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Molecular Recognition of Immobilized Supramolecular Complex of Ironporphyrin and β-CD Polymer as an Analogue for Peroxidase

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Abstract. The immobilized supramolecular inclusion complex of $FeTPPS_4$ and β-CD polymer was applied as a mimesis of peroxidase, and its molecular recognition for twenty substrates was studied. As the space structure of the overall proteinase was mimicked by many β-CD interior cavities, high selectivity was obtained by immobilized mimetic enzyme. *p*-Chlorophenic acid was identified as an optimal substrate in the system tested.

Key words: molecular recognition, β-CD polymer, ironporphyrin, mimetic enzyme.

1. Introduction

Metalloporphyrins were widely used in the enzymatic method of analysis as a mimesis of peroxidase [1]. However, as a chemical model of a prosthetic group in horseradish peroxidase, the free metalloporphyrin was not capable of recognition of the substrates. So it was inferior to natural proteinase in catalytic specificity, and easily inhibited by competitive substrates, probably short of three-dimensional structure of the protein [2]. β-Cyclodextrin (β-CD) is a cyclic oligomer comprised of seven α-D-glucopyranose units linked $1 \rightarrow 4$, as in an amylase. The interior cavity of the doughnut-shaped molecule provides a relatively hydrophobic environment into which various organic substrates with different shape, volume and polarity can be trapped selectively. So β-CD plays an important role in the study of enzymatic model and molecular recognition in supramolecular systems [4–6].

In a previous paper [7], the supramolecular inclusion complex of iron-5,10,15,20-tetrakis-(sulforphenyl)-21H, 23H-porphine ($FeTPPS_4$) and β-cyclodextrin polymer (β-CDP) was synthesized and successfully applied in the enzymatic assay of H_2O_2 as mimesis of peroxidase, which had no reagent blank and could be used repeatedly. A quinoid dye (λ_{max} = 505 nm) was produced when 4-aminoantipyrine (4-AAP) interacted with the *para*- or *ortho*-groups of phenol derivatives catalyzed by the immobilized supramolecular mimetic enzyme, as shown in Scheme 1.

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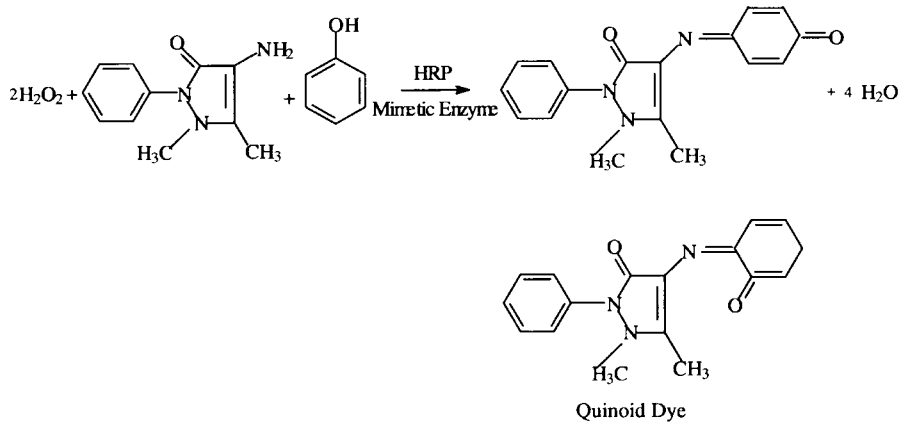

Scheme 1.

In the present work, molecular recognition of twenty-one phenol derivatives was shown by a supramolecular inclusion complex of metalloporphyrin and β-CDP. The selectivity of the method was significantly increased by means of an immobilized mimetic enzyme, and *p*-chlorophenic acid was identified as the optimal substrate in the system. The supramolecular mimetic enzyme was considered as an advanced mimesis, mimicking the space structure of holoenzyme, as well as the prosthetic groups of peroxidase.

2. Experimental

2.1. APPARATUS

The absorbance was measured on a Shimadzu UV-240 spectrophotometer with 10 mm pathlength fused silica cuvettes (Tokyo, Japan). The solution was incubated on a 8HZ-82 type vibrator with a thermostatic bath (Jiangsu Medical Apparatus Factory, China).

