Preparation and catalytic property of L-histidyl group-introduced, crosslinked poly(ethylene imine)s imprinted by a transition-state analogue of an esterolysis reaction

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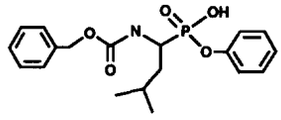
A crosslinked, L-histidyl group-introduced polymer catalyst, which was imprinted by a transition-state analogue of phenyl 1-benzyloxycarbonylamino-3-methylpentylphosphonate in the hydrolysis of *p*-nitrophenyl *N*-carbobenzoxy-L-leucinate, was prepared from poly(2-methyl-2-oxazoline). The catalyst enhanced the hydrolysis of the L-leucine ester, in comparison to the hydrolysis of the L-phenylalanine and L-alanine esters.

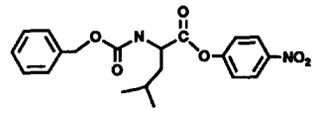
(Keywords: imprinted polymer; catalyst; molecular recognition)

In enzyme catalysis, it has generally been accepted that enzymes recognize and stabilize the transition state of a substrate molecule to make the activation barrier lower[1]. The concept of an 'antibody catalyst', developed by Pollack *et al.*[2], based on antibody formation against a hapten of a transition-state analogue, has received great attention as a strategy for constructing 'tailor-made enzyme-like catalysts'. From a practical point of view, however, most antibody catalysts do not seem to be commercially applicable because of complex purification processes and high cost. Hence, if it is possible to construct a polymer catalyst through molecular imprinting against a template molecule, especially a transition-state analogue, in order to make a catalytically active centre, the enzyme-like polymer catalyst, i.e. plastic catalyst, may be a promising approach. In this respect, we have already reported an appreciably efficient esterolytic catalysis of crosslinked poly(vinyl imidazole), imprinted by a transition-state analogue of methyl *p*-nitrobenzylphosphonate, in the hydrolysis of *p*-nitrophenyl acetate[3]. However, it seems to be difficult to construct the elaborate template polymer catalysts by radical polymerization of vinyl monomers.

This paper describes the preparation and the homogeneous catalytic activity of *N*-carbobenzoxy-L-hystidyl group-introduced, crosslinked poly(ethylene imine) imprinted by a transition-state analogue, phenyl 1-benzyloxycarbonylamino-3-methylpentylphosphonate (**1**), for the hydrolysis of a specific substrate, *p*-nitrophenyl *N*-carbobenzoxy-L-leucinate (Z-L-Leu-PNP) (**2**). The water-soluble catalyst has been derived from poly(2-methyl-2-oxazoline) to control frame polymer chain length and hydrophobicity.



Transition state analogue (1)



Substrate (2)

The template compound **1** was synthesized by stirring a mixture of 13.2 mmol triphenylphosphite, 19.8 mmol 3-methyl-1-butanal, 13.2 mmol benzyl carbamate and acetic acid (2 ml) at 80°C for 1 h. Recrystallization from $CHCl_3$–MeOH (1:1 by volume) gave 2.9 g diphenyl 1-(benzyloxycarbonylamino)-3-methylpentylphosphonate. Hydrolysis of the phosphonate (4.6 mmol) with 0.4 N NaOH (4.6 mmol) at room temperature for 2 days and acidification with 12 N HCl, followed by separation using column chromatography with chloroform–methanol gave 1.0 g of **1**.

The polymer catalyst was prepared by a process shown in *Scheme 1*. The cationic polymerization of 2-methyl-2-oxazoline (47 mmol) using methyl *p*-toluenesulfonate (0.94 mmol) gave 3.8 g poly(2-methyl-2-oxazoline), with number average molecular weight of 6500. The reaction of the polyoxazoline (2.8 g) with 1 N NaOH caused partial hydrolysis of the polymer to eliminate the acetyl group of 12 mol% in **3**, and successive ethylation with $LiAlH_4$ (30 mmol) in 1,2-dichloroethane gave **4** (1.8 g). A catalytically active centre of the imidazole group was introduced by the condensation of *N*-carbobenzoxy-L-

