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Imprinted polymer catalysts for the hydrolysis of *p*-nitrophenyl acetate

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Abstract

Network polymers imprinted with a transition state analogue of hydrolysis were prepared by co-polymerization of vinylimidazole assembled around *p*-nitrophenyl phosphate (transition state analogue) with divinylbenzene as a cross-linker. After removal of the template, the resulting polymers efficiently catalyzed the hydrolysis of *p*-nitrophenyl acetate. The inhibition effect of the template was also examined.

Keywords: Molecular imprinting; Polymer catalyst; Hydrolysis; Transition state analogue

1. Introduction

Recently, the molecular imprinting technique has been extensively utilized for the creation of binding sites in synthetic network polymers, which exhibit efficient molecular recognition, especially in chromatographic separations [1]. However, only several examples of polymer catalysts imprinted with a transition state analogue (TSA) have appeared [2–8]. For the hydrolysis of *p*-nitrophenyl acetate (PNA), *p*-nitrophenyl methylphosphate [3] and methyl hydrogen *p*-nitrobenzylphosphonate [4,5] were used as a TSA to coordinate polyvinylimidazole and vinylimidazole (VI), respectively, by cobalt-ion chelation. However, both polymers contained cobalt ion as a result and it is not clear whether the cobalt ion functions catalytically. In this paper, we describe that highly cross-linked polymers without cobalt ion can be prepared by template polymerization using the electrostatic interaction between VI and *p*-nitrophenyl phosphate (PNP) as a TSA and they are efficient polymer catalysts for the hydrolysis of PNA (Scheme 1).

2. Results and discussion

The network polymers were synthesized using the molecular imprinting technique as shown in Scheme 2.

The template, PNP, was dissolved in acetonitrile and a functional monomer, VI was added to produce an insoluble salt by acid–base reac-

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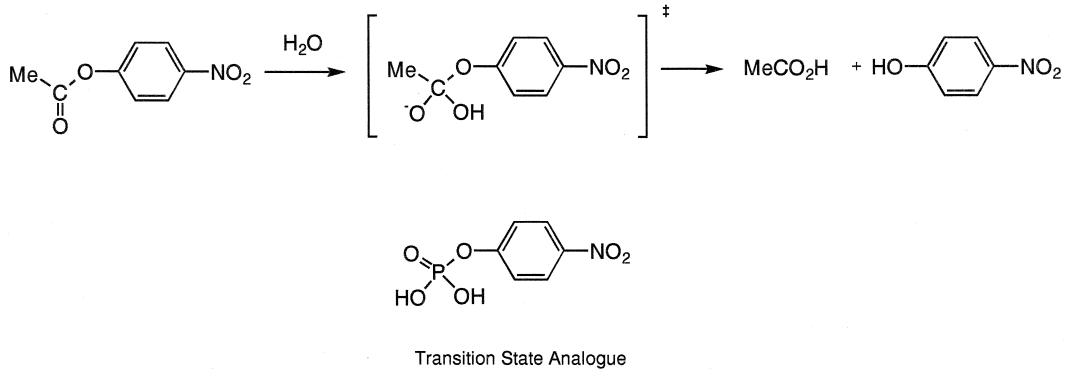
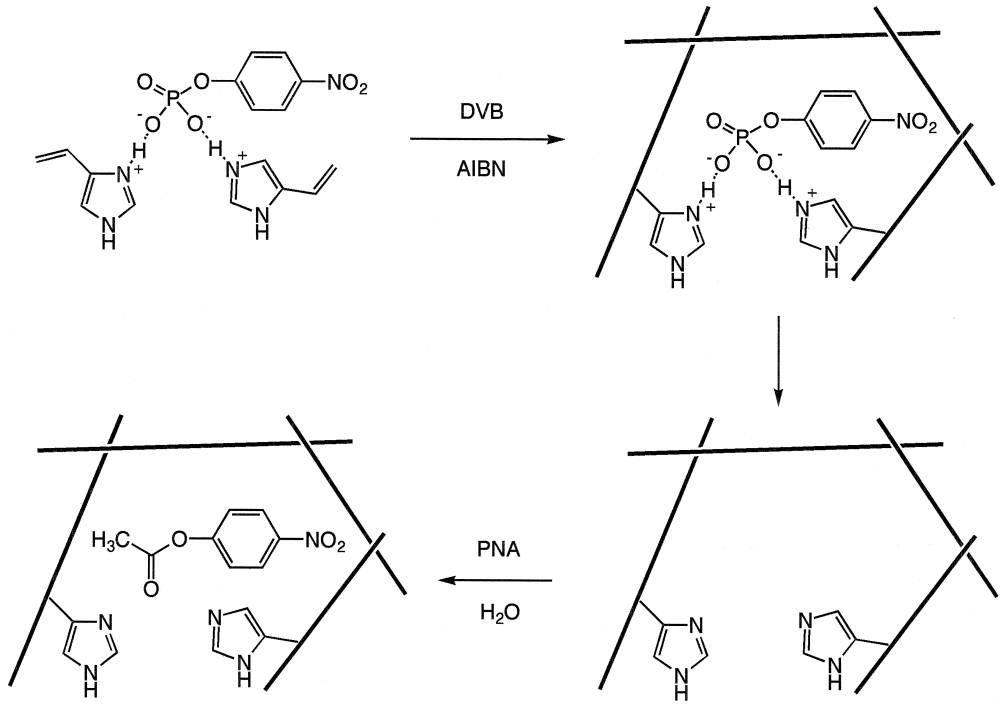


