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An Efficient Model of Cytochrome P-450, Monooxygenase Reactivity; Polymer-Supported Metalloprotoporphyrin IX (M=Fe, Mn)

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Introduction

The elucidation of the monooxygenase systems has been the focus of sustained attention for over two decades.[1-3] Especially, the cytochrome P-450 monooxygenase is the most intensely investigated one. It is mainly due to the fact that these enzyme systems catalyze oxidation steps involved in metabolizing biomolecules and particularly xenobiotics.[4,5] They also provide the possibility of being used as alternative means of organic synthesis.[6-11] Recently, many attempts have been made to reproduce the reactivity in chemical systems with a simple metalloporphyrin as a model compound of heme proteins.

However, two major problems have interfered with the practical application of metalloporphyrin catalysts in synthesis: (1) the catalysts also act as substrates for the oxidant and are destroyed through degradative intermolecular reaction during the catalytic oxidation reaction and (2) it is very hard to recover and reuse the catalysts used.

Therefore, a number of research groups[12-15] have recently designed, prepared and tested catalysts consisting of metalloporphyrins on polymer supports in order to mimick an apoprotein. Thus, these polymer-supported catalysts offer several advantages such as "site isolation"[16] of the catalytic centers on the polymer to prevent degradative intermolecular reaction. However, most of the previous studies have utilized polymer-supported catalysts containing metallotetraphenylporphyrin and metallophthallocyanine.

This paper describes the synthesis and catalytic properties of polymer-supported metalloprotoporphyrin IX (MPPIX, M =metal, PPIX=protoporphyrin IX) (M=Fe, Mn) which is a combination of metalloprotoporphyrin IX and various new polymer supports. The catalytic properties have been tested on epoxidation reactions using cyclohexene as a substrate and hydrogen peroxide as an oxidant in the presence/absence of an axial ligand (pyridine or imidazole). Whether the new catalysts are stable in various systems was also studied.

Experimental

Materials. PPIX, poly(2-vinylpyridine-co-styrene), 1-vinylimidazole, 4-vinylstyrene, styrene and α,α'-azobisiso-

butyronitrile (AIBN) were purchased from Aldrich Chemical Co. The styrene was washed with aqueous NaOH to remove inhibitors, then with water, dried with $MgSO_4$ and distilled under reduced pressure, and passed through a neutral alumina column before use. AIBN was crystallized from acetone and dried under a vacuum at room temperature. Hemin and hydrogen peroxide (30%) were purchased from Wako Pure Chemical Industries, LTD. (Japan) and used without further purification. Iodosylbenzene (PhIO) was prepared from iodobenzene diacetate by a literature method.[17] The standards such as cyclohexene, 1,2-epoxycyclohexane, 2-cyclohexanol, 2-cyclohexanone and decane were obtained from Aldrich Chemical Co. The following solvents were purified according to known methods[18]: acetone, benzene, pyridine and methylene chloride.

Syntheses of Polymer Supports. Polymer supports such as polystyrene (PS), poly(4-vinylpyridine-co-styrene) (PSP4), poly(1-vinylimidazole-co-styrene) (PSI1), and poly (2-vinylpyridine-co-styrene) (PSP2) were prepared using a known method.[12] A typical procedure for PSP4 is described as follows: 3.47 mL (0.03 mole) of freshly distilled styrene, 7.86 mL (0.07 mole) of 4-vinylpyridine and 0.3 g (2 mmole) of AIBN were placed in a Schlenk tube and dissolved in 20 mL of benzene. The mixture was degassed via five freeze-pump-thaw cycles in a vacuum line, and reacted at 60 °C for 24 hr under a nitrogen atmosphere and cooled to room temperature. The reaction mixture was then poured into petroleum ether and the precipitated polymer was filtered, recrystallized from chloroform/petroleum ether, then dried in vacuo to yield the yellowish polymer.

The other two polymer supports,[19] poly(4-hydroxystyrene-co-styrene) [PS(4OH)] and poly(4-hydroxystyrene-co-4-vinylpyridine) [PS(4OH)P4], were synthesized by a two-step reaction: initially 4-acetoxystyrene and styrene (or 4-vinylpyridine) were used to generate poly(4-acetoxystyrene-co-styrene) [PS(4OAc)] (or [PS(4OAc)P4]), and then an excess of acid (12 N HCl) was applied to obtain PS(4OH) (or PS(4OH)P4) by hydrolysis.

