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^{18}O incorporation in the oxidation of *N*-methylcarbazole by lignin peroxidase and a model compound: a mechanistic insight into the oxidative *N*-demethylation of aromatic tertiary amines

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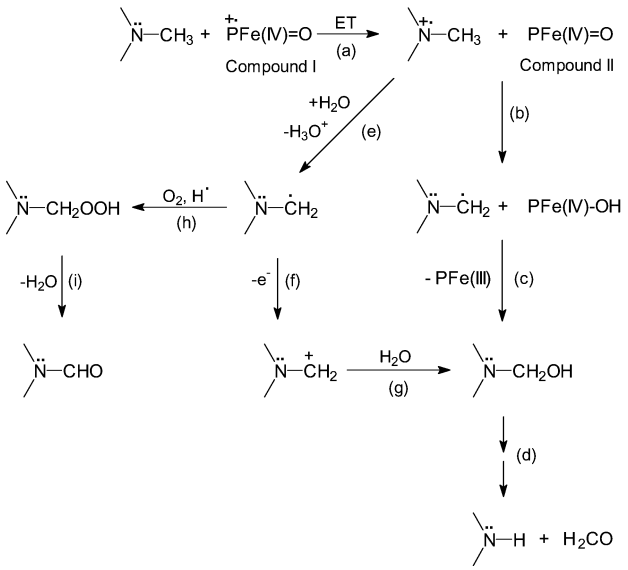
Using ^{18}O labelled reactants and/or solvent, the origin of the oxygen in the products of the oxidation of *N*-methylcarbazole by H_2O_2 catalysed by lignin peroxidase and a model compound has been determined, so getting important information about the mechanism of the oxidative *N*-demethylation of aromatic tertiary amines.

We have recently reported that lignin peroxidase (LiP) and its chemical model (FeTPPSCl)[1] are capable of catalysing the H_2O_2-promoted oxidative *N*-demethylation of aromatic amines, a process of great biological importance.[2]

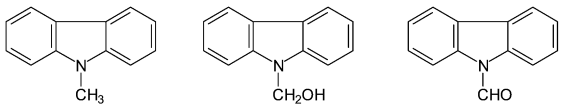
With *N*,*N*-dimethylanilines as substrates, evidence was found suggesting that with both LiP and FeTPPSCl, the mechanism is that shown in Scheme 1, steps a, b, c and d.[3] First, an electron transfer step takes place between the substrate and the active oxidant, *i.e.* the porphyrin radical cation iron(IV)-oxo complex, indicated as $P^{+\cdot}Fe(IV)=O$, leading to an anilinium radical cation and PFe(IV)=O (step a).[4] After the electron transfer, the reaction proceeds through the deprotonation of the radical cation, very probably by PFe(IV)=O itself, to give PFe(IV)–OH and an α-amino carbon radical (step b). The latter is then converted into a carbinolamine by an 'oxygen rebound' step (step c) in which the OH group is transferred from the iron to the carbon of the α-amino carbon radical. Eventually, the carbinolamine is very rapidly converted to the *N*-demethylated product (step d).

Whereas there are few doubts about the electron transfer step, the evidence in favour of steps b and c (Scheme 1) is less compelling. Clearly, a crucial test in this respect would be that of establishing if the oxygen in the carbinolamine intermediate actually comes from the oxidant, H_2O_2, as required by the sequence of the steps b and c. Unfortunately, it is not possible to apply such a test to *N*,*N*-dimethylanilines oxidations since the formed carbinolamines are very unstable and are rapidly converted into formaldehyde which undergoes a fast oxygen exchange with water.



Scheme 1

However, this problem can be overcome by using *N*-methylcarbazole (NMC, **1**) as the substrate because it is known that this compound undergoes oxidation by peroxidases to produce *N*-(hydroxymethyl)carbazole (NHMC, **2**), a stable carbinolamine,[5] very likely by the same electron transfer mechanism (Scheme 1, step a) operating with *N*,*N*-dimethylanilines.[6] We have therefore studied the LiP and FeTPPSCl catalysed oxidation of NMC with H_2O_2 in $H_2{}^{18}O$, with $H_2{}^{18}O_2$ in H_2O or under an $^{18}O_2$ atmosphere and the incorporation of ^{18}O into the products have been determined. The results of this study are reported herewith.



