Calix[6]arene-bismetalloporphyrins as Enzyme Models for P450 V. Catalytic Performance in Epoxidation of Styrene

LI, Dong-Hong(李东红)   **CHEN, Shu-Hua***(陈淑华)   **ZHAO, Hua-Ming**(赵华明)
The Faculty of Chemistry, Sichuan University, Chengdu, Sichuan 610064, China

In this paper the catalytic performance of the calix[6]arene-bis-metalloporphyrins in the epoxidation of styrene was studied. The influence of phase transfer catalyst, pH value, buffer agent, substrate concentration, substituent on the benzene ring of porphyrin, and the central metal ion, *etc.*, on the reaction rates were investigated meticulously. The results showed that the calix[6]arene-bismetalloporphyrins had a much higher catalytic activity than that of the corresponding metalloporphyrins.

Keywords   calix[6]arene-bismetalloporphyrins, cytochrome P450 model compound, styrene, epoxidation

Introduction

Cytochromes P450 are involved in many steps of the biosynthesis or biodegradation of endogenous compounds. They also play a key role in the oxidative metabolism of exogenous compounds such as drugs and other environmental products.[1] Synthetic metalloporphyrins are efficient models of the cytochrome P450 family of monooxygenase enzymes and have been frequently used as catalysts in oxidations of organic substrates.[2,3]

Recent advances in biomimetic chemistry indicated that relevant binuclear metal complexes might be used as models for large number of metalloenzymes, so the study of the porphyrin dimers as the artificial enzymes capable of binding, recognition and catalysis has currently aroused widespread interest. A large number of different types of porphyrin dimers have been reported. Especially, some bismetalloporphyrin as enzyme model compounds showed quite prominent catalytic performance when they maintained appropriate conformation.[4-8]

According to the criterion for designing a good enzyme model, providing hydrophobic binding sites or matrix for substrate are particularly important. In our previous work, we observed that the catalytic performance of metalloporphyrin was increased obviously when it was associated with some compounds which have hydrophobic plane or cavity such as steroids and calix[4]arene.[9,10] Calix[6]arene is made up of six phenol units, having a bigger hydrophobic

cavity than that of calix[4]arene. To take advantage of such feature of calix-[6]arene, we had designed and synthesized a novel type of artificial enzymes by linking calix[6]arene to porphyrin via etheric linkage and then inserting metal ions,[11] as shown in Scheme 1, and studied their catalytic performance in the epoxidation of cyclohexene.[12,13] In this paper, we mainly reported the catalytic performance of calix[6]arene-bismetalloporphyrins in the epoxidation of styrene.

Scheme 1
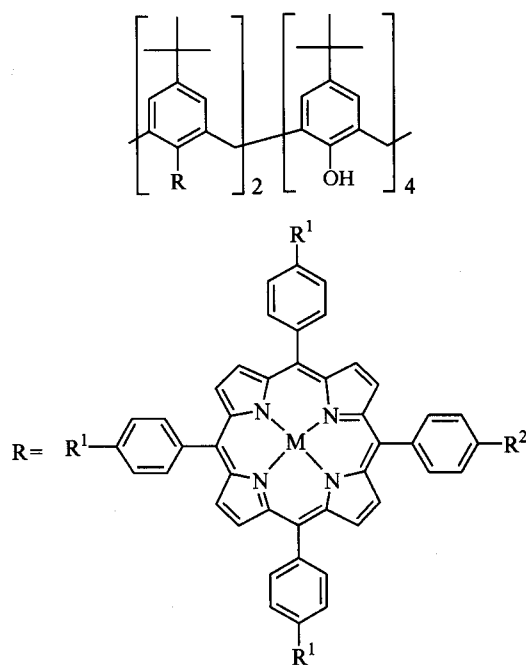
1a: $R^1 = Cl$, $R^2 = OCH_2CH_2CH_2O$, M = Mn(III)Cl;
1b: $R^1 = Cl$, $R^2 = OCH_2CH_2CH_2O$, M = Fe(III)Cl;
1c: $R^1 = Cl$, $R^2 = OCH_2CH_2CH_2O$, M = Co(II);
1d: $R^1 = Cl$, $R^2 = OCH_2CH_2CH_2O$, M = Zn(II);
2: $R^1 = OCH_3$, $R^2 = OCH_2CH_2CH_2O$, M = Mn(III)Cl;
3: $R^1 = H$, $R^2 = OCH_2CH_2CH_2O$, M = Mn(III)Cl;
TCPP: $R^1 = R^2 = Cl$, M = Mn(III)Cl

