New Catalytic Properties of Iron Porphyrins: Model Systems for Cytochrome P450-Catalyzed Dehydration of Aldoximes

Jason Hart-Davis, Pierrette Battioni, Jean-Luc Boucher, and Daniel Mansuy*

Contribution from the Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques (URA 400 CNRS), Université Paris V, 45 rue des Saints-Pères, 75270 Paris, France

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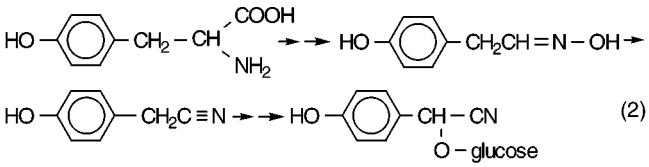
Abstract: Various iron porphyrin systems were found to catalyze the dehydration of aldoximes, such as heptanaldoxime or phenylacetaldoxime, into the corresponding nitriles under mild conditions ($t = 20$ °C, neutral or slightly acidic pH). In all these systems, the presence of both the iron porphyrin catalyst and a reducing agent is required, indicating that the active species is the iron(II) porphyrin. The most efficient systems used either an organosoluble iron porphyrin, such as Fe(OEP), in the presence of a carboxylic acid and zinc amalgam as reducing agent, or a water-soluble heme fragment of cytochrome *c* (microperoxidase MP-11) in the presence of dithionite. The catalytic activity of the systems was greatly increased when using electron-rich iron porphyrins bearing an electron-donating axial ligand, such as imidazole, and a carboxylic acid cocatalyst in close proximity to the iron center. The activity of the best systems was comparable to that of microsomal cytochromes P450 (between 1 and 10 turnovers per min). The intermediate (porphyrin)iron−aldoxime complex formed in those dehydration reactions was isolated in the case of Fe(*meso*-tetra(2,6-dichlorophenyl)-β-octachloroporphyrin) [*meso*-tetra(2,6-dichlorophenyl)-β-octachloroporphyrin = $TDCPCl_8P$] and characterized by elemental analysis and UV−visible and ^{1}H NMR spectroscopy. Comparison of the ^{1}H NMR spectra of Fe($TDCPCl_8P$)(CH_3-$CHNOH)_2$ and Fe($TDCPCl_8P$)(pyridine)$_2$ strongly indicates that acetaldoxime is bound to iron(II) via its nitrogen atom in the former. A mechanism for iron porphyrin-catalyzed dehydration of aldoximes based on all these results is proposed. It involves a partial charge transfer from electron-rich Fe(II) to the aldoxime C═NOH moiety, which favors the departure of its OH group assisted by an acid cocatalyst. This illustrates the potential of iron porphyrins as catalysts for new reactions very different from the redox transformations for which they are well-known.

Introduction

Since their discovery about 40 years ago, cytochromes P450 have been found to be ubiquitous hemeproteins in living organisms. Their main catalytic function is the transfer of one oxygen atom from O_2 into various substrates during monooxygenation reactions requiring the consumption of a reducing agent such as NADPH.[1] More recently, it has been reported that some cytochromes P450 are able to catalyze very different reactions which do not require the consumption of O_2 and a reducing agent.[2] This is the case of the dehydration of *n*-butanaldoxime into *n*-butanonitrile catalyzed by liver microsomal cytochromes P450 (eq 1).[3]

$$C_3H_7CH{=}N{-}OH \xrightarrow{\text{P450 Fe(II)}} C_3H_7C{\equiv}N + H_2O \qquad (1)$$

A detailed study recently showed that this is a general reaction occurring on *Z*-aldoximes with intermediate formation of a 442 nm absorbing complex between the aldoxime and cytochrome P450 Fe(II).[4] The elucidation of the biosynthetic pathway leading to the cyanoglucoside dhurrin from L-tyrosine has indicated a possible biological role for this unusual cytochrome P450 reaction in eq 2.[5]



(2)

Many questions remained about the mechanism of P450-dependent dehydration of aldoximes, concerning for instance the nature of the 442 nm absorbing P450 Fe(II)-aldoxime complex and the role of P450 iron and amino acid residues in the activation of aldoximes toward dehydration. The use of iron porphyrin model systems should be very helpful in that regard. However, although many chemical systems based on iron porphyrins have been described which mimic cytochrome P450-catalyzed monooxygenations[6] or reductions[7] of various substrates, model iron porphyrin-based systems have never been reported for cytochrome P450-catalyzed dehydrations. This paper describes the development of iron porphyrin-based systems that are efficient model catalysts for dehydration of aldoximes under mild conditions. Studies on these systems

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allowed us to determine the structure of the porphyrin Fe(II)−aldoxime complexes that are involved as intermediates in the reaction, and to better understand the detailed mechanism of aldoximes dehydration by cytochromes P450 and their chemical models. As far as coordination chemistry and catalysis are involved, these studies led to the first description of iron complexes bound to oximes via their nitrogen atom and showed that iron porphyrins can efficiently catalyze nonredox reactions.

