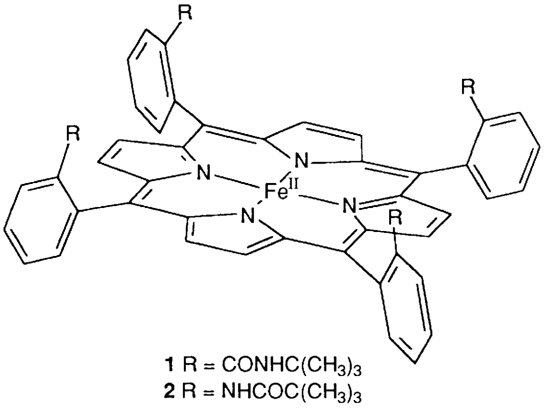
Influence of Polar Amide Groups upon the Kinetics of Ligand Binding with Iron(II) 5,10,15,20-Tetrakis[α,α,α,α-*o*-(*N*-*tert*-butylcarbamoyl)phenyl]porphyrin

C. Tetreau[*,a] **L. Leondiadis,**[b] **D. Lavalette**[a] **and M. Momenteau**[b]
[a] *Institut Curie-Biologie, INSERM, U219, Centre Universitaire, 91405-Orsay, France*
[b] *Institut Curie-Biologie, CNRS URA1387, Centre Universitaire, 91405-Orsay, France*

We have investigated the consequences upon ligand binding of reversing the amide dipoles from PhNHCO in the 'picket-fence' porphyrin **2** to PhCONH in the newly synthesized heme model **1**, by using laser photolysis. **1**-1,2-Me_2Im has been found to be the first example of photodissociation of an iron(II) five-coordinated base–porphyrin complex. Moreover, **1** binds base and gaseous ligands on either porphyrin side, thus displaying little steric discrimination in its five- and six-coordinated states, at variance with the 'picket-fence' porphyrin. The variations of the association and dissociation rate constants both contribute to lower the affinities for binding CO and O_2 on the encumbered side of porphyrin **1** as compared to **2** by two to three orders of magnitude. The main effect is due, however, to an increase of the dissociation rate constants, reflecting the destabilization of the complexes. Linear free energy relationships show that, in spite of these wide differences, both compounds (as well as several 'basket-handle-' and 'picket-fence' derivatives) behave as one reactive family which is thermodynamically homogeneous.

Over the past fifteen years, various model structures have been designed to explore the role of polar, hydrophobic or steric interactions between the ligand and amino acid residues located near the reactive site of hemoproteins.[1,2] Kinetic studies of heme model compounds have been performed to quantify the effects of environment upon ligand binding. Thus, the possibility of favouring oxygen binding by introducing amide groups near the iron centre has been demonstrated with 'picket-fence'[3] and amide 'basket-handle' porphyrins.[4–6] Stabilization of dioxygen as well as of negatively charged species was attributed to polar interactions (hydrogen bonds or dipole–dipole interactions). The polarity of the distal cavity was further increased by inserting a larger number of secondary amide groups in 'amino acid basket-handle' porphyrins.[7] This had no additional stabilizing effects for oxygen, but led to the unusual coordination of water, also stabilized by polar interactions.

It could be anticipated that the structure of the polypeptide backbone near the active site might also modify the strength of polar interactions through changes in the relative orientations of the various polar groups. Compound **1** differs from Collman's 'picket-fence' porphyrin **2** by a reversal of the secondary amide groups of the 'pickets' from PhNHCO to PhCONH. This is expected to affect the polarity in the vicinity of the iron as well as the electronic charge distribution at the core of the porphyrin. A preliminary electrochemical study of redox properties of zinc and copper complexes of the iron-free derivative of compound **1** has revealed a stabilization of positively charged species, while a destabilization was observed with **2**.[8]



1 R = $CONHC(CH_3)_3$
2 R = $NHCOC(CH_3)_3$

In the present study, we have investigated the consequences of reversing the amide dipoles upon the kinetics of binding of O_2, CO and nitrogenous bases to compounds **1** and **2**. The data reveal rather spectacular changes in reactivity; in particular, base and gaseous ligand binding on either face of metalloporphyrin **1** are likely to occur, contrary to the situation encountered with **2**; moreover, we observe with **1** the first case of photolability of an iron(II) five-coordinated nitrogenous base–porphyrin complex.