2.2. MATERIALS

Hydrogen peroxide solution was freshly prepared by dilution of the original solution (H_2O_2 30%, G.R.), which was standardized by titration with standard potassium permanganate. Phenol derivatives (A.R.) were all applied in 0.06 mol/L. 4-AAP (A.R.) was dissolved to give a concentration of 5.0×10^{-3} mol/L in a brown bottle. Kolthoff buffer solutions were used in pH $9.0 \sim 11.6$. $FeTPPS_4$ solution was prepared according to the literature [6] and employed in 1.0×10^{-4} mol/L. The inclusion complex of β-CDP and $FeTPPS_4$ was prepared according to the previous literature [7], with an inclusion content of 6 μmol/g, and a grain size of $0.15 \sim 0.18$ mm ($80 \sim 100$ mesh). All aqueous solutions were prepared in double deionized water.

2.3. PROCEDURE

2.3.1. *Evaluation of apparent absorption factor and inclusion constants for complex formation of β-CDP- FeTPPS$_4$ and phenol derivatives*

150 mg β-CDP-FeTPPS$_4$ resin (host) was added into a mixture of 2 mL phenol derivatives (guest), 2.5 mL buffer solution and 5.5 mL deionized water, and the mixture was incubated on a vibrator until absorption was equilibrated. The concentration of guest in the liquid phase was determined at the maximum absorption wavelength in the range of 250 $\sim$ 300 nm by taking the supernatant. The apparent absorption factor and inclusion constant for the formation of the β-CDP-FeTPPS$_4$ and phenol derivatives were calculated by known formulas [7].

2.3.2. *Examination of the peroxidase-like activity*

Solutions of 2.5 mL of buffer, 1.0 mL of 4-AAP, a certain amount of phenol derivatives, and H_2O_2 were added into a 10.0 mL color tube and diluted to the mark. The mixture was fed into an Erlenmeyer flask containing 150 mg β-CDP-FeTPPS$_4$ resin immediately and incubated at room temperature. The absorbance of the supernatant was measured at maximum absorption wavelength in the range of 480 $\sim$ 505 nm against the reagent blank at suitable time intervals, then the initial rate of catalytic reaction, v_0, was calculated from it. The control value was obtained with the chromogenic solution containing 0.5 mL 1.0×10^{-4} mol/L FeTPPS$_4$, 0.5 mL 4-AAP, and a certain amount of H_2O_2 and phenol derivatives in 10 mL solution, and the initial rate, v_0, was evaluated.

3. Results and Discussion

It was proved that, β-CD included the aromatic group of TPPS$_4$ for 1 : 2 complex formation [8]. The inclusion amount of 1 g β-CDP was 6 μmol FeTPPS$_4$ by experimental result. It could be calculated by a method presented in the literature [9], that 0.51 mmol monomer β-CD was contained in 1 g β-CDP, e.g. only 0.012 mol FeTPPS$_4$ was contained in 1 mol monomer β-CD. So a large quantity of interior cavities were left to form new supramolecular systems by including substrate molecules.

The twenty-one substrates employed were phenol (I), 4-hydroxybenzenesulfonic acid (II), *p*-chlorophenic acid (III), *o*-chlorophenic acid (IV), *m*-chlorophenic acid (V), 2,4-dichlorophenic acid (VI), *p*-bromophenic acid (VII), hydroquinone (VIII), catechol (IX), resorcinol (X), 1,2,4-benzenetriol (XI), *p*-cresol (XII), *o*-cresol (XIII), *m*-cresol (XIV), 4-*tert*-butylphenol (XV), 4-aminophenol (XVI), 2-aminophenol (XVII), 3-aminophenol (XVIII), 4-nitrophenol (XIX), 2-nitrophenol (XX) and 3-nitro-phenol (XXI).

Phenol derivatives were prepared along with their sodium sulfonates in order to dissolve in water, as described in the literature on substrates for natural and mimetic enzymes [11–12]. However, it is shown in Table I that both λ_{max} and the initial rate,

Table I. Comparison of 30% ethanol solution of phenol and aqueous solution of 4-hydroxybenzenesulfonic acid

Substrate	Solvent	λ_{max}	$v_0(\times 10^{-3}\ s^{-1})$	A_{max}
I	Water	505	1.01	0.447
	30% ethanol	505	1.04	0.452
II	Water	500	0.68	0.448

[substrate] = 9.0×10^{-3} mol/L; β-CDP-FeTPPS$_4$: 150 mg; [H_2O_2] = 1.0×10^{-4} mol/L; [4-AAP] = 5.0×10^{-4} mol/L; pH 10.0.

v_0, of phenol sulfonate changed noticeably. On the other hand, significant deviation was not seen in the experiment when phenol dissolved in 30% ethanol solution or water was used. So all substrates were dissolved in 30% ethanol solution in the following experiments.