Results

Dehydration of Aldoximes Catalyzed by Model Iron Porphyrins. It was reported that the active species in aldoxime dehydration was P450 Fe(II), whereas P450 Fe(III) was inactive.[3,4] Therefore, the first models that we studied were based on water soluble iron porphyrins in the presence of a reducing agent under anaerobic conditions. The first system that we tried was based on iron(III) protoporphyrin IX (1 mM) in 0.1 M phosphate buffer pH 7.4 containing sodium dithionite as a reducing agent, since very preliminary results indicated that iron(II)(protoporphyrin IX) was able to catalyze the dehydration of butanaldoxime.[3] First results showed us that heptanaldoxime (10 mM) was completely consumed by this system within 5 h at room temperature. Heptanonitrile was formed during this reaction but only with a low yield (10%) and low initial rate (about 5×10^{-3} mol·mol catalyst^{-1}·min^{-1}). In fact, heptanaldoxime was mainly transformed by this system into unknown nonvolatile products which were not detected by gas chromatography. Preliminary results indicated that they were derived from reactions involving dithionite. We then studied another aqueous system using water-soluble Fe(TPPS) [TPPS = *meso*-tetra(4-sulfonatophenyl)porphyrin] in the presence of Zn amalgam and $CrCl_2$ as reducing agents. At pH between 2 and 7, pentanaldoxime was completely consumed with formation of pentanonitrile (yield between 15 and 25% as a function of pH) and pentanal (yield between 30 and 40%). It is noteworthy that the presence of both the iron porphyrin catalyst and the reducing agent was absolutely required for dehydration of pentanaldoxime to pentanonitrile. The reducing agent alone was able to perform the conversion of pentanaldoxime to pentanal, but the presence of Fe(TPPS) markedly increased the rate of aldehyde formation.

To avoid the formation of aldehyde, biphasic systems, in which the reducing agent is in the aqueous phase and the iron porphyrin and aldoxime in the organic phase, were studied. Such biphasic systems were previously used to mimic the cytochrome P450-dependent reduction of some substrates.[7,8] The best results were obtained with Fe(OEP) [OEP = β-octaethylporphyrin] in CH_2Cl_2 and sodium dithionite in acetate buffer pH 5.2 in the presence of a catalytic amount of methyltrioctylammonium chloride as a phase transfer agent, provided that benzoic acid was used as an acid cocatalyst (Table 1). When using 100 equiv of PhCOOH (relative to 2 mM Fe(OEP)), 20 mM heptanaldoxime was completely converted in 1 day with predominant formation (87% yield) of heptanonitrile and only low amounts of heptanal. By comparison, the same system without PhCOOH only led to 20% conversion of the substrate with the major formation of heptanal (14% yield) (Table 1). However, although this system was selective for dehydration of aldoximes to nitriles, it remained relatively slow (initial catalytic activity of 1 turnover per h).

Table 1. Effect of Benzoic Acid on the Dehydration of Heptanaldoxime by the Biphasic System Fe(OEP) in CH_2Cl_2−Dithionite in H_2O

PhCOOH:Fe(OEP) molar ratio	0	7	30	100
yields (%)[a] (after 24 h)				
aldoxime	81	62	51	<2
nitrile	5	14	40	87
aldehyde	14	24	5	3
initial rate[b] of nitrile formation	0.07	0.09	0.35	1

[a] Conditions: 2 mM Fe(OEP) and 20 mM heptanaldoxime in CH_2Cl_2, and 80 mM sodium dithionite in acetate buffer pH 5.2, in the presence of methyltrioctyl ammonium chloride (1 mg·mL^{-1}), at 20 °C. [b] In mol of product per mol of Fe(OEP) per h.

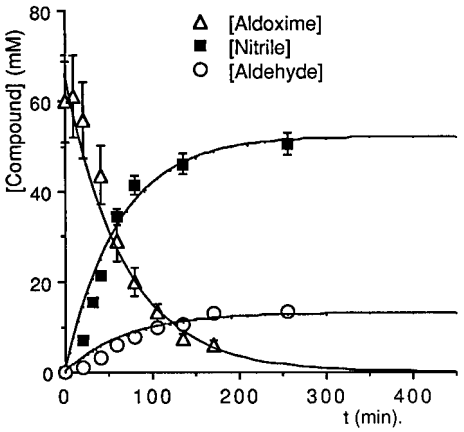

Figure 1. Time-dependent transformation of heptanaldoxime by Fe(OEP) in the presence of Zn amalgam and CH_3COOH. Conditions: 60 mM aldoxime, 0.5 M CH_3COOH, and 2 mM Fe(OEP) in deaerated CH_2Cl_2 (argon). Product concentrations (in mM): mean values ± SD from three experiments.

Finally, the iron porphyrin system that gave the best results consisted of a single liquid phase (CH_2Cl_2) containing Fe(OEP), CH_3COOH, and the aldoxime, in the presence of zinc amalgam as a reducing agent (Figure 1).

Properties of the Fe(OEP)−CH_3COOH−Zn Amalgam System. Table 2 shows the importance of the various components of this system for efficient dehydration of heptanaldoxime at room temperature. In the absence of Zn amalgam, the iron(III) porphyrin, Fe(OEP)Cl, did not catalyze the dehydration of heptanaldoxime at a detectable rate, nor does the reducing agent Zn−Hg in the absence of iron porphyrin. Both species, which together produce the active iron(II) porphyrin, must be present. Replacement of Fe(OEP) by Zn(II)(OEP), Mn(II)(OEP) or Co(II)(OEP), led to systems inactive toward heptanaldoxime dehydration. **This absolute requirement of both the iron porphyrin catalyst and the reducing agent was observed for all the iron porphyrin systems studied**. Addition of a carboxylic acid, such as CH_3COOH, had a dramatic effect on the rate of heptanaldoxime dehydration, and allowed us to achieve the complete conversion of 30 equiv of this aldoxime with a 80:20 nitrile-to-aldehyde ratio (Table 2). Although the transformation of heptanaldoxime to heptanal can be performed in low yields by Zn−Hg in the presence of CH_3COOH (complete system without iron porphyrin), this reaction is accelerated in the presence of the iron porphyrin (Table 2).

