Unusual Co-ordination of Water to Iron(II) Amino Acid Basket-handle Porphyrins

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Iron(II) hanging-imidazole basket-handle porphyrins in which one or two amino-acids [(L)-phenylalanine or (L)-valine] are inserted into one of the two handles, have been prepared in order to increase the steric hindrance and polarity of the distal side cavity. These complexes resulting from reduction of their hematine forms in a heterogeneous two-phase system, organic solvent–aqueous sodium dithionite solution, have been characterized by spectroscopic and magnetic methods. In wet toluene solution, they are found to be a mixture of high ($S = 2$) and low ($S = 0$) spin states at a temperature of 295 K. At a lower temperature of 243 K they give complexes in a pure magnetic low spin state. On the contrary, in solution of a dry solvent, they exhibit a high spin state. This uncommon magnetic property is due to a water molecule present at the sixth co-ordination site of the iron(II) atom stabilized in the distal cavity by hydrogen bond(s). Rate and affinity constants for the unusual water ligation have been measured. Water binding is shown to be controlled by diffusion when toluene is used as solvent. The dissociation rate constants decrease at least 100-fold in the presence of amino acid residues, relative to those of a single hanging-imidazole basket-handle porphyrin. Carbon monoxide and oxygen binding kinetics have been determined and it is concluded that the polarity increase of the distal cavity has no additional stabilizing effects, but the steric effect due to the presence of amino-acid(s) interferes with O_2 and CO ligation.

Iron(II)-basket-handle porphyrins (a-BHP) have been prepared mainly in order to mimic the microenvironment of prosthetic groups in oxygen carrier haemoproteins.[1] Recently we reported the preparation of a new series of this class of superstructured compounds in which amino-acids were inserted in the distal handle whereas the second face of the porphyrin ring was bridged by a chain bearing a hanging-imidazole in order to convert the haem to a five-co-ordinated complex, as in the deoxy form of natural compounds.[2]

The presence of amino-acids in the distal handle allows for an increase in the number of secondary amide groups. This appears to be attractive for two reasons. At first, several studies of a-BHP show that the presence of such chemical groups in the vicinity of the haem produced remarkable environmental effects on the reactivity and co-ordination properties of the central metal such as the stabilization of negative charged species or the oxygenated complexes by polar interactions (dipole–dipole interactions or hydrogen bonds).[3,4] The second effect is a structural one. Increase of amide group number should be able to reinforce the rigidity of the handle and could provide a stronger distal steric hindrance than that observed in simple a-BHP. Indeed, it is now well established that such chemical and structural factors control both the affinities and the discrimination between dioxygen and carbon monoxide in haemoproteins and model compounds.[5–7]

The present paper reports the characterization and the physico-chemical properties of these new iron(II)-basket-handle porphyrins. They differ both from the number of amino-acid residues inserted in the superstructure and from the nature of these residues, (L)-phenylalanine or (L)-valine. The results provide new insights into the co-ordination properties of these iron(II) complexes which are governed by steric and polar effects due to these amino-acids in the distal handle.

From the kinetics of O_2 and CO binding, it can be concluded that the polarity increase of the distal cavity has no additional stabilizing effects as compared to previously studied a-BHPs. The steric effect is similar to that already reported for the 'hybrid' porphyrins.[7] In the case of CO, both the transition state and the final complex are affected, whereas only the transition state of the oxygen adduct is perturbed.

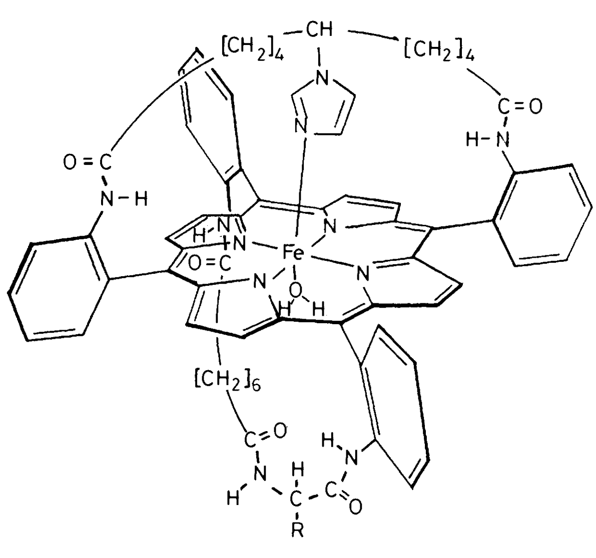
However, the most salient finding of this study was an unexpectedly high affinity of the amino-acid BHPs for water. Optical spectra and ^1H n.m.r. spectroscopy pointed to the possibility of a water molecule being firmly chelated as a sixth ligand. A detailed kinetic investigation was undertaken to measure directly the rate and affinity constants for H_2O complexation. It turned out that water binding is nearly diffusion-controlled in toluene, and that the off-rates are decreased at least 100-fold in presence of the amino-acid residues, leading to moderate, but significant, affinity constants of *ca.* 10^3–10^4 dm^3 mol^{-1}.

