"Redox Tautomerism" in High-Valent Metal–oxo–aquo Complexes. Origin of the Oxygen Atom in Epoxidation Reactions Catalyzed by Water-Soluble Metalloporphyrins

Jean Bernadou,* Anne-Sylvie Fabiano, Anne Robert, and Bernard Meunier*

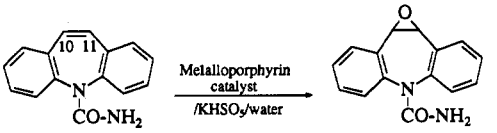
Laboratoire de Chimie de Coordination du CNRS 205 route de Narbonne, 31077 Toulouse Cedex, France

Received June 27, 1994

Most of the studies to determine the origin of the incorporated oxygen atom in olefin epoxidations catalyzed by metalloporphyrins have been performed in organic solvents or biphasic media with hydrophobic complexes. From these data it has been concluded that the oxygen atom originated from the primary oxidant ($PhIO$,[1] $LiOCl$,[2] $KHSO_5$[3]). Although in the case of iodosylbenzene early experiments[1a] suggested that high-valent iron–oxo complexes can exchange the coordinated oxygen atom with labeled water, a recent reevaluation of ^{18}O incorporation in metalloporphyrin-mediated oxygenation reactions carried out in the presence of $H_2{}^{18}O$ indicated that the metal–oxo species does not quickly exchange its oxygen atom with water.[4] The rate of such an exchange reaction is far below the rate of oxygen incorporation into the organic substrate. In the case of oxygenations catalyzed by cytochrome P-450, the source of the incorporated oxygen was molecular oxygen rather than water.[5] Water-soluble manganese and iron porphyrins have water molecules as axial ligands.[6] Does this influence the origin of epoxide oxygen when olefin epoxidations are performed in water with soluble metalloporphyrins activated by a water-soluble oxidant? We report here a possible answer to this question that we obtained by studying the oxidation of drugs catalyzed by water-soluble metalloporphyrins in order to mimic the oxidative metabolism of xenobiotic molecules.[7]

Anionic or cationic metalloporphyrins activated by potassium monopersulfate[8] catalyze the epoxidation of carbamazepine (CBZ, an analgesic and anticonvulsant drug, Scheme 1) to CBZ 10,-11-oxide, which is the main metabolite observed *in vivo*.[9] This epoxidation reaction is one of the rare examples of a metalloporphyrin-mediated oxygenation reaction performed with high yields (75–80%, see below) in aqueous solutions. Most of the drug oxidations previously reported were the result of electron abstraction from the substrate.[7] So the CBZ epoxidation allowed us to investigate the mechanism of the catalytic oxygen transfer in aqueous solution and, in particular, to determine the source of the incorporated oxygen atom.

Scheme 1. Epoxidation of Carbamazepine by a Metalloporphyrin/$KHSO_5$/H_2O System



CBZ CBZ-10,11-oxide

Catalytic epoxidations[10] of CBZ were performed with two different metalloporphyrins, MnTMPyP and FeTDCPPS (a cationic, non-sterically hindered manganese porphyrin and an anionic, sterically hindered iron porphyrin, respectively).[11] We observed high substrate conversions and epoxide yields at pH 5 with MnTMPyP and FeTDCPPS: after 1 h reaction, the conversion in both cases was above 99%, and epoxide yields were 80 and 75%, respectively. The CBZ oxide was unambiguously characterized by comparison of its retention time in HPLC with that of an authentic sample and by its spectroscopic properties.[14,15]

In order to study the origin of the oxygen atom in CBZ oxide, we used the most efficient system, *i.e.*, MnTMPyP/$KHSO_5$ at pH 5 in standard conditions and ^{18}O-labeled water solutions.[16] When the catalyzed epoxidation was performed in aqueous solutions with various contents of $H_2{}^{18}O$, we observed, after extraction of CBZ oxide with dichloromethane and MS analysis,[17] that some ^{18}O had been incorporated in CBZ oxide and that the percentage of CBZ [^{18}O]oxide increased concomitantly as the percentage of $H_2{}^{18}O$ increased in the reaction mixture. *In fact, half of the oxygen atoms incorporated in the epoxide came from the solvent* (Figure 1; a linear correlation was observed with a

(1) (a) McMurry, T. J.; Groves, J. T. In *Cytochrome P-450: Structure, Mechanism and Biochemistry*; Ortiz de Montellano, P. R., Ed.; Plenum Press: New York, 1986; pp 1–28. (b) Groves, J. T.; Stern, M. K. *J. Am. Chem. Soc.* **1988**, *110*, 8628–8638. (c) Heimbrook, D. C.; Carr, S. A.; Mentzer, M. A.; Long, E. C.; Hecht, S. M. *Inorg. Chem.* **1987**, *26*, 3835–3836.

