High Esterolytic Activity of a Novel Water-soluble Polymer Catalyst Imprinted by a Transition-state Analogue

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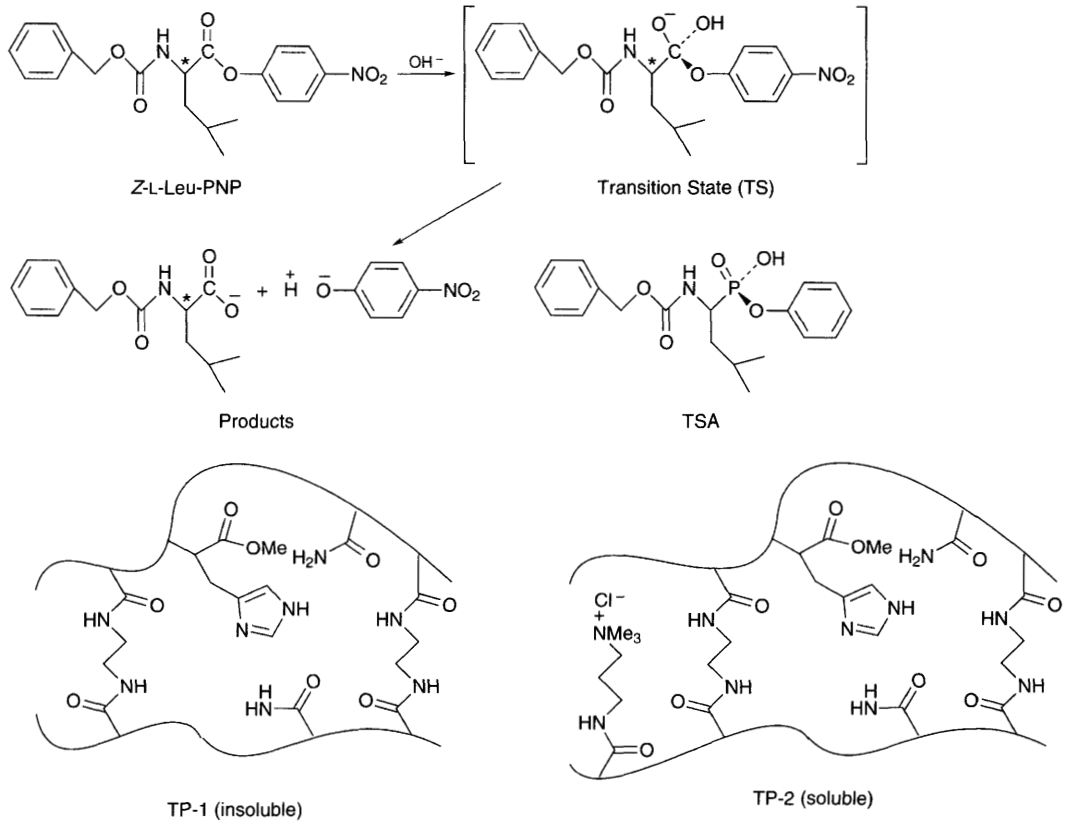
A novel water-soluble polymer catalyst containing a L-histidyl group as a catalytic site, prepared by imprinting of a transition-state analogue of phenyl 1-benzyloxycarbonyl-3-methylpentyl phosphonate, accelerates the substrate-specific esterolysis of *p*-nitrophenyl *N*-(benzyloxycarbonyl)-L-leucinate (Z-L-Leu-PNP) in 10 vol% Me_2O–Tris buffer (pH 7.15) at 293–308 K.

Although polymer catalysts imprinted by transition-state analogues (TSAs) have recently received considerable attention as plastic enzymes,[1–4] the catalysis of water-soluble, TSA-recorded polymers has hitherto been the subject of only limited investigation; there is only one report on the esterolytic activity of a TSA-imprinted poly(ethylene imine)s.[4] This paper reports the catalytic activities of a water-soluble polymer (TP-2) and a water-insoluble one (TP-1), both of which were imprinted using phenyl 1-benzyloxycarbonylamino-3-methylpentyl phosphonate (TSA) for the esterolysis of *p*-nitrophenyl *N*-(benzyloxycarbonyl)-L-leucinate (Z-L-Leu-PNP).