Results and Discussion

The Fe(II) hanging-base basket-handle porphyrins (**1**) and (**2**) were characterized by u.v. visible spectrophotometry and ^1H n.m.r. spectroscopy.

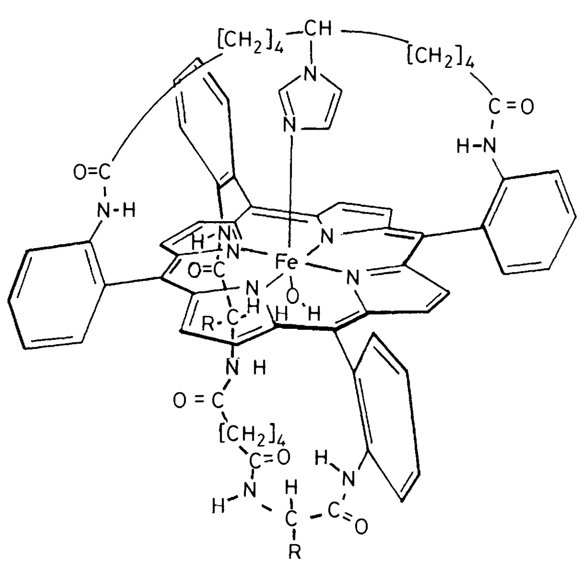
Spectroscopic Properties.—Electronic absorption data of various Fe^{II} porphyrins and their carbon monoxide and dioxygen adducts are presented in Table 1. All compounds obtained after reduction in toluene with aqueous sodium dithionite exhibit nearly identical spectra characterized by a Soret band at 423 nm and two visible bands: a major band at 529 nm and a minor one at 555 nm. These spectra are different from those observed for the well known five-co-ordinated iron(II) complexes of previously described hanging-base basket-handle porphyrins with pure polymethylene distal handle, prepared by the same procedure.[8]

The general shape of these spectra is quite similar to that of the six-co-ordinated complexes of Fe^{II} TPP and Fe^{II} a-BHP with two additional axial ligands.[9] Since the steric hindrance of



(**1a**) R = L - Phe

(**1b**) R = L - Val



(**2a**) R = L - Phe

(**2b**) R = L - Val

Table 1. Absorbance maxima and molecular extinction coefficients (in parentheses) of iron(II)-amino-acid basket handle porphyrins, in toluene at 20 °C.

		$\lambda_{max.}$/nm (ε/dm^3 mmol^{-1} cm^{-1})		
(**1a**)	$Fe^{II}H_2O$	423 (168.5)	528.4 (15.7)	555 (sh)
	$Fe^{II}CO$	426 (208)	541 (10)	
	$Fe^{II}O_2$	425	533	
	$Fe^{II}NMeIm$	429 (210)	536 (15.6)	565 (sh)
(**1b**)	$Fe^{II}H_2O$	423 (119.5)	528 (16.8)	553 (sh)
	$Fe^{II}CO$	426 (145.7)	541 (10.3)	
	$Fe^{II}O_2$	425	533	
	$Fe^{II}NMeIm$	430 (134.5)	536 (16.8)	565 (sh)
(**2a**)	$Fe^{II}H_2O$	424 (180.3)	530 (17.1)	556 (sh)
	$Fe^{II}CO$	425.2 (217)	541 (10.2)	
	$Fe^{II}NMeIm$	427.5 (171)	534.5 (14)	565.5 (sh)
(**2b**)	$Fe^{II}H_2O$	423.7 (184.5)	529 (15.9)	556 (sh)
	$Fe^{II}CO$	424.5 (220.7)	540.9 (9.6)	
	$Fe^{II}NMeIm$	427 (169)	534 (15)	565.5 (sh)