(2) Meunier, B.; Guilmet, E.; De Carvalho, M. E.; Poilblanc, R. *J. Am. Chem. Soc.* **1984**, *106*, 6668–6676.

(3) Robert, A.; Meunier, B. *New J. Chem.* **1988**, *12*, 885–896.

(4) Nam, W.; Valentine, J. S. *J. Am. Chem. Soc.* **1993**, *115*, 1772–1778.

(5) Heimbrook, D. C.; Sligar, S. G. *Biochem. Biophys. Res. Commun.* **1981**, *99*, 530–535.

(6) (a) Prince, S.; Körber, F.; Cooke, P. R.; Lindsay Smith, J. R.; Mazid, M. A. *Acta Crystallogr.* **1993**, *C49*, 1158–1160. (b) Körber, F. C. F.; Lindsay Smith, J. R.; Prince, S.; Rizkallah, P.; Reynolds, C. D.; Shawcross, D. R. *J. Chem. Soc., Dalton Trans.* **1991**, 3291–3299.

(7) (a) Meunier, B. *Chem. Rev.* **1992**, *92*, 1411–1456. (b) Bernadou, J.; Bonnafous, M.; Labat, G.; Loiseau, P.; Meunier, B. *Drug Metab. Dispos.* **1991**, *19*, 360–365. (c) Masumoto, H.; Ohta, S.; Hirobe, M. *Drug Metab. Dispos.* **1991**, *19*, 768–780. (d) Vidal, M.; Bonnafous, M.; Defrance, S.; Loiseau, P.; Bernadou, J.; Meunier, B. *Drug Metab. Dispos.* **1993**, *21*, 811–817. (e) Carrier, M. N.; Battioni, P.; Mansuy, D. *Bull. Soc. Chim. Fr.* **1993**, *130*, 405–416.

(8) Potassium monopersulfate (the triple salt $2KHSO_5{\cdot}KHSO_4{\cdot}K_2SO_4$, Curox) was a gift of Interox.

(9) Frigerio, A.; Fanelli, R.; Biandrate, P.; Passerini, G.; Morselli, P. L.; Garattini, S. *J. Pharm. Sci.* **1972**, *61*, 1144–1147. Morselli, P. L.; Frigerio, A. *Drug Metab. Rev.* **1975**, *4*, 97–113.

(10) All reactions were carried out according to the following standard procedure: the reaction mixture (500 μL) contained 66 mM phosphate buffer (pH 5), 500 μM carbamazepine (introduced as a 5 mM solution in methanol), 5 mM $KHSO_5$, and 10 μM metalloporphyrin; the studies were performed at 20 °C; the catalyst was introduced in five additions (2 μM each) every 15 min; and the first addition initiated the reaction. HPLC analyses of diluted aliquots were performed on a Waters Millipore chromatograph equipped with a 6000A pump, a U6K injector, and a UV-481 detector. CBZ and its epoxide derivative were visualized by using a Nucleosil C18 column, 10 μm (Interchrom), eluted by a mixture of methanol/water, 6:4 (v/v). Detection was at 215 nm. Calculation of conversions of CBZ and yields of epoxide was made by comparison of HPLC profiles with calibrated amounts of benzophenone. Under the above conditions, retention times of CBZ, its epoxide, and benzophenone were 6, 4.5, and 14 min, respectively. CBZ was provided by Aldrich.

(11) MnTMPyP stands for the manganese(III) derivative of *meso*-tetrakis-(4-*N*-methylpyridiniumyl)porphyrin; see ref 6 for its structure and ref 12 for its preparation. FeTDCPPS stands for the iron(III) derivative of *meso*-tetrakis-(2,6-dichloro-3-sulfonatophenyl)porphyrin; see ref 13 for its preparation.

(12) Bernadou, J.; Pratviel, G.; Bennis, F.; Girardet, M.; Meunier, B. *Biochemistry* **1989**, *28*, 7268–7275.

