strong central steric hindrance due to the distal chain.

CO rebinding following photodissociation of the BuS^-–Fe^{II}–CO complex of **2** was exponential and independent of the monitoring wavelength as well as of the thiolate concentration over at least a four-fold range. The kinetics also remained unchanged when dibenzo-18-crown-6 was used instead of 18-crown-6, showing that there was no specific effect associated with the presence of a high amount of crown ether in the toluene medium. Values of the rate and equilibrium parameters are given in Table 1. Previous studies of heme models of cytochromes P-450[13,14] have shown that the affinity constant K_B^{CO} is greatly reduced when the axial ligand is a thiolate group. This effect has been attributed to the σ-donor character of the charged ligand which destabilizes the CO-complex but at the same time stabilizes the O_2 adduct. Traylor *et al.*[14] argued that hydrogen bonding with amino acid residues could modulate the negative charge borne by the sulfur atom thus providing an alternative efficient mechanism to steric discrimination to modify the ratio $K_B^{CO}/K_B^{O_2}$. The oxygenated complexes of the butanethiolate derivatives of porphyrins **1**–**2** oxidized too rapidly to allow their kinetic study. However, the fact that fast oxidation occurs even in presence of a mixture of CO and O_2 (1 : 1) suggests that K_B^{CO} and $K_B^{O_2}$ must be of a comparable order of magnitude, preventing protection by preferential binding of CO. Steric and electronic factors together are thought to contribute to discriminate against CO binding in BuS^-–**2**–CO. With the more severely hindered porphyrin **1**, the decrease of K_B^{CO} seems to be so important as to prevent the formation of the carbonylated complex even at the highest CO concentrations available in practice.

Linear Free Energy Relationships.—In spite of the restricted number of data available, the parameters for CO and O_2 binding with the various five-coordinated complexes of porphyrin **2** appear strongly correlated as shown in Fig. 1 (*a*). A linear relation between rate ($\log k^{\pm}$) and equilibrium constants ($\log K$) means that the change in free energy at the transition state upon going from one molecule to another is a constant weighted average of the corresponding changes in the free energy of reactants and products.[9] Such relations reveal a strong thermodynamic parentage. Even the thiolate-ligated complex obeys the same correlation as the nitrogenous base chelated compounds in spite of its very different reactivity parameters. These findings support the idea that proximal effects can greatly modify the rate parameters as a result of the perturbation of the d_{z^2} iron orbital without appreciably affecting the character of the transition state. The amount of 'reactant' or 'product' character of the transition state appears to be mainly determined by the nature and strength of the distal steric constraint which interferes with the ligand approach at a particular value of the reaction coordinate. The single representative point obtained for CO binding to porphyrin **1** deviates significantly from the correlation shown in Fig. 1 (*a*), but on the contrary fits well with the corresponding correlation of the severely encumbered 'strapped', 'cofacial' and 'cyclophane' porphyrins [Fig. 1(*b*)].[9] The latter group constitutes a homogenous family with a slope of $\log k^+$ *vs.* $\log K$ close to unity indicating that the transition state is almost purely 'product'-like. As a consequence, structural perturbations in this series are only manifested by changes in the association rate constants. A mechanism involving a conformational change occurring in the transition state to permit CO binding