Syntheses of Polymer-Supported MPPIXs

1. Ketone linkage[12] (MPPIX-PS, MPPIX-PSP2, MPPIX-PSI1 and MPPIX-PSP4, here M=Fe and Mn) Fifty milligrams of MPPIX (7.7×10^{-5} mole) and 2 mL of thionyl chloride (2.7×10^{-2} mole) were combined in a 250 mL round bottom flask. When gas evolution subsided, 50 mL of 1,1,2,2-tetrachloroethane and 3 g of polymer (2.9×10^{-2}

mole of repeating polymer units) were added. The mixture was heated to 130 °C while stirring to drive off excessive SOCl₂. After cooling to 10 °C, 2 g of AlCl₃ was added, and the mixture was stirred without further cooling, for 24 hr. The solid was filtered and thoroughly washed with tetrachloroethane and recrystalized in methanol.

2. Ester linkage[19] (MPPIX-PS(4OH) and MPPIX-PS(4OH) P4) Four grams of PS(4OH) polymer and 6 g of 4-(dimethylamino)pyridium 4-toluenesulfonate were combined in a two-neck round bottom flask fitted with a Claisen adapter connected to a septum, and an argon manifold. The system was flushed with argon and 5 mL of dry methylene chloride was added via syringe. 50 mg of FePPIX (7.7× 10⁻⁵ mole) and 51.6 mg (0.25 mmole) of dicyclohexyl-carbodiimide was combined with 1 mL of dry pyridine in a 10 mL vial. This mixture was added to the flask *via* syringe. Stirring at room temperature under argon was continued for 12-24 hr and once the reaction was completed, the solution was poured into 25-50 volumes of rapidly stirred methanol. FePPIX-PS(4OH) was precipitated, collected on a glass funnel, washed with cold methanol, and dried in a vacuum.

MnPPIX-PS(4OH), FePPIX-PS(4OH)P4 and MnPPIX-PS (4OH)P4 were prepared by a similar method. (In the cases of FePPIX-PS(4OH)P4 and MnPPIX-PS(4OH)P4, precipitation was formed in cold acetone).

General Procedure for Epoxidation. In a typical experiment, the catalyst in an amount equivalent to 5×10^{-4} mmole catalytical units was suspended in acetone (8 mL) along with cyclohexene (2.00 mmole) as a substrate and decane (10 μL) as an internal standard. The reaction was initiated by the addition of H₂O₂ (13.8 mmole) to the solution. The reaction was controlled by GC by taking-off aliquots, filtering them, and conducting to analyses. The products were identified by comparison of the retention times with those of the authentic samples, or otherwise proven by GC/ mass. The content of H₂O₂ was determined by iodometric titration. All reactions were carried out at 0 °C in the dark under a nitrogen atmosphere.

Measurements. UV-visible spectra were recorded on a Hewlett Packard 8452A diode array spectrophotometer and IR spectra were obtained on a Midac FT-IR spectrophotometer. GC analysis was carried out on a Hewlett Packard 5890 equipped with FID and an Ultra-2 column (25 m × 0.32 mm, i.d.).

Results and Discussion

Synthesis and Characterization. There are two ways for the chemical attachment of the metalloporphyrin to the polymer supports. One is covalent binding[13] and the other is coordinative binding.[14] Polymer-supported metalloporphyrins with both binding modes were designed. The most common approach, used to prepare metalloporphyrins covalently bound to supports, is to react one or more substituents on the performed porphyrin ring with a functional group on the surface of the support. The fact that PPIX contains a pendant carboxylic acid functional group was used in the synthesis of these polymer-supported MPPIXs, and the formations of ketone and ester linkages were employed.

