Oxidative Cleavage of DNA by Water-Soluble Iron Porphyrin Complex and Potassium Monopersulfate

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Both the oxidative cleavage of DNA and controlled oxidation of hydrocarbons by metal complexes are the subject of continued pursuit.[1] Metalloporphyrins are the often-used metal complexes in the oxidation reactions, for mimicking the reactivities of biological enzymes, *i.e.*, cytochrome P-450, peroxidase, and catalase, and developing synthetic restriction enzymes for sequence specific recognition and efficient DNA cleavage.[2] In the studies, various kinds of oxidants such as H_2O_2, ROOH, PhIO, peracids, NaOCl, and $KHSO_5$ have been used to generate reactive intermediates responsible for the oxidation reactions. Of all the oxygen atom donors, water-soluble $KHSO_5$ (potassium monopersulfate) has been often used as an efficient oxygen atom donor for the reactions in aqueous solution.[3] We herein describe the results of iron porphyrin complex-mediated oxidative DNA cleavage and olefin epoxidation by $KHSO_5$ in aqueous media. An electronegatively-substituted iron porphyrin complex, (*meso*-5,10,15,20-tetrakis(2,3,5,6-tetrafluoro-4-*N,N,N*-trimethylammoniummethylphenyl)porphinato)iron (III) [Fe(TF4TMAP)$^{5+}$], was used in this study, since it has been shown that this complex is an efficient catalyst in olefin epoxidation reactions.[4] The reactivity of the iron complex was followed by monitoring the conversion of supercoiled pBR-322 plasmid DNA (form I) to an open circle (form II) (eq 1). Olefin epoxidation was studied to understand the structure of reactive intermediate generated in the reaction of iron porphyrin complex with $KHSO_5$.

$$\text{Supercoiled DNA} \xrightarrow[\text{buffer}]{\text{Fe(TF}_4\text{TMAP)}^{5+}/\text{KHSO}_5} \text{Relaxed circular DNA} \quad (1)$$

Water-soluble iron porphyrin complexes tested in the oxidative cleavage of DNA by $KHSO_5$ are depicted in Figure 1. Only the Fe(TF$_4$TMAP)$^{5+}$ complex showed the activity of cleaving DNA efficiently and the other two iron complexes were found to be inactive (see Figure 2A).[5] The low reactivity of (*meso*-5,10,15,20-tetrakis(2,6-dichloro-3-sulfonatophenyl)porphinato)iron(III) [Fe(TDCPPS)$^{3-}$] and (*meso*-5,10,15,20-tetrakis(2,4,6-trimethyl-3,5-disulfonatophenyl)porphinato)iron(III) [Fe(TTMDSP)$^{7-}$] in the DNA cleavage reaction was attributed to the overall negative charge of the iron porphyrin complexes,[7] since it has been known that cationic metalloporphyrins are able to bind to DNA in a close vicinity.[8] Other oxidants, *i.e.* *tert*-butyl hydroperoxide and hydrogen peroxide, were studied in the DNA cleavage reaction, and these appear to be less efficient than $KHSO_5$ (Figure 2B). A similar reactivity pattern was observed in manganese porphyrin systems as well.[8(a),9]

Since the nature of the species responsible for the DNA cleavage may be related to the production of reactive radicals formed upon the interaction of $KHSO_5$ with the iron porphyrin complex, alcohol quenching studies were performed to understand whether the cleavage of DNA was induced by radicals.[10] Since the possible radical species generated in the reaction were hydroxyl radical (HO·) and sulfate radical ($SO_4^{\cdot -}$), alcohols added to the reaction solution as a radical scavenger were ethanol and *tert*-butyl alcohol.[10(a)] As the results are shown in Figure 2C, the presence of alcohol in the reaction media did not inhibit the DNA cleavage, suggesting that the reactive species responsible for the oxidative cleavage of DNA were not the diffusible radicals. We, therefore, tentatively propose that a high-valent iron oxo porphyrin complex[11] is a potent intermediate responsible for the oxidative DNA scission.

In order to gain further evidence for the involvement of the high-valent iron oxo porphyrin complex in the DNA cleavage reaction, olefin epoxidations were performed in aqueous solution. When the epoxidation of CBZ by the iron complex and $KHSO_5$ was carried out under the identical reaction conditions used in the DNA cleavage reaction,[6(a),12(b)] high conversion (65%) of CBZ to CBZ-10,11-oxide was observed (eq. 2).[13] Moreover, as observed in the DNA cleavage reaction, addition of alcohols, *i.e.* ethanol and tert-butyl alcohol, to the reaction solution did not reduce the yield of the oxide product, suggesting again that the epoxidizing agent was not radicals but a high-valent iron oxo porphyrin complex.