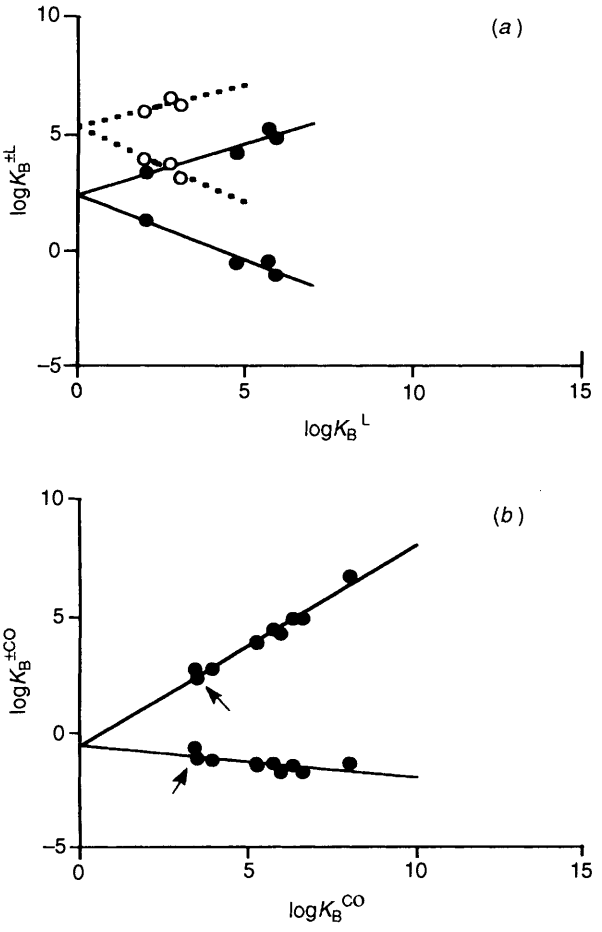


Fig. 1 Linear free energy relationships between rate and equilibrium constants (L = CO or O_2). (*a*) Binding of CO (●) and O_2 (○) with the various nitrogen bases and the thiolate derivatives of compound **2**. Least-square fits: $k_B^{+O_2}$: slope = 0.35, intercept = 5.36; $k_B^{-O_2}$: slope = −0.65, intercept = 5.36; k_B^{+CO}: slope = 0.44, intercept = 2.44; k_B^{-CO}: slope = −0.56, intercept = 2.44. (*b*) CO binding to 1-MeIm-**1**, encumbered 'strapped', 'cofacial' and 'cyclophane' porphyrins of refs. 1 and 11. The arrows indicate the representative points of compound **1**-MeIm. Least-square fits: k_B^{+CO}: slope = 0.86, intercept = −0.56; k_B^{-CO}: slope = −0.14, intercept = −0.54. For comparison, the corresponding data for hemoproteins are: [9] $k_B^{+O_2}$: slope = 0.35, intercept = 5.43; $k_B^{-O_2}$: slope = −0.64, intercept = 5.43; k_B^{+CO}: slope = 0.67, intercept = 0.86; k_B^{-CO}: slope = −0.33, intercept = 0.87. Error bars are smaller than the size of the symbols.

and being retained in the final product has been invoked to account for this type of correlation.[9,11]

Although the 'distal' chain differs by only two CH_2 groups in compounds **1** and **2**, kinetic and equilibrium parameters as well as the LFERs point to a significant change in the amount of central steric hindrance. This view is supported by NMR structural studies of the free bases of compounds **1** and **2**;[15,20] the upfield shift of the resonances of the central methylene protons due to the porphyrin ring current effects is larger in **1** than in **2** (−6.23 *vs.* −5.23 ppm), indicating a significantly shorter distance to the porphyrin plane. The fact that compound **2** obeys a different LFER as the SP-14 'strapped' porphyrin despite the presence of a chain of similar strength also suggests that the aliphatic chain must impose a less severe steric hindrance than do the CO–NH groups of the strap of SP-14.

The LFERs of the derivatives of **2** are intermediate between those of strongly encumbered porphyrins and those of compounds with a small amount of 'peripheral' hindrance; they appear to be close to those of mammalian O_2-carrying hemoproteins; though there are still some differences regarding CO binding, the slope (0.35 for k^+) and intercept (5.36 for k^+) of the correlations for O_2 binding are quite similar to the values previously reported for hemoproteins (slope, 0.35; intercept, 5.43).[9] Preliminary NMR experiments suggest a distorted CO geometry in 1-MeIm–**2**–CO.[21] If this were confirmed, it might be concluded that derivatives of porphyrin **2** simultaneously model both the energetic and structural aspects of ligand binding with O_2-carrying hemo-proteins.