(13) (a) Campestrini, S.; Meunier, B. *Inorg. Chem.* **1992**, *31*, 1999–2006. (b) Pattou, D.; Labat, G.; Defrance, S.; Séris, J. L.; Meunier, B. *Bull. Soc. Chim. Fr.* **1994**, *131*, 78–88.

(14) Unambiguous characterization of CBZ oxide was obtained from the oxidation product isolated from a 100-fold-scale experiment (see ref 10 for standard conditions). Epoxide yield determined by HPLC was 80%. Analytical data: mp 192 ° C; UV (CH_3OH) λ_{max} 212 nm; ^1H NMR (CD_2Cl_2) δ 4.27 (s, $H_{10}H_{11}$, 2H), 4.46 (br s, NH_2, 2H), 7.33–7-53 (m, H_{arom}, 8H); MS (EI) *m/z* (relative intensity) 252 (M^+, 100), 223 (M – CHO, 32), 180 (M – CHO – CONH, 100).

(15) We checked that in standard conditions[10] the epoxide formation resulted effectively from a catalytic reaction. In the absence of metalloporphyrin, no substrate conversion was observed and no CBZ oxide was detected, even after 1 h reaction. When the concentration of $KHSO_5$ was increased to 50 mM, we observed 10% of CBZ conversion and 3% of CBZ oxide after 90 min of reaction.

(16) $H_2{}^{18}O$ (95 atom %) was supplied by Eurisotop (Gif-sur-Yvette, France). Water solutions with various contents of ^{18}O were prepared by dilution with $H_2{}^{16}O$.

(17) The reaction mixture was extracted with 400 μL of dichloromethane, and then the solvent was evaporated to dryness and the sample kept at 4 °C until analysis. Mass spectra were obtained on a NERMAG R10/10H instrument by using the electronic impact method at 70 eV. Samples were diluted in dichloromethane before analyses. The percentage of CBZ [^{18}O]-oxide was deduced from the ratio of molecular peak intensities at *m/z* 254 over 252.

0002-7863/94/1516-9375$04.50/0

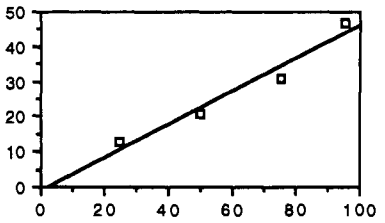
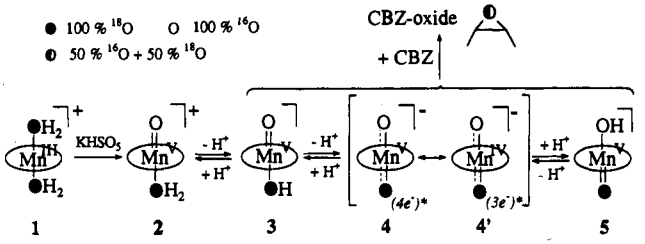


Figure 1. Correlation between the content of $H_2{}^{18}O$ in the reaction mixture and the amount of labeled oxygen found in CBZ oxide (see ref 10 for experimental conditions, MnTMPyP being the catalyst).

Scheme 2. "Redox Tautomerism" Explaination for the Incorporation of 0.5 Mole of ^{18}O per Mole of Epoxide in Experiments Using $H_2{}^{18}O$ and $KHS^{16}O_5$[a].

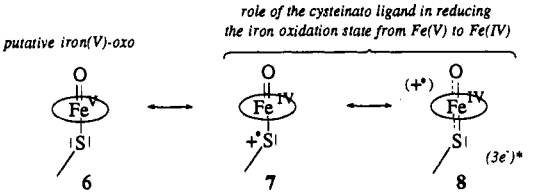


[a] The numbers in parentheses marked with an asterisk reflect the total number of electrons involved in the mesomerism.

slope of 0.47 for the percentage of [^{18}O]oxide versus the percentage of $H_2{}^{18}O$, $R = 0.98$). We checked that the epoxide oxygen atom of a sample of labeled CBZ oxide (47% ^{18}O) did not exchange when exposed to $H_2{}^{16}O$ in the reaction conditions.[18]