the two faces of the porphyrin rings prevents the formation of the intermolecular interaction which would be expected with 'chelated' or 'tailed' compounds,[10–12] axial ligation at the sixth co-ordination position of the iron ion in compounds (**1**) and (**2**) should arise from an unusual ligand. In fact, the presence of a weak field ligand may be postulated because addition of 1-methylimidazole, a strong field ligand, to compounds (**1**) and (**2**) gave no significant difference in the band structure, except for a bathochromic shift of the electronic absorption (430, 534, and 565 nm) (Table 1 and Figure 1). Among the potential additional ligands, water appeared to be good candidate. This was confirmed by ^{1}H n.m.r. experiments as described in the next section.

Proton N.M.R. Spectroscopy.—The spin state of iron(II) porphyrins strongly depends on the axial co-ordination.[13] Furthermore, ^{1}H n.m.r. spectra of iron(II) porphyrin complexes are characteristic of the spin state. Thus, we have shown that iron(II) hanging-base basket-handle porphyrins exhibited spectra characteristic of high-spin ($S = 2$) five co-ordinated complexes indicating an actual co-ordination of the proximal base.[14,15] The general feature of these spectra was the presence of well-resolved downfield-shifted resonances of pyrrolic protons (near 45 ppm) and ligand protons (up to 140 ppm) induced by contact interaction with the paramagnetic centre. Only negligible pseudo-contact shifts to high field were observed. Addition of axial ligand such as imidazole gave new species for which ^{1}H n.m.r. spectra were consistent with those of low-spin hexa-co-ordinated complexes ($S = 0$). In this case the resonances were observed in the diamagnetic range (−1 to 10 ppm), affected only by the ring current of the macrocycle ring.

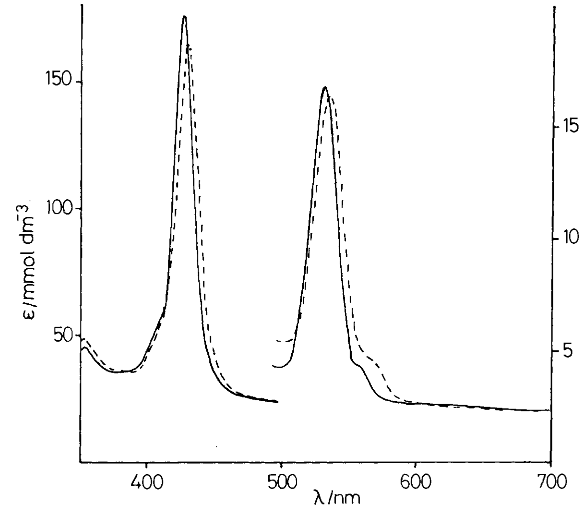


Figure 1. U.v.-visible spectra of the $Fe^{II}H_2O$ (——) and $Fe^{II}NMeIm$ (– – –) complexes of compound (**2a**) in toluene at 20 °C.

The spectrum at 295 K of compound (**1a**) inserting one (L)-phenylalanine residue in wet toluene show resonances between 0 and 24 ppm [Figure 2(*a*)]. This spectrum is modified at lower temperature. On decreasing the temperature down to 243 K, all the resonances shift to the diamagnetic range and the resulting spectrum is identical to those of iron(II) complexes in six-co-ordinated low-spin state. This magnetic behaviour clearly indicates that two iron(II) complexes differently co-ordinated are in fast exchange at 295 K; these consist of a five-co-ordinated ($S = 2$) and a six-co-ordinated ($S = 1$) iron(II) species in equilibrium. On lowering the temperature, the latter becomes predominant. On the contrary, compound (**2a**) containing two amino-acid residues in the distal handle is found to be in a diagmagnetic low-spin state even at 295 K [Figure 2(*b*)].

Iron(II) complexes of compounds (**1**) and (**2**) in anhydrous solution (see Experimental section) exhibit n.m.r. spectra in which the same resonances are downfield shifted to 80 ppm and assigned to protons of the hanging imidazole ring [Figure 2(*c*)]. Thus normal five-co-ordinated iron(II) complexes with a magnetic high-spin state ($S = 2$) characteristic of deoxy haemoproteins and deoxy Fe(II) a-BHP are obtained under these conditions. The spectra observed in wet conditions can be restored by addition of heavy water.