Experimental

Preparation of the Five-coordinated Nitrogen Base–Fe^{II} *and* CO–Fe^{II} *Complexes.*—The synthesis and characterization of porphyrins **1** and **2** were performed as described in ref. 15. The Fe^{II} forms were obtained by reduction of Fe^{III} derivatives using sodium dithionite in wet toluene under anaerobic conditions.[22] The five-coordinated B–Fe^{II} and CO–Fe^{II} complexes were obtained by addition of a deaerated base solution or by bubbling CO through the porphyrin solution respectively. The solubilities of oxygen and carbon monoxide in toluene at 20 °C were taken as 7.2×10^{-3} mol dm^{-3} atm^{-1} and 5.3×10^{-3} mol dm^{-3} atm^{-1} respectively.[23]

Preparation of the Five-coordinated Thiolate–Fe^{II} *Complexes.*—Potassium butane thiolate (BuSK) was prepared by action of potassium hydride on butanethiol.[13] KH (1 g; 50% in oil, Aldrich) was washed twice with carefully deoxygenated dry toluene and about 4 cm^3 of butanethiol (Aldrich) were added under argon. Excess thiol and solvent were removed under reduced pressure (2 h). The resultant white powder was stored in a dry box (2 ppm O_2).

To enhance the reactivity of the thiolate anion and to increase its solubility in toluene, a crown ether complex was prepared, BuS⊂. A small amount of thiolate was added to an equivalent amount of 18-crown-6 (Aldrich) in dry deoxygenated toluene; the mixture was stirred for 3 h before use. The solubility of the thiolate salt in toluene was rather poor, even in the presence of crown ether. Although the exact concentration of a saturated solution is not known, it must be of the order of a few mmol dm^{-3}, since it is higher[24] than that in dibenzo-18-crown-6 where a value of 2 mmol dm^{-3} has been reported.[13]

A deaerated solution of porphyrin **1** or **2** (10^{-4} mol dm^{-3} in toluene) was stirred for a few min with aq. sodium dithionite in borate buffer, pH 8 under argon. The organic phase was separated from the mixture and dried by bubbling argon for 2 h. The five-coordinated thiolate–heme complex was finally prepared by adding an equal volume of a saturated solution of thiolate–crown ether complex. Because of the extreme oxygen-sensitivity of porphyrin and thiolate, all transfers were made under argon.

Kinetic Measurements.—The experimental set-up was as previously described,[25] except for some modifications performed in order to improve the accuracy. The new Q-switched Nd:Yag laser source ('Quantel') had a pulse width of 10 ns, and its energy could be varied between 1 and 450 mJ at 532 nm. The detection system was as before except for digital recording and in-line data processing. Absorbance changes as small as 10^{-3} can be measured with a time constant of 50 ns. The transient absorption changes were recorded on a Lecroy 9450 digital oscilloscope and the data were transferred to an Apple MacIntosh II-CI computer *via* an IEEE-488 interface for conversion of the recorded signals into transient absorbance changes.

The rate constants were obtained from the kinetics of direct rebinding following laser photodissociation of the CO or O_2 complexes of porphyrins **1** and **2** or of their base-derivatives. Alternatively, the photo-competitive rebinding technique was

used to investigate the oxygen rate parameters[16] whenever possible. All kinetic experiments were performed under pseudo-first order conditions. For the study of nitrogen-ligated derivatives, rate constants have been determined by varying the base concentration over *ca.* four orders of magnitude until a range could be found within which the CO and O_2 binding rates remained constant. This procedure has previously been applied[2] to prevent spoiling of the kinetics by various additional processes, such as base elimination or hemochrome formation, which can occur when the base is added as a free ligand in the solution.[18] The following conditions were found to be satisfactory: [1-MeIm] = 0.5–2, [Py] = 1–3.5, [1,2-Me_2Im] = 1–2.5 mol dm^{-3}. Since a large excess of BuS^- was required to ensure penta-coordination of porphyrins **1** and **2** and to avoid auto-oxidation, its range of concentration variation was only of a factor of four, obtained by dilution of the saturated solution. Unless otherwise stated, the estimated errors on the kinetic and equilibrium constants were of the order of ± 25%.

Spectrophotometric Titrations.—Carbon monoxide affinities K^{CO} and K_B^{CO} were obtained by spectrophotometric titration. Determined argon–CO mixtures were bubbled in the reduced solution of porphyrin. The absorption changes were followed in the Soret band. Measurements were performed at different base concentrations chosen in the range in which kinetic experiments have shown neither base elimination nor hemochrome formation. Values of k^{-CO} and k_B^{-CO} were calculated as the ratio k^+/K. For 1-MeIm–**1**, k_B^{CO} was derived from the kinetic measurements of k_B^{+CO} and k_B^{-CO}.

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