1 **2** **3**

The oxidations were carried out in 50 mM sodium tartrate buffered aqueous solution (pH = 3.5) under the experimental conditions detailed in the footnotes of Table 1. It was found that both the enzymatic and the biomimetic systems are able to catalyse the oxidation of NMC leading to *N*-hydroxymethylcarbazole (**2**) with yields of 15% and 34%, respectively.[9] However, in the case of the oxidation with LiP also *N*-

Table 1 Products yields and percentage of ^{18}O incorporation in the LiP and FeTPPSCl catalysed oxidations of NMC by H_2O_2[a]

		^{18}O incorporation (%)[b]	
Catalyst	Labelled reagent	NHMC	NFC
LiP	$H_2{}^{18}O$	94	0[c]
LiP	$H_2{}^{18}O_2$	0[c]	0[c]
LiP	$^{18}O_2$	0[c]	95
FeTPPSCl	$H_2{}^{18}O$	0[c]	—[d]
FeTPPSCl	$H_2{}^{18}O_2$	100	—[d]

[a] Experimental procedures are as follows: hydrogen peroxide (3 μmol) was added, over a period of 1 h, to a stirred solution of the substrate (3 μmol) and LiP (2.5 U, 2.6 nmol) in 1 mL of 50 mM sodium tartrate-buffered solution with 5% acetonitrile as cosolvent, pH = 3.5, at 25 °C, under argon or $^{18}O_2$ atmosphere. The oxidations catalysed by FeTPPSCl were carried out using the same procedure in the presence of imidazole in order to minimise any possible oxo–hydroxo tautomerism.[10] The substrate/FeTPPSCl/imidazole ratio used in these experiments was 1:0.025:1. Product analysis was performed by GC, GC-MS and HPLC on the NHMC trimethylsilyl derivative. [b] Determined by GC-MS analysis of NFC and the trimethylsilyl derivative of NHMC by the ratio of the intensities of the $(m + 2)/z$ and m/z peaks, corrected for the ^{13}C contribution. [c] The $(m + 2)/m$ area ratios were the same (0.5%) as those measured under the same experimental conditions but using unlabelled reagents. [d] No NFC was detected.

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formylcarbazole (NFC, **3**) was formed with a yield of 2%. It was verified that either in the absence of LiP or FeTPPSCl or in the absence of H_2O_2 no products were formed.

The percent of ^{18}O incorporation in the products when the oxidation of NMC was carried out under a variety of conditions is reported in Table 1.

From these data, it appears that in the LiP-promoted reaction, NHMC is formed without any incorporation from the oxidant $H_2{}^{18}O_2$. Clearly, oxygen must derive from the solvent and accordingly, almost complete (94%) incorporation of ^{18}O was observed when the reaction was run in $H_2{}^{18}O$. No ^{18}O incorporation was instead observed in the other oxidation product, NFC, formed in this reaction, which clearly indicates that such a species does not derive from NHMC but probably by reaction of the α-amino carbon radical with O_2 present in the medium (*vide infra*). Accordingly, when the reaction was carried out in the presence of $^{18}O_2$ the yield of NFC increased to *ca*. 10% with almost complete ^{18}O incorporation in NFC and no ^{18}O incorporation in NHMC.

A completely different situation holds for the biomimetic system: in this case a complete incorporation of labelled oxygen in NHMC is observed when the reaction is carried out in the presence of $H_2{}^{18}O_2$, whereas no ^{18}O incorporation is observed from $H_2{}^{18}O$. Therefore, although both LiP and FeTPPSCl are able to catalyse *N*-demethylation reactions, the mechanisms involved appear to be different.