Clearly these unusual magnetic properties suggest that a water molecule can be present at the sixth co-ordination site of iron(II) in these complexes. Water has already been considered as a potential ligand for iron(II) porphyrins. Numerous investigations by a variety of physical methods of anionic or cationic iron(II) porphyrins in water or in mixtures of organic solvent–water suggests water as an axial ligand.[16–18] Furthermore, Rougée and Brault have shown that water can play the role of weak field axial ligand during the titration of deuterohaem by carbon monoxide in wet toluene and measured the value of the affinity constant of water in these conditions to be *ca.* 0.1 $dm^3 mol^{-1}$ at 295 K.[19]

The uncommon co-ordination of water to the sixth co-ordination position of iron(II) in compounds (**1**) and (**2**) may be attributed to the polarity of the distal cavity associated with the presence of amino-acid residues. The co-ordination of the ferrous ion by the oxygen atom of a water molecule could be stabilized in the cavity by hydrogen bonding between the hydrogen atoms of the water molecule and nearest oxygen atoms of 'peptide' linkages formed by amino group of amino acids and carboxylic groups of the central chain.[2] Similar water co-ordination has been mentioned in a preceding paper concerning iron(III) derivatives of these compounds. The water molecule should be even more strongly stabilized in compounds (**2**) than in compounds (**1**) because of the possibility of hydrogen interactions alternatively with the carbonyl groups of the 'peptide linkages'. We cannot, therefore, exclude the possibility of water participation in two hydrogen bonds in the former one; Drieding molecular models do not confirm such a fact.

CO and O_2 Complexes.—Addition of pure CO or O_2 to wet organic solutions of iron(II) complexes of compounds (**1**) and (**2**) immediately produced new species which were characterized as carbonylated and oxygenated adducts respectively on the basis of their absorption spectra (Table 1). Whereas carbonylated derivatives were stable, O_2 adducts were sensitive to fast oxidation.

The changes in the visible spectra accompanying an increase in CO pressure were not simple. No well defined isosbestic points were observed, indicating the presence of several absorbing species in solution. This behaviour differs from that of the iron(II) five-co-ordinated complexes for which the binding of CO corresponds to a simple equilibrium. At low concentration of CO, the formation of hexa-co-ordinated species involves a competition between CO and the co-ordinated water molecule. In fact, at a pressure of CO above 7×10^{-6} Torr,* the