To explain the constant ratio of 0.5 for the incorporation of oxygen from the solvent, we propose a "redox tautomerism" mechanism involving a coordinated water molecule on the metalloporphyrin catalyst (Scheme 2). MnTMPyP can exist in aqueous solution with one or two metal-bound water molecules as axial ligands (**1** in Scheme 2). Conversion of the Mn(III) complex **1** to the Mn(V)–oxo **2** should lower the pK_a value of the ligated water molecule, allowing, at the pH of the reaction, its conversion into a hydroxo ligand (**3**; see ref 20 for discussions on the pK_a values of aquo and hydroxo ligands in high-valent metalloporphyrins). Removal of a proton from this hydroxo ligand results in the formation of the stabilized anion **4** with 4e⁻ delocalized on both metal–oxygen bonds (**4'** is a mesomeric form with 3 e⁻ delocalized and the manganese at the formal oxidation state IV).[21] This anion can be protonated with the same probability on either of the two metal–oxo-like bonds, giving rise to either form **3** or **5**, which then reacts with CBZ to produce CBZ oxide containing either ^{16}O or ^{18}O, respectively, in the ratio 1:1. The tautomeric equilibrium between **3** and **5** can not only localize the oxidizing entity on one or the other face of the activated metalloporphyrin but can also, via electron delocalization along the two axial positions, contribute to stabilize the high-valent metal–oxo through a mesomeric equilibrium between **4** and **4'**, this latter form involving a lower oxidation state of manganese. Such a "redox tautomerism" along the axial ligands in high-valent metal–oxo–aquo complexes suggests that, in the active form of cytochrome P-450, the proximal cysteinato ligand is probably a noninnocent ligand:[22] it may be able to provide an electron to Fe^V in the putative iron(V)-oxo **6** to reduce the formal oxidation state of the iron center to Fe^{IV}, creating a radical cation on the sulfur atom (form **7** on Scheme 3; alternatively, 3 e⁻ are delocalized along the O–Fe–S axis in the mesomeric form **8**). In the case of peroxidases, this electron is provided by the porphyrin ligand itself, not by the imidazole ring of the proximal histidine, which is less oxidizable than cysteine. Since most high-valent P-450 models studied up to now were based on hydrophobic metalloporphyrins with poorly oxidizable axial ligands (chloride, pyridine or imidazole), it is not surprising that all these oxometalloporphyrins have been shown to exhibit a radical cation on the macrocycle like in peroxidase compound I.[23]

Scheme 3. One Proposal for the Stabilized Form of Activated Cytochromes P450 Involving Electron Donation from the Proximal Cysteinato Ligand[a]



[a] The numbers in parentheses marked with an asterisk reflect the total number of electrons involved in the mesomerism.

Acknowledgment. The authors thank Dr. Monique Bonnafous for preliminary experiments, Dr. Alexander Sorokin and Suzy Richelme for MS data, and CIBA-GEIGY (Rueil-Malmaison, France) for a gift of CBZ 10,11-oxide. Prof. Robert Balahura (Guelph University, Ontario, Canada) is acknowledged for fruitful discussions.

(18) It must be noted that $KHSO_5$ does not exchange oxygen atoms with water; see ref 3 for experiments with unlabeled monopersulfate and labeled water and ref 20 for experiments with labeled monopersulfate and unlabeled water. In addition, these data on CBZ epoxidation indicate that an oxidation of a coordinated water molecule in compound **1** by $KHSO_5$ can be excluded (the oxygen atom of the Mn–oxo bonds should be exclusively ^{18}O, not ^{16}O).

(19) Thompson, R. C.; Wieland, P.; Appelman, E. H. *Inorg. Chem.* **1979**, *18*, 1974–1977.

(20) Jeon, S.; Bruice, T. C. *Inorg. Chem.* **1992**, *31*, 4843–4848.

(21) The conversion of **3** to **5** does not neccessarily involve **4** and **4'** as discrete deprotonated intermediates but might also proceed via a hydrogen-bonded water molecule in a more concerted pathway.

(22) Champion, P. M. *J. Am. Chem. Soc.* **1989**, *111*, 3433–3434.

(23) (a) Groves, J. T.; Haushalter, R. C.; Nakamura, M.; Nemo, T. E.; Evans, B. J. *J. Am. Chem. Soc.* **1981**, *103*, 2884–2886. (b) Mandon, D.; Weiss, R.; Jayaral, K.; Gold, A.; Terner, J.; Bill, E.; Trautwein, A. X. *Inorg. Chem.* **1992**, *31*, 4404–4409.