Effects of the Nature of the Iron Porphyrin and the Carboxylic Acid on Aldoxime Dehydration by Iron Porphyrin−RCOOH−Zn/Hg Systems. Table 3 shows the variation

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Table 2. Characteristics of the Dehydration of Heptanaldoxime Catalyzed by Iron Octaethylporphyrin in Dichloromethane in the Presence of Zinc Amalgam

system	yields after 5 h (%)		initial rate of formation of nitrile (mol·h^{-1}/mol of Fe(OEP))
	heptanonitrile	heptanal	
complete system (CS)[a]	17	0 (<0.5)	4
CS−Zn/Hg	0 (<0.5)	0 (<0.5)	(−)
CS−Fe(OEP)(Cl)	0 (<0.5)	0 (<0.5)	(−)
CS + CH_3CO_2H (0.5 M)	80	20	20
CS + CH_3CO_2H−Fe(OEP)	0 (<0.5)	0.5	(−)

[a] The complete system consists of 2 mM Fe(OEP)Cl in CH_2Cl_2 in the presence of 0.25 g of Zn amalgam (containing 1% Zn by mass) per mL of incubate and 60 mM heptanaldoxime. All reactions were carried out at 20 °C in deaerated CH_2Cl_2 under argon.

Table 3. Effect of the Nature of Porphyrin and Carboxylic Acid on the Rates of Dehydration of Heptanaldoxime into Heptanonitrile by the Iron Porphyrin−Zn/Hg−RCOOH System

iron porphyrin	initial rates, acid		
	CH_3CO_2H	CH_2ClCO_2H	$CHCl_2CO_2H$
Fe(OEP)	20	100	-
Fe(TPP)	0.4	14	200
Fe(TDCPP)	0.02	2	100
Fe(TFPP)	0.01	0.1	30
Fe(TDCPCl_8P)	0.002	0.02	1

[a] Conditions as in Table 2; [RCOOH] = 0.5 M in all cases. Initial rates are expressed in mol of nitrile produced per mol of iron porphyrin catalyst per h.

of the initial rates of dehydration of heptanaldoxime by such systems (expressed in mol of nitrile produced per mol of iron porphyrin catalyst per h, under the conditions of Table 2: 2 mM iron porphyrin, 60 mM heptanaldoxime in CH_2Cl_2 in the presence of Zn amalgam) as a function of the nature of the iron porphyrin catalyst and of the carboxylic acid.

The nature of the porphyrin ring has a great influence on the initial rate of dehydration, electron-rich iron porphyrins such as Fe(OEP) giving much higher catalytic activities (by a factor of 10^4) than iron porphyrins bearing electron-withdrawing substituents such as Fe(TFPP) [TFPP = *meso*-(tetrapentafluorophenyl)porphyrin] or Fe[TDCPCl_8P] (TDCPCl_8P = *meso*-tetra(2,6-dichlorophenyl) β-octachloroporphyrin) (Table 3). In fact, by plotting log(v_o) (v_o = catalytic activity at $t = 0$ for heptanaldoxime dehydration by the iron porphyrin−Zn/Hg−$CH_2ClCOOH$ system) against the Fe(III)/Fe(II) electrode potential of the iron porphyrins in CH_2Cl_2, one observed a good straight line correlation (Figure 2). This clearly shows that the electron richness of the iron(II) porphyrin, which should be the active species as in cytochrome P450-dependent reactions, is very important for aldoxime dehydration.

Table 3 also shows that, for a given iron porphyrin catalyst, an increase in the strength of the added carboxylic acid leads to a spectacular increase of the initial rate of dehydration of heptanaldoxime. For instance, in the case of Fe[TDCPP]Cl (TPCPP = *meso*-tetra(2,6-dichlorophenyl)porphyrin), the use of $CH_2ClCOOH$ or $CHCl_2COOH$ instead of CH_3COOH respectively led to a 10^2- or (5×10^3)-fold increase of this initial rate.

This important role of a carboxylic acid in the dehydration reaction was further shown in experiments by using an iron porphyrin related to Fe(TPP) [TPP = *meso*-tetraphenylporphyrin] in which a COOH substituent has been introduced in the ortho position of only one *meso*-phenyl group. This COOH substituent was expected to facilitate aldoxime dehydration by an intramolecular effect on the aldoxime bound to iron(II). Accordingly, dehydration of heptanaldoxime catalyzed by this substituted Fe(TPP), under conditions identical with those described in Table 3, but without externally added carboxylic

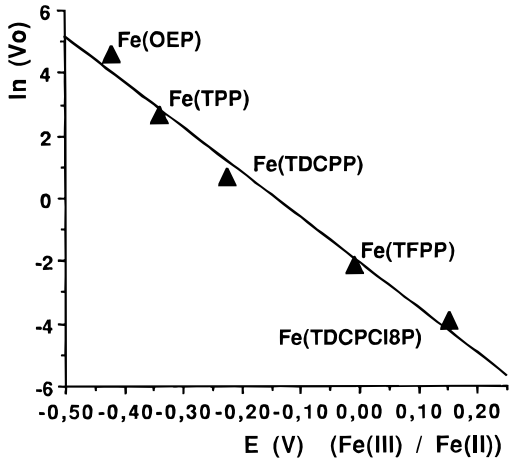

Figure 2. Correlation between the catalytic activity of various iron porphyrins with respect to heptanaldoxime dehydration and their Fe(III)/Fe(II) redox potentials. v_o is the initial rate expressed in mol of nitrile produced per mol of iron porphyrin per h under the conditions of Table 3 with $CH_2ClCOOH$ as carboxylic acid; redox potentials were measured for Fe(porphyrin)Cl complexes (1 mM in CH_2Cl_2 with 0.1 M nBu_4BF_4 at 20 °C, relative to the saturated calomel electrode).

acid, occurred with a catalytic activity of 40 turnovers·h^{-1}. This activity is 100-fold greater than that observed for Fe(TPP) itself in the presence of 0.5 M CH_3COOH. Moreover, the final yield of heptanonitrile observed with this substituted Fe(TPP) was very high (~95%).