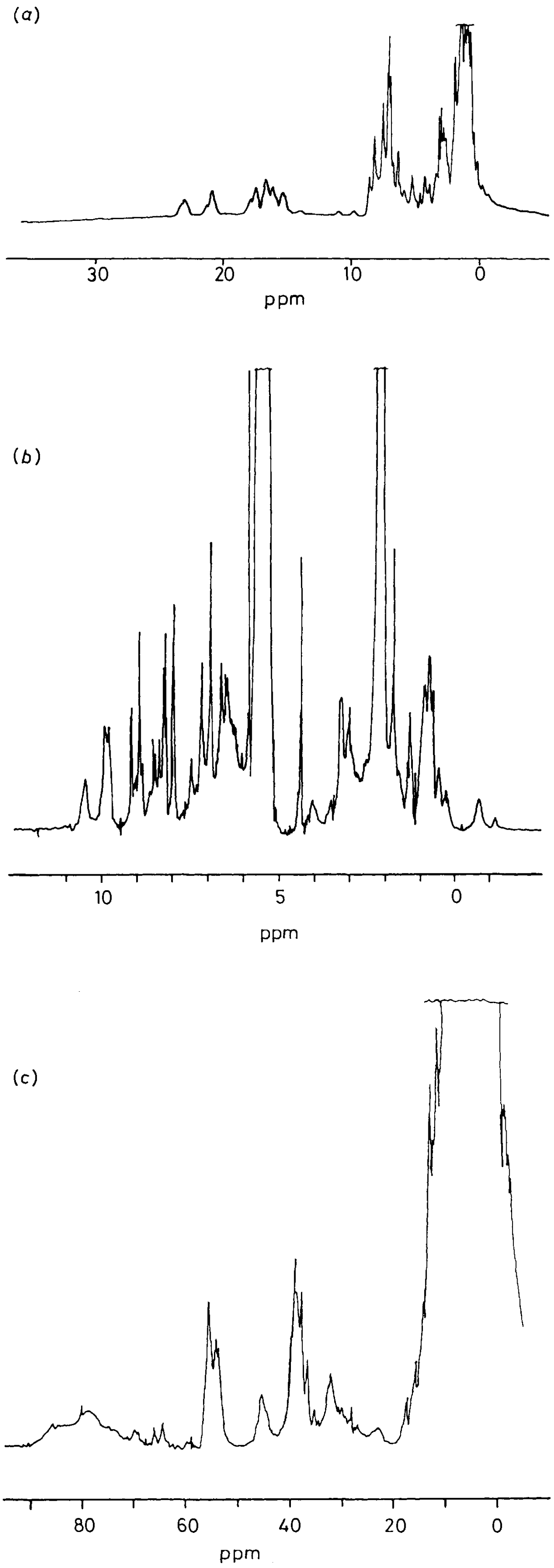


Figure 2. 400 MHz proton n.m.r. spectra of compounds (**1a**) and (**2a**) in wet CD_2Cl_2 [Figures 2(*a*) and 2(*b*)] and compound (**1a**) in dry CD_2Cl_2 [Figure 2(*c*)] at 295 K.

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* 1 Torr = 101 325/760 Pa.

substitution of water occurs, generating monocarbonylated derivatives. However, affinity constants can be determined from the ratio of kinetic rate constants as presented below.

Kinetic Rates and Equilibrium Constants.—(*a*) *Water binding.* The rate constants for binding or dissociating ligands were obtained from the kinetics of equilibrium relaxation following laser photodissociation. The apparatus has already been described.[20]

The measurements were based on the technique of photo-triggered competitive rebinding[21] which is briefly summarized as follows. In the presence of CO plus another ligand L (H_2O or O_2), the carboxyhaemochrome is the dominant species. Photodissociation yields the five-co-ordinated species P. CO and L compete for rebinding with P; this first step leads to the transient formation of PL and is followed by an exchange reaction which ultimately restores PCO. The overall reaction scheme is:

$$\mathrm{PCO} + \mathrm{L} \xleftarrow{k'^{+}_{\mathrm{CO}}} \mathrm{P} + \mathrm{CO} + \mathrm{L} \underset{k^{-}_{\mathrm{L}}}{\overset{k'^{+}_{\mathrm{L}}}{\rightleftharpoons}} \mathrm{PL} + \mathrm{CO}$$

Scheme.

in which primed symbols denote second-order rate constants. Both steps can be followed accurately at the isosbestic wavelength for P and PCO.

The fast (τ) and slow (θ) relaxation times obey the equations:[22]

$$\frac{1}{\tau} = k'^{+}_{\mathrm{CO}}[\mathrm{CO}] + k^{-}_{\mathrm{L}} + k'^{+}_{\mathrm{L}}[\mathrm{L}] \qquad (1)$$

$$\theta = \frac{1}{k'^{+}_{\mathrm{CO}}[\mathrm{CO}]} + \frac{1}{k^{-}_{\mathrm{L}}}\left[1 + \frac{k'^{+}_{\mathrm{L}}[\mathrm{L}]}{k'^{+}_{\mathrm{CO}}[\mathrm{CO}]}\right] \qquad (2)$$

Laser photolysis of carboxyhaemochromes of compounds (**1**) and (**2**) in wet toluene led to a transient absorbance change, even at the isosbestic wavelength for P and PCO. This signal was distinctly biphasic, with a fast step followed by a much slower step which restored the initial absorbance. The dependence of both components upon the H_2O concentration—which will be discussed below—allows us to attribute them to the transient fixation of water followed by the exchange H_2O/CO, according to the Scheme. This reaction had not been observed previously in the kinetic studies of BHPs and 'hybrid' models.[7,20]

Equations (1) and (2) assume that the kinetics are exponential. This was indeed found to be the case for the fast rebinding phase [equation (1)] but the slower exchange kinetics showed a distinct deviation from exponential behaviour. However, it could be accurately described by a sum of two exponentials, in which the major component represented at least 80% of the total amplitude. Moreover, several arguments to be developed below show unambiguously that this major component could be attributed to the pure exchange reaction [Scheme, equation (2)]. However, the Scheme does not provide a complete description of the complicated kinetics: the origin of the residual component is not yet understood and requires further investigations.