The TP-1 and TP-2 catalysts possessing a L-histidyl group as a catalytic site were prepared by radical polymerization as follows. Equivalent amounts (0.26 mmol) of methyl *N*-acryloyl-L-histidinate (His monomer) and TSA[4] were mixed in DMSO (12 cm^{-3}) for 1 h at room temperature under N_2,† followed by the addition of acrylamide (2.65 or 1.33 mmol), *N*-acryloyl-*N*-(*tert*-butyloxycarbonyl)-1,2-diamine (0.0 or 1.33 mmol), a cross-linker, *N*,*N*′-ethylenebis(prop-2-eneamide) (0.265 mmol), and AIBN (0.08 mmol) to the Me_2SO solution, and then polymerized at 60 °C to produce polymers (Polymer 1 and Polymer 2) possessing the same cross-linker content (8.4%). The complete removal of TSA from Polymer 1 with 5 vol% Et_3N–MeOH gave TP-1, while the treatment of Polymer 2 with 4 mol dm^{-3} HCl in 1,4-dioxane for 8 h and then with $Bu^n{}_3N$ and MeI in DMF for 36 h at room temperature resulted in TP-2 including TSA, from which TSA was washed out with ethyl acetate–MeOH–Et_3N (10 : 5 : 3 *v*/*v*) and 60 vol% ethyl acetate–MeOH. The TP-2 catalyst possesses the randomly distributed quaternary trimethylammonium group through its framework and was found to be very soluble in water.

The hydrolyses of amino acid esters (10.0 μmol dm^{-3}) by the polymer catalysts (His unit concentration = 65.1–112.0 μmol dm^{-3}) or His monomer (100.0 μmol dm^{-3}) were carried out in 10 vol% Me_2SO–Tris buffer (pH 7.15) at 293–308 K, and the pseudo-first-order reaction rate constants obtained with and without the catalyst (k_{cat} and k_{uncat} respectively) were determined by monitoring the amount of PNP anion produced spectrophotometrically at 400 nm.‡ The second-order catalytic rate constant k_{cat}^{app} was evaluated by the equation $k_{cat}^{app} = (k_{cat} - k_{uncat})/[His]$, where [His] denotes the concentration of His units in the catalyst. These kinetic parameters, especially k_{cat}^{app} values, listed in Table 1 indicate that both the polymer catalysts exhibited higher catalytic activities than His monomer in the



Scheme 1 Transition-state analogue (TSA) and TSA-imprinted polymers (assumed structures) for the esterolysis of Z-L-Leu-PNP

Table 1 Catalytic activities of TSA-imprinted polymer catalysts for the esterolysis of Z-L-Leu-PNP[a]

Catalyst	$10^5\ k_{cat}$/ s^{-1}	k_{cat}/k_{uncat}	$10^2\ k_{cat}^{app}$/ $mol^{-1}\ dm^3\ s^{-1}$	Relative activity[b]	$\Delta H^{\ddagger}$/ kcal mol^{-1}	$\Delta S^{\ddagger}$/ cal $mol^{-1}\ K^{-1}$	$\Delta G^{\ddagger}$ (303 K)/ kcal mol^{-1}
None	4.00	—	—	—	22.6	−4.35	23.9
His monomer	5.73	1.4	17.3	1.0	4.52	−47.1	18.5
TP-1	10.2	2.6	95.2	5.5	20.2	7.85	17.8
TP-2	47.0	11.8	383	22.1	31.9	49.4	16.9

[a] [His] = 100.0, 65.1 and 112.0 μmol dm^{-3} for His monomer, TP-1 and TP-2, respectively, and [Z-L-Leu-PNP] = 10.0 μmol dm^{-3} in 10 vol% Me_2SO–Tris buffer (pH 7.15) at 303 K. [b] Obtained from k_{cat}^{app} ratio.