The results concerning the FeTPPSCl promoted oxidation of NMC fully confirm the conclusions of our previous study of the oxidation of *N*,*N*-dimethylanilines by H_2O_2 catalysed by this soluble iron porphyrin.[3] Accordingly, the finding that oxygen in the carbinolamine derives exclusively from H_2O_2 is exactly what is expected by the operation of path c in Scheme 1 and implicitly also confirms that PFe(IV)=O must be, as suggested, the deprotonating agent towards the substrate radical cation (path b).[11]

On the contrary, the results obtained in the LiP catalysed oxidations indicate that the oxygen in NHMC derives for the largest part from the medium. This excludes an oxygen rebound mechanism for the formation of the carbinolamine and also renders unlikely that the deprotonation of the radical cation is induced by PFe(IV)=O. Probably, the radical cation is deprotonated by the medium and the α-amino carbon radical produces the carbinolamine through the intermediacy of a carbocation (Scheme 1, paths e, f and g). The formation of NFC also indicates that, in competition with the oxidation to carbocation, the radical can also react with O_2. A hydroperoxide is formed which, by water loss, is converted to NFC (Scheme 1, paths h and i).[14]

Thus, LiP and FeTPPSCl appear to catalyse the oxidation of NMC by mechanisms which substantially diverge after the electron transfer step. This conclusion contrasts with the previous one concerning the oxidation of *N*,*N*-dimethylanilines. In that case, strong evidence was found that the same mechanism holds for the two catalysts.[3]

The origin of this discrepancy probably resides in the larger steric requirements of NMC with respect to *N*,*N*-dimethylanilines, which can be a very important factor in oxidations promoted by LiP, due to the very restricted heme accessibility in this enzyme.[15] Thus, it can be much more difficult for the former substrate to approach the enzyme active site up to a distance where deprotonation of the radical cation by Compound II followed by oxygen rebound (paths a, b, c, Scheme 1) is possible. More likely, as already stated, the NMC radical cation, which can be formed by a long range electron transfer, is deprotonated by the medium to a carbon radical which is then converted to a carbocation (paths e and f, Scheme 1) or reacts with molecular oxygen (path h).

It is reasonable that the substrate steric requirements play a much less important role in the oxidation promoted by FeTPPSCl for what concerns the approach of the substrate to the oxygen of the iron–oxo complex. Thus, deprotonation of the radical cation by PFe(IV)=O and oxygen rebound occur with both NMC and *N*,*N*-dimethylanilines.

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Notes and references

1 (FeTPPSCl) = 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine-*p*,*p*′,*p*″,*p*‴-tetrasulfonic acid iron (III) chloride.

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6 The oxidation potential of NMC (1.27 V, *vs* NHE in MeCN)[7] is in the range of those of the *N*,*N*-dimethylanilines previously investigated. On the other hand, it is well established that LiP catalyses the oxidation of aromatic compounds, with a redox potential ≤1.4 V, by an ET mechanism; see, for example, ref. 8.

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9 In the reaction of *N*,*N*-dimethylanilines with an oxidation potential similar to that of NMC the yields of *N*-demethylated products were *ca*. 25 % with LiP and 10 % with FeTPPSCl (ref. 3b).

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11 It is possible that deprotonation and oxygen rebound take place in a P$^{+\cdot}$Fe(IV)=O-substrate complex (presumably of charge transfer, CT, type) since a complete masking of the kinetic deuterium isotope effect (KDIE) was observed in the oxidative *N*-demethylation of *N*-methyl-*N*-trideuteromethyl-2,4,6-trichloroaniline by FeTPPSCl which exhibits a k_H/k_D value of 0.98 ± 0.05, while the same reaction performed with *N*,*N*-bis(dideuteromethyl)-2,4,6-trichloroaniline showed a k_H/k_D value of 3.08 ± 0.04. For the interpretation of KDIE masking see ref. 3a. The formation of a CT complex between reactants has also been suggested for the metallo-porphyrin catalysed epoxidation of alkenes (ref. 12) and side chain oxidation of aromatic compounds (ref. 13).

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