Catalytic reactions were carried out at the optimal pH for each substrate, and the absorbances were measured at the maximum absorption wavelength of the quinoid dye. The liquid phase mimetic enzyme and aqueous FeTPPS$_4$ were also used in the same catalytic system as the control sample, under its optimal reaction conditions.

3.1. PHENOL AND HALOGENATED PHENOLIC ACID AS SUBSTRATES

As shown in Table II, there were no remarkable differences in initial rate (V_{01}) and sensitivity (F_1) for the oxidation of phenol and halogenated phenolic acid in the aqueous FeTPPS$_4$ mimetic enzyme systems. However, the initial rates (V_{02}) of the oxidation reactions catalyzed by β-CDP-FeTPPS$_4$ inclusion complexes differed significantly, but the sensitivities (F_2) had insignificant differences. It was shown that the mimetic enzyme bound substrate selectively. The initial rate was inhibited when the mimetic enzyme united poorly with the substrate, but the sensitivity of the product varied slightly due to the constant product.

The order of ratio of V_{02}/V_{01} was as follows: *p*-chlorophenic acid > *m*-chlorophenic acid > phenol > *o*-chlorophenic acid > 2,4-dichlorophenic acid > *p*-bromophenic acid, the same as the sequence of absorption factor and inclusion equilibrium constants (β). It was indicated that the firmer the substrates contacted with the β-CDP-FeTPPS$_4$, the higher the catalytic activity displayed. The hydrophobic interior cavity of β-CD expressed favorable selectivity to shape, volume and polarity of various substrates, so as to improve the catalytic specificity of the mimetic enzyme. This is in accordance with the lock-key theory for compound formation of natural enzyme and substrate. So it was a kind of mimetic enzyme similar to natural enzymes. It could be concluded from Table II that *p*-chlorophenic acid was the optimal substrate in the β-CDP-FeTPPS$_4$ mimetic enzyme system.

Table II. Substrate behavior of phenol and halogenated phenolic acid

Substrate	$FeTPPS_4$[a]			β-CD-$FeTPPS_4$[b]				
	λ_{max} (nm)	V_{01} ($\times 10^3$ s^{-1})	F_1[c]	λ_{max} (nm)	V_{02} ($\times 10^3$ s^{-1})	F_2[c]	V_{02}/V_{01}	β
I	505	3.50	1	505	1.04	1	0.296	33.2
III	505	3.53	1.32	505	3.02	1.44	0.856	286
IV	505	3.72	1.21	505	0.86	1.06	0.228	25.3
V	492	3.36	1.12	492	2.61	1.16	0.777	81.5
VI	505	3.72	1.37	505	0.78	1.30	0.209	20.5
VII	505	3.51	1.28	505	0.66	1.03	0.188	20.0

[a] $[FeTPPS_4] = 5.0 \times 10^{-6}$ mol/L; $[substrate]_0 = 6.0 \times 10^{-3}$ mol/L; $[4\text{-AAP}] = 2.5 \times 10^{-4}$ mol/L; $[H_2O_2] = 1.0 \times 10^{-4}$ mol/L.
[b] $[substrate]_0 = 9.0 \times 10^{-3}$ mol/L; β-CD-$FeTPPS_4$: 150 mg; $[4\text{-AAP}] = 5.0 \times 10^{-4}$ mol/L; $[H_2O_2] = 1.0 \times 10^{-4}$ mol/L.
[c] $F = A_{substrate(\lambda_{max})}/A_{phenol(\lambda_{max})}$.