Scheme 1.

tion. Then the resulting salt was co-polymerized with divinylbenzene (DVB) as a cross-linker using azobis(isobutyronitrile) (AIBN) as a radical initiator at 60°C for 24 h under a N_2 atmosphere. Continuous extraction of crude solid with acetonitrile, phosphate buffer and methanol afforded the insoluble macroporous polymer. A non-printed control polymer containing the same ratio of VI and DVB but without PNP (template) was also prepared.

The polymer-catalyzed hydrolysis of PNA was carried out in Tris–HCl buffer, pH 7.0 at



Scheme 2.

25°C. The pseudo-first-order rate constants (k_{obs}) were determined spectrometrically at 400 nm. The results are summarized in Table 1.

The content of cross-linking agent affected the catalytic activity. The imprinted polymer P2 was found to be the most active polymer with the optimal active sites, although the other polymers P1 or P3 contained different total equivalents of imidazole per gram of polymer. The rate of hydrolysis catalyzed by the imprinted polymer P2 was 85 times faster than that observed in the uncatalyzed hydrolysis (k_{uncat}). Moreover, the catalytic activity of the imprinted polymer P2 was two times higher than that of the control polymer P4 (the corresponding non-printed polymer). The imprinted polymers using other cross-linking agent, ethylene glycol methacrylate, N,N'-methylenebisacrylamide, showed relatively low catalytic activity.

Furthermore, the inhibition effect of the template, PNP was also investigated. As shown in Fig. 1, the hydrolytic activity of the imprinted polymer P2 was fairly inhibited, as the concentration of the template, PNP was increased. This suggests that the catalytic site which contains imidazole group and/or oxy anion hole which stabilizes the tetrahedron intermediate [9] should be formed by molecular imprinting of PNP as a template.

In conclusion, we have demonstrated that the network polymers imprinted with a TSA of hydrolysis can be prepared by co-polymerization of VI prearranged around PNP (TSA) as a template with DVB and effectively catalyzed the hydrolysis of PNA two times faster than non-printed polymer and 85 times faster than the uncatalyzed hydrolysis. We have also observed that the template, PNP exhibits inhibition of the imprinted polymer P2, which provided evidence for the creation of the imidazole-containing active site and/or oxy anion hole within the network polymer. Although the catalytic activities are not high enough, compared with that of catalytic antibodies for the same hydrolysis of PNA [10,11], it is promising that the imprinted polymers work as an artificial cat-