The Fe(OEP)−CH_3COOH−Zn/Hg system was found to dehydrate various alkylaldoximes such as pentanaldoxime, heptanaldoxime, and phenylacetaldoxime with similar efficiencies (20 turnovers·h^{-1} for 2 mM Fe(OEP), 0.5 M CH_3COOH, and 0.06 M aldoxime in CH_2Cl_2 at 20 °C). However, it was inactive for benzaldoxime under identical conditions. The much lower aptitude of aromatic aldoximes toward dehydration, when compared to alkylaldoximes, was already mentioned in cytochrome P450-catalyzed reactions.[4] The same report also showed that only *Z*-arylaldoximes were dehydrated.[4] Therefore, we have measured (^{1}H NMR) the relative amounts of *Z*- and *E*-benzaldoxime after having submitted *Z*-benzaldoxime to dehydration by the Fe(OEP)−Zn/Hg−CH_3COOH system. After 3 h at 20 °C in that medium, the *Z*:*E* ratio was greatly in favor of the *E* isomer (12:88), explaining at least in part the lack of reaction found with *Z*-benzaldoxime. However, it was possible to observe the dehydration of benzaldoxime by using the best system described in Table 3, which used Fe(TPP) in the presence of a stronger acid, $CHCl_2COOH$. Under those conditions, *Z*-benzaldoxime was transformed into benzonitrile in a 48% yield after 1 day at 20 °C and into benzaldehyde as a minor product (11%). The catalytic activity observed for this reaction, 2 turnovers·h^{-1}, was much lower than that found for heptanaldoxime dehydration (Table 3).

Effects of Iron Axial Ligands on Iron Porphyrin-

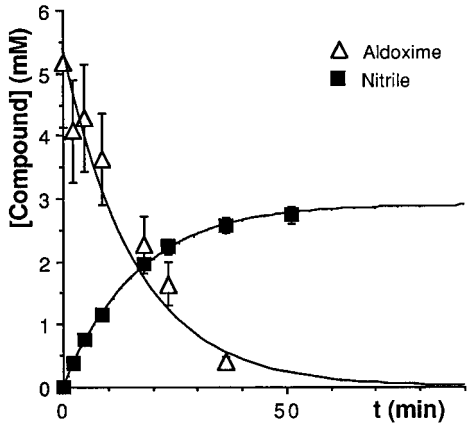

Figure 3. Time-dependent dehydration of heptanaldoxime into heptanonitrile by ferrous microperoxidase MP-11. Conditions: 5.2 mM aldoxime, 25 mM MP-11, 20 mM $Na_2S_2O_4$ in a deaerated mixture of 20:80 ethanol:0.1 M aqueous phosphate buffer (pH 7.4) at 20 °C. Product concentrations: mean values ± SD from four experiments.

Catalyzed Dehydration of Aldoximes. The presence of electron-donating axial ligands such as imidazole has a very positive effect upon the catalysis of oxygenation reactions by iron porphyrins.[6] Addition of 0.1 to 0.5 equiv of 1-methylimidazole, with respect to iron porphyrin, in the Fe(OEP)−Zn/Hg system (conditions of entry 1 of Table 2) resulted in a 3- to 4-fold increase of the initial rate of dehydration of heptanaldoxime. However, addition of more than 0.5 equiv of 1-methylimidazole led to an inhibition of heptanaldoxime dehydration. This inhibition was not unexpected as imidazole ligands have a high affinity for iron(II) porphyrins and led to the formation of iron(II) complexes, Fe(porphyrin)$(Im)_2$, in which both axial positions are blocked.[9]

We thus decided to use microperoxidase MP-11, a heme undecapeptide readily obtained by proteolysis of cytochrome *c*, in which a histidine imidazole coordinates the heme iron from one side, leaving the sixth coordination site available for binding by external ligands.[10] In this system, the intramolecular nature of the imidazole ligand considerably reduces the possibility of blocking of the iron via the formation of Fe(porphyrin)-$(imidazole)_2$ complexes.

A typical reaction, in which MP-11, reduced to the ferrous state by sodium dithionite, dehydrates heptanaldoxime into heptanonitrile, is shown in Figure 3. It seems likely that the difference between aldoxime consumption and nitrile production as analyzed by GC can be accounted for by the conversion to aldehyde. The aldehyde would be transformed into "bisulfite type" adducts under the reaction conditions, since addition of HCl to reaction aliquots, hydrolyzing such adducts, allowed us to regenerate the missing aldehyde in the appropriate quantities.

The initial rate of nitrile production in the reaction shown in Figure 3, divided by the concentration of MP-11, gives an activity of 7 catalytic cycles per min. This is faster than any of the systems of Table 3 operating in dichloromethane (maximum 200 h^{-1}, i.e., 3 min^{-1}), very much faster than the best aqueous systems that we have tested (maximum 0.05 min^{-1}, see Table 4), and is almost comparable to the rates observed for cytochrome P450 enzymes,[3,4] albeit at higher catalyst concentrations in the case of MP-11. An extremely positive role of an electron-donating axial ligand in the dehydration of aldoximes is thus indicated. It is also possible that the hydrophobic peptide chain surrounding the porphyrin in MP-11 helps to maintain a high local concentration of *n*-heptanaldoxime, and that this effect is partly responsible for the high rate observed.