Also, metalloporphyrin can attach, by coordination, to ligands on the surface of the support as a proximal ligand
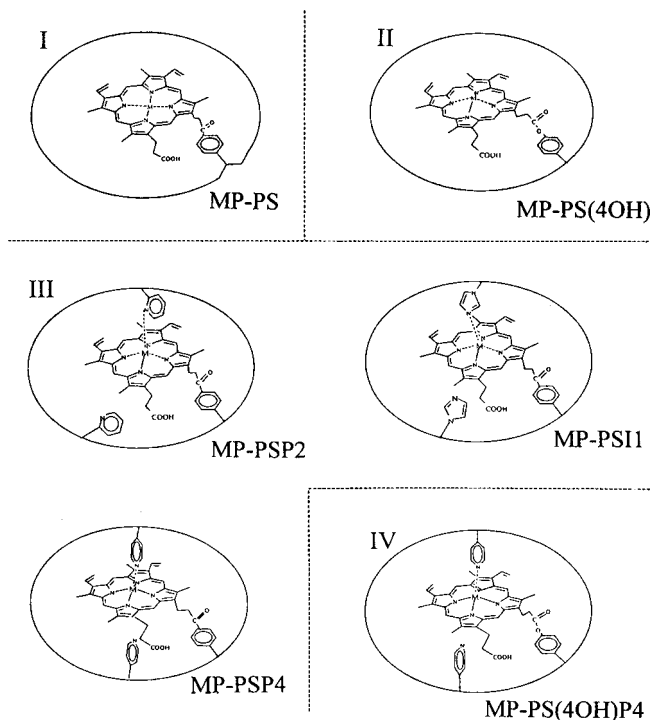
Figure 1. Idealized structures of synthetic polymer-supported metallprotoporphyrin IX (M=Fe, Mn) and MP = MPPIX.

and/or a distal ligand. Some of the synthetic polymer-supported metalloporphyrins have a nitrogen atom as an axial ligand. The polymer-supported metalloporphyrins, which are used here, are classed into four groups (Figure 1): (1) the first group having ketone linkage between MPPIX and polymer support (MPPIX-PS), (2) the second group containing ester linkage (MPPIX-PS(4OH)), (3) The third group combining ketone linkage with proximal ligand (MPPIX-PSP2, MPPIX-PSI1, MPPIX-PSP4), and (4) the forth group including ester linkage and proximal ligand (MPPIX-PS(4OH) P4).

The resulting catalysts were characterized by common spectroscopic techniques such as UV/Vis, FT-IR and AA. The absorption maxima, λ_max, of FePPIXCl and MnPPIXCl were centered at 398 and 464 nm, respectively.[20] It is a very intense absorption band due to a π-π* transition.[21] In all metalloporphyrins, it is shown near 400 nm which is called the Soret or B band. However, there are no absorption bands above 300 nm on polymer supports such as PS, PSP2, PSP4, PSI1, PS(4OH) and PS(4OH)P4. After the polymer-supported MPPIX is formed between polymer support and MPPIX, the Soret band reappeared near 400 nm which is a typical band for MPPIX. In the case of polymer-supported MnPPIX series, the absorption maxima due to a π-π* transition showed around 462-472 nm. The absorption maxima of polymer-supported FePPIX series showed around 396-404 nm. The Soret bands of all catalysts are summarized in Table 1.

A characteristic carbonyl (-CO) stretching band was observed in the region between 1618-1638 cm⁻¹ for catalysts with ketone linkage (MPPIX-PS, MPPIX-PSP2, MPPIX-PSI 1, MPPIX-PSP4), as compared with 1703 cm⁻¹ for the parent FePPIX and 1723 cm⁻¹ for MnPPIX. In general, the

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Table 1. Physical properties of polymer-supported metalloproto-porphyrin IX complexes

|	λ_{max}^{a} (nm)	$v(C=O)$ (cm^{-1})	$v(C-O)$ (cm^{-1})	content^{c} (mole/mole)
MnPPIX($\equiv$MnP)	464	1723	-	-
MnP-PS	462	1632	-	0.148
MnP-PSP2	464	1618	-	0.849
MnP-PSI1	464	1630	-	0.080
MnP-PSP4	462	1638	-	0.221
MnP-PS(4OH)	472^{b}	1765	1214	0.186
MnP-PS(4OH)P4	464	1752	1204	0.134
FePPIX($\equiv$FeP)	398	1703	-	-
FeP-PS	396	1634	-	0.121
FeP-PSP2	400	1617	-	0.519
FeP-PSI1	396	1626	-	0.153
FeP-PSP4	400	1638	-	0.209
FeP-PS(4OH)	404^{b}	1763	1204	0.543
FeP-PS(4OH)P4	400	1750	1221	0.303

