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Synthesis and coordination properties of superstructured iron-porphyrins

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Abstract - Superstructured iron (II)-porphyrins have been designed to closely approach the properties of oxygen carrier hemoproteins and yield useful information concerning their biological structure and function. "Basket-handle" porphyrins and "hybrid" porphyrins allow one to modify the constraint exerted on a chelated "proximal" base as well as to alter the chemical and steric environment of the distal side of the heme which controls selectivity of O_2 and CO binding. The presence of amide groups in the vicinity of the heme ring gives the possibility of forming a cavity to stabilize dioxygen or hydroxyl ion on iron (II) and carbon monoxide on iron (I).

I. INTRODUCTION

The functional differentiation of hemoproteins (hemoglobin, myoglobin, cytochromes, catalase and peroxidases) having the same prosthetic group (protoheme IX) arises from differences in axial ligation to the heme iron and from differences in the protein environment surrounding the heme. Over the last decade, a number of superstructured metalloporphyrins have been synthesized in order to gain a molecular level understanding of the properties and the structure-function relationships in natural systems (ref. 1-3).

The main results of our research in this field have focused on the mechanism by which the oxygen carrier hemoproteins (hemoglobin and myoglobin) regulate dioxygen and carbon monoxide binding.

Hemoglobin (Hb) is a tetramer composed of two similar protein chains (globins) of unequal length which reversibly binds oxygen under conditions of high dioxygen pressure (ref. 4). In the tissues where oxygen is required, the oxygen-hemoglobin complex is dissociated. The oxygen is then transferred to another hemoprotein, myoglobin (Mb) which is a monomeric compound (ref. 4). In both proteins the heme is tightly bound to the globin through a single coordinate bond between an imidazole ring from a "proximal" histidine residue (His F8) and the iron atom of the heme. In the deoxy form, the iron atom is in a high spin five-coordinated Fe (II) state and lies out of the porphyrin plane toward the proximal side (ref. 5). The protein surrounding the sixth-coordination site of the iron forms a "distal" hydrophobic cavity and is used for reversible binding of dioxygen or other ligands. Upon oxygenation, the iron atom moves into the porphyrin plane forming a low spin six-coordinated complex. The O_2 molecule is bound in a bent geometry (ref. 6). Recent X-ray studies of HbO_2 (ref. 7) and neutron diffraction studies of MbO_2 (ref. 8) have provided strong direct evidence of H-bonding between the imidazole ring of the "distal" histidine (His E7) and bound O_2. Furthermore, structural studies of carbonmonoxy derivatives have shown close nonbonded contacts between the bound CO and aminoacid residues in the vicinity of the distal site (His E7 and Val E11) resulting in a bent or tilted geometry for this ligand (ref. 9).

From these structural and chemical features considered necessary in the affinity and kinetic control of gaseous ligand binding, it seemed to us that a realist design of synthetic active site models of dioxygen carrier hemoproteins must involve the following requirements :

1 - resistance to irreversible oxidation of iron (II) upon dioxygen binding
2 - five-coordination of the iron (II) by a proximal base
3 - chemical environment control of the binding site
4 - distal steric interference to O_2 and CO binding.

This paper describes the synthesis of superstructured iron-porphyrins which incorporate these features and reports their reactivities with dioxygen and carbon monoxide. The introduction of amide groups in such superstructures has induced, in some cases, unusual behaviour in redox properties and coordination chemistry of iron porphyrins. This feature is also discussed.

II. PREVENTION OF HEME OXIDATION

The first difficulty is the reproduction of the redox stability of the iron (II) upon exposure to dioxygen. In solution simple iron (II) porphyrins cannot reversibly bind dioxygen, except at low temperature. At room temperature and in the absence of a large excess of nitrogenous ligand six-coordinated iron (II) oxygen complex is immediately converted to a μ-oxo-dimer via a μ-peroxo-dimer and an iron (IV)-oxo species

following a binuclear reaction (ref. 10,11). This reaction does not occur in hemoproteins because the polypeptide chain surrounding the heme prevents the close approach of two hemes and the subsequent oxidation.

$$\text{B-PFe(II)} + O_2 \rightleftharpoons \text{B-PFe(II)} - O_2 \qquad \text{Reversible dioxygen binding}$$

$$\text{B-PFe(II)} - O_2 + \text{B-PFe(II)} \longrightarrow \text{B-PFe} - O_2 - \text{PFe} - \text{B} \longrightarrow \text{B-PFe(IV)} = O \longrightarrow \text{PFe(III)} - O - \text{PFe(III)}$$

Irreversible autoxidation

To inhibit such an undesirable reaction, different modified porphyrins have been proposed. Each of these porphyrins is sterically encumbered on one side, thereby directing the binding of a nitrogenous base to the other side. Such an approach has been followed by many groups to produce an array of different model porphyrins : picket fence, cyclophane and capped (ref. 1-3). Thus the bulky substituents cover one side of the heme in such a way that no irreversible autoxidation takes place as long as the other side is occupied by an axial ligand which favors reversible oxygenation.

However, in non polar organic solvents, their lifetimes depend largely on the nature and the concentration of the exogenous base. For example, at low concentration of imidazole, the undesired μ-oxo dimer formation can still take place on the unprotected side of the heme.

Steric encumbrance on both faces of the porphyrin may prevent this bimolecular oxidation pathway but still allowing ligand fixation. This strategy have been independly proposed by Battersby (ref. 12) and ourselves (ref. 13). In our case this new class of so-called "basket-handle" porphyrins (BHP) is derived from meso-tetraphenyl-porphyrin in which the two opposite phenyl groups are linked by a convenient chain to each side of the macrocycle ring. We have synthesized two series of such compounds which differ by the fixation mode of the handles to the macrocycle, ether or amide group.