Comparison of the Efficiency of the Various Iron Porphyrin Systems Studied. Table 4 compares some characteristics of the various iron porphyrin-based systems that we developed for mimicking cytochrome P450-catalyzed dehydration of aldoximes. The best systems in terms of selectivity of transformation of aldoximes to nitriles are those in which the iron porphyrin catalyst and aldoximes are in an organic phase (CH_2Cl_2), either with only one liquid phase (organosoluble iron porphyrin−RCOOH−Zn/Hg) or with two liquid phases (organosoluble iron porphyrin−PhCOOH in CH_2Cl_2, and $Na_2S_2O_4$ in water). Such systems led to yields of nitrile formation between 80 and 90% and only minor formation of aldehyde. On the contrary, aqueous systems of water-soluble porphyrins (Fe(TPPS)−$CrCl_2$−Zn/Hg) led to markedly lower nitrile yields because of an increased formation of aldehyde or its reaction products with dithionite. However, the most efficient systems in terms of initial reaction rates are microperoxidase MP-11 reduced by dithionite and the organosoluble system with an iron porphyrin in CH_2Cl_2 reduced by Zn/Hg in the presence of RCOOH. Catalytic activities observed with these systems reach several turnovers·min^{-1} (with 0.5 M $CHCl_2COOH$ for the second system) and are similar to those previously reported for microsomal cytochrome P450-catalyzed dehydration of alkylaldoximes.[3,4]

Formation and Characterization of Iron Porphyrin−Aldoxime Complexes. During the reactions of heptanaldoxime with iron porphyrins in the presence of a reducing agent described above, the intermediate formation of a complex, which exhibits a visible spectrum similar to those of iron(II)(porphyrin)$(pyridine)_2$ complexes, was always detected. To be able to isolate and characterize such a complex, we chose to use the electron-poor iron porphyrin, Fe($TDCPCl_8P$), whose capacity to dehydrate aldoximes into nitriles was found to be particularly low (Table 3). Moreover, preliminary experiments also showed us that this iron(II) porphyrin exhibited the highest affinity for alkylaldoximes. Addition of 10 equiv of acetaldoxime to a CH_2Cl_2 solution of Fe(III)($TDCPCl_8P$)Cl, previously reduced by a solution of $Na_2S_2O_4$ in 0.1 M phosphate buffer pH 7.4 in the presence of a phase transfer agent (1 h at 20 °C under argon), led to the immediate formation of a complex absorbing at 444, 550, and 581 nm. Evaporation of CH_2Cl_2 and repeated washing of the complex by ether led to a complex whose elemental analysis indicated the stoichiometry of two acetaldoxime ligands for one iron porphyrin. This stoichiometry was confirmed by the ^1H NMR spectrum of the complex which showed that it is a diamagnetic Fe(II) species, with an upfield shift of the ligand protons due to the porphyrin ring current (Table 5). Clear multiplets were seen for the protons of bound acetaldoxime ligands, showing that ligand exchange is slow on the NMR time scale at room temperature. Furthermore, the complex is air-stable without an excess of aldoxime ligand at concentrations in the millimolar range. The addition of 2 equiv of pyridine to this complex completely displaced the acetaldoxime ligands and gave rise to a pyridine complex Fe($TDCPCl_8P$)$(py)_2$, whose ^1H NMR and UV−visible characteristics are given in Table 5.

Comparison of the ^1H NMR spectra of Fe($TDCPCl_8P$)$(CH_3CHNOH)_2$ and Fe($TDCPCl_8P$)$(pyridine)_2$ strongly suggests that the aldoxime ligands are bound to the iron via their nitrogen atom. Actually, because of the current shift of the porphyrin ring, the ortho and meta protons of bound pyridine are shifted

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Table 4. Comparison of Different Types of Iron Porphyrin Systems Capable of Dehydrating Aldoximes into the Corresponding Nitriles

type of system[a]	iron porphyrin	catalytic activity[b]	final yields(%)[c] OX	N	A
1. CH_2Cl_2, Zn−Hg, CH_3CO_2H (0.5 M)	Fe(OEP), 2mM	0.3	<1	80	20
2. $CH_2Cl_2 + H_2O$, $Na_2S_2O_4$, $PhCO_2H$ (0.2 M)	Fe(OEP), 2 mM	0.02	<2	87	3
3. aqueous buffer, $CrCl_2$/Zn−Hg	Fe(TPPS), 2 mM	0.05	<1	15	37
4. aqueous buffer, $Na_2S_2O_4$	MP-11, 10 μM	6	<1	80	

[a] Conditions used for systems 1 and 2 as in Tables 2 and 1, respectively. Conditions of system 3: 2 mM Fe(TPPS) and 20 mM pentanaldoxime in 0.2 M acetate buffer pH 4.2 in the presence of 0.25 g of Zn amalgam and 5 mg of $CrCl_2$ per mL of incubate. Conditions of system 4: 10 μM MP-11 and 5 mM heptanaldoxime in a 80:20 0.1 M phosphate buffer pH 7.4: ethanol mixture in the presence of 20 mM sodium dithionite at 20 °C under argon. [b] Expressed in mol of nitrile produced per mol of iron porphyrin and per min at $t = 0$. [c] OX stands for aldoxime (heptanaldoxime, except for system 3 in which pentanaldoxime was used), N for nitrile, and A for aldehyde.

Table 5. ^{1}H NMR and UV−Visible Characteristics of Fe(TDCPCl$_8$P)(L)$_2$ Complexes

ligand L	CH_3CH═NOH	C_5H_5N
RMN ^{1}H[a]	7.6 (12H, m, *meso*-Ar)	7.7 (12H, m, *meso*-Ar)
	+1.62 (2H, q, 5.5, C**H**═NOH)	+3.28 (4H, d, 5.5, *ortho*)
	($\Delta\delta = -5.32$)	($\Delta\delta = -5.32$)
	−0.25 (6H, d, 5.5, −C**H$_3$**)	+5.25 (4H, t, 6.8, *meta*)
	($\Delta\delta = -2.11$)	($\Delta\delta = -2.01$)
	+1.40 (2H, s, ═NO**H**)	+6.15 (2H, t, 6.5, *para*)
		($\Delta\delta = -1.51$)
λ_{max} in nm (ϵ in mM^{-1}·cm^{-1})	444 (230)	446 (190)
	550 (40)	552 (26)
	581 (27)	580 sh. (17)

[a] δ in ppm (integration, multiplicity, coupling constant J in Hz, assignment). $\Delta\delta$ is the difference in chemical shift between a given proton of a coordinated ligand and the same proton in the free ligand. ^{1}H NMR spectra recorded in CD_2Cl_2 (acetaldoxime complex) and $CDCl_3$ (pyridine complex).

