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Cytochrome P-450 and synthetic models

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Abstract - Chemical model systems based on iron-porphyrins have been used in order to better understand the detailed molecular mechanisms of the reactions catalyzed by cytochromes P-450. Many porphyrin complexes containing different kinds of Fe-O, Fe-N or Fe-C bonds have been isolated from reactions mimicking those of cytochromes P-450, and completely characterized. This has largely contributed to show the existence of an important organometallic chemistry of cytochrome P-450 during its reactions with substrates. The participation of pyrrole nitrogens of the heme in several cytochrome-P-450 reactions has also been better understood thanks to the isolation of N-alkyl-porphyrin metallacyclic complexes. Catalytically active model systems using Fe- or Mn-porphyrins have been recently obtained. A proper choice of the porphyrin and axial ligands of the metal has led to very efficient systems which reproduce the cytochrome P-450 reactions, and particularly the monooxygenation of hydrocarbons by C_6H_5IO, ClO^-, H_2O_2 or O_2 itself in the presence of a reducing agent.

INTRODUCTION

Cytochrome P-450-dependent monooxygenases are widely distributed in living organisms. They catalyse the transfer of an oxygen atom from dioxygen into a substrate, the second oxygen atom being reduced into water thanks to the use of two electrons and two protons (eq.1).

$$RH + O_2 + 2H^+ + 2e^- \xrightarrow{\text{P-450}} ROH + H_2O \qquad \text{(eq. 1)}$$

These cytochromes P-450 are involved in many steps of the biosynthesis or biodegradation of endogenous compounds such as steroids, fatty acids, prostaglandins and leukotrienes. They play also a key role in the oxidative metabolism of exogenous compounds such as drugs and other environmental products, allowing their elimination from living organisms. These hemoproteins have been the subject of a huge amount of studies by researchers of many disciplins such as chemistry, biochemistry, pharmacology, toxicology and endocrinology. Thanks to these studies and, more particularly, thanks to a recent X-ray analysis of Pseudomonas Putida cytochrome P-450 (ref. 1), very much is known about the structure and function of cytochromes P-450 (for recent reviews, see ref. 2-4). Many spectroscopic studies have led to a good knowledge of the intermediate complexes involved in the catalytic cycle of dioxygen activation and substrate oxidation by cytochrome P-450. However, because of the high molecular weight of cytochromes P-450 (about 50 kD), it is still difficult to study the detailed mechanisms of substrate oxidations and to determine the molecular structure of the iron-metabolite complexes formed occasionally during the oxidation of particular classes of substrates. A possible approach to solve these problems is to use biomimetic chemical systems based on iron-porphyrins. During the last ten years, a great deal of work has been done in that direction and one can classify the studies that have been performed on iron-porphyrin biomimetic systems into two main groups depending on the objective of these studies. A first objective was to prepare and completely characterize iron-porphyrin complexes capable not only to mimic as well as possible the specific spectroscopic properties of the different iron intermediate complexes of the catalytic cycle of cytochrome P-450, but also to prepare model complexes exhibiting the particular coordination structures that were expected to occur in cytochrome P-450-iron-metabolite complexes (for previous reviews on these points, see ref. 5-7). A second objective of the use of iron-porphyrin models was to build up catalytically active chemical systems able to reproduce the various reactions catalyzed by cytochromes P-450. This strategy should have at least three main consequences : (i) to find homogenous catalysts for the selective hydroxylation of alkanes or of aromatic hydrocarbons under mild conditions, these two problems having so far no good answer in chemistry, (ii) to design efficient catalysts for regioselective and asymmetric oxidations in fine organic chemistry, and (iii) to have simple chemical systems for the preparation of the primary oxidized metabolites of a drug or of an exogenous compound in large amounts.

I. USE OF IRON-PORPHYRINS TO UNDERSTAND CYTOCHROME P-450 REACTIONS

I.1 Catalytic cycle of cytochrome P-450 and the iron-porphyrin models for its intermediate complexes

In its resting state, two forms of cytochrome P-450 are in equilibrium : a hexacoordinate low-spin iron(III) complex bearing two axial ligands, a cysteinate and presumably an OH-containing residue, and a pentacoordinate high-spin iron(III) complex with the cysteinate as only axial ligand. The binding of a substrate which occurs, in general, on a protein binding hydrophobic site close to the heme, leads to a shift of this equilibrium towards the pentacoordinate state. The high-spin enzyme-substrate complex is then reduced by one electron coming from NADPH via an electron transfer chain. The high-spin pentacoordinate ferrous complex derived from this step is able to bind many ligands such as CO, isocyanides, nitrogenous bases, phosphines and dioxygen. It is also able to transfer its electron to some substrates such as polyhalogenated compounds, nitroaromatics and amine-oxides, and this is the starting point of a possible reductive metabolism of these substrates (Fig. 1). The binding of O_2 to cytochrome P-450-Fe(II) leads to a relatively stable hexacoordinate low-spin complex. Model iron-porphyrin complexes for these four intermediates of the cytochrome P-450 catalytic cycle have been prepared and completely characterized by X-ray analysis (for reviews on that point, see ref. 5-7). This has largely contributed to give a detailed view of the variations of the coordination and spin state of the iron along the catalytic cycle of cytochrome P-450. Unfortunately, the only intermediate of this cycle which is not well known presently is the really oxidizing species . It is derived from a one-electron reduction of the ferrous-dioxygen intermediate and has a lifetime so short that it could not be studied so far by any spectroscopic technique. Our knowledge about its possible nature and about the final steps of the catalytic cycle is based on indirect evidence coming from studies on the characteristics of the oxidation reactions and on comparisons with better known active oxygen complexes derived from other hemoproteins or from iron-porphyrins. From all these data, the most likely mechanism for O_2 activation by cytochrome P-450 involves (i) an heterolytic cleavage of the O-O bond of a possible Fe(III)-O-O-H intermediate formed by one-electron reduction of the Fe(II)-O_2 complex, (ii) the formation of a high-valent iron-oxo complex derived formally from a two-electron oxidation of the ferric state and the binding of an oxygen atom to the iron, and (iii) the transfer of the oxygen atom of this iron-oxo complex into the substrate. Accordingly, single oxygen-atom donors such as C_6H_5IO, H_2O_2 or $NaIO_4$ can replace O_2 and NADPH for the cytochrome P-450-catalyzed oxidations of many substrates (ref. 2-4). The active oxygen complex of cytochrome P-450 oxidizes a wide range of compounds and performs diverse kinds of reactions such as the hydroxylation of C-H bonds, the epoxidation of double or triple bonds, the epoxidation and hydroxylation of aromatic rings and the transfer of its oxygen atom to sulfur, phosphorous or iodine atoms present in organic molecules. A high-valent porphyrin-iron-oxo complex corresponding formally to a Fe(V)=O structure

