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Water-Soluble Metalloporphyrins as Mimics of Heme-containing Enzymes

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Abstract: This letter compared catalase-, peroxidase- and cytochrome P450-like catalytic activities of 15 water-soluble metalloporphyrins produced from Fe, Mn and Co ions and 5 porphyrins. The metalloporphyrins with Fe and Mn as central ions show relatively high catalytic activities of catalase and peroxidase at pH 11.0. Only Mn-*meso*-tetrakis (4-N-methylpyridinium) porpho-phine of the 15 metalloporphyrins exhibits high cytochrome P450-like activity. Effects of imidazole on the catalytic reactions were also studied.

Keywords: Metalloporphyrin, biomimetic catalysis, peroxidase, catalase, cytochrome P450.

Hemin is a naturally occurring metalloporphyrin (M-P), which is the common prosthetic group of heme-containing enzymes. Many M-Ps with different ligand structures and central metal ions were synthesized to mimic heme-containing enzymes[1-3]. To improve the catalytic capability and specificity of mimic enzyme, the effect of axial ligand was studied as well[4-8]. Similar to the active center of all heme-containing enzymes, M-P should show more or less catalytic activities of heme-containing enzymes, and the activities depend on the porphyrin structures and central metal ions. However, no study was conducted on the activities of the complex in a single report. In this communication the catalase-, peroxidase-, and cytochrome P-450-like catalytic activities of 15 water-soluble M-Ps were studied and some useful results were obtained. The reactions utilized in this study were decomposition of hydrogen peroxide, oxidation of homovanillic acid (HVA) with hydrogen peroxide, and oxidation of styrene with oxone. These were typical reactions catalyzed by catalase, peroxidase and cytochrome P-450, respectively.

Experimental

According to the procedures reported[9-12], four porphyrins were synthesized, *i.e.*, *meso*-tetrakis (*p*-sulfonatophenyl) porphophine ($TPPS_4$), *meso*-tetrakis (4-carboxyphenyl) porpho-phine (TCPP), *meso*-tetrakis [4-(N, N, N-trimethyl) aminophenyl] porphophine (TTMAPP) and *meso*-tetrakis (4-N-methylpyridinium) porphophine (TMPyP), and fifteen metallopor-phyrins were prepared by use of Fe, Mn, Co ions and porphyrin IX

(Sigma) plus four synthesized porphyrins[13]. The measurements of the enzyme-like catalytic activities were performed as follows:

Determination of catalase-like activity

Buffer solution (3.80 mL) and 100 μL M-P of 1.0×10^{-4} mol/L were mixed. H_2O_2 solution (100 μL) of 5.0×10^{-3} mol/L was then added under stirring. After 2 min, 100 μL of the mixed solution was taken out and placed into a mixture composed of 1.70 mL PBS (0.2 mol/L, pH 7.0), 100 μL horseradish peroxidase of 0.15 mg/mL and 100 μL homovanillic acid (HVA) of 2.0×10^{-3} mol/L. After 30 min, the fluorescence intensity was measured at $\lambda_{ex}/\lambda_{em}$=315/425 nm with a Perkin Elemer LS-50B luminescence spectrometer. From the intensity the percentage of H_2O_2 decomposition can be calculated.

Measurement of peroxidase-like activity

$NaHCO_3$-Na_2CO_3 buffer solution (1.70 mL), 100 μL HVA of 1.0×10^{-3} mol/L and 100 μL H_2O_2 of 1.0×10^{-4} mol/L were, in turn, added in a quartz cell with 10 mm long light path. Then, 100 μL M-P solution of 2.0×10^{-6} mol/L was added under stirring to initiate the reaction. The kinetic curve was recorded at $\lambda_{ex}/\lambda_{em}$=315/425 nm for reaction rate calculation.

Measurement of cytochrome P450 activity

Styrene solution (2.0 mL) of 0.05 mol/L in acetonitrile and 1.0 mL M-P solution of 1.0×10^{-4} mol/L were mixed together. Then, 1.0 mL oxone solution of 0.1 mol/L in 0.2 mol/L PBS (pH 6.8) was added under stirring. The reaction products were analyzed on a Shimidzu GC-9A (equipped with an SE-30 capillary column, FID detector and HP3394 integrator) by direct injection of 2.0 μL of the reaction mixture at a reaction time of 10 min and their structures were confirmed by using an HP 5971 GC-MS.

Results and discussion

The pH influences on the decomposition rate of H_2O_2 in the presence of M-Ps were studied and the results were depicted in **Figure 1**. Both negatively charged Fe-TCPP and positively charged Fe-TTMAPP showed similar activity-pH profiles. Relatively high activities were found in the pH range of 10.5~11.5. The data of catalase-like activities of 15 M-Ps in the presence and absence of imidazole at pH 10.5 indicated that the order of such activity for the M-Ps with the same central ion is: TMPyP≈TTMAPP> TCPP≈$TPPS_4$>porpthyrin IX; for the M-Ps with the same porphyrin ligand, the order is Fe>Mn>Co. The decomposition rate of H_2O_2 in the presence of Fe-TMPyP is the highest, 263 nmol/min, close to that of Fe-TTMAPP, 246 nmol/min, greater than that of Mn-TMPyP, 192 nmol/min, and much larger than that of Co-TMPyP, 16.9 nmol/min.