Both τ^{-1} and θ were found to vary linearly as a function of the water concentration (Figure 3); moreover, the slope and the intercept of the plot of τ^{-1} against $[H_2O]$ [Figure 3(*a*)] were independent of the CO concentration within errors; in contrast, they exhibited a $[CO]^{-1}$ dependence in the plot of θ against $[H_2O]$ [Figure 3(*b*)]; these findings are in good agreement with equations (1) and (2), provided that $k'^{+}_{\mathrm{CO}}[\mathrm{CO}] \ll k^{-}_{\mathrm{H_2O}}$, a condition which is indeed satisfied for compounds (**1**) and (**2**). $k^{-}_{\mathrm{H_2O}}$ and $k'^{+}_{\mathrm{H_2O}}$ were obtained respectively as the intercept and the slope of the straight line of Figure 3(*a*) [equation (1)]; k'^{+}_{CO} and $k'^{+}_{\mathrm{H_2O}}$ were derived from Figure 3(*b*) according to equation (2). Both values of $k'^{+}_{\mathrm{H_2O}}$ are in reasonable agreement. The consistency of these results confirms the attribution of the major component of the slow kinetics to the exchange reaction described by equation (2). Furthermore, the extrapolated value of θ derived from Figure 3(*b*) was found to be equal to the direct CO rebinding time measured at the lowest water content (*ca.* 10^{-5} mol dm^{-3}), as expected from equation (2).

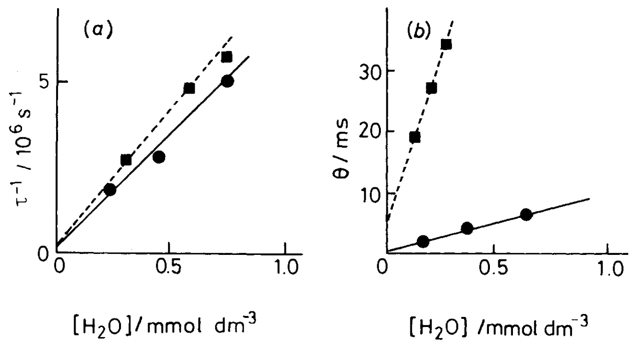

Figure 3. Plots of (*a*) τ^{-1} and (*b*) θ as a function of the water concentration in toluene, 20 °C, for the CO/H_2O transient exchange following photodissociation of the carboxyhaemochrome of compound (**2a**). (——), [CO] = 7 × 10^{-3} mol dm^{-3}; (- - -), [CO] = 7 × 10^{-4} mol dm^{-3}. The estimated errors are *ca.* ±10%, due principally to the difficulty of controlling accurately the water concentration.

Affinity and rate constants are reported in Table 2. The values for the hanging-imidazole basket-handle porphyrin, a-BHP (C_{11}-Im)(C_{12})[23] are also given for comparison. Compounds (**1**) and (**2**) have the same proximal handle as compound a-BHP, but differ from it by their distal face which is more polar and more sterically encumbered, because of the presence of one or two amino acids. The increase of steric hindrance results in part from a shorter length and from a greater rigidity of the handle, but it can be mainly attributed to a more packed conformation of the handle at a closer distance from the iron due to specific interactions within the chain.[2]

Laser photolysis of the carboxyhaemochrome of a-BHP (C_{11}-Im)(C_{12}) in wet toluene did not lead to any transient absorbance change at the isosbestic wavelength for P and PCO. Water binding to this molecule probably occurs too fast for detection. The association rate constant $k'^{+}_{\mathrm{H_2O}}$ for compounds (**1**) and (**2**) is too close to the diffusion limit to be enhanced significantly in compound a-BHP. Thus, the failure to detect any transient H_2O complex may be attributed entirely to a greater dissociation rate constant. A lower limit of $k^{-}_{\mathrm{H_2O}} >$ 300 × 10^5 s^{-1} can be estimated from the time resolution of the laser apparatus. The decrease of $k^{-}_{\mathrm{H_2O}}$ by at least two orders of magnitude in compounds (**1**) and (**2**) reflects the stabilization of the water complex associated with the amino-acid(s) insertion in the distal cavity. N.m.r. spectroscopy has evidenced[2] the ligation of a water molecule to the sixth axial co-ordination site of the iron ion which could probably be attributed to an hydrogen bond formation between one of the two protons of water and the carbonyl group of the amino-acid(s). This stabilization is even greater with compounds (**2a**) and (**2b**) where two amino-acids are inserted, as apparent from the smaller values of $k^{-}_{\mathrm{H_2O}}$ (Table 2). As already observed,[20] hydrogen bonding has no influence upon the association rate constants which do not differ significantly within the series (**1**) and (**2**) and are very close to the diffusion limit of 1.6 × 10^{10} dm^3 mol^{-1} s^{-1}. A small energy barrier implies a compensation between the energetic cost required for orientating the partners in a favourable configuration for bond formation and the enthalpic stabilization. Assuming, reasonably, $k'^{+}_{\mathrm{H_2O}}$ *ca.* 6 × 10^9