The synthesis of compounds bearing ether anchoring chains (e-BHP) were performed with 5,10,15,20-tetrakis (o-hydroxyphenyl) porphyrin (mixture of the four atropisomers) (scheme 1). Alkylation with the appropriate dibromo-derivative under conditions of high dilution in dimethylformamide at 100°C, followed by chromatography led to the isolation of three porphyrin isomers : cross-trans-linked, adjacent-trans-linked and adjacent-cis-linked (ref. 14). Pure polymethylene or arylene-p-bis-alkylene chains were used as handles. These changes in the length and in the nature of the bridge modify the cavity size and the environment of the iron coordination site. In each case, the most important compound is expected to be the cross-trans-linked isomer.

The precursor of the amide-basket handle porphyrins (a-BHP) is the alternating $\alpha\beta\alpha\beta$ atropisomer of the 5,10,15,20-tetrakis (o-aminophenyl)porphyrin (scheme 2). A coupling reaction with the appropriate diacid chloride in dry tetrahydrofuran at room temperature in the presence of base gives the desired compound. The possibility of using only one atropisomer offered an easy synthesis of the cross-trans-linked isomer under mild conditions and eliminated the separation difficulties of three isomers which were obtained with e-BHP (ref. 15).

The degree of steric hindrance is illustrated by the rates of metallation and oxidation of the various isomers (ref. 13). The adjacent-cis-linked isomer has one face unhindered and is easily metallated. In contrast the other isomers where both faces are hindered undergo iron insertion reluctantly. Either in the presence or in the absence of nitrogenous base, the iron (II) complexes of e-BHP and a-BHP exhibit a remarkable redox stability towards oxidation when they are exposed to 1 atmosphere of dioxygen, even at room temperature. For example, in the absence of 1-methylimidazole, the cross-trans-linked isomers of iron (II)-e-BHP has a half life ($t_{1/2}$) for oxidation to the hematin derivatives [Fe (III)-OH^-] of 1.5-10.5 minutes compared to 7-5.4 seconds for oxidation of the adjacent-cis-linked isomers to the μ-oxo-dimers. In fact, these single-face hindered hemes exhibit $t_{1/2}$ comparable to that of the "picket fence" porphyrin under identical conditions.

In toluene solution, iron (II) complexes of both-faces hindered porphyrins show ^1H NMR spectra which are characteristic of four-coordinated intermediate spin (S = 1) species (ref. 16) (figure 1A). They exhibit a large magnetic anisotropy which induces strong paramagnetic shifts upfield of the methylene protons by pseudocontact interactions with the central metal ion. The analysis of these shifts allowed us to determine both the distance and the orientation of the protons with respect to the porphyrin core.