^{a} in acetone. ^{b} in methanol. ^{c} The analysis of metalloporphyrin content (%) in each polymer-supported metalloporphyrin (mole contents of metalloporphyrin vs polymer in polymer-supported metalloporphyrin).

carbonyl stretching wavenumber is observed between 1820-1660 cm^{-1}, the basic value[22] of carbonyl for ketone appears at 1715 cm^{-1}. The carbonyl stretching band of newly synthesized catalysts with ketone linkage shifts to lower wavenumbers due to the conjugation effect and intramolecular hydrogen bonding.

Figure 2 shows the series of IR spectra of an MnP-PS (4OH)P4 catalyst. Trace A shows the IR spectrum of MnPPIX, which exhibits the band typical of carbonyl at 1723 cm^{-1}. Trace B shows the IR spectrum of PS(4OAc)P4. This shows the bands at 1750 cm^{-1} for carbonyl stretching and at 1200 cm^{-1} for C-O stretching vibration. After a reaction with conc. HCl, the bands (C=O and C-O) had disappeared, and a band at 3443 cm^{-1} caused by -OH was observed (not shown in the Figure 2). As seen in trace D, two new bands appear at 1752 cm^{-1} for C=O and 1204 cm^{-1} for C-O due to ester linkage for MnPPIX-PS(4OH)P4 . The carbonyl stretching wavenumbers of all catalysts are also summarized in Table 1.

The contents of MPPIX in each catalyst were analyzed and calculated on the basis of the content of metal by AA data, and mole contents of MPPIX vs polymer in catalysts are as summarized in Table 1.

Catalytic Oxidation. There are few reports on oxidations with H$_2$O$_2$ catalyzed by polymer-supported metalloporphyrins. The main reason for this is probably the difficulty in preparing polymer-supported catalysts with a good donor ligand which is necessary to generate and stabilize the high-valent oxometalloporphyrin π-cation radical species.[23] Campestrini and Meunier[24] investigated the oxidation of cyclooctene with H$_2$O$_2$ in CH$_3$CN with combined ligand and electrostatically bound Mn^{III} catalysts, but no reaction occurred according to their report. Hirobe and his coworkers[25,26] reported that iron(III)porphyrins, covalently bound to poly-L-glutamate, catalyzed oxidation of styrene to styrene oxide by H$_2$O$_2$, but the yields were low (<10 catalyst turnover). And there is no report on oxidation ca-
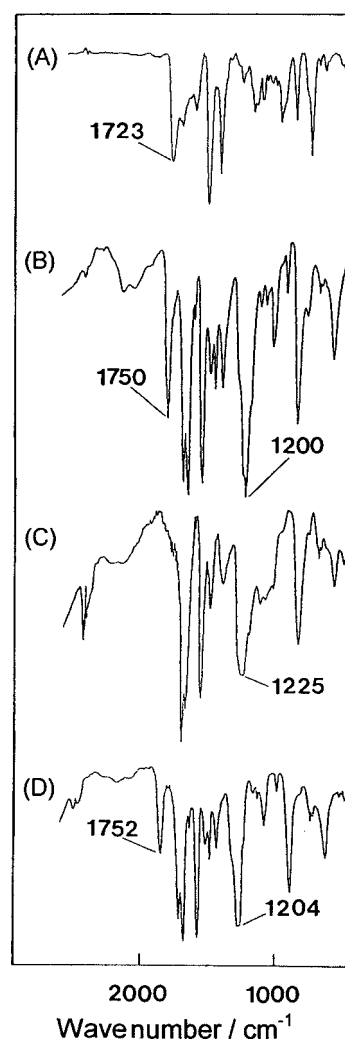
Figure 2. IR spectra of (a) MnPPIX, (b) PS(4OAc)P4, (c) PS (4OH)P4 and (d) MnPPIX-PS(4OH)P4.

talyzed by FePPIX due to a bleach, even though it is the real active site for cytochrome P-450. Substituted tetraphenylporphyrin and phthallocyanine types were usually used. Especially, it is known that catalysts halogenated on the porphyrin β-pyrrol positions (tetraphenylporphyrin type) such as tetrakis(2,6-dichlorophenyl)porphyrin and tetrakis (pentafluorophenyl)porphyrin are good catalysts.