3.2. POLYPHENOLS AS SUBSTRATES

Almost all polyphenols could be substrates of oxidation reactions in the H_2O_2/HRP system [12]. Significant differences of sensitivities and initial rates were not seen when three kinds of diphenols were used as the substrates of monomeric $FeTPPS_4$ mimetic enzyme, but a fairly high catalytic activity was shown by 1,2,4-benzenetriol. However, catalytic activities changed a great deal when polyphenols were used as the substrates of the β-CDP-$FeTPPS_4$ mimetic enzyme, as shown in Table III. Hydroquinone lost catalytic activity, while the activity of resorcinol declined appreciably; only 1,2,4-benzenetriol increased slightly, in spite of a rather high absorption factor and inclusion constant (β). It should be explained that metalloporphyrin not only catalyzed the formation of quinoid dye between polyphenols and 4-AAP, but also oxidized itself to quinone that absorbed at 420 nm. The main product of hydroquinone catalyzed by immobilized mimetic enzyme was 1,4-benzoquinone, but two products (quinoid dye and quinone) existed at the same time in the catalytic systems for the other substrates. It was concluded that molecular recognition for the immobilized mimetic enzyme of substrate depended not only on the amount of absorption factor and inclusion constants, but also on various other factors such as molecular structure, polarity and electrostatic interaction.

3.3. METHYL PHENOLS AS SUBSTRATES

Substrate behaviors of *p*-cresol, *o*-cresol, *m*-cresol in the catalytic reaction were studied. The peroxidase-based coupling reaction with cresol and 4-AAP took place *ortho* to the phenol with the methyl group *para* to it, so it was understood that the sensitivity of *p*-cresol was lower than that of *o*-cresol and *m*-cresol by using

Table III. Substrate behavior of polyphenols

Substrate	$FeTPPS_4$[a]		β-CD-$FeTPPS_4$[b]		
	λ_{max} (nm)	V_{01} ($\times 10^3$ s^{-1})	λ_{max} (nm)	V_{02} ($\times 10^3$ s^{-1})	β
VIII	487	0.72	–	0	41.2
IX	505	0.56	505	0.51	30.7
X	490	0.50	490	0.38	32.1
XI	505	1.24	505	1.33	254

[a] $[FeTPPS_4] = 5.0 \times 10^{-6}$ mol/L, $[substrate]_0 = 6.0 \times 10^{-3}$ mol/L, [4-AAP] = 2.5×10^{-4} mol/L, $[H_2O_2] = 3.0 \times 10^{-4}$ mol/L.
[b] $[substrate]_0 = 9.0 \times 10^{-3}$ mol/L, β-CD-$FeTPPS_4$: 150 mg, [4-AAP] = 5.0×10^{-4} mol/L, $[H_2O_2] = 3.0 \times 10^{-4}$ mol/L.

Table IV. Substrate behavior of methylphenols

Substrate	$FeTPPS_4$[a]		β-CD-$FeTPPS_4$[b]		
	λ_{max} (nm)	V_{01} ($\times 10^3$ s^{-1})	λ_{max} (nm)	V_{02} ($\times 10^3$ s^{-1})	β
XII	495	0.445	495	0.090	96.0
XIII	495	1.17	495	0.087	9.71
XIV	490	1.17	490	0.725	43.1

[a] $[FeTPPS_4] = 5.0 \times 10^{-6}$ mol/L, $[substrate]_0 = 4.0 \times 10^{-3}$ mol/L, [4-AAP] = 2.5×10^{-4} mol/L, $[H_2O_2] = 3.0 \times 10^{-4}$ mol/L.
[b] $[substrate]_0 = 6.0 \times 10^{-3}$ mol/L, β-CD-$FeTPPS_4$: 150 mg, [4-AAP] = 5.0×10^{-4} mol/L, $[H_2O_2] = 3.0 \times 10^{-4}$ mol/L.

the $FeTPPS_4$ monomer mimetic enzyme. The sensitivities of methyl phenols in immobilized β-CD-$FeTPPS_4$ mimetic enzyme systems were lower than those in the monomer $FeTPPS_4$ mimetic enzyme system, so high absorption factor and inclusion constants did not lead to a relevant high initial rate for *p*-cresol as substrate. However, molecular recognition by β-CD-$FeTPPS_4$ was displayed for the *o*-cresol and *m*-cresol which had the same sensitivity in the monomer mimetic enzyme system, as shown in Table IV.