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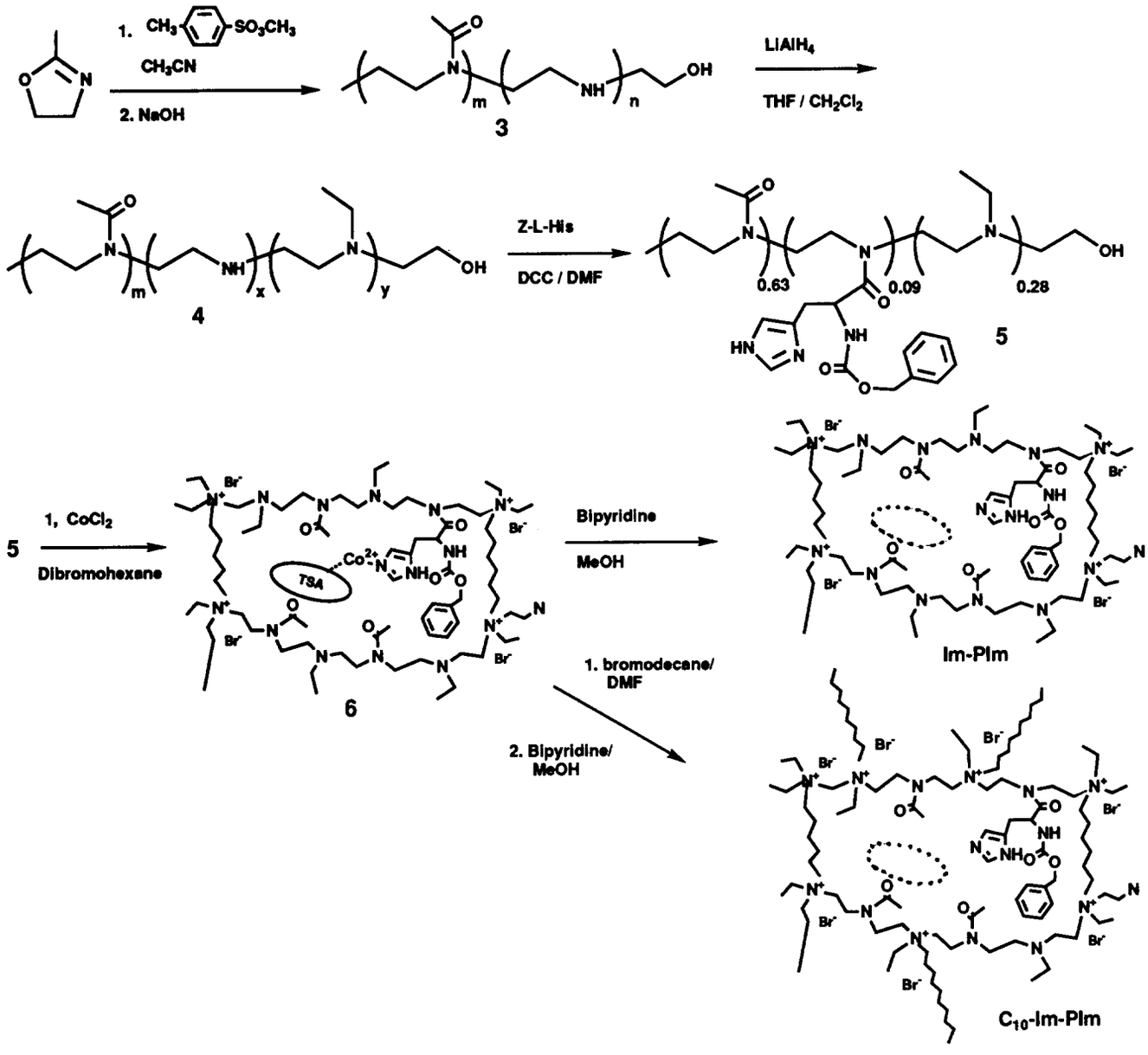