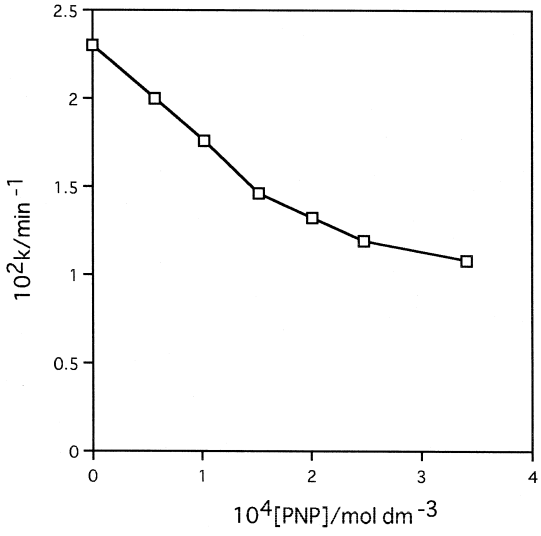


Fig. 1. Inhibition of PNA for the hydrolysis of PNA catalyzed by imprinted polymer P2.

Table 1
Kinetic parameters for the hydrolysis of PNA catalyzed by the network polymers[a]

Polymer	Ratio (PNP:VI:DVB)	10^4 [Im][b] (mol dm^{-3})	10^2 k_{obs} (min^{-1})	k_{obs}/k^c_{uncat}	k^d_{cat} (min^{-1} mol^{-1} dm^3)
P1	1:10:10	14.9	1.5	56	9.9
P2	1:10:20	9.4	2.3	85	24.1
P3	1:10:30	6.9	1.6	59	22.7
P4[e]	0:10:20	9.4	1.1	41	11.4

[a][polymer] = 3.3×10^{-1} g dm^{-3} and [PNA] = 1.0×10^{-5} mol dm^{-3} in 20% (v/v) MeOH/H_2O (Tris–HCl buffer, pH 7.0) at 25°C.
[b]Theoritical concentration of imidazole in the polymer.
[c]2.7×10^{-4} min^{-1}.
[d]$k_{cat} = (k_{obs} - k_{uncat})/[Im]$.
[e]Non-printed polymer.

alytic antibody which has advantages of stability and ease to prepare.

3. Experimental

UV spectra were determined on a Shimadzu UV-260 spectrophotometer. PNA, AIBN, and DVB (55%, mixture of isomers, remainder mainly 3- and 4-ethylvinylbenzene) were obtained from Wako Pure Chemical. PNP was prepared by acidification of disodium PNP (Wako) with conc. HCl and extraction with ether. 4-(5)-Vinylimidazole was prepared from urokanic acid [12].

3.1. Typical preparation of polymers

To a solution of 4-(5)-vinylimidazole (471 mg, 5 mmol) in acetonitrile (3 ml) was added a template molecule (a TSA), PNP (219 mg, 0.5 mmol) in acetonitrile (0.5 ml) with stirring at room temperature for 30 min. Then, the resulting salt was co-polymerized with DVB (1.4 ml, 10 mmol) using AIBN (20 mg) at 60°C for 24 h under a N_2 atmosphere. Continuous extraction of crude solid with acetonitrile, phosphate buffer and methanol afforded the insoluble macroporous polymer which was dried at 60°C at reduced pressure for 24 h. The control non-printed polymer containing the same ratio of VI and DVB but without PNP was prepared as the similar method above.

3.2. Kinetic studies

The hydrolysis was carried out in the suspended solution of the polymer and 20% (v/v) MeOH–Tris–HCl buffer (0.05 mol dm^{-3}), pH 7.0 with stirring at 25°C by injecting an acetonitrile solution of PNA. The final concentration of PNA was 1×10^{-5} mol dm^{-3} and that of the polymer was 3.3×10^{-1} g dm^{-3}. At regular time interval, an aliquot of the reaction mixture was removed, filtered and monitored the release of *p*-nitorophenolate at 400 nm. The pseudo-first-order rate constants were obtained from linear plots of $\ln[A_\infty/(A_\infty - A_t)]$ vs. time. Triplicate runs showed a measurement error of less than 5%.

To examine the inhibition effect of PNP (template), the similar kinetic studies were carried out in the presence of an appropriate aliquot of a stock solution of PNP in acetonitrile ($0.57 - 3.4 \times 10^{-4}$ mol dm^{-3}). It was confirmed by another run that PNP was not hydrolyzed by the polymers in this condition (pH 7.0).

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