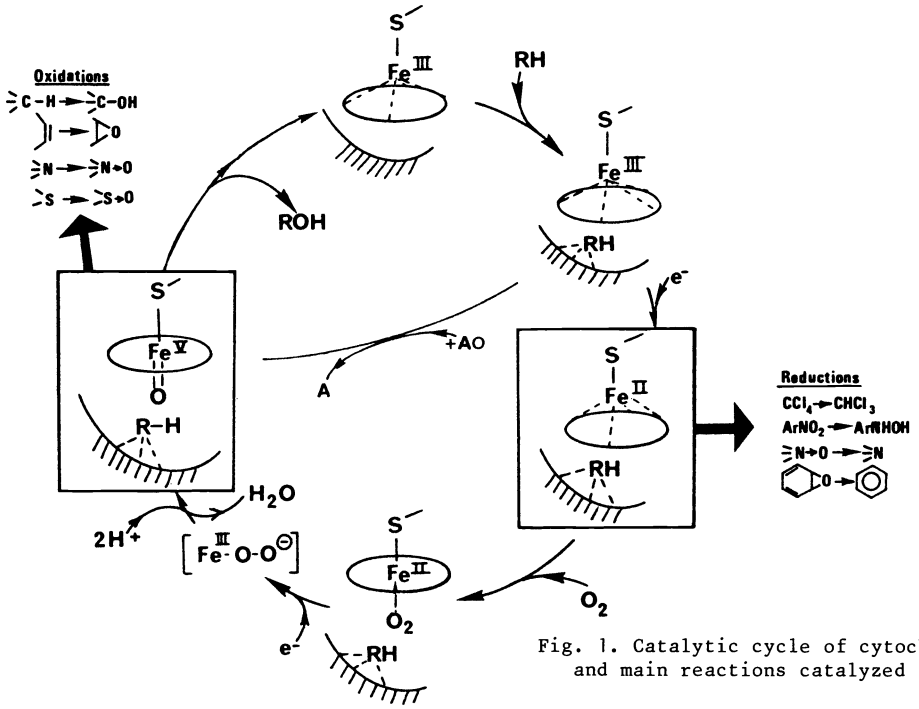


Fig. 1. Catalytic cycle of cytochrome P-450 and main reactions catalyzed

has been recently prepared by reaction of metachloroperbenzoic acid with Fe (tetramesityl-porphyrin = TMP)(Cl) (ref. 8) and studied by ^1H NMR, EPR, Mössbauer and EXAFS spectroscopy (ref. 9,10). All its characteristics are compatible with a (porphyrin-radical cation) Fe(IV) = O structure and similar to those of horseradish peroxidase compound I. Interestingly, this complex transfers its oxygen atom to alkenes almost quantitatively, as the equivalent complex of cytochrome P-450 does (Fig. 2). Recently, this complex has been prepared by electrochemical oxidation of (TMP)Fe(III)-OH (ref. 11,12).

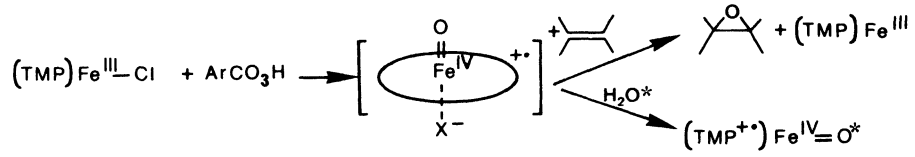
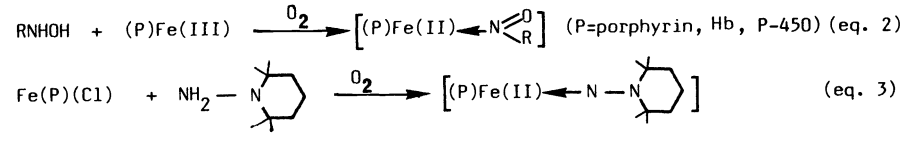


Fig. 2. Formation and properties of high-valent iron-oxo complexes (TMP = meso-tetramesityl-porphyrin)

I.2 Iron-porphyrin models for cytochrome P-450-iron-metabolite complexes containing Fe—N bonds

The intermediate complexes of the cytochrome P-450 catalytic cycle exhibit different types of Fe-O bonds such as those found in the [Fe(II)←O_2] or [Fe(V) = O] complexes. Nitrogen analogues of these complexes have been prepared by oxidation of nitrogenous compounds in the presence of iron-porphyrins. Oxidation of alkylhydroxylamines RNHOH by Fe(III)-porphyrins leads to very stable Fe(II)-nitrosoalkane complexes (ref. 13) (eq. 2).Various spectroscopic studies of these complexes including an X-ray analysis show that the RNO ligand is bound to the iron by its nitrogen atom and underline the great analogy between the Fe(II)-RNO and Fe(II)-O_2 bonds in porphyrin complexes (ref. 14). Such Fe(II)-RNO complexes are also formed upon oxidation of alkylhydroxylamines by metmyoglobin or methemoglobin (ref. 15) and upon metabolic oxidation of several amines by cytochromes P-450 (ref. 16-17). Nitrogen analogues of porphyrin-iron-oxo complexes have been prepared by oxidation of 1,1-disubstituted-hydrazines by ferric-porphyrins (ref. 18) (eq. 3). The X-ray structure determination of one of these complexes proved the existence of porphyrin-iron-nitrene complexes (ref. 18).