Results and Discussion

The bimolecular association rate constants k_B^{+CO}, $k_B^{+O_2}$ and k^{+B} and the first-order dissociation rates $k_B^{-O_2}$, k^{-B} (defined according to refs. 9 and 10) were obtained from the kinetics of equilibrium relaxation following laser photodissociation of the respective carboxyhemochromes, oxyhemochromes, and five-coordinated base–porphyrin complexes.

Photodissociation of Six-coordinated Complexes.—O_2 and CO were found to rebind with all base complexes of porphyrin **2** according to mono-exponential kinetics; the derived rate constants (Table 1) are in agreement with those previously reported at the same temperature.[11] On the basis of X-ray crystal structure determinations,[12] these rates have been attributed to O_2 or CO ligation on the encumbered side. In contrast, all re-binding kinetics for **1** showed a distinct deviation from exponential behaviour and could be accurately described only by a sum of two exponentials. The initial difference spectrum for either component of the biphasic kinetics was found to reproduce reasonably well the calculated difference spectrum between the five- and six-coordinated complexes of porphyrin **1**. Moreover, the relative amplitude and rate constant of both exponential phases remained constant when base and ligand concentrations were varied over a *ca.* ten-fold range (Fig. 1). These various observations imply the presence of two distinct six-coordinated complexes leading, after photodissociation, to O_2 and CO re-binding at two different rates.

Since chemical characterization of **1** gives no evidence for the presence of more than one chemical (or conformational) species, we must assume the existence of two isomeric species, due to

Table 1 Kinetic rate parameters and equilibrium constants for CO and O_2 binding to compounds **1** and **2**[a]

Compound	Isomer	k_B^{+CO}/ 10^7 dm^3 mol^{-1} s^{-1}	k_B^{-CO}/s^{-1}	K_B^{CO}/ 10^{-5} dm^3 mol^{-1}	$k_B^{+O_2}$/ 10^8 dm^3 mol^{-1} s^{-1}	$k_B^{-O_2}$/s^{-1}	$K_B^{+O_2}$/ 10^{-3} dm^3 mol^{-1}
1-1,2-Me_2Im	A	0.02 (35%)[b]	1.6	1.1	—	—	—
1-1,2-Me_2Im	B	0.11 (65%)	10.0	1.1	—	—	—
2-1,2-Me_2Im	B	0.16 (100%)	0.13	120	—	—	—
1-1-MeIm	A	0.07 (43%)	—	—	0.16 (75%)	11 000	1.46
1-1-MeIm	B	0.30 (57%)	—	—	0.55 (25%)	35 000	1.57
2-1-MeIm	B	3.90 (100%)	—	—	8.20 (100%)	127	6400
1-DCHIm	A	0.01 (25%)	—	—	—	—	—
1-DCHIm	B	0.17 (75%)	—	—	—	—	—
2-DCHIm	B	3.10 (100%)	—	—	—	—	—

[a] Solvent toluene, temperatures: 25 °C (CO) and 5 °C (O_2). [b] The values in parentheses give the relative amplitude of each 'component' contributing to the kinetics.

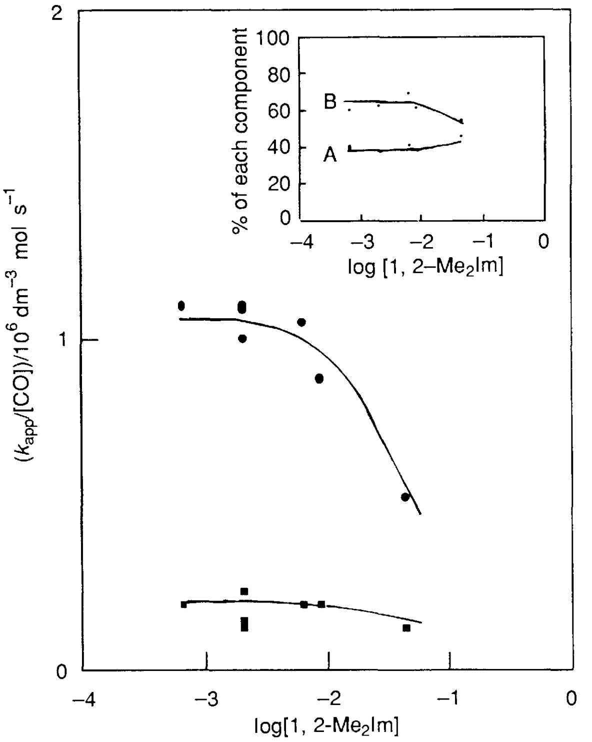
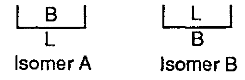


