Inorganica Chimica Acta, 74 (1983) 275–278 275

An ESR Spin Trapping Investigation on the Photoreduction of Chlorohemin in Mixed Solvents

ANDREA MALDOTTI, CARLO BARTOCCI, ROSSANO AMADELLI and VITTORIO CARASSITI

Centro di Studio sulla Fotochimica e Reattività degli Stati Eccitati dei Composti di Coordinazione del C.N.R., Istituto Chimico dell'Università di Ferrara, Ferrara, Italy

Received January 7, 1983

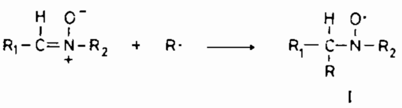
Radical scavenging by nitrone compounds is used in conjunction with ESR spectroscopy in order to obtain evidence for radical formation in the photoreduction of chlorohemin in aqueous alcoholic mixed solvents. The formation of adducts between hydrogen atoms and the spin traps appears to be the most important process in aerated and in pyridine-containing solutions. Evidence for the formation of hydroxyethyl adducts is obtained when the alcoholic component of the solvent is ethanol. In deaerated solutions not containing pyridine, no evidence is obtained for radical formation. The results are interpreted in terms of hydrogen abstraction by the nitrone from an alkoxy radical formed together with Fe(II) in the primary photoreduction. The abstration process strongly competes with cage re-oxidation, as well as with the diffusion of the alkoxy radical in the solvent bulk where it would rapidly react to give hydroxy radicals. The role of pyridine and oxygen may respectively be stabilizing and oxidizing the Fe(II) species before it undergoes cage re-oxidation.

Introduction

The study of redox processes involving the central atom in iron porphyrin complexes is of considerable interest because of the role that cytochromes, heme containing proteins, play in electron transfer processes occurring in the respiratory chain.

The photochemical behavior of Fe(III)–protoporphyrinIX chloride (Chlorohemin, Fe(III)PPCl) has been previously studied in different solvents [1, 2]. Irradiation of a deaerated solution of Fe(III)PPCl in water/alcohol/pyridine [1] and pure pyridine [2] solvent systems caused Fe(III) to be reduced to Fe(II) to give bis-pyridine Fe(II) protoporphyrinIX as final product. The obtained experimental results indicated that an electron transfer from axial ligand to central iron atom was responsible for the primary photoreduction.

The use of the spin-trapping technique appears to be particularly suitable in this field in order to show the formation of short-lived radical intermediates as a consequence of the photoredox process. The use of nitrones as spin traps has been extensively investigated by Janzen [3]. This method is based on the ability of the nitrone in trapping a radical to give a paramagnetic nitroxide (eqn. 1)



$$R_1-\overset{H}{C}=\underset{+}{\overset{O^-}{N}}-R_2 + R\cdot \longrightarrow R_1-\underset{R}{\overset{H}{C}}-\overset{O\cdot}{N}-R_2 \qquad (1)$$

I

which is stable enough to be studied by ESR technique. In some instances, the nature of the trapped radical can be identified from the parameters obtainable from the ESR spectra of nitroxide adduct I.

In a recent study Bartocci *et al.* [2] have reported that during the photoreduction of chlorohemin in a pyridine solution containing phenyl-tert-butyl nitrone (PBN) as a spin trap, a paramagnetic species was formed which gave an ESR spectrum typical of a PBN–pyridyl radical adduct. A mechanism was proposed whereby a pyridine radical cation was formed in the primary photochemical act which consisted in an electron transfer from axially bound pyridine to central iron. Pyridyl radical was then formed by a fast transfer of a proton to pyridine solvent.

In the present study, the spin trapping technique is used with the aim to clarify the mechanism of the photoreduction of chlorohemin in aqueous alcoholic solvent systems, also in the light of the conclusions which have been previously reached on this field by different authors [1, 4].

Experimental

Materials

Fe(III)PPCl was obtained from Fluka and recrystallized following the reported [5] glacial acetic

0020-1693/83/$3.00

acid method. Aldrich products phenyl-tert-butyl nitrone and 5,5′-dimethylpyrroline-N-oxide (DMPO) were used without further purification. The solvents used were commercial products of spectrograde quality.