Stable cytochrome P-450-iron-metabolite complexes are formed upon oxidation of similar 1,1-dialkyl-hydrazines (ref. 19). It is likely that they also involve iron-nitrene bonds. As iron-nitrene complexes, the nitrogen analogues of iron-oxo complexes, do exist, it is likely that they could transfer their nitrene moiety into hydrocarbons as iron-oxo complexes transfer their oxygen atom into hydrocarbons. Accordingly, the potential nitrene donor, PhI = N-tosyl, associated with a catalytic amount of an iron-porphyrin, transfers its N-tosyl moiety into alkane C-H bonds (ref. 20) and to alkene double bonds (ref. 21). This transfer may occur efficiently and in a stereospecific manner thanks to a proper choice of the iron-porphyrin catalyst (ref. 22) (Fig.3). Interestingly, certain cytochromes P-450 were recently found able to catalyze the insertion of the N-tosyl group of PhIN-tosyl into C-H bonds of alkanes (ref. 23)

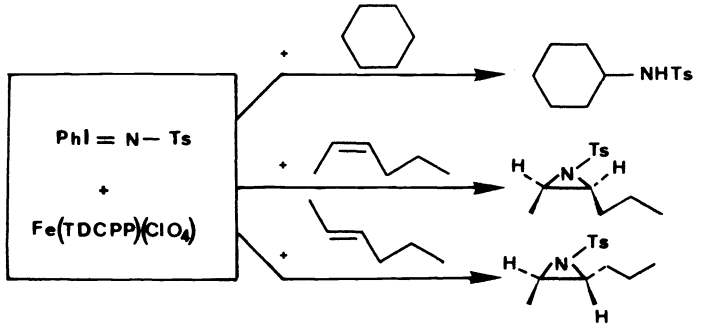


Fig. 3. Transfer of nitrenes into hydrocarbons catalyzed by iron-porphyrins (TDCPP = meso-tetra-2,6-dichlorophenyl-porphyrin ; Ts = SO_2-C$_6$H$_4$-CH_3)

I.3 Iron-porphyrin models for cytochrome P-450-iron-metabolite complexes containing Fe—C bonds

During the microsomal reductive metabolism of compounds containing reactive carbon-halogen bonds, such as CCl_4 or other polyhalogenomethanes (ref. 24), benzyl halides (ref. 25) and halothane, $CF_3CHClBr$ (ref. 26), cytochrome P-450-iron-metabolite complexes characterized by unusually redshifted Soret peaks (450-480 nm) are formed. Model reactions between such halogenated compounds and iron-porphyrins in the presence of a reducing agent in excess have led to the isolation and complete characterization of many complexes containing either σ-alkyl Fe(III) or carbene-Fe(II) bonds (ref. 27). For instance, the reduction of CCl_4 or of 1,1,1-trichloro-2,2-bis-parachlorophenyl-ethane (DDT) under these conditions leads respectively to an iron(II)-carbene (CCl_2) and to an iron(II)-vinylidene complex (eq. 4 and 5), whose structures have been proved by an X-ray structure analysis (ref. 28,29). When applied to CI_4 (ref. 30) or $PhCH_2SCCl_3$ (ref. 31), such reactions gave porphyrin-iron(II) complexes bearing a μ-carbido or a thiocarbonyl ligand and exhibiting very short Fe-C bonds (ref. 32,33)(eq. 6 and 7). Porphyrin-Fe(III)-σ-alkyl complexes are obtained upon reduction of benzyl halides (ref. 34) and $CF_3CHClBr$ (ref. 35)(eq. 8 and 9). The σ-alkyl complex derived from $CF_3CHClBr$ is stable under the used reductive conditions, whereas the corresponding Fe(III)-σ-alkyl complexes that could be formed as intermediates in the reduction of many polyhalogenomethanes like CCl_4 undergo the loss of a second halogen atom and lead eventually to Fe(II)-carbene complexes (ref. 35). Interestingly, very similar reactions seem to occur in the case of cytochrome P-450 since its iron-metabolite complex derived from CCl_4 reduction exhibits no EPR signals and releases CO upon hydrolysis as expected for a diamagnetic [Fe(II)←CCl_2]complex (ref. 24), whereas its iron-metabolite complex derived from $CF_3CHClBr$ reduction exhibits EPR signals expected for a σ-alkyl Fe(III)-$CHClCF_3$ complex (ref. 36).

$$(P)Fe(II) + CCl_4 \xrightarrow[-2Cl^-]{+2e^-} \left[(P)Fe(II) \leftarrow CCl_2\right] \qquad \text{(eq. 4)}$$

$$(P)Fe(II) + Cl_3CCHAr_2 \xrightarrow[-2Cl^- \; -HCl]{+2e^-} \left[(P)Fe(II) \leftarrow C{=}CAr_2\right] \qquad \text{(eq. 5)}$$

$$2(P)Fe(II) + CI_4 \xrightarrow[-4I^-]{+4e^-} \left[(P)Fe{=}C{=}Fe(P)\right] \qquad \text{(eq. 6)}$$

$$(P)Fe(II) + RSCCl_3 \xrightarrow[-2Cl^-]{+2e^-} \left[(P)Fe(II)\leftarrow C\begin{matrix}SR\\Cl\end{matrix}\right] \rightarrow \left[(P)Fe(II)\leftarrow CS\right] \qquad \text{(eq. 7)}$$

$$(P)Fe(II) + ArCH_2X \xrightarrow[-X^-]{+e^-} \left[(P)Fe(III) - CH_2Ar\right] \qquad \text{(eq. 8)}$$

$$(P)Fe(II) + CF_3CHClBr \xrightarrow[-Br^-]{+e^-} \left[(P)Fe(III) - CHClCF_3\right] \qquad \text{(eq. 9)}$$

Cytochrome P-450-iron-metabolite complexes, exhibiting spectral characteristics (Soret peaks between 470 and 480 nm) and reactivity very similar to those derived from halothane or benzyl halides reduction, are formed upon oxidative metabolism of hydrazines $RNHNH_2$ (ref. 37,38). Oxidation of these hydrazines in the presence of iron-porphyrins leads successively to iron(II)-diazene and iron(III)-σ-alkyl complexes (ref. 39)(eq.10). The same reactions seem to occur in the presence of myoglobin and hemoglobin and the eventual formation of a stable myoglobin-Fe(III)-Ph complex upon oxidation of $PhNHNH_2$ was definitively established thanks to an X-ray structure analysis of this complex (ref. 40). Thus, a similar σ-alkyl (or aryl)-Fe(III) structure appears highly probable for the cytochrome P-450-iron-metabolite complexes formed upon oxidation of hydrazines $RNHNH_2$.

$$(P)Fe(III) + RNHNH_2 \xrightarrow{ox.} \left[(P)Fe(II) \leftarrow NH{=}NR\right] \xrightarrow[-N_2]{ox.} \left[(P)Fe(III){-}R\right] \qquad \text{(eq. 10)}$$

The formation of cytochrome P-450 complexes involving an iron-carbon bond has also been proposed during the hydroxylation of C-H bonds (ref.6). Indirect evidence suggests that the very stable iron-metabolite complexes formed upon oxidative metabolism of benzodioxole derivatives are iron-carbene complexes (ref. 6,41). Model iron-porphyrin complexes exhibiting the proposed iron-carbene bond have been prepared and characterized (ref. 42) (eq. 11). A mechanism has been proposed for the formation of iron-carbene complexes upon the oxidation of benzodioxole derivatives by cytochrome P-450 (ref. 6). It involves the intermediate formation of a high-valent-σ-alkyl iron complex which leads eventually to the stable iron-carbene complex by elimination of water (Fig.4). From these data, it is tempting to speculate that, in a more general manner, the cytochrome P-450-dependent hydroxylation of compounds containing C-H bonds involves the intermediate formation of transient σ-alkyl complexes (ref. 6, 43). These complexes would be formed by combination of the two species derived from the abstraction of