We also carried out the oxidation of *cis*-stilbene by Fe(TF$_4$TMAP)$^{5+}$ and $KHSO_5$, since analysis of the product distributions (*i.e.* the ratio of *cis*-stilbene oxide to *trans*-stil-

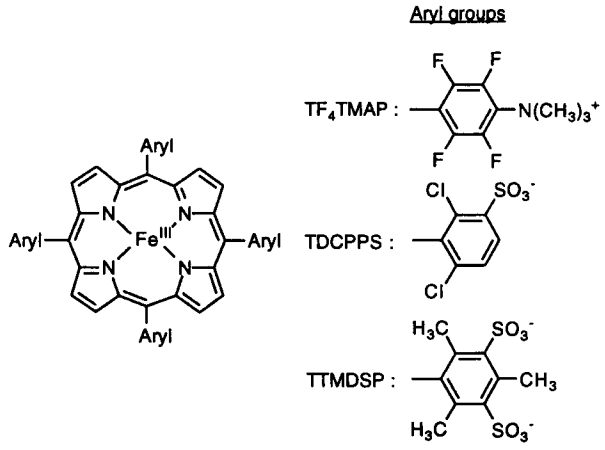


Figure 1. Structure of water-soluble iron porphyrin complexes.

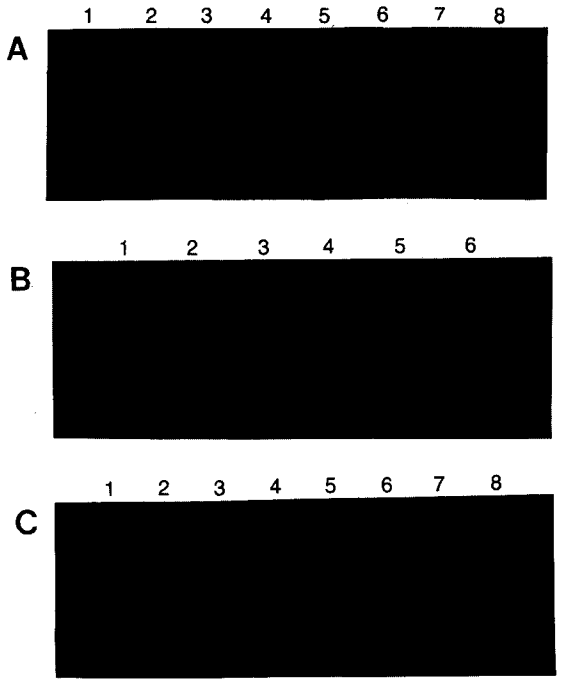
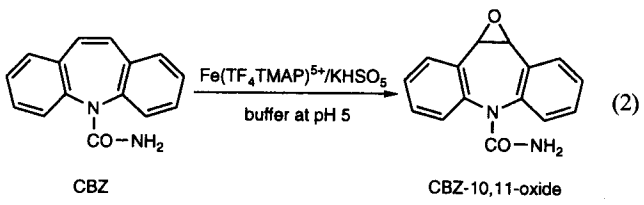


Figure 2. Reactions of oxidative cleavage of supercoiled pBR 322 plasmid DNA. (A) Cleavage of supercoiled plasmid DNA (18.7 μM bp) by various kinds of iron porphyrin complexes (0.5 μM) with KHSO5 (40 μM). Lane 1, DNA control; lane 2, $KHSO_5$ control; lane 3, Fe(TDCPPS) control; lane 4, Fe (TDCPPS)+$KHSO_5$; lane 5, Fe(TF_4TMAP) control; lane 6, Fe (TF_4TMAP)+$KHSO_5$; lane 7, Fe(TTMDSP) control; lane 8, Fe (TTMDSP)+$KHSO_5$. (B) Cleavage of supercoiled plasmid DNA (18.7 μM bp) by Fe(TF_4TMAP) (0.5 μM) with various oxidants. Lane 1, DNA control; lane 2, $KHSO_5$ control; lane 3, Fe (TF_4TMAP) control; lane 4, Fe(TF_4TMAP)+$KHSO_5$ (40 μM); lane 5, Fe(TF_4TMAP)+t-BuOOH (150 mM); lane 6, Fe(TF_4 TMAP)+H_2O_2 (150 μM). (C) Effect of radical scavengers on the DNA cleavage reaction by Fe(TF_4TMAP) (0.5 μM) with $KHSO_5$ (40 μM). Lane 1, DNA control; lane 2, $KHSO_5$ control; lane 3, Fe(TF_4TMAP) control; lane 4, Fe(TF_4TMAP)+$KHSO_5$; lane 5, Fe(TF_4TMAP)+$KHSO_5$+ethanol (50 mM); lane 6, Fe(TF_4TMAP)+ $KHSO_5$+ethanol (100 mM); lane 7, Fe(TF_4TMAP)+$KHSO_5$+*tert*-butanol (50 mM); lane 8, Fe(TF_4TMAP)+$KHSO_5$+*tert*-butanol (100 mM).