Fig. 1 Apparent second-order rate constants *vs.* base concentration for CO re-binding with isomers A (■) and B (●) of **1**-1,2-Me_2Im in toluene at 25 °C, with [CO] = 7.2 × 10^{-3} mol dm^{-3}. The inset at the top shows that the ratio of both components of the biphasic kinetics remains independent of the base concentration, within errors.



L = CO or O_2, B = base

unsymmetrical ligand binding on both faces of porphyrin **1**, giving two isomers A and B.

The fact that hemochromes of compounds **1** and **2** can be easily formed even with encumbered bases such as 1,5-dicyclohexylimidazole (DCHIm) suggests that, contrary to expectation, the steric interaction between the hindered base and the pickets is not too severe and might be compensated by stabilizing electronic factors. Nitrogenous base binding on the picket side has already been invoked, although not definitely proved, in a series of substituted 'picket-fence' derivatives.[13]

The absorption spectra of the various complexes of **1** showed no anomaly, thereby indicating that isomers A and B cannot be distinguished spectroscopically. Fig. 2 shows that the formation of both carboxyhemochromes of 1,2-dimethylimidazole (1,2-Me_2Im) could even be titrated and that clean isosbestic points were found at 402, 435, 550 and 635 nm. Moreover, the titration obeyed the law expected for equilibrium between two different species.[9,10] This implies that both equilibrium constants associated with ligation on either face of the porphyrin must have close values; indeed, if this were not the case, we would have observed either the sequential formation of carboxyhemochromes A and B at different CO concentrations, or a deviation from the usual law of titration. Because the method cannot discriminate between two close values of K_B^{CO}, the value given in Table 1 should be considered as an estimation of the order of magnitude rather than as an accurate one.

Before attempting a comparison with 'picket-fence' porphyrin **2**, one must assign each rate constant to a particular porphyrin side. Table 1 shows that one of the association rate constants is systematically smaller than the other, by a factor of 5–10. In isomer B, the gaseous ligand is constrained (distal constraints), whereas in isomer A, the base will experience a proximal constraint. Since both effects may contribute to the destabilization of the iron d_{z^2} orbital, thereby directly affecting the kinetic rates, we cannot determine which of these two antagonist factors will predominate. However, we speculated that a comparison between 1-methylimidazole (1-MeIm) and DCHIm would permit such an assignment. Indeed, the latter base is expected to give a stronger steric interaction with the pickets than the former, therefore affecting much more the binding of CO on the opposite side; by contrast, both bases should bind unconstrained with the free porphyrin side, and CO binding on the encumbered face should not be significantly affected by the cyclohexyl substituents. In agreement with this expectation, Table 1 shows that one constant k_B^{+CO} is less reduced than the other compared to their values for 1-MeIm. We therefore attribute the higher rate constants to gaseous ligand binding on the encumbered face of the porphyrin (isomer B) and the smaller ones to ligation on the free side (isomer A). It appears that the constraints exerted upon the proximal base are more effective than steric distal interaction in decreasing the reactivity of O_2 and CO with compound **1**. This result appears reasonable, taking into account the relative encumbrance of the base and gaseous ligands.