Apparatus and Procedure

Two standard mixed solvents were used: A, 40% v/v alcohol/water; B, 20% v/v pyridine, 40% v/v alcohol, 40% v/v water. The alcohols used were ethanol and 2-propanol. The concentration of the Fe(III)-PPCl solutions was 5×10^{-4} *M*, that of PBN and DMPO was 5×10^{-2} *M*. X-band ESR spectra were recorded with a Bruker 220 SE Spectrometer which was calibrated using $\alpha\alpha'$-diphenyl-picryl-hydrazil (DPPH). The irradiation was carried out at room temperature (295 °K) using a 150 W xenon lamp. Light was filtered using a device which could cut off the light of wavelength lower than 360 nm. The filtered light was focussed on the ESR cavity by quartz lenses. The test solutions were contained in a ESR quartz capillary (internal diameter 1.5 mm) which could be connected to a vacuum line for deaeration. Solutions could be degassed to less than 10^{-5} torr by means of at least five vacuum line freeze–thaw pump cycles.

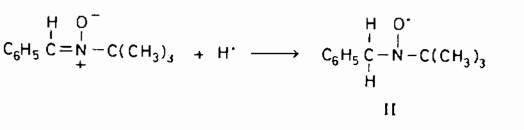
Results

When solutions of Fe(III)PPCl in solvents A and B containing PBN or DMPO were stored in the dark for several hours, no appreciable ESR signals or changes in the electronic absorption spectra were observed: an indication that the reaction between the spin traps and the porphyrin complex did not proceed at a significant rate.

Irradiation in the ESR cavity of Fe(III)PPCl solutions containing spin traps gave the results reported below:

Solvent A (Alcohol: ethanol, spin trap: PBN, pH = 10)

The spectrum reported in Fig. 1 appeared after some minutes of irradiation. This can be interpreted as resulting from the superimposition of two distinct signals. A rather intense signal consisting of a 1:1:1 triplet of 1:2:1 triplets which can be attributed to nitroxide II formed upon trapping by PBN of an H• radical according to eqn. 2:



$$C_6H_5\overset{H}{C}{=}\underset{+}{\overset{O^-}{N}}{-}C(CH_3)_3 + H^\bullet \longrightarrow \underset{\text{II}}{C_6H_5\underset{H}{\overset{H}{C}}{-}\overset{O^\bullet}{N}{-}C(CH_3)_3} \qquad (2)$$

The values of the hyperfine splitting constants have been found to be $a_N = 16.5$ G and $a_H = 9.2$ G. The second signal observed was weaker than the first and consisted of a 1:1:1 triplet of doublets. The values of the hyperfine splitting constants for this last signal ($a_N = 15.5$ G, $a_H = 3.7$ G) are consistent with the trapping by PBN of a hydroxyethyl radical to form the nitroxide adduct III [6]:

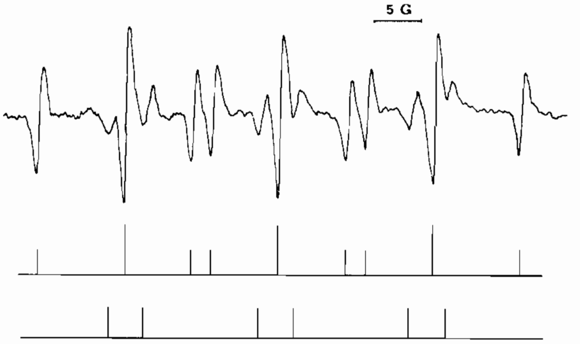
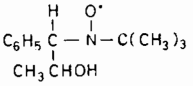


Fig. 1. ESR spectrum, with the stick-diagram reconstructions, obtained after 15 minutes of irradiation at 298 K of a 5×10^{-4} *M* Fe(III)PPCl solution containing 5×10^{-2} *M* PBN in solvent A (alcohol = ethanol).



$$C_6H_5\underset{CH_3CHOH}{\overset{H}{C}}{-}\overset{O^\bullet}{N}{-}C(CH_3)_3$$

III

Solvent A (alcohol: 2-propanol, spin trap: PBN, pH = 10)

An ESR spectrum, which consisted only of a triplet of triplets, was obtained when solutions were irradiated, indicating that only hydrogen radicals were trapped by PBN to give adduct II.