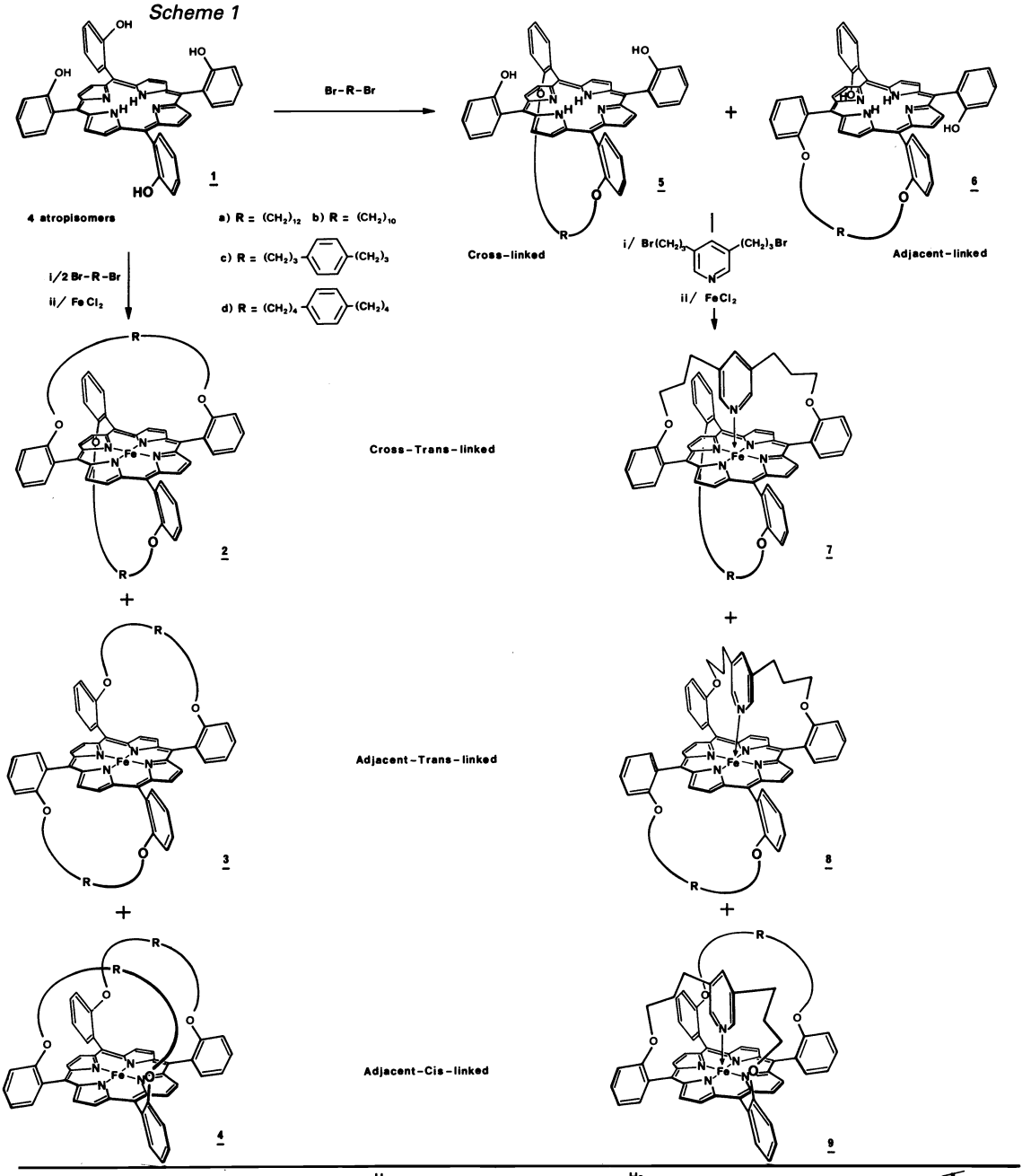
III. FIVE-COORDINATION OF HEME

The second major problem in studying iron (II) porphyrins as models of the active site of dioxygen carrier hemoproteins is the preference of the metal to give six-coordination. On addition of strongly coordinating N-donor ligands, four-coordinated iron (II) porphyrins readily add two molecules of ligand to give the symmetrical six-coordinated complexes (hemochromes) (ref. 17). This is due to greater equilibrium constant K_2 for the addition of the second ligand than K_1 for the first addition :

$$\text{PFe (II)} + B \overset{K_1}{\rightleftharpoons} \text{PFe (II)} - B + B \overset{K_2}{\rightleftharpoons} B - \text{PFe (II)} - B$$

Indeed the low spin six-coordinated complexes are favoured thermodynamically by crystal field stabilization energy. However, when a sterically hindered axial ligand such as 2-methylimidazole is used, five-coordinated high-spin iron (II) complex can be obtained, because the steric interaction between the methyl group and the porphyrin ring would prevent the iron of moving into the porphyrin plane (ref. 18,19).

Scheme 1



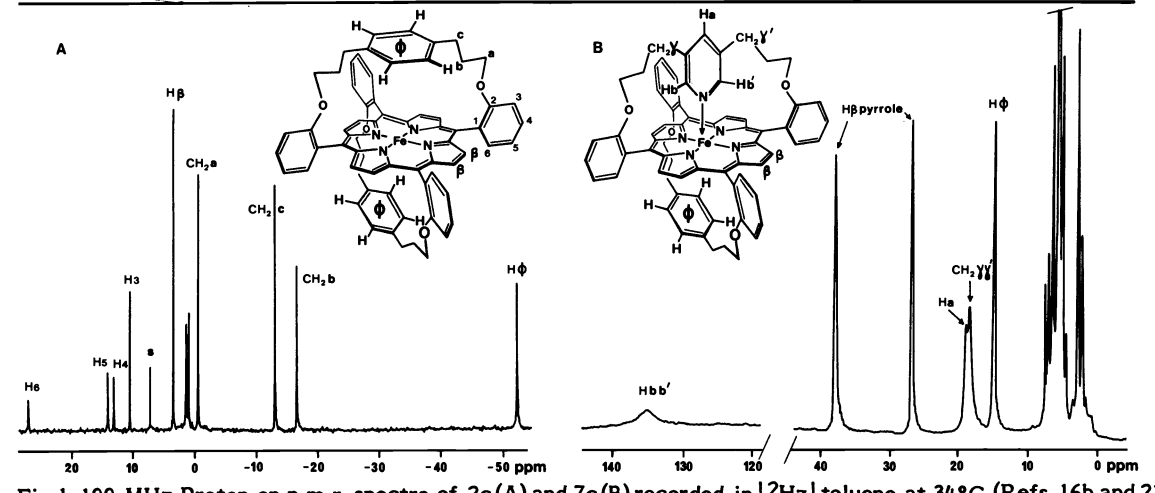
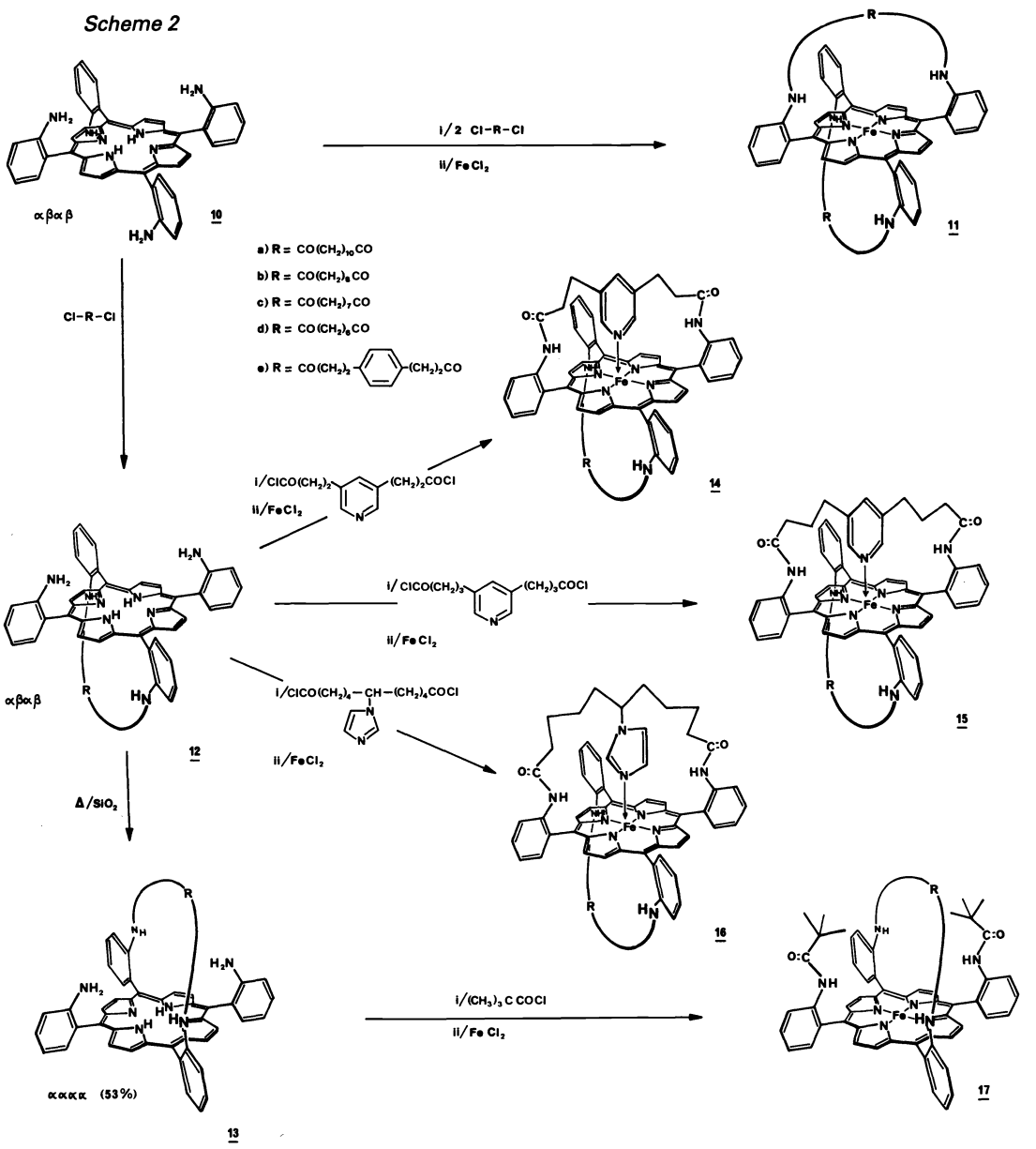