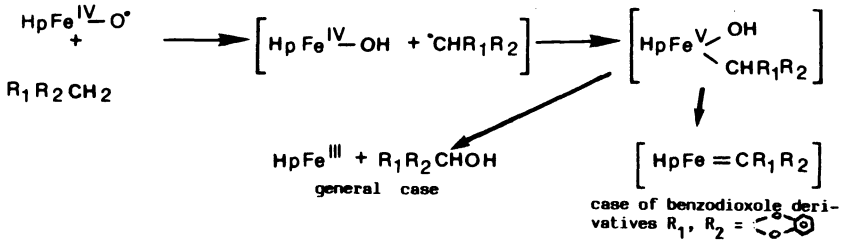
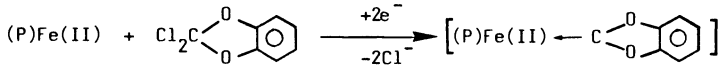


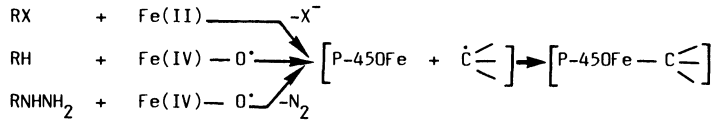
Fig. 4. Possible mechanism for C-H bond hydroxylations by cytochrome P-450, and for the formation of a carbene complex in the case of benzodioxole derivatives

the C-H hydrogen atom by the high-valent iron-oxo complex, the substrate-derived free radical and the cytochrome P-450-Fe(IV)-OH intermediate (Fig.4). In general, these σ-alkyl complexes would undergo a cis-elimination of their OH and alkyl ligands with formation of the hydroxylated metabolite and regeneration of cytochrome P-450Fe(III). The formation of these σ-alkyl complexes could be a way by which the substrate-derived free radicals would be controlled by the cytochrome P-450-iron inside the hydrophobic pocket of this hemoprotein. Such an efficient control would explain the high stereospecificity observed for some cytochrome P-450 dependent hydroxylations.



(eq. 11)

Although the actual structures of the aforementioned cytochrome P-450-iron-metabolite complexes remain to be established, by X-ray analysis for instance, the present evidence based on spectral and chemical properties of these complexes and the data accumulated on model reactions using iron-porphyrins strongly suggest the existence of an **important organometallic chemistry** of cytochrome P-450. During its reactions with many substrates, complexes involving an iron-alkyl or an iron-carbene bond are formed. Some of them are stable and have been studied by spectral methods (ref. 24-26, 36-38, 41 for instance, see also reviews 6 and 44). However, most of them could be formed as transient intermediates during hydroxylation of C-H bonds or epoxidation of double bonds, as discussed previously (ref. 43). This would lead to a possible common situation for the very diverse cytochrome P-450 reactions, where a free radical derived from the substrate by a reductive or oxidative activation is formed in close proximity of the iron and is more or less efficiently controlled by iron-carbon bond formation (eq. 12).



(eq. 12)

A recent peculiar result on the epoxidation of propene by a purified cytochrome P-450 illustrates well the importance of this organometallic chemistry of cytochrome P-450. The deuterium-hydrogen exchange observed during the epoxidation of trans-1-deutero-propene has been explained by the mechanism indicated on Fig.5 (ref. 45). Its first step is the formation of a four-membered metallacycle after addition of the iron-oxo species to the double bond and combination of the intermediate free radical with the iron as proposed in eq. 12. Indirect evidence for the existence of such metallacycles in model iron-porphyrin systems has been

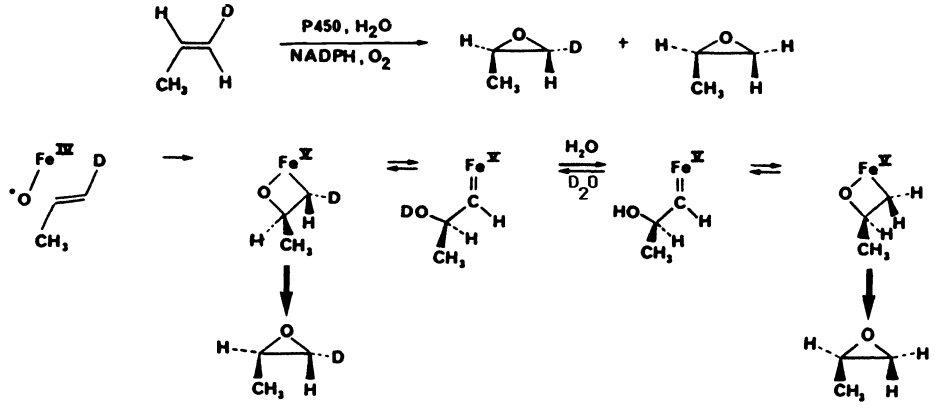
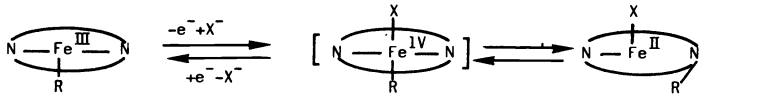


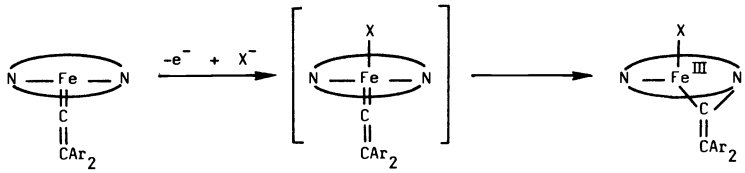
Fig. 5. Hydrogen-Deuterium exchange during propene epoxidation by cytochrome P-450 : a possible mechanism

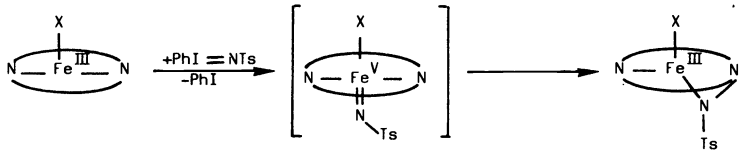
published (ref. 46,47). This metallacycle could be in equilibrium with a carbene complex after α-elimination of D^+. After fast D/H exchange at the level of the OD(H) function and nucleophilic addition of OH(D) on the Fe = C double bond, it is also in equilibrium with the corresponding metallacycle where the deuterium atom has been replaced by a hydrogen atom. By reductive elimination these two metallacycles lead to a mixture of the deuterated or non-deuterated epoxide. This mechanism which implies the intermediate formation of iron-carbon σ bonds and iron-carbene bonds is consistent with the results described in chapter I-3, and rationalizes experimental data that are difficult to explain otherwise.