Table 2. Rate and equilibrium constants for CO and O_2 binding to porphyrins (**1**) and (**2**).[a]

	H_2O			CO			O_2		
	$k^+/10^9$ $dm^3\ mol^{-1}\ s^{-1}$	$k^-/10^5$ s^{-1}	$K/10^3$ $dm^3\ mol^{-1}$	$k^+/10^6$ $dm^3\ mol^{-1}\ s^{-1}$	$k^-/10^{-3}$ s^{-1}	$K/10^8$ $dm^3\ mol^{-1}$	$k^+/10^7$ $dm^3\ mol^{-1}\ s^{-1}$	k^-/s^{-1}	$K/10^5$ $dm^3\ mol^{-1}$
(**1a**)	5.9	25.0	2.4	2.3	20.9	1.1	2.2	60	3.6
(**1b**)	9	12.0	7.5	3.0	27.3	1.1	1.8	25	7.2
(**2a**)	6.3	3.0	21.1	0.3					
(**2b**)	7.0	5.4	12.9	0.5					
Fe^{II}-a(BHP) (C_{11}-Im)(C_{12})	—	>300	<0.2	40	6.7	60	31.4	620	5.1

[a] Solvent, toluene; temp., 20 °C.

$dm^3\ mol^{-1}\ s^{-1}$ for a-BHP leads to $K_{H_2O} < 200\ dm^3\ mol^{-1}$. This allows us to estimate to at least 6–11 kJ mol^{-1} the gain in free energy associated with hydrogen-bond formation in the water complexes of (**1**) and (**2**).

(*b*) *Oxygen and carbon monoxide binding*. In the presence of CO/O_2 mixtures, the photodissociation of carboxyhaemochrome leads to the transient formation of the oxyhaemochrome. In principle, the oxygen rate constants $k'^{+}_{O_2}$ and $k^{-}_{O_2}$ could be obtained from the kinetics in a similar way as for water; however, due to the residual water content in toluene, the rebinding kinetics can be vitiated since H_2O competes for the formation of the six-co-ordinated complex; the concentration ratio of both transient species PH_2O and PO_2 depends on all kinetic rate constants k'^{+}_{CO}, $k'^{+}_{H_2O}$, $k^{-}_{H_2O}$, $k'^{+}_{O_2}$ and $k^{-}_{O_2}$. For compounds (**1a**) and (**1b**) the gas concentrations could be adjusted so that the water fixation remained negligible; the kinetic rate constants $k'^{+}_{O_2}$ and $k^{-}_{O_2}$ were independent of the O_2/CO ratio over a tenfold range of variation, and remained unaffected when a small amount of water (2×10^{-4} mol dm^{-3}) was added to the residual content of 10^{-5} mol dm^{-3}. Unfortunately, it has not been possible to find similar favourable conditions for compounds (**2a**) and (**2b**). Nevertheless, some interesting observations can be made upon examination of Table 2.

As compared to a-BHP (C_{11}-Im)(C_{12}), the affinity of compounds (**1a**) and (**1b**) for carbon monoxide, K_{CO}, is reduced by a factor of *ca.* 55, while that for oxygen, K_{O_2}, remains almost unaffected. The reducton of K_{CO} results mainly from the decrease of the association rate constants (by a factor of *ca.* 13–17) together with a smaller increase of the dissociation rate constants (*ca.* 3–4); in contrast, the simultaneous decrease by a factor of *ca.* 10–25 of both rate constants $k'^{+}_{O_2}$ and $k^{-}_{O_2}$ leads to an almost constant oxygen affinity. These variations can be attributed to the increase of steric hindrance; qualitatively similar effects have been reported previously within the 'hybrid' model series.[7] The steric interaction is similar for both ligands in the transition state, but it relaxes in the case of oxygen as bond formation leads to a bent conformation whereas it persists for CO which is known to adopt a linear geometry in such complex.