bene oxide) may give us clues to exclude the possibility of radical species as reactive intermediate.[14] In the *cis*-stilbene epoxidation reaction, *cis*-stilbene was oxidized to *cis*-stilbene oxide predominantly with only a trace amount of *trans*-stilbene oxide formation.[15] This result strongly supports that



(2)

high-valent iron oxo porphyrin complex was generated as a reactive intermediate in the reaction of Fe(TF_4TMAP)$^{5+}$ and $KHSO_5$. If the *cis*-stilbene oxidation was mediated by a radical species, then *trans*-stilbene oxide should be yielded as the major product.[14,16]

In conclusion, we have shown that a water-soluble cationic iron porphyrin complex associated with $KHSO_5$ was able to induce DNA cleavage efficiently. On the basis of the results obtained in the alcohol quenching studies and olefin epoxidations, the reactive intermediate responsible for the DNA cleavage was proposed to be a high-valent iron oxo porphyrin complex, not radical species.

Experimental

Materials. All chemicals obtained from Aldrich Chemical Co. were of the best available purity and used without further purification. Potassium monopersulfate, available as $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$ (Oxone), was obtained from Aldrich. H_2O_2 (30%) and *tert*-butyl hydroperoxide (70%) were purchased from Fluka and Sigma, respectively. CBZ-10,11 oxide was synthesized by the published method[6(a)] and used as an authentic sample for the determination of product yield. [Fe(TF_4TMAP)]$(CF_3SO_3)_5$, [Fe(TDCPPS)]Na_3, and [Fe(TTMDSP)]Na_7 were obtained from Mid-Century Chemicals. pBR322 supercoiled plasmid DNA was isolated from *E. coli* and purified by equilibrium centrifugation in CsCl-Ethidium Bromide concentration gradients.[17]

DNA Cleavage Experiments. In a typical DNA cleavage assay, preincubation of supercoiled pBR322 DNA (18.7 μM in base pairs) with iron porphyrin complex (0.5 μM) was performed for 1 min in sodium acetate (50 mM, pH 5), and the digestion time in the presence of $KHSO_5$ was 10 min at 25 °C. The extent of DNA cleavage was monitored by agarose gel electrophoresis. Loading buffer was added before the mixtures were run in 1% agarose slab horizontal gel containing 1 μg/mL ethidium bromide at constant current in Tris-borate buffer. Bands were located by UV light and photographed using SL-5G-Photographic system.

General Epoxidation Reactions. HPLC analyses of the reaction solution were performed on Orom *Vintage* 2000 high performance liquid chromatography equipped with a variable wavelength detector. Reaction mixtures were separated by using C18 column, eluted by a mixture of methanol-water (70 : 30, v/v) at a flow rate of 1.2 mL/min. Detection was at 215 nm.

In a typical experiment for CBZ epoxidation, $KHSO_5$ (2 mM) was added to a reaction solution containing [Fe (TF_4TMAP)]$(CH_3SO_3)_5$ (0.08 mM) and CBZ (CBZ=carbamazepine, 1 mM, introduced as a 100 mM solution in methanol) in 5 mL of 250 mM acetate buffer (pH 5). The reaction mixture was stirred for 30 min at room temperature and analyzed by HPLC. For the epoxidation of *cis*-stilbene, a solvent mixture (5 mL) consisting of 50% H_2O (250 mM acetate buffer, pH 5), 40% CH_3CN, and 10% CH_3OH was used to make the reaction solution homogeneous. All reaction procedures were the same as described in the CBZ oxidation reaction except that *cis*-stilbene (1 mM) was used instead of CBZ.

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ence and Engineering Foundation (Grant No. 93-05-00-04) (W.N.) is gratefully acknowledged.