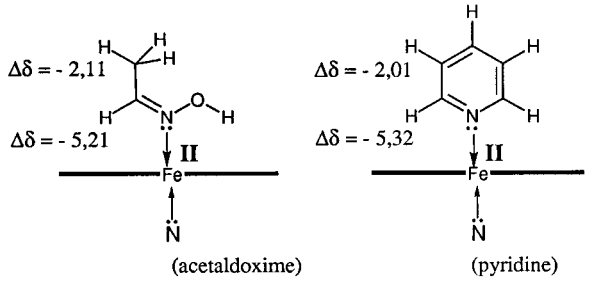

Figure 4. Analogy between the proposed coordination modes of pyridine and acetaldoxime ligands with the upfield ^{1}H NMR shift values induced by coordination to Fe(II)(TDCPCl$_8$P). $\Delta\delta$ (in ppm) = δ for bound ligands − δ for free ligand.

by $\Delta\delta = -5.32$ and -2.01 ppm relative to those of free pyridine. The CH and CH_3 protons of bound acetaldoxime are shifted upfield relative to free acetaldoxime with very similar $\Delta\delta$ values (-5.32 and -2.11 ppm). The structure of the Fe(TDCPCl$_8$P)(CH_3CHNOH) complex which is in agreement with these data is compared in Figure 4 to that of Fe(TDCPCl$_8$P)(pyridine)$_2$. This structure involving an iron−nitrogen bond leads to a positioning of the C*H*═N and C*H*$_3$ protons relative to the porphyrin ring almost identical to that of the ortho and meta pyridine protons in Fe(TDCPCl$_8$P)(pyridine)$_2$. This could well explain the chemical shifts observed for the two complexes. To the best of our knowledge, this is the first described example of an iron−aldoxime complex with such an Fe−N binding mode.

Discussion

The aforementioned results show that iron(II) porphyrins are good catalysts for the dehydration of aldoximes to the corresponding nitriles under very mild conditions. As previously observed in the case of cytochromes P450,[3,4] the ferric state is unable to catalyze this reaction. Efficient biomimetic systems for cytochrome P450-catalyzed dehydration of aldoximes have been obtained, either in water by using dithionite-reduced microperoxidase MP-11 or in CH_2Cl_2 by using an organosoluble iron(II) porphyrin in the presence of a carboxylic acid.

All these systems require both an iron porphyrin catalyst and a reducing agent to generate the active iron(II) state. However, in most cases, a relatively large excess of reductant must be used for complete consumption of the aldoxime. For instance, in reactions of the MP-11−dithionite system, the use of a large excess of dithionite (MP-11:dithionite:heptanaldoxime molar ratio of 1:100:20) leads to a complete conversion of the aldoxime and a 60% yield of nitrile, and keeps MP-11 iron in its ferrous state even after the end of the reaction. Using a smaller excess of dithionite (MP-11:dithionite:aldoxime = 1:4:20) does lead to heptanonitrile, however, in a smaller yield (37%), but only to a partial substrate conversion (60%); under these conditions MP-11 was in its ferric state at the end of the reaction. Moreover, Fe(II)(OEP), produced under rigorous anaerobic conditions in the medium used for the Fe(OEP)−CH_3COOH−Zn/Hg system (entry 1 of Table 4) but in the absence of any reducing agent, leads to dehydration of heptanaldoxime to heptanonitrile in a 4% yield (based on starting aldoxime). This yield corresponds to the formation of 1.2 mol of nitrile per mol of Fe(OEP). The iron catalyst was in its ferric state at the end of the reaction.

The need of an excess of reductant (relative to iron porphyrin) is presumably due to the consumption of this reductant by side reactions. We have not tried to identify all these reactions as their nature should depend on the system used. However, we have mentioned above that dithionite itself seems to react with aldehydes with formation of "bisulfite type" adducts. Moreover, in a very general manner, the formation of aldehydes from aldoximes could derive from an in situ reduction to the corresponding imines and hydrolysis of the latter products to aldehydes. Accordingly, control experiments with the biomimetic systems without iron−porphyrin failed to give nitriles but led to the formation of small amounts of aldehydes, although with much lower rates. Thus, aldehydes could be derived either from a direct reduction of aldoximes by the reductant or from an iron(II) porphyrin-catalyzed reduction. Whatever these side

reactions may be (reduction of oximes to aldehydes, direct reaction between aldehydes and dithionite, oxidation of iron(II) by air traces, ...), they consume electrons and explain why excess reductant is necessary for completion of aldoxime dehydration.

The mechanism that was postulated for cytochrome P450-catalyzed dehydration of aldoximes involves the formation of a 442 nm absorbing P450−aldoxime complex as a key intermediate.[4] It was proposed that this complex is derived from the binding of the nitrogen atom of aldoximes to P450 Fe(II). This proposition was based on the position of its Soret peak, which is similar to those of complexes between P450 Fe(II) and nitrogen-containing ligands, and on stereochemical data showing that only *Z*-aldoximes form such 442 nm absorbing complexes. Here we show that aldoximes readily bind to iron(II) porphyrins with formation of Fe(II)(porphyrin)(aldoxime)$_2$ complexes in which aldoxime binds to the iron via its nitrogen atom. These data give a firm basis for the structure proposed for P450−aldoxime complexes and describe the first example of iron−aldoxime complexes exhibiting such a coordination mode.[11]