Fig.1. 100-MHz Proton on n.m.r. spectra of 2c(A) and 7c(B) recorded in $|^2Hz|$ toluene at 34°C (Refs. 16b and 23).

Scheme 2



In order to obtain five-coordinated species of iron (II)-BHP, pyridine or imidazole have been inserted into one of the two handles. This doubly covalent attachment of the base should :

- ensure a high degree of iron five-coordination
- eliminate the need of having excess of free ligand in solution
- inhibit the intramolecular association observed with "chelated" hemes or "tailed" hemes developed by Traylor, Collman and ourselves (ref. 20-22).

Thus, the preparation of such integrated compounds marks an important advance and a further refinement in the design of dioxygen carrier models because incorporation of a N-donor ligand into one handle simulates the "proximal" base of the natural system whereas the steric hindrance provided by the second handle creates a protected "distal" cavity for dioxygen ligation (ref. 15,23).

These "hanging-base basket-handle" porphyrins have been synthesized in both the ether and amide series by two-step procedures. The first step involved formation of mono-bridged porphyrins containing the "distal" protection chain. The doubly-bridged porphyrins were obtained in a second step by coupling the pyridine or imidazole chain with a mono-bridged intermediate. As the mixture of four atropisomers was used in the preparation of the ether compounds, condensation of tetrakis (o-hydroxyphenyl) porphyrin with one equivalent of dibromoalkane gave a mixture of two singly-handled intermediates depending on whether adjacent or opposite meso-phenyl groups were linked. This mixture was then reacted with dibromopyridine derivative and the desired cross-trans-linked isomer isolated by preparative t.l.c. Similar amide-compounds were prepared from α β α β -tetrakis (o-aminophenyl) porphyrin.

Following iron insertion and reduction, both visible absorption and ^1H NMR spectra of ether and amide compounds were consistent with five-coordinated high spin (S = 2) iron (II) complexes (ref. 23,24). A typical NMR spectrum of such a complex recorded at 307K is presented in Figure 1B. The spectrum exhibits 1) downfield shifted pyrrolic resonances by contact interaction, 2) negligible pseudocontact shifts of methylene protons of the handles ; geminal protons are still equivalent and appear in the diamagnetic range, except for the first methylene group of the chain attached to the pyridine which is affected by some spin delocalisation, 3) the protons of the pyridine moiety having a large dowfield shift (up to 135 ppm) for the bb' protons and 18 ppm for the a proton, confirming that the pyridine is actually coordinated to the iron (II).

IV. DIOXYGEN AND CARBON MONOXIDE BINDING

With the "hanging-base basket-handle" porphyrins, we have a series of nine heme models (compounds 7a-d, 14a,b,e, 15a, 16a) which differ by the mode of attachment of the handles, the length and rigidity of the handles varying in discrete steps and the nature of the proximal base. These numerous chemical and stereochemical factors which can be individually controlled should permit the evaluation of the influence of proximal base restraint, distal steric hindrance or distal site polarity upon dioxygen and carbon monoxide binding. Furthermore, as these systems should not require exogenous base, determination of the equilibrium and kinetic constants of O_2 and CO binding can be easily obtained either by direct photometric titration or by the technique of flash-laser photo-triggered ligand replacement introduced by Gibson for investigating hemoproteins (ref. 25,26). The comparison of these data (Table 1) reveals that these model compounds are undoubtedly of considerable importance in the qualitative assessment of structure-function relationships in the hemoproteins (ref. 27).

Differentiation of central and peripheral steric effects

There is a great influence from a distal inserted phenyl group in lowering O_2 and CO binding (compounds 7d and 14e). This results from a decrease of the association rate and an increase of the dissociation rate. But the decrease of O_2 binding is greater than that of CO binding in the ether series. An opposite situation prevails in the amide series. These kinetic results suggest a distinction between central and peripheral steric effects in the amide and ether series respectively due to a sideways displacement of the phenyl group as proposed by several authors (ref. 28,29). The greater rigidity of the amide linkage should reduce the amplitude of the lateral displacement of the phenyl residue. This should lower carbon monoxide affinity, by increasing its destabilization relative to dioxygen since the former prefers to bind in a linear manner. On the contrary, in the ether series a peripheral steric effect could decrease the dioxygen affinity more than CO since dioxygen adopts an end-on bent geometry.