I.4 N-alkylporphyrin-iron complexes involving metallacyclic structures: participation of pyrrole nitrogens in some cytochrome P-450 reactions

It is now clear that in many cytochrome P-450 reactions, green pigments coming from N-alkylation of its heme are formed (ref. 44). Is has been shown that the one-electron oxidation of porphyrin-Fe(III)-alkyl (or aryl) complexes led to the migration of their alkyl ligand to a pyrrole nitrogen (ref. 48)(eq. 13). The propensity of high-valent iron-porphyrin complexes to undergo a migration of an axial ligand from the iron to a pyrrole nitrogen has been also shown in the case of vinylidene (ref. 49) and nitrene (ref. 50) complexes. The corresponding final complexes showing Fe-C-N or Fe-N-N structures have been isolated and completely characterized (eq. 14, 15). Interestingly, their oxa-analog with a Fe-O-N structure has been recently described (ref. 51)


(eq. 13)


(eq. 14)


(eq. 15)

The N-alkylporphyrins isolated from the iron-porphyrin-catalyzed oxidation of monosubstituted alkenes (ref. 52) could also derive from the migration of the σ-alkyl ligand of a possible four-membered metallacycle from the iron to a pyrrole nitrogen (Fig. 6).

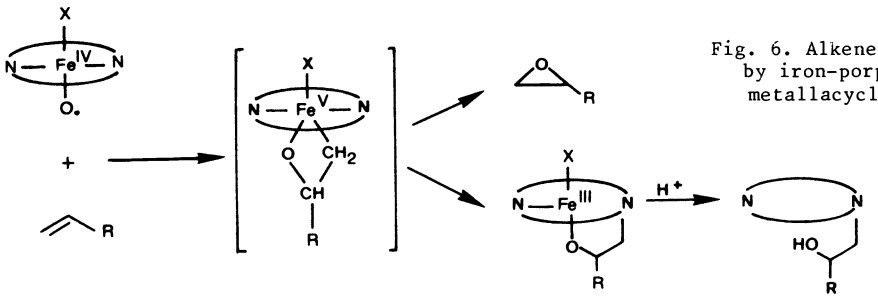
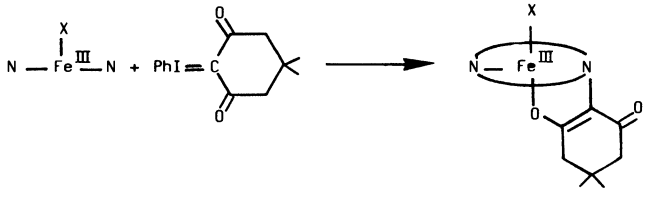


Fig. 6. Alkene oxidation catalyzed by iron-porphyrins. Possible metallacyclic intermediates

Preliminary spectral studies are in agreement with the resulting metallacyclic Fe - O - C - C - N structure for the final complex formed in these reactions (ref. 52a). Iron-porphyrin complexes involving such a metallacyclic structure have been prepared by reaction between


(eq. 16)

iron-(III)-porphyrins and an iodonium ylid PhI = CRR' (eq. 16), and have been completely characterized (ref. 53). All these results on iron-porphyrin model systems strongly suggest that in many situations, where a high-valent iron intermediate complex containing a σ-alkyl (or aryl) or a carbene ligand is involved, N-alkylporphyrins may be formed. In fact, it is likely that the various N-alkylporphyrins isolated after oxidative metabolism of mono-substituted alkenes or alkynes, dihydropyridines or dihydroquinolines, monosubstituted hydrazines or 1-amino-benzotriazole (ref. 44) result from such migrations of an alkyl or aryl ligand of a high-valent iron intermediate from the iron to a pyrrole nitrogen. The mechanism proposed recently for the oxidative activation of a sydnone derivative to a species that N-alkylates the heme of cytochrome P-450 is given on Fig 7 (ref. 44). It is based on several reactions that were proved on model systems, such as the formation of an iron-carbene complex (formally a Fe(V) = CHR or Fe(III)←CHR complex) analogous to the intermediate of eq. 14, its isomerisation into a brigded Fe-C-N complex analogous to the final complex of eq. 14 that was completely characterized, and the formation of a N-substituted porphyrin after acidic demetallation, as observed for the model bridged complex (ref. 54).

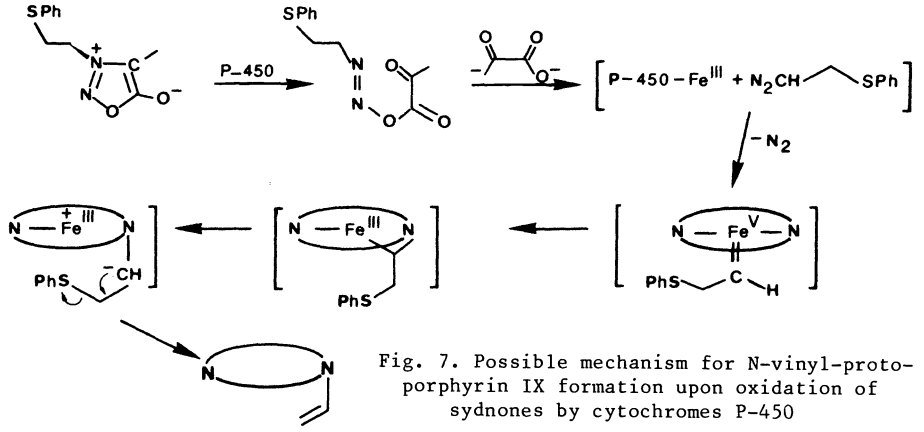


Fig. 7. Possible mechanism for N-vinyl-proto-porphyrin IX formation upon oxidation of sydnones by cytochromes P-450

I.5 The very rich coordination chemistry of cytochrome P-450 and iron porphyrins

The iron-ligand structures that have been found so far in model iron-porphyrin complexes are schematically depicted in Fig. 8. Most of these structures have been definitively established by X-ray analysis. An always growing body of evidence indicates that most of these structures are also involved in cytochrome P-450-dependent reactions. Thus, model studies have largely contributed to establish this exceptional richness of the coordination chemistry of cytochrome P-450 and to understand the detailed mechanisms of cytochrome P-450 reactions.