Examination of Table 1 reveals that the iron coordination properties are very sensitive to the reversion of the local polarity near the binding site. The CO and O_2 affinities of **1** are, respectively, two and three orders of magnitude lower than those of **2**. The variations of the association and dissociation rate constants both contribute to lower the affinity; however, the most striking effect is observed with the dissociation rate: its increase, by at least two orders of magnitude clearly reflects the destabilization of the complex. All association rates (Table 1) are decreased at least ten-fold, except with 1,2-Me_2Im as a proximal base. In this case, one may reasonably assume that the

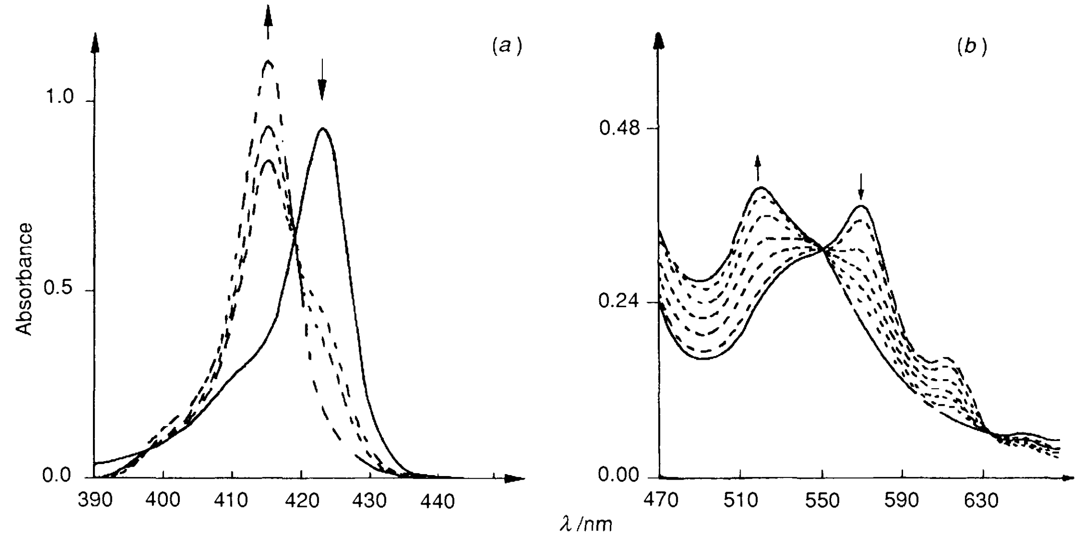


Fig. 2 UV–VIS spectral changes occurring upon titration of **1**-1,2-Me_2Im by CO in toluene. [**1**] = 5 × 10^{-5} mol dm^{-3}, [1,2-Me_2Im] = 2.5 × 10^{-3} mol dm^{-3}.

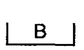
Table 2 Kinetic rate parameters and equilibrium constants for 1,2-Me_2Im binding to compounds **1** and **2**[a]

Compound	k^{+B}/ 10^8 dm^3 mol^{-1} s^{-1}	$k^{-B}/10^3$ s^{-1}	K^B/ 10^4 dm^3 mol^{-1}
1	1.3	25	0.52[b]–2.4[c]
2	3.6	9	4[c]

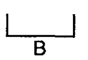
[a] Solvent toluene, temperature 25 °C. [b] Determined kinetically, as k^{+B}/k^{-B}. [c] Obtained by spectrophotometric titration.

rate constant is predominantly governed by the severe steric interaction between the 2-methyl group of the imidazole and the porphyrin plane.

The assumption that the quantum yields of photodissociation do not differ much for isomers A and B appears reasonable, at least for carboxyhemochromes which are known to photodissociate with a yield of approximately unity. Therefore, the concentrations of isomers A and B are expected to be of a similar order of magnitude, considering the relative weight of each component of the biphasic kinetics (Table 1). Since the equilibrium constants for the formation of both six-coordinated species are also roughly equal, we may conclude that the equilibrium constants for the formation of the five-coordinated base complexes are also of a similar order of magnitude. Therefore, compound **1** must already exist as two



Isomer A



Isomer B

different isomers in its five-coordinated base-complex. Further arguments to be developed in the next section also suggest that isomers A and B are formed in comparable proportions, in contrast to the situation encountered with **2**, which gives rise only to isomer B. The reason why base binding with porphyrin **1** does not occur preferentially on the free side as it does with **2** is not presently clearly understood. The possibility that the picket could adopt a conformation leading to a more open cavity cannot be ruled out, although NMR data do not give any evidence in favour of this hypothesis; an alternative explanation might be that base stabilization within the cavity could result from hydrogen bond formation between the proton located at the α-position of the nitrogen of the imidazole bound to the iron and the carbonyl group of a carbamoyl picket; although this second hypothesis must await experimental support, NMR data have established that the carbonyl groups occupy an inward position favourable to hydrogen bond formation.[8]