Table 2 Substrate-specificity of TP-2 in the esterolyses of Z-L-Ala-PNP, Z-L-Leu-PNP and Z-L-Phe-PNP[a]

	Z-L-Ala-PNP	Z-L-Leu-PNP	Z-L-Phe-PNP
k_{cat}/k_{uncat}	2.21	5.78	1.79
$10^2\ k_{cat}^{app}$/mol $dm^{-3}\ s^{-1}$	82.8	174	60.8

[a] Reaction conditions are the same as those in Table 1 except 10 vol% MeCN–Tris buffer (pH 7.15).

esterolysis of Z-L-Leu-PNP, and the order of relative catalytic activities (TP-2 ≫ TP-1 > His monomer > None) indicates the high catalytic efficiency of TP-2.

The esterolysis of Z-L-Leu-PNP with or without the catalysts at 293–308 K gave the activation parameters (Table 1) in good linear Eyring relationships (correlation coefficient = 0.993–0.999). As Table 1 indicates, the order of the $\Delta G^{\ddagger}$ values reflects that of the catalytic activities mentioned above; TP-2 lowered the $\Delta G^{\ddagger}$ value from 23.9 (uncatalysed reaction) to 16.9 kcal mol^{-1} (1 cal = 4.184 J), and the smallest $\Delta G^{\ddagger}$ value of TP-2 results from the largest $\Delta S^{\ddagger}$ value. Therefore, the high esterolytic activity of TP-2 is presumably due to the possible reaction between the histidyl group and the incorporated substrate in its reaction cavity. In this regard, the TP-2-catalysed esterolysis of Z-L-Leu-PNP was appreciably inhibited by the addition of TSA into the present reaction system; for example, the k_{cat}^{app} value at 289 K was decreased from 159 to 124, 76.3 or 31.6 $dm^3\ mol^{-1}\ s^{-1}$ under [TSP]/[substrate] = 1.0, 2.0 or 3.0, respectively. The substrate specificity of TP-2 was also examined in the hydrolyses of Z-L-Ala-PNP, Z-L-Leu-PNP, and Z-L-Phe-PNP in 10 vol% MeCN–Tris buffer (pH 7.15) at 303 K (Table 2).§ The k_{cat}/k_{uncat} or k_{cat}^{app} values for the esterolysis of Z-L-Leu-PNP were considerably larger than those for Z-L-Ala-PNP and Z-L-Phe-PNP. Moreover, the hydrolysis of Z-D-Leu-PNP by TP-2 under the same conditions as those in Table 1 resulted in $10^5\ k_{cat}$ = 3.81 s^{-1} and k_{cat}/k_{uncat} = 0.95; the ratio of k_{cat}/k_{uncat} for Z-L-Leu-PNP to that of the other enantiomer is estimated to be 12.4. Therefore, it is possible to demonstrate that the present soluble, TSA-imprinted polymer catalyst of TP-2 facilitated the efficient esterolysis of Z-L-Leu-PNP in its reaction cavity through substrate specificity.

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Footnotes

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† This procedure was carried out to induce some interaction between His monomer and TSA before the polymerization; 400 MHz ^1H NMR analysis of the [$^2H_6$]Me_2SO solution of the two molecules suggested hydrogen bond formation between them, because the chemical shifts of the imidazolyl NH proton in His monomer and the amide C(=O)NH proton in TSA changed from δ 6.60 to 7.20 and from δ 7.53 to 6.90, respectively.

‡ In the case of the esterolysis with the water-insoluble TP-1 catalyst, aliquots of the reaction mixture were analysed every 10 min after centrifuging TP-1 from the aliquots.

§ The 10 vol% MeCN–Tris buffer solution was used due to the low solubility of Z-L-Phe-PNP in the 10 vol% Me_2SO–Tris buffer.

References

1 D. K. Robinson and K. Mosbach, *J. Chem. Soc., Chem. Commun.*, 1989, 969.
2 K. Ohkubo, Y. Urata, S. Hirota, Y. Honda and T. Sagawa, *J. Mol. Cat.*, 1994, **87**, L21.
3 K. Ohkubo, Y. Urata, S. Hirota, Y. Honda, Y. Fujishita and T. Sagawa, 1994, **93**, 189.
4 K. Ohkubo, Y. Urata, Y. Honda, Y. Nakashima and K. Yoshinaga, *Polymer Commun.*, 1994, **35**, 5372.