Constraints on the proximal side

The strain effect engendered by the proximal handle length can greatly affect the equilibrium constants of CO and O_2 in the amide series (compounds 14a and 15a). The increase in the number of methylene carbon atoms in the chain connecting the pyridine ring to the macrocycle (from C_3 to C_4) decreases the equilibrium constant by a factor of 10, principally because of a larger dissociation rate. This finding is similar to the effect of the introduction of a methyl group at the 2 position of 1-methylimidazole when used as the proximal base in iron (II) "picket fence" compounds (ref. 30).

Table 1. Equilibrium and kinetic rate constants for the binding of CO and O_2 with "basket-handle" and "hybrid" porphyrins. solvent : toluene ; temperature : 20°C ; Im : 1-methylimidazole.
* Hb : hemoglobin (ref. 32) ; Mb : Horse myoglobin (ref. 33) in aqueous solution.

Compound	$10^{-7}x\ k^{CO}_B$ ($M^{-1}\ s^{-1}$)	$10^{3}x\ k^{-CO}_B$ (s^{-1})	$P_{1/2}^{CO}$ (Torr)	$10^{-7}x\ k^{O_2}_B$ ($M^{-1}\ s^{-1}$)	$10^{-3}x\ k^{-O_2}_B$ (s^{-1})	$P_{1/2}^{O_2}$ (Torr)	M
7a	6.8	69	$1.1\ 10^{-4}$	30	40	18	132000
7b	4.7	55	$1.2\ 10^{-4}$	27	42	21	132000
7c	4.7	196	$4.4\ 10^{-4}$	16	220	200	343000
7d	3	180	$6.2\ 10^{-4}$	10	400	700	680000
14a	3.5	30.4	$9\ 10^{-5}$	36	5	2	16400
14b	1	35.7	$3.7\ 10^{-4}$	15	2.8	2.6	5200
14c	0.93	82	$9.6\ 10^{-4}$	12	11	12.5	9649
15a	1.9	156	$8.5\ 10^{-4}$	30	47	21.8	18700
16a	4	6.7	$1.7\ 10^{-5}$	31.4	0.62	0.29	12000
17a	6.3	2.7	$4.44\ 10^{-6}$	62.3	0.13	0.03	4960
17b	0.18	2	$1.2\ 10^{-4}$	3	0.027	0.13	814
17c	0.1	2.63	$2.8\ 10^{-4}$	2.1	0.005	0.033	89
17d	0.008	8.16	$1.06\ 10^{-2}$	0.22	0.002	0.10	7
Hb*	0.65	10	$1.1\ 10^{-3}$	5.9	0.012	0.11	130
Mb*	0.05	17	$1.9\ 10^{-2}$	1.4	0.011	0.7	23

^1H NMR spectroscopy provided useful information on this surprising result (ref. 15). The spectrum of the pyridine C_4 derivative shows that the pyridine ring is confined in two equivalent planes passing through the pyrrole nitrogen and it undergoes a flip-flop movement. On the contrary, the pyridine ring in the C_3 compound could execute a continous libration around its average position in a plane directed along the meso carbon atoms. Thus the constraint imposed on the proximal base in the pyridine C_4 derivative may oppose the necessary re-organisation of the proximal base accompanying the movement of the iron atom towards the porphyrin plane on ligation with O_2 and CO. This interpretation follows the ideas of Perutz according to whom the constraints exerted upon the base by its environment (T state) restrains the transition to the low spin R state and leads to reduced ligand affinities in hemoglobin (ref. 31).

Distal polar effect

Changing the attachment mode of both the proximal and distal handles strongly modifies the O_2 affinity. It was found that the amide derivative had an affinity for O_2 an order of magnitude greater than did the ether analog (compounds 7a and 14a). This was due exclusively to a ten-fold reduction in O_2 dissociation rate. On the contrary, the CO affinities are not significantly modified.

This increase in stability of the "amide" oxygenated species was attributed to the presence of the two amide linkages and the possibility of hydrogen-bonding with the terminal oxygen atom of the liganded oxygen molecule. The low temperature (-27°C) ^1H NMR spectra were used to provide evidence for such an interaction (ref. 34) (Figure 2). The spectrum of the carbonylated iron (II) amide complex exhibits a C_2 symmetry with the pyrrolic protons equivalent, indicating a linear geometry of the CO molecule (ref. 35,36). All the amide protons appear as a single resonance. Replacing CO by O_2 gives a large inequivalence of the pyrrolic protons indicating a preferential orientation of the bent oxygen molecule (ref. 37) toward two opposite methene bridges. The observed C_2 symmetry results from a fast exchange between these two opposite positions. Examination of amide proton resonances confirms the direct interaction of O_2 with amide groups of the distal chain. The amide proton resonances, which are easily assigned by deuterium exchange, form two groups of two equivalent protons corresponding to the "distal" and the "proximal" chains at 8.2 and 6 ppm respectively, whereas they appear in the carbon monoxide derivative at 6.4 ppm, a position comparable to that of the diamagnetic zinc complex.

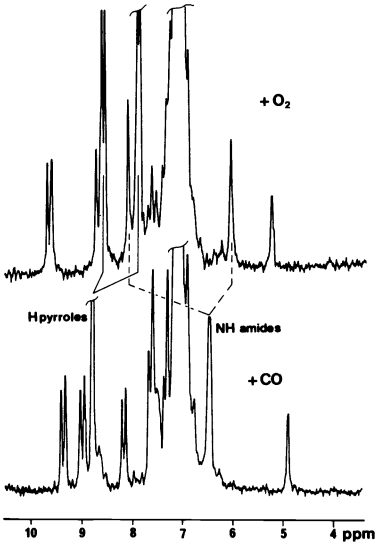


Fig. 2 100-MHz Proton n.m.r. spectra of CO (bottom) and O_2 (top) complexes of compound 15a in [$^2H_8$] toluene at -27°C (Ref. 27).