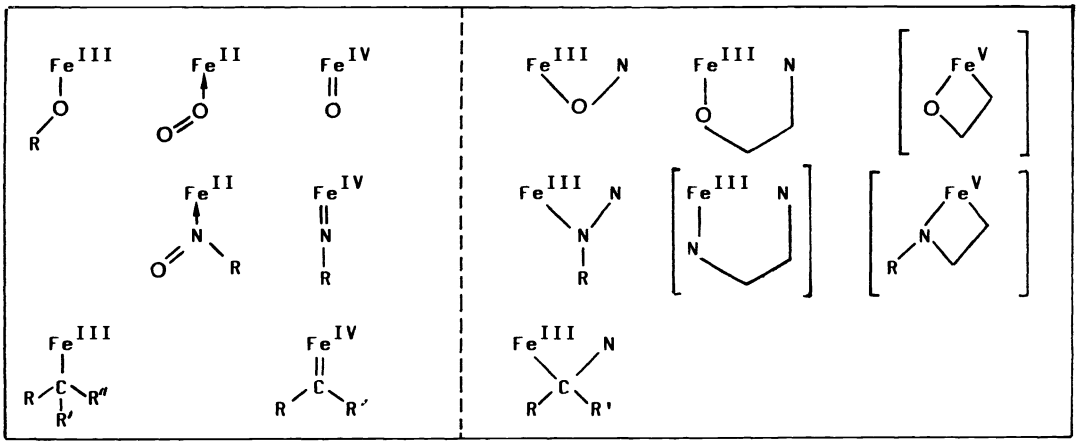


Fig. 8. Different coordination structures proved for iron-porphyrin complexes and proposed for cytochrome P-450 complexes (structures into brackets have been proposed but not established)

II. CATALYTICALLY ACTIVE METALLOPORPHYRINIC MODELS FOR CYTOCHROME P-450

II.1 How to build up catalytically active models for cytochrome P-450?

The following analysis will be restricted to reported model systems based on a metalloporphyrin catalyst. An ideal model system would associate to the metalloporphyrin (preferably an iron-porphyrin) a thiolate ligand to mimic the axial cysteinate ligand of cytochrome P-450, a reducing agent and a proton donor and O_2 itself as the oxygen atom donor (eq.1). However, because of the high oxidizing activity of the active oxygen metal-oxo intermediates in such systems, the use of thiolate ligands that are very reactive toward oxidizing agents seems very difficult. The long catalytic cycle of substrate oxidation by cytochrome P-450, using O_2 and NADPH, appears difficult to mimic for two main reasons. The monooxygenase system allows one a complete separation of the iron-oxo species from the reducing agent (NADPH), since the electrons from NADPH are transferred to the heme via an electron transfer chain. In chemical models, it seems difficult to separate the metal-oxo active species from the reducing agent in excess. This explains the very low yields of substrate oxidation based on the reducing agent observed so far with model systems using O_2 itself (see below). The other role of the protein is to regulate the sequence of the different steps of the catalytic cycle. With a simple chemical system, it appears by far easier to mimic the shortened catalytic cycle (Fig.1) that uses single oxygen atom donors AO. However, the level of difficulty to generate a high-valent iron-oxo complex upon reaction of an iron-porphyrin with an oxygen atom donor will depend on the nature of this oxidant. It is likely that oxidants containing only one oxygen atom linked to a leaving group such as PhIO or ClO^- should transfer more easily their oxygen atom to metalloporphyrins than oxidants containing a O - O bond, such as alkylhydroperoxides or H_2O_2, for which two modes of cleavage of this O-O bond (homolytic and heterolytic) are possible. These considerations explain the order of presentation of model systems in the following.

The reactions to be performed by these systems are mainly the hydroxylation of alkanes, the epoxidation of alkenes and the hydroxylation of aromatic rings as well as the transfer of an oxygen atom to N,S or P heteroatoms (Fig. 1) under mild conditions. Very efficient model systems usable in preparative chemistry should have the three following characteristics : (i) a good catalytic activity (around 10 turnovers per min, a mean value for cytochromes P-450), (ii) a good stability of the metalloporphyrin catalyst in the oxidizing medium, and (iii) a good selectivity for the oxidation reactions (regioselectivity and stereospecificity). The aim of the following chapters is not to give a detailed review of the results obtained this last six years in that field (for such reviews, see ref. 22,55,56) but only to describe its general evolution and to underline its progress.

II.2 Model systems using oxidants containing a single oxygen atom

Since the first report by Groves et al. (ref. 57) showing that iodosylbenzene, PhIO, in the presence of catalytic amounts of iron-meso-tetraarylporphyrins was able to epoxidize alkenes and to hydroxylate alkanes, a great deal of work has been devoted to iodosoarene-iron-porphyrin systems. It is now clear that these systems are able to perform all the reactions catalyzed by cytochromes P-450 with very similar characteristics. For instance, alkane hydroxylation occurs with high isotopic effects (k_H/K_D of about 13 for cyclohexane (ref. 58)), retention of configuration of the C-H bond (for hydroxylation of cis-decaline into 9-decalol (ref. 58,59)) and preferential reaction on tertiary C-H bonds (ref. 58) (Fig.9).