Solvent A (alcohol: ethanol, spin trap: DMPO, pH 10)

The spectrum reported in Fig. 2 was obtained after two minutes of irradiation. In this spectrum two distinct signals can be identified. One, very intense, consists of a triplet of triplets ($a_N = 16.5$ G, $a_H = 22$ G) which is typical of a paramagnetic adduct between DMPO and a hydrogen radical [7] (eqn. 3):

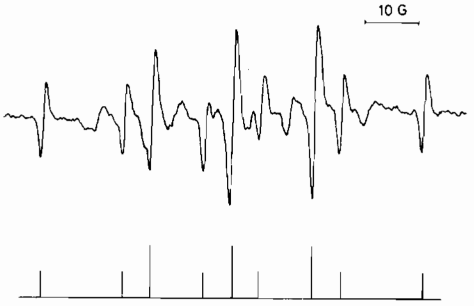
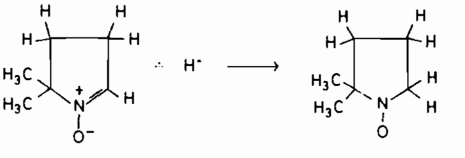


Fig. 2. ESR spectrum, with the stick-diagram reconstruction, obtained after 15 minutes of irradiation at 298 K of a 5×10^{-4} *M* Fe(III) PPCl solution containing 5×10^{-2} *M* DMPO in solvent A (alcohol = ethanol).


(3)

IV

The other signal, although difficult to analyze, indicates that besides hydrogen atoms, other species, which are likely to be hydroxyethyl radicals, were trapped by DMPO.

Solvent A (alcohol: 2-propanol, spin trap: DMPO, pH = 10)

The ESR spectrum obtained in this case consists only of a triplet of triplets, with coupling constants identical to those obtained in aqueous ethanol solutions.

Solvent B (alcohol: ethanol or 2-propanol, spin trap: PBN, pH = 10)

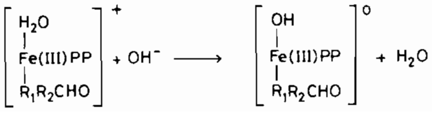
In these solvent systems the ESR spectra recorded while irradiating the solution are quite similar to those obtained in solvent A, thus suggesting that the same radical species are trapped by PBN irrespective of the presence of pyridine in the solution.

When oxygen-free solutions of chlorohemin were irradiated, a rather different behaviour was observed depending on whether the solvent used was A or B: the ESR spectra recorded during the irradiation of deaerated, pyridine containing solutions (solvent B) were exactly the same as those obtained in aerated solutions free of pyridine (solvent A; on the other hand, no appreciable ESR signal was obtained on irradiation of solutions which contained neither pyridine or oxygen (solvent A).

No evidence for the formation of paramagnetic radical adducts with PBN as well as with DMPO was obtained during the irradiation of Fe(III)PPCl solutions which did not contain alcohol. This is in agreement with the results obtained in a previous investigation [1], where no photoreduction of chlorohemin was observed in aqueous alkaline solutions.

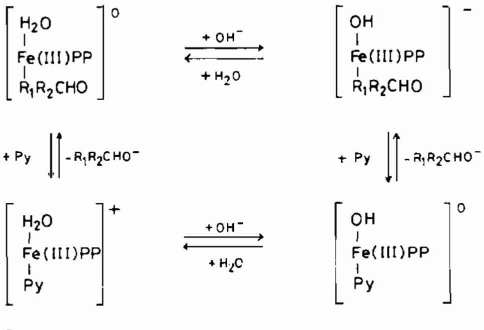
Discussion

One of the equilibria established when chlorohemin is dissolved in solvent A is likely to be that between the Fe(III)PP complex containing alcoholate and H_2O in the axial positions and its alkaline form (eqn. 4):



$$\left[\begin{matrix} H_2O \\ | \\ Fe(III)PP \\ | \\ R_1R_2CHO \end{matrix}\right]^{+} + OH^- \longrightarrow \left[\begin{matrix} OH \\ | \\ Fe(III)PP \\ | \\ R_1R_2CHO \end{matrix}\right]^{0} + H_2O \quad (4)$$

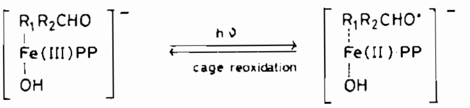
In solvent B the system is complicated by the presence of pyridine, which is known to be a potential axial ligand for Fe(III) protoporphyrin [8]. For this case the equilibria established in solution are shown in Scheme 1:



Scheme 1

On this basis, and in accordance with what has been reported in previous papers [1, 2], the reduction observed upon irradiation of Fe(III)PPCl in solvent A can be attributed to an electron transfer from the alcoholate axial ligand to the central iron atom. On the other hand, the possibility should be considered that in solvent B an intramolecular electron transfer from pyridine to iron may occur in competition with that from alcoholate. However, the lack of detection of pyridyl radicals in spin trapping experiments (see Results) indicates that the electron transfer from pyridine, although possible under particular conditions [2], is quite inefficient in solvent B. The primary process in the photoreduction of Fe(III)PPCl in aqueous alcoholic solutions containing pyridine should, therefore, consist essentially of an electron transfer from the axial alcoholate to the central atom.