Experimental

Materials

Styrene, toluene, benzaldehyde and dichloromethane were purified prior to use. Styrene epoxide used as standard sample in GC (made in Japan). Benzyldimethyltetradecylammonium chloride (phase-transfer catalyst) is made in Switzerland. Chloros was diluted from 1.0 to 0.35 mol/L. The accurate concentration was titrated with the iodimetry. Sodium hypochlorite buffer solution was prepared as follows: 0.1 g of sodium borate was added to 25 mL of sodium hypochlorite solution, and the pH was adjusted with 10% sodium hydroxide or 10% hydrochloride. Other chemicals used were of reagent grade. Calix[6]arene-bismetalloporphyrins 1, 2, 3 and TCPP were synthesized in our laboratory and their structures were confirmed by means of NMR (600 MHz), MS (FAB), IR and UV-Vis spectrometry.[11]

Catalytic epoxidation of styrene and products analysis

All reactions were carried out in a 10 mL Schlenk tube equipped with a stirrer at (20 ± 0.2) ℃. 0.30 mmol of styrene, 0.015 mmol of benzyldimethyltetradecylammonium chloride (phase-transfer catalyst), 0.15 mmol of toluene (standard for GC analysis), 2.0×10^{-3} mmol of catalyst, and 2.0×10^{-2} mmol of axial ligand were added successively to 2 mL of dichloromethane. Then 3.0 mL of sodium hypochlorite (0.37 mol/L) was added batch-wise to the organic phase. Magnetic stirring was stopped before each aliquot (2 μL) was withdrawn from the organic phase for product analysis. Analytical condition of GC: oven temperature 150 ℃, column temperature 98 ℃, flowing speed of the hydrogen gas 28 mL/min, 6201 red carrier, SE-30 as fixed phase. The content of each component was gotten directly from a CDMC-1E chromatograph digital computer with the method of internal standard. Under these analytical conditions, the retention time of toluene, styrene, benzaldehyde and styrene epoxide were 0.93, 1.58, 2.23 and 3.42 min respectively.

Results and discussion

Olefin epoxidation catalyzed by manganese(III) porphyrins with NaOCl has been well-established.[14,15] Thus we chose aqueous-organic two-phase conditions with styrene as substrate to examine the performance of synthesized calixarene-porphyrin complexes as model of cytochrome P450 monooxygenase. 1a was chosen as a typical catalyst for the epoxidation. For comparison to the model system, reactions were conducted with TCPP under the same conditions.

Effects of various factors, such as phase-transfer catalyst, pH value, buffer solution, and the substituent in the meso-phenyl of porphyrins, etc., on the epoxidation were

investigated.

Comparison of catalytic performance

Under the same reaction conditions (20 ℃, N-methylimidazole as axial ligand, pH = 13.0), we compared the catalytic performance of TCPP and compound 1a. As shown in Fig. 1, the catalytic performance of 1a was obviously higher than the TCPP. After 155 min, the yields of styrene epoxide were 26% and 94.4% catalyzed by TCPP and 1a respectively, and the yield was also up to 84.5% as the concentration of 1a was reduced to 5.0×10^{-4} mol/L (so the content of porphyrin Mn(III) in TCPP and 1a is equivalent). From the results it is suggested that the excellent catalytic property of model compound may be attributed to the hydrophobic action of calixarene and the cooperative action of two metalloporphyrins.
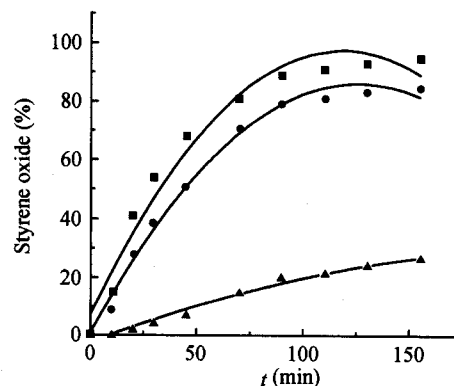

Fig. 1   Comparison of catalytic performance. ■—1.0×10^{-3} mol/L 1a; ●— 5.0×10^{-4} mol/L 1a; ▲—1.0×10^{-3} mol/L TCPP.