Efficient model systems for aldoxime dehydration require the following features: (i) the use of electron-rich iron porphyrin catalysts (see Table 3), (ii) the presence of a carboxylic acid, as shown in Table 3 and from data obtained with Fe(TPP) bearing a COOH ortho substituent on a *meso*-phenyl group, and (iii) the presence of an electron-donating axial ligand of the iron, as shown by the spectacular increase of the reaction rate when using MP-11 instead of iron protoporphyrin(IX) as catalysts. On the basis of these results, it seems that an ideal catalyst for aldoxime dehydration would be based on an electron-rich iron(II) porphyrin involving an electron-donating axial ligand and a free axial binding position and, in close proximity to the iron center, an acid cocatalyst and a site of specific recognition and binding for the aldoxime. This may be the case of some cytochromes P450 which have a very electron-rich cysteinate proximal ligand of the iron and, on the distal side of the heme, an acid residue from the protein and a hydrophobic protein site for aldoxime binding. This should occur in the cytochrome P450 responsible for the dehydration of an aldoxime intermediate involved in the biosynthesis of dhurrin from L-tyrosine (eq 2).[5]

With reference to the possible mechanisms of aldoxime dehydration by iron(II) porphyrins and by cytochromes P450, it is important to understand the origin of the activation of aldoximes toward dehydration by the iron(II) porphyrins. In view of the fact that electron-donating substituents on the porphyrin ring and electron-donating axial ligands strongly favor aldoxime dehydration, it seems that iron(II) activates bound aldoxime by increasing the electron density in the C=N−OH moiety. Accumulation of a partial negative charge on the C=N carbon in the transition state would then favor the departure of the OH group of aldoxime (Figure 6). This departure should be greatly assisted by protonation of the OH group by an acid cocatalyst, in agreement with the spectacular effect of the COOH group of carboxylic acids added in excess in the medium, or introduced in close proximity to the iron on a *meso*-aryl porphyrin substituent (Figure 5). The species that would be generated by loss of the OH group should have a very acidic hydrogen atom on the carbon bearing the nitrogen atom, because of its β-position relative to positively charged iron (Figure 6). It should

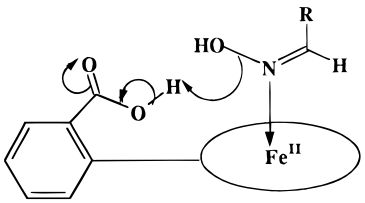

Figure 5. Intramolecular acid catalysis possibly involved in aldoxime dehydration catalyzed by iron(II) [*meso*-(*ortho*-carboxyphenyltriphenyl)-porphyrin].

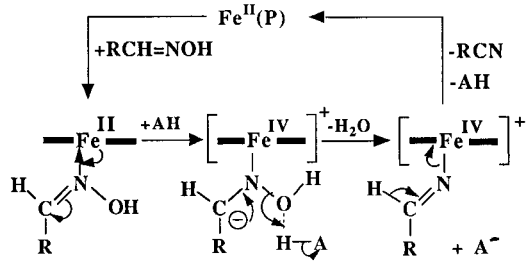

Figure 6. Possible mechanism for iron(II) porphyrin-catalyzed dehydration of aldoximes.

be very easily removed by any weak base present in the medium (pyrrole nitrogens are well located and possible candidates for this reaction), in a fast step leading to the nitrile product and regenerating the iron(II) porphyrin catalyst.

This type of activation of the aldoxime is analogous to that described for pentammineruthenium(II) ions, capable of dehydrating aldoximes and of eliminating alcohols from the corresponding oxime ethers, for whom Ru 4d → N(π*) back-bonding was evoked as a mechanistic explanation.[12] In the case of Ru(II), the nitrile formed has a good affinity for the metal center and so the complex $[(H_3N)_5Ru(NCR)]^{2+}$ is obtained in a stoichiometric reaction.

Whatever its detailed mechanism may be, this reaction of aldoxime dehydration illustrates the potential of iron porphyrins as catalysts for new reactions very different from the redox transformations for which they are well-known.

Experimental Section

General Aspects. UV−visible spectra were recorded on a Cary 210 spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker WN 250 MHz. Mass spectra were recorded in the laboratory of Prof. J. C. Tabet at the Université Paris VI. Elemental analyses were performed by the Service de Microanalyze du CNRS at Gif-Sur-Yvette, France. GC analysis was carried out with an Intersmat IGC 120 FL with a filled glass column (10% FFAP on a WAW 80/100 chromosorb mesh). Solvents were obtained from SDS (France) (synthesis quality) and used as obtained for catalysis experiments.

The aldoximes used (acetaldoxime, *n*-pentanaldoxime, *n*-heptanaldoxime) were prepared by literature methods[13] from the correspondingly aldehydes, which were obtained from the Aldrich chemical company, and the purity of the aldoximes was verified by ^{1}H NMR. Normal preparations gave *Z*:*E* isomer mixtures with the *Z* isomer predominating (75% to 95%). GC calibration for catalysis studies was performed by using bromobenzene as an internal standard for *n*-pentanaldoxime, *n*-pentanonitrile, and *n*-pentanal, and iodobenzene in the case of the

(11) For other modes of coordination of oximes to iron complexes in general, see: King, R. B.; Douglas, W. M. *Inorg. Chem.* **1974**, *13* (*6*), 1339−1342. (b) Khare, G. P.; Doedens, R. J. *Inorg. Chem.* **1976,** *15* (*1*), 86−90. (c) Aime, S.; Gervasio, G.; Milone, L.; Rossetti, P. L.; Stanghellini, *J. Chem. Soc., Dalton Trans.* **1978**, 534−540.

(12) (a) Guengerich, C. P.; Schug, K. *Inorg. Chem.* **1983**, *22*, 1401−1402. (b) Geno, M. J. K.; Dawson, J. H. *Inorg. Chem.* **1984**, *23*, 509−510. Os(II) complexes were also described to catalyze the dehydration of aldoximes into nitriles: Daniel, T.; Knaup, W.; Dziallas, M.; Werner, H. *Chem. Ber.* **1993**, *126*, 1981−1993.