The results reported in this work show that irradiation of Fe(III)PPCl produces two main species which can be trapped by a radical scavenger and thus revealed by the ESR technique: hydrogen atoms and hydroxyalkyl radicals. The trapping of a hydrogen atom is unequivocally demonstrated by the triplet of triplets ESR spectrum (Fig. 1). The triplet of doublets in ethanol-containing solutions has coupling constants which are characteristic of a trapped hydroxyethyl radical [6]. On the basis of the above reported results, one may speculate [1] that the above mentioned radicals originate from an alkoxy-radical formed in the primary photochemical step, *i.e.*, the electron transfer from the coordinated alcoholate to Fe(III). Due to the high pH value of the solutions used, it is likely that the photoreducible species is the basic form of the iron porphyrin complex which has a hydroxyl group bound to the metal in one of the two axial positions. The primary photochemical process may be represented as follows (eqn. 5):



$$\left[\begin{array}{l}R_1R_2CHO\\ |\\ Fe(III)PP\\ |\\ OH\end{array}\right]^- \underset{\text{cage reoxidation}}{\overset{h\nu}{\rightleftarrows}} \left[\begin{array}{l}R_1R_2CHO^{\cdot}\\ \vdots\\ Fe(II)PP\\ |\\ OH\end{array}\right]^- \qquad (5)$$

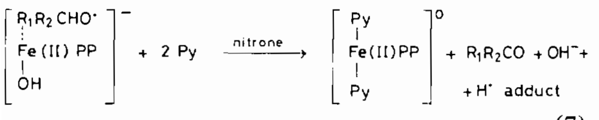
The photoreduction step may be followed by a fast reoxidation, inside the solvent cage, of the reduced form, if this is not stabilized.

Maillard and Giannotti [9] have studied the photochemistry of cobalamines and B_{12} coenzyme in the presence of PBN and DMPO. They observed the reduction of Co(III) by charge transfer from a corrine equatorial ligand to the metal. The charge transfer is accompanied by the expulsion of a hydrogen atom from a methin group of corrine itself. In the case of Fe(III)PPCl, this mechanism would imply that a porphyrin → Fe(III) charge transfer is responsible for the photoreduction. However, this mechanism may be ruled out because it is likely [10] that a fast deactivation to low-lying iron d–d states occurs prior to the photoreduction step. Moreover, irradiation of Fe(III)PPCl in the absence of alcohol does not lead to the formation of any trapped radical.

The experimental results show that the trapping of radicals in the absence of dissolved oxygen is observed only when the solution contains pyridine (solvent B) whereas, in aerated solutions, there is evidence of trapped radicals in both solvents A and B. A possible explanation is that oxygen and pyridine can lead to reactions which, strongly competing with cage reoxidation (eqn. 5), allow either the diffusion of the alkoxy radicals in the bulk or its direct reaction with the radical scavengers.

The alkoxy radical can follow three reaction pathways in the presence of PBN or DMPO: *i*) it can be trapped as it is, *ii*) it can react with another molecule of alcohol to yield hydroxyalkyl radical (eqn. 6) which can in turn form a paramagnetic adduct with the spin trap, *iii*) it can be oxidized to aldehyde (or ketone) giving a α-hydrogen atom to the spin trap (eqn. 7):

$$R_1R_2CHO^{\cdot} + R_1R_2CHOH \longrightarrow R_1R_2CHOH + R_1R_2\dot{C}OH \qquad (6)$$

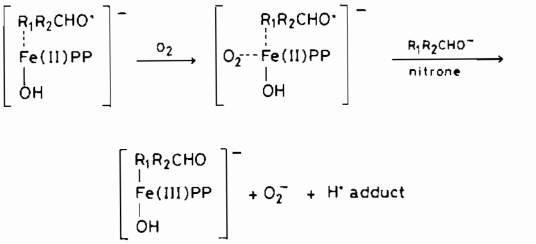


$$\left[\begin{array}{l}R_1R_2CHO^{\cdot}\\ \vdots\\ Fe(II)PP\\ |\\ OH\end{array}\right]^- + 2\,Py \xrightarrow{\text{nitrone}} \left[\begin{array}{l}Py\\ |\\ Fe(II)PP\\ |\\ Py\end{array}\right]^0 + R_1R_2CO + OH^- + H^{\cdot}\text{ adduct} \qquad (7)$$