Ring current shift calculations from the ^1H NMR spectrum of the zinc complex allowed an estimate of the position of the distal amide protons and hence the nitrogen atoms. The distance between one of the amide nitrogen atoms and the terminal oxygen atom in a bent configuration may be close to 3 Å. This is consistent with an intramolecular hydrogen bond. Thus the direct interaction of an amide proton with the oxygen molecule is of the same general nature as those involving "distal" imidazole (E7) recently observed in neutron diffraction studies of oxymyoglobin (ref. 8). The relative strength of these hydrogen bonds could contribute to the control of the affinity of dioxygen for a-BHP and for hemoproteins exclusively by a decrease in dissociation rates. The greater stability of the a-BHP in comparison of the e-BHP corresponds to a gain in free energy of 5.4 $kJ.mol^{-1}$.

Proximal base electronic effect

The replacement of pyridine by an imidazole group enhances the affinity of the five-coordinated complex for O_2 and CO by a factor of 7 and 5 respectively (compounds 14a and 16a). The general trend is consistent with the greater basicity of imidazole. Thus the better model compound for dioxygen carrier hemoprotein active sites is that incorporating a pendant imidazole in the amide series (ref. 15). It is able to bind dioxygen giving a relatively stable oxygenated species (lifetime is about one day in dry toluene under 1 atm of dioxygen). However, even though the O_2 equilibrium constant is similar to those of hemoproteins, the imidazole model reacts 10 times faster with O_2 and dissociates about 100 times faster than the natural system.

Influence of the central steric hindrance

A comparison of the affinities of all "hanging-base basket-handle" models for CO with those of myoglobin or isolated hemoglobin chains shows that the synthetic compounds react 100 times faster than observed in hemoproteins, as fast as flat-open hemes. This suggests a weak central steric interaction which is proposed as the factor responsible both for the non linear geometry of the CO moiety (ref. 9) and for the low M ratio of the equilibrium constants for CO and O_2 ($M = K^{CO}/K^{O2}$). Such a low ratio has been correlated with the partial detoxification of carbon monoxide inhalation in respiring organisms. The question of steric differentiation between carbon monoxide and dioxygen in both-faces hindered hemes described above may be explained by the fact that the distal handle could be displaced sideways from its average position in the Fe-C (meso) plane preventing a steric effect from controlling the CO affinity. To solve this problem we have synthesized a series of single-face hindered porphyrins designed to hold the distal handle in a central position and thus to increase the central steric hindrance for gaseous ligands (ref. 38) (scheme 2). These so-called "hybrid" models (compounds 17a-d) have two pivalamido pickets (as "picket fence" porphyrins) on each side of an amide handle of variable length linked in a cross-trans configuration (as a-BHP). In this connection, Collman has prepared a series of "pocket" hemes containing a phenyl ring strapped unsymmetrically over the porphyrin plane (ref. 39). Some of these strongly encumbered systems showed a considerable decrease in CO affinity (ref. 40).

Except for the first compound of this series (compound 17a) bearing the largest handle, these iron (II) derivatives behave spectroscopically (U.V-visible, RMN) as five-coordinated hemes in the presence of 1-methylimidazole. The systematic decrease of the available space for iron-bound CO produces a decrease of affinity constants for this ligand by a factor 10-100 as compared with a-BHP. These reductions in affinity appear entirely in the association rates and is therefore attributed to central steric effects. No major change in the CO dissociation rates is observed. On the contrary their O_2 affinities are slightly higher than those of our a-BHP analogs. But the most remarkable fact is that O_2 affinities are changed by less than five-fold within the "hybrid" series whereas association and dissociation rate constants are simultaneously affected by factors as large as 300 and 60 respectively. The values of the partition coefficients, M, for the four "hybrid" complexes decrease from 5000 to 7 for the least encumbered compound to the most hindered one revealing a direct correlation with increased steric hindrance. These values are in reasonable agreement with those of hemoproteins (Table 1).

It has been suggested that the CO stretching frequency is indicative of steric effects in hemoproteins and the subsequent CO configuration. The υ CO in our four "hybrid" compounds (1958 to 1948 cm^{-1}) are intermediate between those of hemoproteins (1950-1945 cm^{-1}) (ref. 41,4) and single hemes or "picket fence" iron (II) porphyrins (1970 cm^{-1}) (ref. 42) in which CO binds to the iron atom in a linear fashion. A comparison with a-BHP complexes shows that the change of υ CO is discontinuous upon going from a-BHP to the hybrid series without a correlation change in affinity. We obtained crystals of the carbonyl derivative of complex 17a. X-ray analysis indicates that the Fe-C-O group is essentially linear as in flat open heme carbon monoxide crystals while the υ CO is lower (1958 cm^{-1}) (ref. 43). Contrary to our expectation no steric effect induces a Fe-C-O deformation ; all contacts with the terminal oxygen atom and polymethylene chain are larger than 4 Å. Thus, the relative importance of other factors such as electronic distribution within the heme, polarity of the distal cavity and/or distortion of the porphyrin core upon a particular ligand stretching frequency must be also taken into account.

On the other hand, these compounds have allowed us to answer the controversial question as to whether pivalamido pickets in oxygenated-iron (II) "picket fence" porphyrin could also undergo amide NH-hydrogen bonding with bound dioxygen by using IR spectroscopy in homogenous solution at ambient temperature (ref. 44,45). The spectra of the four "hybrid" complexes, in deoxy or carbonylated forms are characterized by an intense band from the N-H stretching vibration of both pickets and handle amide groups at 3420-22 cm^{-1}. By contrast, the IR spectra of the oxygenated adducts reveal two bands at 3420 and at 3372 to 3358 cm^{-1}. This last band is absent in the spectrum of the same "picket fence" derivative. The additional band at lower frequency is assigned to the N-H bond of one of the amide groups of the handle whose stretching frequency is shifted of about 50 cm^{-1} by intramolecular hydrogen interaction with the coordinated dioxygen and confirms the ^1H NMR data for oxygenated a-BHP complexes reported above. This shift is slightly dependent upon the length of the distal handle, presumably reflecting a variable degree of strain on the H-bonding interaction. These data provide more direct evidence that such H-bonding is absent with picket fence amides, confirming structural data in the crystalline state.