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n-heptanaldoxime systems. Heptanonitrile was prepared as described.[4] Octaethylporphyrin was obtained from Aldrich, and the porphyrins $TPPH_2$,[14] $TDCPPH_2$,[15] $TFPPH_2$,[15] and $TDCPCl_8PH_2$[16] were obtained by literature methods. In all cases, iron insertion was performed in DMF as previously described,[17] giving (porphyrin)$Fe^{III}Cl$ complexes, dissolved in this form in dichloromethane in catalysis experiments. Iron(III) *meso*-tetra(*p*-sulfonatophenyl)porphyrin (in the form of a sodium sulfonate salt) was obtained from Interchim (France) and microperoxidase MP-11 from Sigma.

Preparation of the Fe(TDCPCl$_8$P)(CH$_3$CHNOH)$_2$ Complex. To a stirred CH_2Cl_2 deaerated solution (30 mL) of Fe(III)(TDCPCl$_8$P)Cl (0.12 g, 0.1 mmol) was added a deaerated phosphate buffer solution (15 mL, pH 7.4, 0.1 M) of $Na_2S_2O_4$ (0.1 M) containing 2 mg of octadecyltrimethylammonium chloride. The mixture was stirred for 2 h during which it turned from brown to red purple while some precipitation of Fe(II)(TDCPCl$_8$P) occurred. A deaerated solution (3 mL) of $CH_3CH{=}NOH$ (0.07 g, 1.2 mmol) was added to the biphasic medium leading to a red CH_2Cl_2 solution. The water phase was extracted with deaerated CH_2Cl_2. The solvent was removed through a strong flow of argon. The resulting solid was purified by several washings with deaerated $(CH_3CH_2)_2O$ and dried under argon (90% of isolated products). Anal. Calcd for $C_{48}H_{22}Cl_{16}FeN_6O_2$, $0.5CH_2Cl_2$: C, 42.17; H, 1.66; N, 6.08. Found: C, 42.20; H, 1.93; N, 5.79. ^{1}NMR ($CDCl_3$): δ 7.6 (m, 12H, *m*, *p*-H of *meso*-aryl), 1.62 (q, $J = 5.5$ Hz, 2H, H of $CH{=}N$), 1.40 (s, 2H, H of NOH), -0.25 (d, $J = 5.5$ Hz, 6H, H of CH_3). UV−vis spectrum: λ, nm (ϵ, $mM^{-1}\,cm^{-1}$) in CH_2Cl_2 : 444 (230), 550 (40), 581 (27).

Typical Procedure for Dehydration of Aldoximes by Aqueous Systems with Water-Soluble Porphyrin Catalysts (Fe(TPPS) or MP-11). (a) Fe(TPPS) as catalyst: An acetate buffer solution (4.5 mL, pH 4.2, 0.2 M) of Fe(TPPS) (8.5 mg, 9 μmol) and pentanaldoxime (9 mg, 90 μmol) was deaerated; an aliquot of 0.5 mL was withdrawn for an initial measurement. The resulting solution was transferred under argon to a mixture of $CrCl_2$ and Zn amalgam (5 and 250 mg respectively per mL of incubate). At different reaction times, the stirring was stopped to allow an uptake of an aliquot (0.5 mL) which was extracted with 1 mL of CH_2Cl_2 containing PhI as GC internal standard. The organic phase was directly analyzed by GC. Concentrations of heptanonitrile and heptanal were determined after their corresponding response factors. Similar reactions were performed in the same conditions with heptanaldoxime and *Z*-benzaldoxime.

(b) MP-11 as catalyst: Deaerated solutions of MP-11 (1 mM) in phosphate buffer (pH 7.4, 0.1 M), of $Na_2S_2O_4$ (0.1 M) in phosphate buffer, and of heptanaldoxime in EtOH (25 mM) were prepared. Reactions of Table 4 and Figure 3 were performed by mixing under argon known amounts of these solutions. At different reaction times, the stirring was stopped to allow an uptake of an aliquot (0.1 mL) that was extracted with 1 mL of CH_2Cl_2 containing PhI as GC internal standard. The organic phase was directly analyzed by GC. For example, reaction of Table 4 was performed by mixing under argon 20 μL of MP-11 solution, 1180 μL of phosphate buffer, 400 μL of heptanaldoxime solution, and 400 μL of $Na_2S_2O_4$ solution.

Typical Procedure for Dehydration of Aldoximes by the Biphasic System with Fe(OEP) and PhCO$_2$H in CH$_2$Cl$_2$ and Na$_2$S$_2$O$_4$ in Water. To a deaerated acetate buffer solution (5 mL, pH 5.2, 0.2 M) containing Aliquat 336 (methyltrioctylammonium chloride) (1 $g{\cdot}L^{-1}$) was added under argon a deaerated CH_2Cl_2 solution (6.25 mL) of Fe(OEP)Cl (7.9 mg, 12.5 μmol) containing heptanaldoxime (16.2 mg, 0.125 mmol), benzoic acid (0.122 g, 1 mmol), and PhI (16.5 μL, 0.0147 mmol). A 0.5 mL aliquot was withdrawn for an initial measurement. The biphasic solution was transferred on deaerated $Na_2S_2O_4$ (0.07 g, 0.4 mmol) with vigorous stirring. At different reaction times, the stirring was stopped to allow an uptake of an aliquot (0.5 mL) which was extracted with 1 mL of CH_2Cl_2 and directly analyzed by GC.

Typical Procedure for Dehydration of Aldoximes by Systems by Using an Organosoluble Iron Porphyrin and Zn Amalgam in the Presence of a Carboxylic Acid. A deaerated CH_2Cl_2 solution (4 mL) of iron porphyrin (8 μmol) containing the aldoxime (0.24 mmol), the carboxylic acid (2 mmol), and the internal standard was added under argon to 1 g of Zn amalgam (1%). An aliquot was taken just before stirring. At different reaction times, the stirring was stopped to take an aliquot (0.1 mL) which was directly analyzed by GC.

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