3.4. OTHER SUBSTRATES

Three aminophenols, three nitrophenols and 4-*tert*-butylphenol were studied as the substrates for mimetic enzymes. *p*-, *o*-, *m*-Aminophenol and 4-*tert*-butylpnenol could not be used as the substrates for $FeTPPS_4$ monomer because of low sensitivity. A higher reagent blank was seen due to oxidation of aminophenols

Table V. Substrate behavior of other substrates

Substrate	$FeTPPS_4$[a]		β-CD-$FeTPPS_4$[b]	
	λ_{max} (nm)	A	λ_{max} (nm)	A
XVI	490	0.142	490	0.122
XVII	480	0.137	480	0.101
XVIII	480	0.091	480	0.088

[a] $[FeTPPS_4] = 5.0 \times 10^{-6}$ mol/L, $[substrate]_0 = 4.0 \times 10^{-3}$ mol/L, [4-AAP] = 2.5×10^{-4} mol/L, $[H_2O_2] = 3.0 \times 10^{-4}$ mol/L.
[b] $[substrate]_0 = 6.0 \times 10^{-3}$ mol/L, β-CD-$FeTPPS_4$: 150 mg, [4-AAP] = 5.0×10^{-4} mol/L, $[H_2O_2] = 3.0 \times 10^{-4}$ mol/L.

which were themselves catalyzed by a mimetic enzyme, similar to hydroquinone. Selectivity was not satisfactory for aminophenols as substrates of immobilized β-CD-$FeTPPS_4$, as shown in Table V. So aminophenols were not suggested to be the substrates for peroxidase.

4. Conclusion

1. A supramolecular system was formed when the β-CDP-$FeTPPS_4$ inclusion complex bound with the substrate molecule. This kind of immobilized supramolecular mimetic enzyme, with excellent catalytic activity and specificity, possessed similarity to a natural holoenzyme to some extent.
2. The higher activities for the halogenated phenolic acids were observed for both $FeTPPS_4$ monomer and immobilized supramolecular β-CDP-$FeTPPS_4$. Methyl phenols and polyphenols could be substitutes of halogenated phenolic acids; however the use of aminophenols and others were not recommended.
3. Selectivity of the substrates depended mainly on the absorption factor and inclusion action (such as halogenated phenolic acid) and specificity of $FeTPPS_4$ itself to the substrate molecule (such as methylphenol); so supramolecular β-CDP-$FeTPPS_4$ mimetic enzyme, with double selectivity, is an advanced mimetic enzyme.
4. The products of catalytic reactions might be changed by using β-CDP-$FeTPPS_4$ (such as polyphenol and aminophenol).
5. The optimal substrate for immobilized β-CDP-$FeTPPS_4$ mimetic enzyme was *p*-chlorophenolic acid.

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References

1. Y. Saito, M. Mifune, S. Nakashima, Y. Tanaka, M. Chikuma, and H. Tanaka: *Chem. Pharm. Bull*. **34**, 5016 (1986).
2. Y.X. Ci, W.B. Chang, and L. Chen: *Chem. J. Chin. Univ.* **16**, 683 (1995).
3. J. Szejtli: *Cyclodextrin Technology*, p. 79, Kluwer, Dordrecht (1988).
4. J.M. Lehn: *Angew. Chem. Int. Ed. Eng.* **29**, 1304 (1990).
5. I. Tabushi, N. Shimizu, T. Sugimoto, M. Shiozuka, and K. Yamamura: *J. Am. Chem. Soc.* **99**, 7100 (1977).
6. R. Breslow: *Acc. Chem. Res.* **28** 146 (1995).
7. Y. Saito, M. Satouchi, M. Mifune, T. Tai, J. Odo, Y. Tanaka, M. Chikuma, H. Tanaka: *Bull. Chem. Soc. Jpn.* **60**, 2227 (1987).
8. M. Zhu, L.Y. Mao, X.M. Huang, R. Li, and H.X. Shen: *Chin. Chem. Lett.* **7**(9), 868 (1996).
9. D.L. Dick, T.V.S. Rao, D. Sukumaran, D. Sukumaran, and D.S. Lawrence: *J. Am. Chem. Soc.* **114**, 2664 (1992).
10. M. Komiyama and H. Hirai: *Polym. J*. **19**, 773 (1987).
11. J.D. Artiss, R.J. Thibert, J.M. Mcintosh, and B. Zak: *Microchem. J*. **26**, 487 (1981).
12. F. Wang, X.W. Wu, S.S. Shong, and Y.X. Ci: *Fresenius' J. Anal. Chem*. **344**, 556 (1992).
13. Wako Pure Chemical Industries, Ltd,.: *JP pat* 58187858.