V. MOLECULAR ENVIRONMENT EFFECT IN REDOX AND COORDINATION CHEMISTRY OF IRON PORPHYRINS

The presence of amide linkage groups in superstructured porphyrins to create a protected side in which dioxygen is stabilized by polar interactions also exerts a pronunced influence on the redox and coordination chemistry of the central metal ion. Two lines of investigation have confirmed the strong tendency of iron-a-BHP to stabilize negatively charged complexes compared to e-BHP.

The first one concerns the stabilization of Fe (II) OH^- and Fe (II) Cl^- complexes (ref. 46,47). Binding of halides to Fe (II) porphyrins had been observed electrochemically (ref. 48), but there was no data concerning the possible formation of corresponding-iron (II) complexes with OH^- and the stability of the Fe-OH bond in both the iron (III) and iron (II) oxidation states. The efficient protection of iron porphyrin complexes by a basket handle in a cross-trans configuration against the formation of μ -oxo-dimers allows electrochemical investigation of hydroxo-iron (III) species.

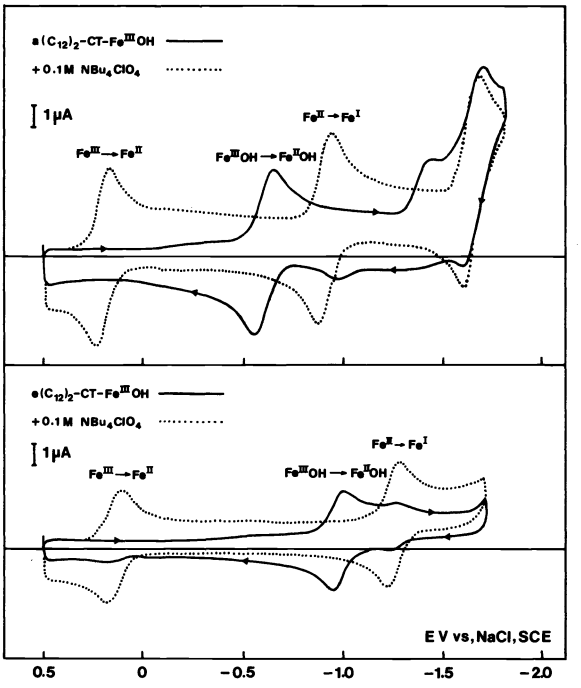


Fig. 3 Cyclic voltammetry of hydroxy complexes (——) and of the neutralized complexes (-------) in PhCN 0.1 M N Bu_4 ClO_4 (sweep rate O.1 V/s) : Compounds 11a-Fe (III) OH (top) and 2a-Fe (III) OH (bottom) (Ref. 47).

Cyclic voltammograms of a- and e-BHP Fe (III) OH^- complexes in benzonitrile with or without tetramethylammonium perchlorate exhibit successive reductions of Fe (III) into Fe (II) and Fe (I) complexes as is commonly observed with flat-open iron porphyrins (Figure 3). The reversible Fe (III)-Fe (II) wave, in the absence of ClO^-_4 undergoes a dramatic negative shift compared to the same wave obtained upon neutralization of the hydroxo complexes by perchlorate salt (0.81 and 1.15 V for the a- and e-BHP complexes, respectively). Whereas with a-BHP no trace of reduction of uncomplexed Fe (II)⟶Fe (I) appears on the cathodic scan beyond the $Fe(III)OH^-/Fe(II)OH^-$, small waves corresponding to the reduction and oxidation of uncomplexed Fe (II) appear on the cathodic and anodic traces with the e-BHP. This shows that the $Fe(II)OH^-$ complex of the a-BHP is very stable but it is less in the case of the ether-linked complex. The association constant (K_{OH^-}) determined by spectroelectrochemistry is a thousand times larger in the amide complex (2.3×10^6 M^{-1}) than in the ether complex (1.6×10^3 M^{-1}). This difference is, however, lower than that with the chloro complexes for which the ratio of association constants is 10^5 (a-BHP : 2.5×10^5 M^{-1} and e-BHP : 2.2 M^{-1}). Thus the effects of the anchoring groups appear opposite. In e-BHP these effects are essentially due to steric hindrance to solvation of negatively charged species by the handle whereas in a-BHP the observed effects could be related to the specific influence of NH-CO groups acting as a "built-in" acceptor solvent.

The second example is provided by the fixation of carbon monoxide to iron (I) (ref. 49). Electrochemical reduction of TPP-Fe (II) or e-BHP-Fe (II) in dimethylformamide (DMF) gives two reversible waves corresponding to the Fe (II)/ Fe $(I)^-$ and Fe $(I)^-$/Fe $(I)^{2-}$ couples, respectively. Upon addition of CO, the first wave, while remaining reversible, is shifted negatively by 290 mv in this complexing media. The second wave remains almost the same while a small cathodic wave appears at more negative potentials. The first wave corresponds to the formation of a complex formed upon electron transfer to Fe (II)-CO, formally the Fe $(I)^-$ CO. But under the reaction conditions this last complex is not stable and gives an Fe (I) species which is further reduced as revealed by the presence of the second reversible wave (Figure 4) (scheme 3).