Michaelis menten behaviour

We had investigated the influence of styrene concentration for initial velocity of epoxidation reaction and found that the initial velocity increase linearly as the styrene concentration changed from zero to 0.25 mol/L, and then trended to level off as the concentration exceeded 0.25 mol/L (as shown in Fig. 2). This is characteristic of enzyme-driven reaction, in which the substrate tends to become saturated by the catalyst. This showed that the calix[6]arene-bismetalloporphyrins 1a was a versatile model of P450. Plotting the reciprocal of the initial velocity vs. the reciprocal of the olefin concentrations gave a straight line (Fig. 3, $\gamma = 0.997$), from which the kinetic power, the maximum velocities, Michaelis contant and first-order velocity constant were obtained as $1.446 \times 10^{-3} s^{-1}$, 2.45×10^{-4} mol·L^{-1}·s^{-1}, 0.1696 mol·L^{-1} and 0.245 s^{-1} respectively, according to Lieweaver-Burk equation.

Affecting factors for catalytic activity

The influence of phase transfer catalyst (benzyldi-

methyltetradecylammonium chloride) on the epoxidation of styrene is shown in Fig. 4. The inductive period was shortened and the yield of styrene epoxide was increased rapidly by adding the phase-transfer catalyst. For instance, at 155 min, the yield of styrene epoxide was increased by 75.5% (from 19% to 94.5%). This indicated that the benzyldimethyltetradecylammonium chloride plays
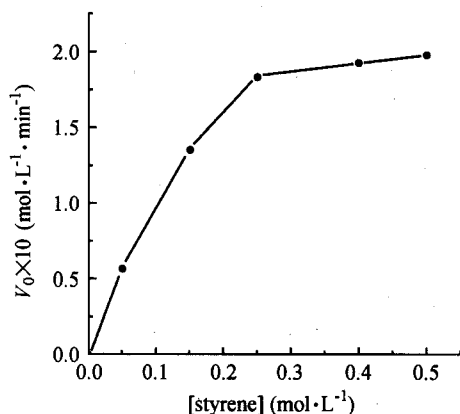
the auxocatalysis role for the styrene epoxidation as sodium hypochloride being used as oxidant in water-dichloromethane two-phase system.

The influence of pH value on the epoxidation of styrene is shown in Table 1. It can be seen from the table that when the pH value was too high or too low, the yield and the selectivity decreased. Because there was a balance in this system, as shown in formula 1: $OCl^- + Cl^- + H_2O \rightleftharpoons Cl_2 + 2OH^-$. This implies that, the pH value increased along with the reaction procession. In order to control the increasing of pH value, we added buffer—$Na_2B_2O_7$ to the system, and compared the catalytic result with no buffer in this system. The result is shown in Fig. 5. From which we found that the yield of styrene epoxide was increased effectively in the presence of the buffer.

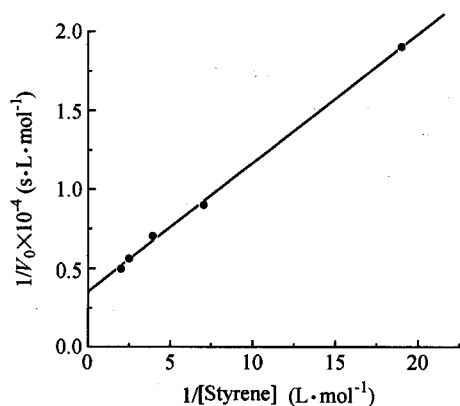**Fig. 2** Plot of initial velocity vs. styrene concentration.

Fig. 3 Reciprocal plot of the initial velocity (V_0) vs. the reciprocal of styrene concentration.

Table 1 Influence of pH value on styrene epoxidation

pH value	Conversion (%)	Yield (%)	Selectivity (%)
10.0	97.66	72.76	74.47
11.8	90.22	82.02	91.07
13.0	100	94.40	94.40
14.0	100	69.46	69.46
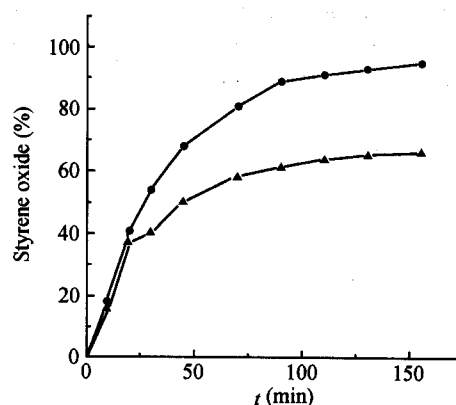
Fig. 5 Influence of buffer solution on the epoxidation of styrene. ■—the buffer solution is in presence; ▲—the buffer solution is in absence.