A previous comparative study of various porphyrins has shown that polarity and electronic effects due to the insertion of suitable groups in the vicinity of the iron can also favour oxygen binding; in particular, hydrogen-bond stabilization of oxygen has been demonstrated in amide basket-handle porphyrins.[20] However, hydrogen bonding affects only the dissociation rate;[20] the fact that both the 'on' and the 'off' rates of compounds (**1a**) and (**1b**) are decreased by a roughly similar amount as compared with compound a-BHP suggests that a change in the hydrogen-bond strength is not involved, but that the effect is entirely a consequence of the change of steric hindrance. The insertion of amino-acid(s) does not prevent a favourable conformation of the chain for H-bond formation between oxygen and the proton of the amide group linking the handle to the macrocycle. Finally, the kinetic results suggest that oxygen, unlike water, does not give rise to any specific interaction with the amino-acid, and that the polarity increase of the distal cavity has no additional stabilizing effects.

Materials and Methods.—Materials. Chloro-iron(III) hanging imidazole basket handle porphyrins, inserting one or two amino-acids [(L)-phenylalanine or (L)-valine] in the superstructures, were synthesized and characterized by methods previously described.[2] Chloro-iron(II)-a-BHP (C_{11}-Im)(C_{12}) was synthesized following the method previously described.[23]

Iron(II) complexes used in this work were obtained by reduction using the classic two-phase system.[8] The haemin-chloride dissolved in toluene was vigorously shaken with an aqueous sodium dithionite solution under argon. After separation of the two layers, the reduced organic solution was transferred anaerobically into the optical cuvette or the n.m.r. tube through a latex septum. Such transfer under argon minimized the risk of oxidation.

In order to prepare solutions of iron(II) complexes in dry solvent, reduced compounds were first prepared using the techniques described above. The organic solution was then transferred into a glass tube from which the solvent was evaporated to dryness. The remaining solid, heated at 70 °C, was dried under vacuum (10^{-2} mm Hg) for 3 h, and then dissolved in anhydrous methylene dichloride which was distilled from CaH_2 and kept over 4 Å molecular sieves in an inert atmosphere glovebox.

Optical and ^1H *n.m.r. measurements.* Electronic spectra in the Soret and visible regions were recorded using a Varian DMS-100 spectrometer. Proton n.m.r. spectra were obtained at 400 MHz in the indicated deuteriated solvents using a Bruker AM-400 instrument.

Determination of kinetic rate constants. Because of its expected interference with ligand rebinding in the kinetic measurements, water was carefully eliminated after the usual reduction procedure with aqueous dithionite. The wet toluene solution was therefore transferred anaerobically to a vessel and dry CO was bubbled through. The residual water concentration, as estimated from the kinetic measurements (see below) reaching the limiting value of *ca.* 10^{-5} mol dm^{-3} after 2 h. This concentration was *ca.* two orders of magnitude smaller than that obtained after reduction under anhydrous conditions using toluene dried on molecular sieves. Known concentrations of water in toluene were further obtained by adding small aliquots of wet toluene to the Fe(II) solution in the optical cell; the concentrations were calculated using $[H_2O] = 2.4 \times 10^{-2}$ mol dm^{-3} for the solubility of water in toluene at 20 °C. Water and gaseous ligands were always added in a large excess (3–7 mmol dm^{-3}) as compared to the porphyrin concentration

(5×10^{-5} mol dm^{-3}) in order to satisfy pseudo first-order kinetics.

The rate constants k'^{+}_{CO}, $k'^{+}_{H_2O}$, $k^{-}_{H_2O}$, $k^{+}_{O_2}$, and $k^{-}_{O_2}$ were obtained from the kinetics of equilibrium relaxation following laser photodissociation. The apparatus has been already described.[20]

Determination of equilibrium constants. The partition coefficient $M = K_{CO}/K_{O_2}$ was determined by photometric titration of the oxyhaemochrome against the carboxyhaemochrome. The absorption changes were followed in the Soret band. The samples were carefully dried by bubbling CO through the solution, as described above. In these conditions, the water content is low enough compared to the gaseous ligand concentrations, and there is no appreciable (H_2O)-six-co-ordinated species present in the solution; moreover, the oxyhaemochrome does not undergo appreciable autoxidation during the timescale of the experiment. The carbon monoxide affinities K_{CO} were calculated using M and the values of K_{O_2} determined kinetically. The dissociation rate constants k^{-}_{CO} were calculated as k'^{+}_{CO}/K_{CO}.

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