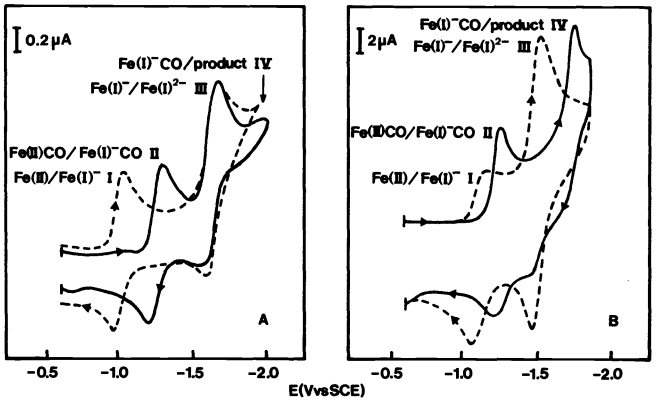
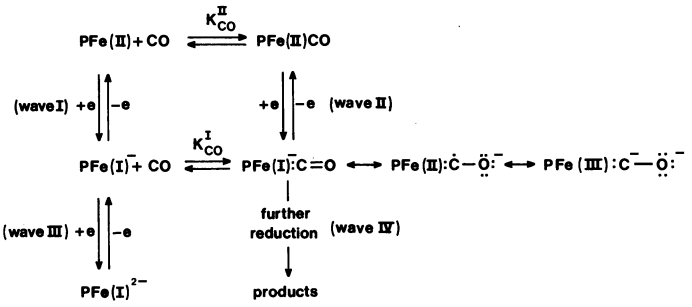


Fig. 4 Effect of CO on the cyclic voltammetry of iron (II) porphyrins at 20°C : (------), in the absence of CO, (——) in the presence of CO (1 atm pressure).

A : TPP-Fe (II) in DMF + 0.1 M NBu_4 BF_4 at 0.1 $V.s^{-1}$.
B : 16 a in PhCN + 0.1 M Bu_4NBF_4 at 0.2 $V.s^{-1}$ (Ref. 49).

Scheme 3



The same investigation using amide linkage substituent (a-BHP or "picket fence" porphyrin) showed that the wave Fe (II) CO/ Fe (I)$^-$ CO is reversible but it is less shifted in reduction potential. This reflects a ratio of the CO affinities for iron (II) and iron (I) significantly smaller than that observed with the preceding compounds (Figure 4). The second wave corresponding to the reduction of free Fe (I) is completely disappears at the expense of the wave at more negative potentials which features the futher reduction of the Fe (I)$^-$ CO complex. The comparison of the various porphyrins investigated in the presence of a given axial ligand shows that the stability of the Fe (I)$^-$ CO complex is greatly enhanced by the presence of amide-linked basket-handle. We found the following order of Fe (I)$^-$ CO stability in DMF : e-BHP < TPP < a-BHP < a-picket fence porphyrin. Another important effect is that of axial ligation : the presence of a strong complexing agent such as 1-methylimidazole strongly increases the Fe (I)$^-$ CO affinity. In this context, the integrated molecule bearing a pendant imidazole ligand in the amide series (16a) appears ideally suited for obtaining good fixation of CO on iron (I) even in a poor complexing solvent such as benzonitrile.

Combined use of cyclic voltametry, UV-visible, IR and ESR spectroscopies has provided evidence for carbonylated-iron (I) porphyrin adducts when an appropriate superstructure is attached to the porphyrin ring and when the reaction is carried out in a complexing medium. Thus the CO stretching frequency changes significantly in going from Fe (II) CO to Fe (I)$^-$ CO complex in benzonitrile in the presence of 1-methylimidazole toward smaller wavenumber (1966 to 1931 cm^{-1}). Such a shift is indicative of a substantial transfer of electron density from the iron atom to the CO molecule. A similar conclusion derives from ESR data. The ESR spectrum of the Fe (I)$^-$ CO at 77K appears as that of a low spin complex with three bands at g : 2.004, 1.995 and 1.842. It is considerably different from the spectrum of the Fe (I) $^-$ complex (ref. 50) but exhibits some resemblance with that of a low spin iron (III) complex (ref. 51). In addition, the absence of hyperfine coupling with the ^{13}CO and with the bound nitrogen of the 1-methylimidazole indicates the absence of a significant unpaired electron density in the $d_{z}2$ orbital of iron atom.

VI. CONCLUSION

Several conclusions emerge from the preceding studies :

1) The double protection of iron (II) ether-and amide-"basket-handle" porphyrin complexes, in a cross-trans configuration, efficiently prevents the formation of μ -oxo-dimers upon exposure to dioxygen.

2) Closely related "hanging-base" derivatives have been developed in which the structural parameters could be varied relatively softly. Kinetic data of ligand binding allowed us to appreciate with some confidence the relative importance of factors such as the hydrogen bond stabilization, the indirect constraints on the proximal base and the distinction between "proximal" and "distal" effects. "Hybrid" compounds have shown that CO affinity is more sensitive to the steric effects than is O_2 affinity. But it seems to us that a better understanding of the ligand binding properties of both the models and the biological systems will ultimately depend on a number of additionnal experiments : structural data on the O_2 and CO adducts, more complete thermodynamical data and synthesis of high-affinity O_2 complexes. This requires the preparation of new integrated and superstructured molecular systems that should show ligand binding characteristics greatly enhanced over those presently observed.

3) Superstructures (basket-handle or picket fence) have a significant influence on the redox and coordination chemistry of iron porphyrins. Stabilization of negatively charged species by the NH-CO linkage groups contributes to the strong coordination of Fe (II) by OH^- and Cl^- and that of Fe (I) by CO. These results allow speculation that polar interactions similar to those observed in the a-BHP may take place in hemoproteins in which a number of peptide bonds belonging to the surrounding protein chains might contribute to the redox and coordination properties of the prosthetic group.

4) Both-faces hindered porphyrins also allow the investigation of all possible spin states of ferric and ferrous porphyrins and the analysis of the various stereochemical parameters which govern the electronic and magnetic properties of the active site of hemoproteins.

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