The influence of substituent in the *meso*-phenyl of porphyrin is shown in Table 2. From which it was remarkable that the yield and selectivity of the epoxidation reaction degraded in accordance with the decreasing sequence of electron withdrawing power of the substituent on phenyl group in porphyrin *i. e.* Cl > H > OCH_3. This indicated that the stability of porphyrin became the main factor as the styrene being oxidized by strong oxidant sodium hypochloride. As a electron withdrawing group, Cl atom (− I effect > + C effect) could decreases the electron cloud density of porphyrin, so the auto-oxidation of porphyrin was decreased, and the metal porphyrin showed higher catalytic activity.
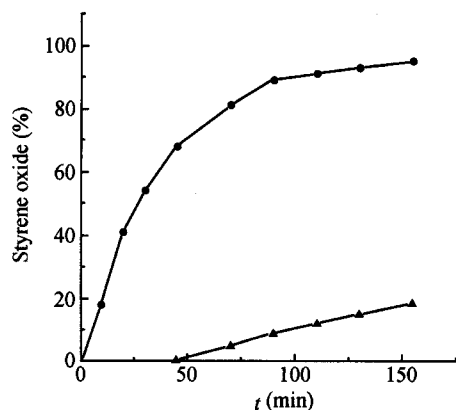

Fig. 4 Influence of phase transfer catalyst on the epoxidation of styrene. ●—the phase transfer catalyst is in presence; ▲—the phase transfer catalyst is in absence.

Table 2 Influence of substituent on styrene epoxidation

Substituent	Conversion (%)	Yield (%)	Selectivity (%)
p-Cl	100	94.4	94.4
H	92.58	79.36	85.72
p-OCH$_3$	61.27	38.35	62.57

Under the same condition (20 ℃, N-methylimidazole as axial ligand, pH = 13.0, benzyldimethyltetradecylammonium chloride as phase transfer catalyst), when the epoxidation of styrene was catalyzed by Co(II)-porphyrin (**1c**) and Zn(II)-porphyrin (**1d**), no styrene epoxide was detected by GC analysis. In other word, under these conditions we used, the **1c** and **1d** had no catalytic activity for styrene epoxidation. And catalyzed by Fe(III)-porphyrin (**1b**), the yield of styrene epoxide was lower than that catalyzed by **1a**.

Conclusion

Calix[6]arene-bis(Mn)$^{3+}$ porphyrins had much higher catalytic activity than the corresponding metalloporphyrins. This fact reveals that how important it is to provide a hydrophobic binding site or microenvironment in designing enzyme model, and when two metalloporphyrins maintained an appropriate conformation, they have cooperative action in catalysis to enhance their catalytic activity.

References

1   Mansuy, D. *Pure Appl. Chem.* **1987**, *59*, 759.

2   Baldwin, J. E. P. *Top. Curr. Chem.* **1984**, *121*, 181.

3   Meunier, B. *Bull. Soc. Chim. Fr.* **1983**, 345.

4   Collman, J. P.; Elliott, C. M.; Halbert, T. R.; Tovrog, B. S. *Proc. Natl. Acad. Sci. U. S. A.* **1977**, *74*, 18.

5   Narata, Y.; Sasayama, M. *Chem. Lett.* **1994**, 2411.

6   Guo, C.-C. *Acta Chim. Sinica* **1994**, *52*, 367 (in Chinese).

7   (a) Chen, S.-H.; Li, S.-J. *Chin. Chem. Lett.* **1992**, *3*, 801.
     (b) Chen, S.-H.; Li, S.-J.; Wang, Y.-L. *Chin. J. Chem.* **2000**, *18*, 201.

8   Chen, S.-H.; Chen, Y.; Li, H.-G. *Atca Chim. Sinica* **1997**, *55*, 716 (in Chinese).

9   Yuan, L.-H.; Chen, S.-H.; Zhao, H.-M. *J. Mol. Catal.* **1997**, *11*, 283.

10 Chen, S.-H. *Chin. J. Chem.* **1999**, *17*, 309.

11 Li, D.-H.; Chen, S.-H.; Zhao, H.-M. *J. Sichuan Univ. (Nat. Sci. Ed.)* **1999**, *36*, 913 (in Chinese).

12 Li, D.-H.; Chen, S.-H.; Zhao, H.-M. *J. Mol. Catal.* **1999**, *13*, 81 (in Chinese).

13 Li, D.-H.; Chen, S.-H.; Zhao, H.-M. *Acta Chim. Sinica* **1999**, *57*, 869 (in Chinese).

14 Miyamoto, T. K.; Takagi, S.; Hasegawa, T.; Takahashi, E.; Okude, K.; Banno, I.; Sasaki, Y. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 1649.

15 Meunier, B.; Carvalho, M. E.; Bortolini, O.; Momenteau, M. *Inorg. Chem.* **1988**, *27*, 161.

(E0211265 CHENG, B.; ZHENG, G. C.)