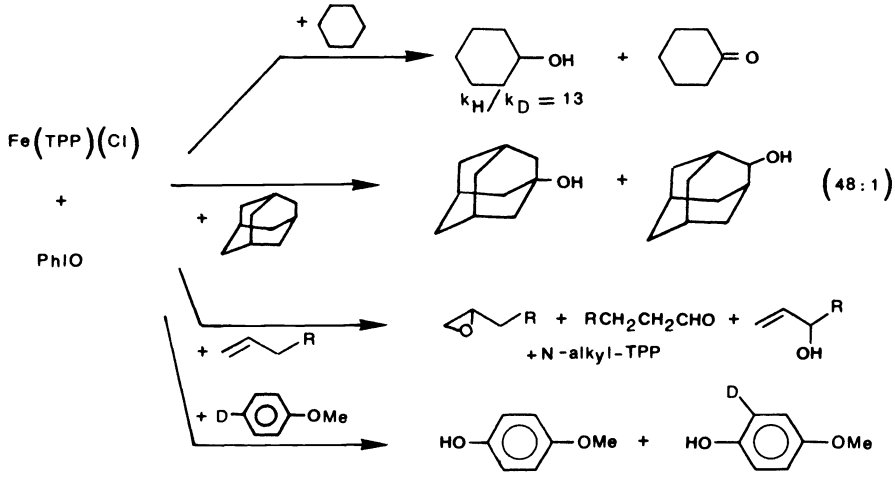


Fig. 9. Some characteristics of oxidations by PhIO catalyzed by iron-porphyrins

The four reactions observed upon cytochrome P-450-dependent oxidation of alkenes-i.e. the stereospecific epoxidation of the double bond (ref. 60,61), the hydroxylation of allylic C-H bonds (ref. 62), the minor formation of aldehydes RCH_2CHO from $RCH=CH_2$ (ref. 60, 63, 64) and the transformation of the iron-porphyrin catalyst into a N-alkyl-porphyrin (ref. 52, 65) - have also been reproduced by these model systems. Moreover, hydroxylation of aromatic hydrocarbons occurs with migration of the hydrogen (or deuterium) atom present at the hydroxylation site in the ortho position ("NIH shift") (72% retention of deteurium in para-methoxy-phenol formed by hydroxylation of para-deutero-anisole (ref. 66)) (Fig.9). Simple iron-tetraaryl-porphyrins such as Fe(TPP = meso-tetra-phenylporphyrin)(Cl) are oxidatively destroyed at least in part during the oxidation reactions. Thus, major improvements of the half-times and also of the catalytic activities of these systems were obtained by using tetraaryl-porphyrins containing halogen-substituted aryl groups such as the pentafluorophenyl (ref. 66) or 2,6-dichlorophenyl (ref. 67) groups. For instance, the iron-porphyrin, Fe(TDCPP = tetra-2,6-dichlorophenyl-porphyrin)(Cl), catalyzes alkene epoxidation by C_6F_5IO with an initial rate as high as 300 turnovers per second, and more than 10,000 moles of epoxide are obtained per mole of catalyst (ref. 68). The catalytic activity of porphyrin complexes of different metal ions has been evaluated. Fe(III)- and Mn(III)-porphyrins appear as the best catalysts. In the design of modified metalloporphyrin catalysts for selective oxidations, two series of results are especially interesting. With the very hindered Mn(III)-tetra-[2,4,6-triphenyl]-phenyl-porphyrin catalyst, the hydroxylation of linear alkanes occurs mainly in ω and ω-1 positions (ref. 69). By comparison, the hydroxylation of n-hexane by PhIO catalyzed by the non-hindered catalyst Mn(TPP)(Cl) occurs mainly in ω-2 position. With iron-porphyrins bearing either pickets containing chiral binaphtyl groups (ref. 70) or chiral "basket-handles" (ref. 71) on both sides of the porphyrin ring, asymmetric epoxidation of styrenes is obtained with an enantiomeric excess up to 50% (Fig. 10).

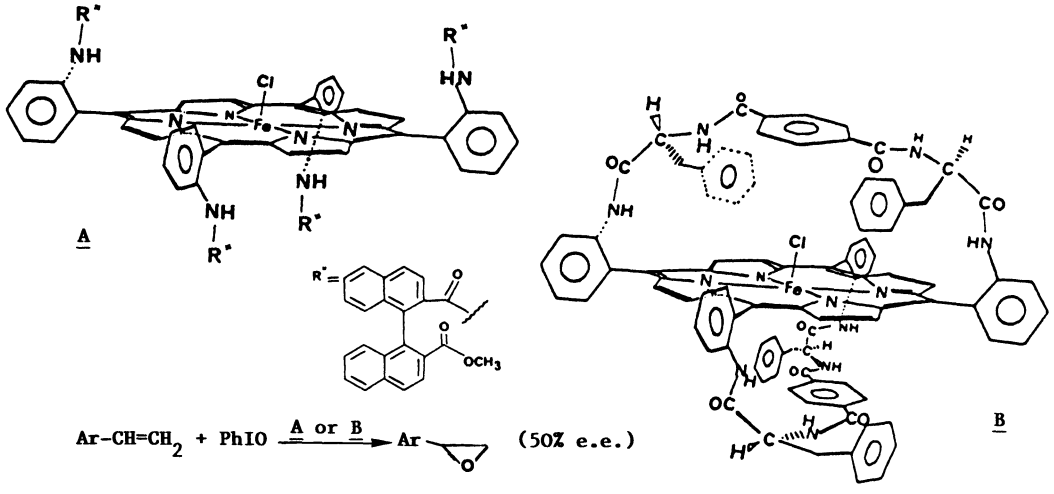
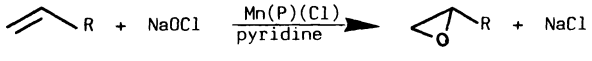


Fig. 10. Asymmetric epoxidation of styrene catalyzed by chiral-porphyrins

Other oxidants containing a single oxygen atom such as hypochlorites (for a review, see ref. 55) or tertiary amine-oxides (ref. 72) associated with Fe- or Mn-porphyrins lead to results similar to those obtained with PhIO. In particular, in the presence of Mn(III)-porphyrins and axial ligands such as pyridines or imidazoles, NaOCl becomes able to epoxidize a large series of alkenes (ref. 73, 74), with initial rates up to ten turnovers per second (eq.17).

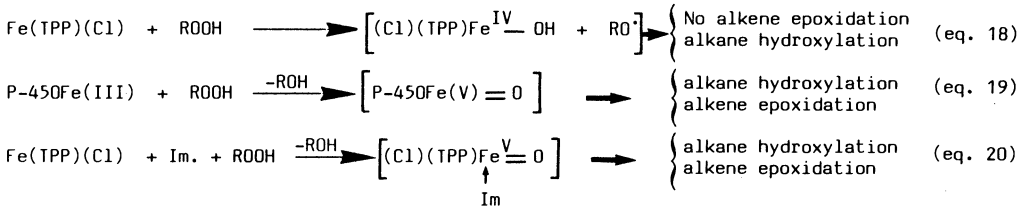


(eq. 17)

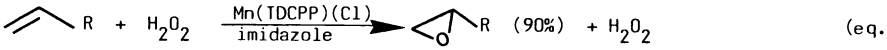
II.3 Model systems using alkylhydroperoxides or H_2O_2