The presence of imidazole has some effects on the catalase-like activity of some M-Ps, but the effects are much weaker than those on peroxidase-like activity (see below).

Figure 1 Effect of pH on H_2O_2 decomposition in the presence of metalloporphyrins (n=3)

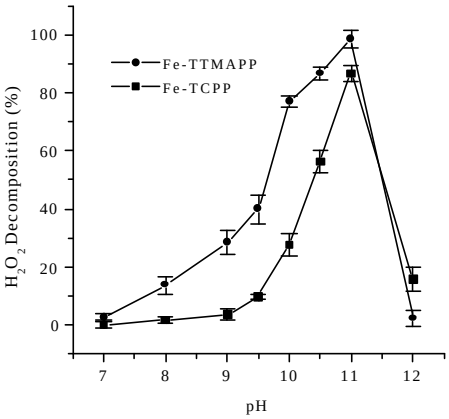
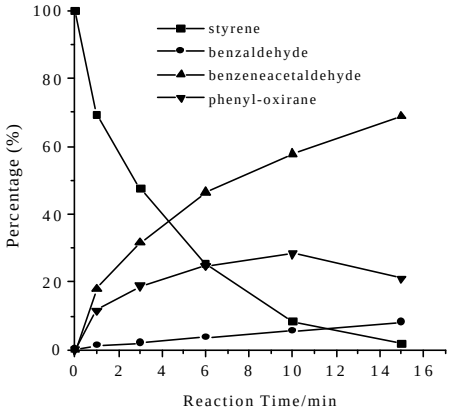


Figure 2 The percentage of species in Mn-TMPyP-catalyzedstyrene epoxidation system by oxone



The reaction rates of the M-P-catalyzed oxidation of HVA with H_2O_2 appeared dependent on pH. Both anionic and cationic M-Ps have highest catalytic activity at the pH range of 10.5~11.0. **Table 1** indicated that for the M-Ps with same central ion, the order of peroxidase-like activity is TMPyP≈TTMAPP>TCPP≈$TPPS_4$>porpthyrin IX, and for the M-Ps with the same porphyrin structure, the order is Fe>Mn>Co. Furthermore, imidazole shows great influence on this catalytic reaction.

We explored the epoxidation of styrene with oxone in the present and absence of M-Ps in water-acetonitrile (1:1) solution. The data showed that all tested water-soluble M-Ps, except for Mn-TMPyP, are not effective mimics of cytochrome P450 with oxone as an oxidant in water-acetonitrile solution, since the conversion rates are lower than 10%. Although Mn-TMPyP was found to give out a total conversion of 96%, its specificity was not good enough. The much higher activity of Mn-TMPyP than other M-Ps is interesting and worthy of further study. **Figure 2** showed the kinetics of styrene oxidation in the presence of Mn-TMPyP. Total conversion was completed in 15 min.

From the experimental data and discussion we could draw some conclusions. When M-Ps are used as mimics of heme-containing enzymes, if the oxidants are the same, they should have the same order in catalytic activities of catalase and peroxidase. Equally important is that different ligand will show different modulation effect on catalase and peroxidase-like activities, which is in good agreement with the fact that these enzymes have a different environment around the active center, porphyrin IX

Table 1 The initial rates of HVA oxidation with H_2O_2 catalyzed by metalloporphyrins

Metalloporphyrin	Relative velocity (Fluorescence intensity/s)	
	Absence of imidazole	Presence of imidazole
Mn-$TPPS_4$	0.24±0.01	0.69±0.01
Fe-$TPPS_4$	1.24±0.02	1.54±0.02
Co-$TPPS_4$	Extremely slow	a
Mn-TCPP	0.27±0.06	0.59±0.03
Fe-TCPP	3.80±0.03	5.58±0.28
Co-TCPP	Extremely slow	a
Mn-porphyrin Ⅸ	0.15±0.01	0.40±0.02
Hemin	0.44±0.01	1.20±0.05
Co-porphyrin Ⅸ	Extremely slow	a
Mn-TTMAPP	2.81±0.01	3.76±0.01
Fe-TTMAPP	12.00±0.38	14.00±0.59
Co-TTMAPP	Very slow	a
Mn-TMPyP	5.11±0.22	11.28±2.12
Fe-TMPyP	13.13±0.21	15.34±0.34
Co-TMPyP	Very slow	a

[a] Insignificantly different from that in the absence of imidazole. c(M-P)=1.0×10^{-7} mol/L, c(HVA)=5.0×10^{-5} mol/L, c(H_2O_2)=5.0×10^{-6} mol/L, c(M-P)/c(imidazole)=1:1000. The reaction was at room temperature and at pH 10.5. The data were based on the three replicate measurements.

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