Scheme 1 Preparation of Im-PIm and C_{10}-Im-PIm

histidine (Z-L-His) (0.5 mmol) with **4** (0.2 g) employing *N,N'*-dicyclohexylcarbodiimide (0.25 mmol) in dimethylformamide (DMF). The resulting polymer **5** was composed of 0.63 parts of *N*-acetylethyleneimine, 0.09 parts of *N*-(Z-L-His)ethyleneimine and 0.28 parts of *N*-ethylethyleneimine, estimated by ^{1}H n.m.r. analysis. The imprinting of the transition-state analogue (**1**) and simultaneous crosslinking were carried out by stirring a mixture of 170 mg **5**, 0.17 mmol **1**, equimolar $CoCl_2 \cdot 6H_2O$ and 1,6-dibromohexane in DMF (2 ml) at 45°C for 3 days. The crosslinked polymer was purified by precipitation with hexane from the DMF solution. Alkylation of the resulting polymer was conducted by heating with 1-bromodecane (0.095 mmol) in DMF (2 ml). The extraction of Co^{2+} and **1** from the crosslinked polymers with bipyridine in methanol at room temperature gave Im-PIm and C_{10}-Im-PIm catalyst shown in *Scheme 1*.

Hydrolyses of *p*-nitrophenyl amino acid esters were carried out at 25°C in pH 7.15 CH_3CN–Tris buffer solution (1:9 by volume). The reaction was followed spectrophotometrically by monitoring the absorption of produced *p*-nitrophenolate anion at 400 nm. Catalytic activity was estimated by apparent pseudo-first-order rate constant (k_{obs}).

Table 1 Kinetic parameters for Im-PIm and C_{10}-Im-PIm-catalysed hydrolysis of *p*-nitrophenyl esters[a]

	Im-PIm			C_{10}-Im-PIm		
	Phe	Leu	Ala	Phe	Leu	Ala
$10^3\ k_{obs}$ (min^{-1})	36.9	6.9	7.2	22.2	10.4	11.0
$10^3\ k_{uncat}$ (min^{-1})	22.2	1.2	1.8	22.2	1.2	1.8
k_{obs}/k_{uncat}	1.7	5.8	4.0	1.0	8.7	6.1

[a] [Z-L-His] in Im-PIm and in C_{10}-Im-PIm = 8.0×10^{-4} mol dm^{-3}; [substrate] = 2.0×10^{-5} mol dm^{-3} in CH_3CN–H_2O (1:9 by volume) in Tris-HCl buffer, including 0.5 mol dm^{-3} NaCl at 298 K, pH 7.15. Phe, Leu and Ala represent Z-L-Phe-PNP, Z-L-Leu-PNP and Z-L-Ala-PNP, respectively

Im-PIm enhanced the catalytic hydrolysis of Z-L-Leu-PNP more than those of *p*-nitrophenyl Z-L-alanate (Z-L-Ala-PNP) and Z-L-phenylalanate (Z-L-Phe-PNP), as shown by the ratio of k_{obs} to the rate constant (k_{uncat}) for the uncatalysed reaction in *Table 1*. The rate enhancement in the reaction of Z-L-Leu-PNP by Im-PIm was five times larger than that of Z-L-Phe-PNP. These results show that the cavity formed by imprinting an analogue of **1** in Im-PIm has specificity for subtrate recognition in the hydrolysis of the esters. C_{10}-Im-PIm, prepared by alkylation of Im-PIm, effectively promoted the reaction of Z-L-Leu-PNP about nine times faster than the uncatalysed system. Presumably, the hydrophobicity increased by the C_{10} part in the catalyst made the substrate inclusion fast in the 10 vol% MeCN–H_2O solution. Interestingly, the alkylated catalyst did not exhibit catalytic enhancement for hydrolysis of Z-L-Phe-PNP. Probably, steric hindrance of the decyl group protects the catalytic site from the approaching bulky Z-L-Phe-PNP substrate. However, C_{10}-Im-PIm affected the reaction of Z-L-Ala-PNP as well as that of Z-L-Leu-PNP. Therefore, the cavity, imprinted by a transition-state analogue in the hydrolysis of Z-L-Leu-PNP, can recognize the difference between phenyl and isopropyl groups of the substrate, but cannot effectively differentiate Z-L-Ala-PNP from Z-L-Leu-PNP. Probably, the flexibility of the polymer frame of the catalyst led to incomplete substrate specificity.

Research to improve the catalytic activity and substrate specificity by changing the frameworks of Im-PIm and C_{10}-Im-PIm is now in progress.

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