References

1. (a) Ortiz de Montellano, P. R., Ed. *Cytochrome P-450: Structure, Mechanism, and Biochemistry*; Plenum Press: New York, 1986. (b) Reedijk, J., Ed. Bioinorganic Catalysis; Marcel Dekker, Inc.: New York, 1993. (c) Barton, D. H. R.; Martell, A. E.; Sawyer, D. T., Eds. *The Activation of Dioxygen and Homogeneous Catalytic Oxidation*; Plenum Press: New York, 1993. (d) Tullius, T. D., Ed. Metal-DNA Chemistry; ACS Symposium Series 402; American Chemical Society: Washington, DC, 1989. (e) Sigman, D. S.; Mazumder, A.; Perrin, D. M. *Chem. Rev.* **1993**, *93*, 2295-2316.
2. (a) Meunier, B. *Chem. Rev.* **1992**, *92*, 1411-1456 and references therein. (b) Montanari, F.; Casella, L., Eds. *Metalloporphyrins Catalyzed Oxidations*; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1993. (c) Meunier, B.; Pratviel, G.; Bernadou, J. in *Bioinorganic Chemistry*; Kessissoglou, D. P., Ed., Kluwer Academic Publishers: Dordrecht, The Netherlands, 1995, pp 201-208.
3. (a) Meunier, B. *New. J. Chem.* **1992**, *16*, 203-211 and references therein. (b) Pitie, M.; Bernadou, J.; Meunier, B. *J. Am. Chem. Soc.* **1995**, *117*, 2935-2936. (c) Pratviel, G.; Duarte, V.; Bernadou, J.; Meunier, B. *J. Am. Chem. Soc.* **1993**, *115*, 7939-7943.
4. (a) La, T.; Richards, R.; Miskelly, G. M. *Inorg. Chem.* **1994**, *33*, 3159-3163. (b) Lee, Y. J.; Lee, K. A.; Nam, W., manuscript in preparation.
5. The DNA cleavage reaction was carried out at pH 5 since the reactivity of the iron complex was found to depend on the pH value of the reaction solution. A similar pH effect on the reactivity of catalyst was observed in metalloporphyrin (M=Mn, Fe) complex-catalyzed oxidation reactions.[6]
6. (a) Bernadou, J.; Fabiano, A.-S.; Robert, A.; Meunier, B. *J. Am. Chem. Soc.* **1994**, *116*, 9375-9376. (b) Labat, G.; Meunier, B. *J. Org. Chem.* **1989**, *54*, 5008-5011. (c) Labat, G.; Meunier, B. *New. J. Chem.* **1989**, *13*, 801-804.
7. We have observed that $Fe(TDCPPS)^{3+}$ is a better catalyst than $Fe(TF4TMAP)^{5+}$ in the olefin epoxidation of CBZ (CBZ=carbamazepine) by $KHSO_5$. Yang, S. J.; Nam, W., manuscript in preparation.
8. (a) Bernadou, J.; Pratviel, G.; Bennis, F.; Girardet, M.; Meunier, B. *Biochemistry* **1989**, *28*, 7268-7275. (b) Pasternack, R. F.; Gibbs, E. J. in *Metal-DNA Chemistry*; Tullius, T. D., Ed., ACS Symposium Series 402; American Chemical Society: Washington, DC, 1989, pp 59-73.
9. Fouquet, E.; Pratviel, G.; Bernadou, J.; Meunier, B. *J. Chem. Soc., Chem. Commun.* **1987**, 1169-1171.
10. (a) Muller, J. G.; Zheng, P.; Rokita, S. E.; Burrows, C. J. *J. Am. Chem. Soc.* **1996**, *118*, 2320-2325. (b) Daniels, J. S.; Gates, K. S. *J. Am. Chem. Soc.* **1996**, *118*, 3380-3385. (c) Kawanishi, S.; Yamamoto, K.; Inoue, S. *Biochem. Pharm.* **1989**, *38*, 3491-3496. (d) Mottley, C.; Mason, R. P. *Arch. Biochem. Biophys.* **1988**, *267*, 681-689. (e) Gilbert, B. C.; Stell, J. K. *J. Chem. Soc., Perkin Trans 2* **1990**, 1281-1288.
11. Another possible DNA-cleaving agent is an iron porphyrin complex containing HSO_5^- ligand, $(TF_4TMAP)Fe^{III}\text{-}OOSO_3H$.[12]
12. (a) Sorokin, A.; Robert, A.; Meunier, B. *J. Am. Chem. Soc.* **1993**, *115*, 7293-7299. (b) Nam, W.; Hwang, W.; Ahn, J. M.; Yi, S.-Y.; Jhon, G.-J. *Bull. Korean Chem. Soc.* **1996**, *17*, 414-416.
13. As in the DNA cleavage reaction, other oxidants such as H_2O_2 and *tert*-butyl hydroperoxide did not give the CBZ-10,11 oxide product in the epoxidation of CBZ by the iron porphyrin complex.
14. (a) Castellino, A. J.; Bruice, T. C. *J. Am. Chem. Soc.* **1988**, *110*, 158-162. (b) He, G.-X.; Bruice, T. C. *J. Am. Chem. Soc.* **1991**, *113*, 2747-2753.
15. *cis*-Stilbene oxide (0.94 mM, 94% based on the starting *cis*-stilbene) and *trans*-stilbene oxide (0.02 mM, 2% based on cis-stilbene) were found to be formed as products in this reaction.
16. (a) Labeque, R.; Marnett, L. J. *J. Am. Chem. Soc.* **1989**, *111*, 6621-6627. (b) Catalano, C. E.; Ortiz de Montellano, P. R. *Biochemistry* **1987**, *26*, 8373-8380.
17. Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning: A Laboratory Manuals*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1989.