Photodissociation of the Five-coordinated Complex of **1** *with* 1,2-Me_2Im.—Photodeligation, which appears as a general property of six-coordinated ferrohemes, has never been previously reported for any iron(II) five-coordinated porphyrin–base complex. In the present work, we observed that the adduct of compound **1** with 1,2-Me_2Im could be photodissociated by a laser pulse, whereas no such reaction was observed with **2**. That the base deligation was indeed involved was demonstrated by the following two arguments: (*a*) the rate of re-binding was directly proportional to the base concentration; and (*b*) the difference spectrum recorded at $t = 0$ followed the calculated difference spectrum between the four- and five-coordinated complexes (in particular, no signal was detected at the isosbestic wavelengths of both species).

Since hemochromes are known to be photosensitive,[14] their formation must be prevented in order to avoid spoiling the re-binding kinetics. With such an encumbered base as 1,2-Me_2Im, the concentration can be raised up to several 10^{-3} mol dm^{-3} without observing bis-ligation; in contrast, hemochrome formation, even at the lowest concentrations, has not permitted confirmation as to whether the five-coordinated complexes of 1-MeIm and DCHIm with **1** were also photosensitive or not.

Affinity and rate constants for 1,2-Me_2Im binding to **1** are given in Table 2. The dissociation rate, which was obtained as the intercept of the linear plot of the relaxation rate *vs.* [B], was too small compared to the product k^{+B}[B] to be determined accurately; the estimated error was *ca.* 100%. Even by taking this into account, the difference between the value K^B = 5.2 × 10^3 dm^3 mol^{-1} derived from the kinetics as k^{+B}/k^{-B}, and the value K^B = 2.4 × 10^4 dm^3 mol^{-1} determined independently by spectrophotometric titration appears significant. In fact, the discrepancy is only apparent because the latter value must be attributed to the sum of two equilibrium constants corresponding to base binding on either face of compound **1**, both isomers being titrated simultaneously. The observation of exponential re-binding kinetics argues against the eventuality that both complexes could be simultaneously photodissociated by the laser pulse, but does not provide a definitive argument against it. If the alternative hypothesis holds, then the ratio 5.2 × 10^3:(2.4 × 10^4 − 5.2 × 10^3) = 0.28 would be equal to the concentration ratio of both isomers; its value is consistent with the previous estimation that both must be formed in concentrations of a comparable order of magnitude.

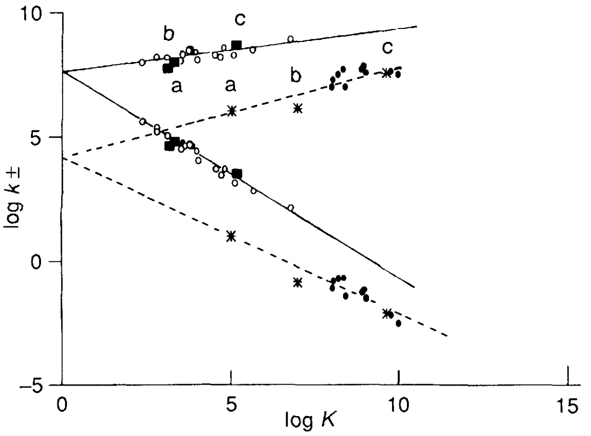


Fig. 3 LFER between rate and equilibrium constants for binding of CO and O_2 with compounds **1**, **2**, 'basket-handle' porphyrins of ref. 5 and 'picket-fence' derivatives of ref. 13. (*a*) **1**-1,2-Me_2Im; (*b*) **2**-1,2-Me_2Im; (*c*) **2**-1-MeIm. Least-square fits: $k_B^{+O_2}$; slope = 0.18, intercept = 7.56, correlation coefficient $r = 0.79$: $k_B^{-O_2}$; slope = −0.82, intercept = 7.56, $r = 0.98$: k_B^{+CO}; slope = 0.37, intercept = 4.12, $r = 0.85$: k_B^{-CO}; slope = −0.63, intercept = 4.12, $r = 0.94$. - - - ●*, CO; — — ○■, O_2.