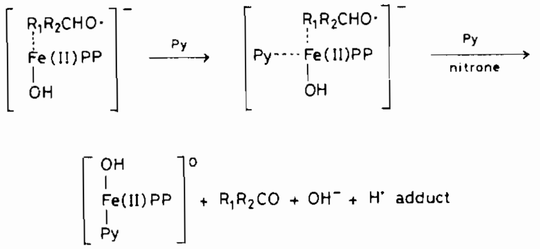
Since evidence of formation of alkoxy radical adducts was not obtained with our experimental conditions, the process indicated under point *i* can be ruled out. As far as points *ii* and *iii* are concerned, the high intensity of the triplets' triplet signals is an indication that abstraction of hydrogen (eqn. 7) is by far the most efficient process.

The efficiency of trapping of short-lived radicals by radical scavengers depends on various factors, such as the steady-state concentration of the radicals, the kinetic constants for the radical–spin traps adduct formation, and the stability of the adducts formed. The above results indicate that the alkoxy radical has a strong tendency to be oxidized yielding a hydrogen atom to the spin trap instead of being trapped directly or reacting with another alcohol molecule. A possible explanation of this fact is that the alkoxy radical, formed in the primary photochemical act, interacts inside the solvent cage with both the central metal in the complex and the spin trap. The interaction with the metal could still be strong enough to prevent the trap from reacting with alkoxy radical, and could allow this radical to be oxidized with production of a hydrogen radical which is trapped instead.

In the framework of the above proposed mechanism, the way in which oxygen and pyridine prevent cage re-oxidation (eqn. 5) may be that of reacting with Fe(II) when this is still bound to some extent to the alkoxy radical (eqns. 8 and 9):



$$\left[\begin{array}{l}R_1R_2CHO^{\cdot}\\ \vdots\\ Fe(II)PP\\ |\\ OH\end{array}\right]^- \xrightarrow{O_2} \left[\begin{array}{l}\quad R_1R_2CHO^{\cdot}\\ \quad\vdots\\ O_2^-\cdots Fe(II)PP\\ \quad |\\ \quad OH\end{array}\right]^- \xrightarrow[\text{nitrone}]{R_1R_2CHO^-} \left[\begin{array}{l}R_1R_2CHO\\ |\\ Fe(III)PP\\ |\\ OH\end{array}\right]^- + O_2^- + H^{\cdot}\text{ adduct} \qquad (8)$$



$$\left[\begin{array}{l}R_1R_2CHO^{\cdot}\\ \vdots\\ Fe(II)PP\\ |\\ OH\end{array}\right]^- \xrightarrow{Py} \left[\begin{array}{l}\quad R_1R_2CHO^{\cdot}\\ \quad\vdots\\ Py\cdots Fe(II)PP\\ \quad |\\ \quad OH\end{array}\right]^- \xrightarrow[\text{nitrone}]{Py} \left[\begin{array}{l}OH\\ |\\ Fe(II)PP\\ |\\ Py\end{array}\right]^0 + R_1R_2CO + OH^- + H^{\cdot}\text{ adduct} \qquad (9)$$

References

1 C. Bartocci, F. Scandola, A. Ferri and V. Carassiti, *J. Am. Chem. Soc., 102,* 7067 (1980).

2 C. Bartocci, A. Maldotti, O. Traverso, C. A. Bignozzi and V. Carassiti, *Polyhedron, 2,* 97 (1982).

3 E. G. Janzen, *Acc. Chem. Res., 4,* 31 (1971).

4 C. Bizet, P. Morliere, D. Brault, O. Delgado, M. Bazin and R. Santus, *Photochem. Photobiol., 34,* 315 (1981).

5 J. H. Furhop and K. M. Smith, in 'Porphyrins and Metalloporphyrins' (Edited by K. M. Smith), p. 809, Elsevier, Amsterdam, 1975.

6 A. Ledwidth, P. J. Russel and L. M. Sutcliffe, *Proc. Roy. Soc. London, A332,* 151 (1973).

7 P. Maillard, J. C. Massot and C. Giannotti, *J. Organometal. Chem., 159,* 219 (1978).

8 C. Bartocci, F. Scandola, A. Ferri and V. Carassiti, *Inorg. Chim. Acta, 37,* L473 (1979).

9 P. Maillard and C. Giannotti, *J. Organometal. Chem., 182,* 225 (1979).

10 F. R. Hopf and D. G. Whitten, ref. 5, p. 667.