Here, we report the catalytic oxidation of cyclohexene with H$_2$O$_2$ catalyzed by newly synthesized catalysts. It afforded a mixture of products (Scheme 1). 1,2-epoxycyclohexane (product A) was identified as the main component in the product mixture. Further products were identified as 2-cyclohexenol (product B) and 2-cyclohexenone (product C). Equimolar amounts (5 × 10^{-4} mmole) of MPPIX in each polymer were used for catalytic oxidation. All reactions
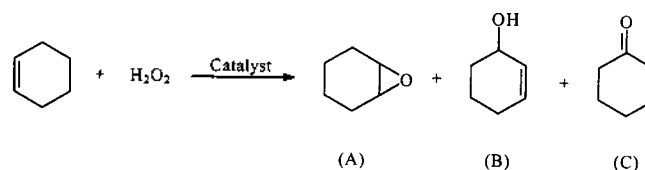
Scheme 1

Table 2. The catalytic dependence of the oxidation products in the reaction of cyclohexene with H_2O_2

Catalysts		Yields of products (mmole $\times 10^{-2}$)				turnover No.	PR^d
metallo-porphyrin	polymer support	oxide[a]	ol[b]	one[c]	total		
MnPPIX	PS	0.73	0.31	0.30	1.3	26	1.2
	PSP2	0.74	0.33	0.31	1.4	28	1.2
	PSl1	0.89	0.37	0.39	1.7	34	1.2
	PSP4	0.92	0.38	0.41	1.7	34	1.2
	PS(4OH)	0.93	0.38	0.39	1.7	34	1.2
	PS(4OH)P4	1.03	0.33	0.33	1.7	34	1.6
FePPIX	PS	0.58	0.29	0.32	1.2	24	1.0
	PSP2	0.62	0.25	0.34	1.2	24	1.1
	PSl1	0.64	0.22	0.29	1.2	24	1.2
	PSP4	0.83	0.32	0.36	1.5	30	1.2
	PS(4OH)	0.86	0.35	0.29	1.5	30	1.3
	PS(4OH)P4	0.87	0.38	0.32	1.6	31	1.2

[a] 1,2-epoxycyclohexane. [b] 2-cyclohexenol. [c] 2-cyclohexenone. [d] product ratio (=oxide/(ol+one)). Experimental conditions; H_2O_2, 13.76 mmole; 5×10^{-4} mmole of MPPIX in each polymer-supported MPPIX; cyclohexene, 2 mmole; acetone, 8 mL; at 0 °C; in anaerobic condition; reaction time, 1 hr.

were performed at 0 °C under an anaerobic condition. Control experiments using a catalyst in the absence of the oxidant gave no oxidation products and using only the oxidant without the catalyst gave traces of oxidation products.

Table 2 shows the distribution of the oxidation products depending on the catalysts. It was found that, with the use of catalysts in the series of the same metal ion, the amount of 1,2-epoxycyclohexane, the amount of total oxidation products and the turnover number increased in the following order: PS < PSP2 < PSl1 < PSP4 < PS(4OH) < PS(4OH)P4. In general, the catalysts with ester linkage show better oxidation activities than those with ketone linkage, and in the case of MPPIX-PS, exhibit the lowest oxidation products. This can be explained by the effects of axial ligands. The Mn-containing catalysts were also found to be more active than Fe-analogs. The product ratio (PR) according to the catalysts used was calculated by the formula: PR=(oxide)/(ol+one). On the basis of the observed results, we come to the following conclusions: (1) Syntheses of models of the cytochrome P-450, monooxygenase systems based on the ketone or ester linkage are simple and efficient. (2) FePPIX, while bleached by H_2O_2 occuring no reaction, becomes an active catalyst when it is based on polymer supports.

Under conditions of catalytic oxidation, the ketone or ester linkage between MPPIX and polymer supports is sufficiently stable, and allows performance of a relatively high turnover number of the catalyst. These results suggest the possibility of modeling the active site of the monooxygenase systems by imposing conditions similar to the pro-

tein matrix by use of a synthetic polymer.

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