Whereas reaction of PhIO with Fe(TPP)(Cl) leads to a high valent iron-oxo complex capable of epoxidizing alkenes and of hydroxylating alkanes, alkylhydroperoxides ROOH (R = cumyl or t-butyl), in the presence of the same catalyst, Fe(TPP)(Cl), hydroxylate alkanes but fail to epoxidize alkenes (ref. 75). Thus different active oxygen complexes are involved in the PhIO- and ROOH-dependent systems. Reaction of cumylhydroperoxide with Fe(TPP)(Cl) does not lead to a heterolytic cleavage of the alkylhydroperoxide O-O bond that would have given the Fe(V)=O species characteristic of the PhIO system, but to a homolytic cleavage of this bond with formation of the cumyloxy radical as the active species (ref. 75)(eq. 18). On the contrary, cytochrome P-450 is able to catalyze alkene epoxidation by cumylhydroperoxide (eq. 19). This suggests that its strong electron-donating cysteinate ligand could play a key role by

favoring an heterolytic cleavage of the O-O bond (ref.22, 56). Imidazole seems to play a similar role since its addition to the Fe(TPP)(Cl)- or Mn(TPP)(Cl)-cumylhydroperoxide system

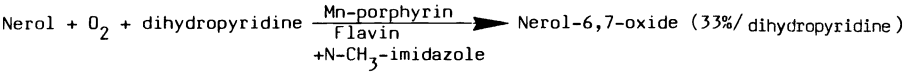


makes them able to epoxidize alkenes such as cyclooctene, cis-stilbene and 2-methyl-hept-2-ene with yields between 20 and 50% (eq. 20) (ref. 76). Accordingly, kinetic studies on reactions of various peroxidic compounds with Fe(III)and Mn(III)-porphyrins show that the heterolytic cleavage of their O-O bond and formation of a high-valent metal-oxo species occur more easily in the presence of nitrogenous bases such as imidazole (ref. 77). It was also found that, in the presence of imidazole, Mn(III)-porphyrins catalyze the epoxidation of alkenes and the hydroxylation of alkanes by the readily available oxidant H_2O_2 in a very efficient manner. Stereospecific epoxidation of various alkenes are obtained with high yields and good rates (up to 100 turnovers per min) (ref. 78) (eq. 21). Alkanes are oxidized with good conversions and yields (ref. 79).

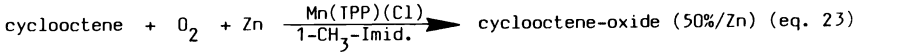


II.4 Model systems using O_2 and a reducing agent

Various systems using catalytic amounts of Fe(III)- or Mn(III)-porphyrins and various reducing agents such as borohydrides (ref. 80,81), sodium ascorbate (ref. 82), H_2 in the presence of Pt (ref. 83) or electrons coming from an electrode (ref. 84) have been found to perform the oxidation of hydrocarbons by O_2 itself. A detailed analysis of alkene epoxidation by one of these systems (ref. 83) shows that a crucial step is the heterolytic cleavage of the O-O bond of a metal- $O-O^-$ intermediate. The presence of protons or of an acid anhydride facilitates greatly this step. In fact, its was found recently that the heterolytic cleavage of the O-O bond in porphyrin-Fe(III)-O-O-COR complexes involved a very low enthalpy of activation (4 Kcal per mole) (ref. 85). This easier cleavage of the O-O bond of Fe(or Mn)-superoxo intermediates after acylation of the terminal oxygen atom by acyl chlorides or anhydrides was successfully used in systems based on Mn- (ref. 86) or Fe-porphyrins (ref. 87). These O_2-dependent systems reproduce qualitatively the main reactions of cytochrome P-450 and particularly the hydroxylation of alkanes under mild conditions. However, their efficacy, in terms of catalytic activity (0.1 to 36 turnovers per h) and of yields based on the reducing agent (0.1 to 5%) remains inferior to that of the enzymatic system. The very low yields based on the reducing agent in model systems is due to a reduction of the metal-oxo species by the reducing agent which is faster than its reaction with substrates because of the lack of separation between this oxidizing species and the reducing agent. The best yield reported so far, 56%, was obtained with an electrochemical system and relatively slow arrival of the electrons as the rate of cyclooctene epoxidation remained low (about 2 turnovers per h) (ref. 84). Very recently two systems were reported to give good yields based on the reducing agent (up to 50%) and higher rates (up to 9 turnovers per min). The first one employs a dihydropyridine as a reducing agent in the presence of a flavin mononucleotide, and a water soluble Mn-porphyrin and N-methyl-imidazole as catalysts. It epoxidizes nerol with good rates (9 turnovers per min) and yields based on the reducing agent (up to 33%) (ref. 88)(eq.22). The



second system uses Zn powder as a reducing agent, O_2 as the oxygen atom donor, CH_3COOH as a source of protons and Mn(TPP)(Cl) and N-methyl-imidazole as catalysts (ref. 89). It epoxidizes various alkenes with rates between 0.5 and 3.5 turnovers per min and good yields based on the reducing agent (up to 50%) (eq. 23). Moreover, it hydroxylates alkanes with rates (around 0.5 turnover per min) and yields (around 15%) which remain satisfactory. . It is noteworthy that the presence of N-methylimidazole is absolutely required in this system and that, in a more general manner, imidazoles in most of the other reported systems led to a clear improvement of their efficacy.



CONCLUSION

The use of iron-porphyrins to mimic cytochrome P-450 reactions has led to the isolation and complete characterization of many complexes containing different kinds of Fe-O, Fe-N or Fe-C bonds, as well as of N-alkylporphyrin metallacyclic complexes. This has largely contributed (i) to show the existence of an important **organometallic chemistry of cytochrome P-450** during its reactions with substrates, (ii) to reveal the **exceptional richness of the coordination chemistry of cytochrome P-450** and iron-porphyrins, and (iii) to better understand the detailed molecular mechanisms of cytochrome P-450 reactions. Major progress has been made in the obtaining of efficient catalytically active model systems for cytochrome P-450. Thanks to the use of pyridine or imidazole cocatalysts associated with Fe(III)- or Mn(III)-porphyrins, various systems using not only iodosoarenes but also readily available oxidants such as ClO^-, alkylhydroperoxides and H_2O_2 have been found able to reproduce most cytochrome P-450 reactions. Thanks to the use of Fe- or Mn-tetrameso-aryl-porphyrins very resistant toward oxidative degradation, catalytic activities as high as 10 to 300 turnovers per second have been obtained for alkene epoxidation, the catalysts being able to effect at least 100,000 turnovers. In fact, these catalysts are more potent and more resistant than cytochrome P-450 in the presence of the same oxidants. These systems can be used now for preparative organic chemistry as they lead to complete substrate conversions. Important efforts remain to be done to improve the efficacy of the model systems using O_2 itself as oxygen atom donor and to make the catalysts capable of performing selective oxidations. In that regard, it is noteworthy that first very encouraging results on regioselective hydroxylation of linear alkanes and asymmetric epoxidation of alkenes have been obtained already.

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