Since **2**-1,2-Me_2Im gave rise to no photodissociation upon laser excitation, the kinetic parameters for base binding with **2** could not be determined in a similar way as for **1**. An indirect estimation of the dissociation rate k^{-B} was made from the base elimination process which occurred after laser photolysis of the carboxyhemochrome, at low CO concentration. K^B was obtained by spectrophotometric titration, and k^{+B} derived as the product $K^B k^{-B}$ (Table 2). Because isomers **1**-A and **1**-B cannot be distinguished spectroscopically, there is no obvious means to determine which one gives rise to the photodissociation reaction. Therefore, a discussion of kinetic data in Table 2 cannot yet be safely attempted. In any case, the enhancement of photolability observed with **1**-1,2-Me_2Im should still reflect changes in the electronic distribution at the core of the porphyrin leading to a destabilization of the d_{z^2} iron orbital.

Linear Free Energy Relationships.—The usefulness of linear free energy relationships (LFER) for objectively comparing the reactivity of whole series of related molecules has been previously stressed.[5] To attempt such a comparison between compounds **1** and **2**, we have plotted in Fig. 3 log k^+ and log k^- *vs.* log K for CO and O_2 binding on the 'picket side' of each porphyrin. Fig. 3 clearly shows that compounds **1** and **2** obey, within errors, the correlations which have been previously established with ten 'basket-handle' and six 'picket-fence' derivatives. These series of compounds differ either by the amount of distal or proximal constraints or by the changes in the dipolar environment provided by the superstructure. Thus, in spite of the fairly wide range of values covered by the equilibrium constants, all of them, including compound **1**, behave as one reactive family. This observation is entirely consistent with the idea developed previously[5] that the several factors contributing to a destabilization of the d_{z^2} iron orbital are thermodynamically equivalent. The correlations shown in Fig. 3 are representative of the behaviour expected for compounds presenting a small 'peripheral' steric hindrance; therefore, the fact that compound **1** obeys these correlations also suggests that the reversed picket superstructure does not provide a more severe steric hindrance above the Fe–L axis than does compound **2**. Thus, the substantial modulation of iron(II) coordination properties can be attributed almost exclusively to changes in dipolar interactions and charge distribution resulting from the reversal of the amide groups of the pickets. Further work is in progress to compare the redox properties of compounds **1** and **2**.

Experimental

The synthesis and characterization of porphyrins **1**[8] and **2**[12] have been described previously. The Fe^{II} forms used in this work were obtained by reduction of iron(III) derivatives using sodium dithionite in wet toluene under anaerobic conditions.[15] The five-coordinated complexes were obtained by addition of a deaerated solution of base. The solubilities of oxygen and carbon monoxide in toluene at 25 °C were taken as 7.2×10^{-3} and 5.3×10^{-3} mol dm^{-3} atm^{-1} respectively.*[,16] In the presence of a large excess of 1-MeIm, the oxygenated complexes of compounds **1** and **2** were stable enough towards oxidation (at 5 °C) to allow a direct study by laser photolysis. In contrast, the oxygen adduct of **1**-1,2-Me_2Im gave fast irreversible autoxidation and could not be studied in this way, nor by the alternative technique of competitive rebinding,[16] because of kinetic complications arising from the binding of ligands on both faces of the porphyrin (see discussion above).

The apparatus and methods have already been described.[14] The rate constants were obtained from the kinetics of direct rebinding following laser photodissociation of the carboxy- and oxy-hemochromes and of the five-coordinated base porphyrin complex. All kinetic experiments were performed under pseudo-first-order conditions. 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[11] to prevent spoiling of the kinetics by the various processes (base elimination[10] or hemochrome formation) which can occur when the base is added as a free ligand in the solution.

Equilibrium rate constants were obtained either by photometric titration or from the kinetics, as k^+/k^-. For 1,2-Me_2Im complexes, CO affinities were determined by photometric titration, k_B^{-CO} was derived as k_B^{+CO}/K_B^{CO}. Since K_B^B is greater than K^B for 1-MeIm, hemochrome formation could not be prevented and precluded the measurement of K_B^{CO}. Oxygen affinities were obtained from the kinetic method.

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* 1 atm (atmosphere) ≡ 101 325 Pa.

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