

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

Katsutoshi Ohkubo,^{*a} Yoshio Funakoshi,^b Yasuo Urata,^b Shogo Hirota,^b Satoshi Usui^b and Takashi Sagawa^b

^a Institute for Fundamental Research of Organic Chemistry, Kyushu University, Fukuoka 812, Japan

^b Department of Applied Chemistry, Faculty of Engineering, Kumamoto University, Kumamoto 860, Japan

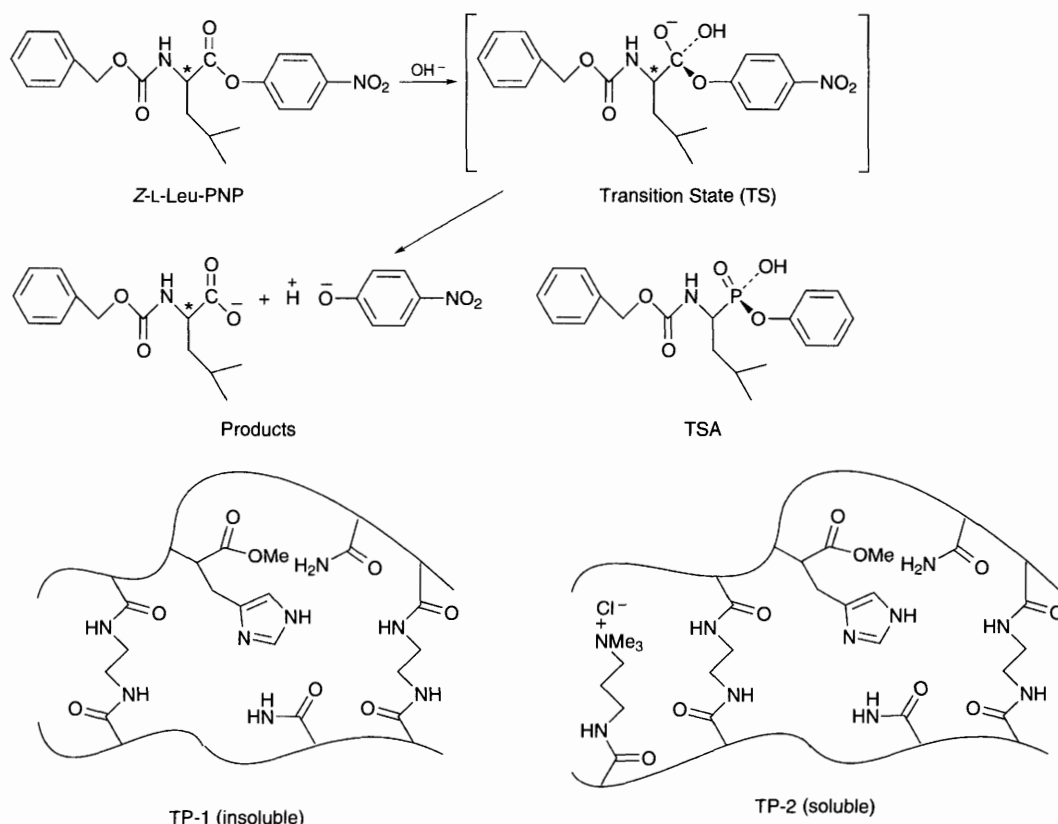
A novel water-soluble polymer catalyst containing a L-histidyl group as a catalytic site, prepared by imprinting of a transition-state analogue of phenyl 1-benzyloxycarbonyl-3-methylpentyl phosphonate, accelerates the substrate-specific esterolysis of *p*-nitrophenyl *N*-(benzyloxycarbonyl)-L-leucinate (Z-L-Leu-PNP) in 10 vol% Me₂O–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 TSA⁴ were mixed in DMSO (12 cm⁻³) for 1 h at room temperature under N₂,[†] 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 Me₂SO 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% Et₃N–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 Bu₃N 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–Et₃N (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% Me₂SO–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	$10^5 k_{\text{cat}}/$ s^{-1}	$k_{\text{cat}}/k_{\text{uncat}}$	$10^2 k_{\text{cat}}^{\text{app}}/$ $\text{mol}^{-1} \text{dm}^3 \text{s}^{-1}$	Relative activity ^b	$\Delta H^\ddagger/$ kcal mol^{-1}	$\Delta S^\ddagger/$ $\text{cal mol}^{-1} \text{K}^{-1}$	$\Delta G^\ddagger (303 \text{ K})/$ 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 $\mu\text{mol dm}^{-3}$ for His monomer, TP-1 and TP-2, respectively, and [Z-L-Leu-PNP] = 10.0 $\mu\text{mol dm}^{-3}$ in 10 vol% Me₂SO-Tris buffer (pH 7.15) at 303 K. ^b Obtained from $k_{\text{cat}}^{\text{app}}$ 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
$k_{\text{cat}}/k_{\text{uncat}}$	2.21	5.78	1.79
$10^2 k_{\text{cat}}^{\text{app}}/\text{mol dm}^{-3} \text{s}^{-1}$	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 \gg 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 ΔG^\ddagger values reflects that of the catalytic activities mentioned above; TP-2 lowered the ΔG^\ddagger value from 23.9 (uncatalysed reaction) to 16.9 kcal mol^{-1} (1 cal = 4.184 J), and the smallest ΔG^\ddagger value of TP-2 results from the largest ΔS^\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 $k_{\text{cat}}^{\text{app}}$ value at 289 K was decreased from 159 to 124, 76.3 or 31.6 $\text{dm}^3 \text{mol}^{-1} \text{s}^{-1}$ 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 $k_{\text{cat}}/k_{\text{uncat}}$ or $k_{\text{cat}}^{\text{app}}$ 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 $10^5 k_{\text{cat}} = 3.81 \text{ s}^{-1}$ and $k_{\text{cat}}/k_{\text{uncat}} = 0.95$; the ratio of $k_{\text{cat}}/k_{\text{uncat}}$ 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.

Received, 12th June 1995; Com. 5/03747F

Footnotes

* E-mail ohkuboscc@mbox.uc.kyushu-u.ac.jp

† This procedure was carried out to induce some interaction between His monomer and TSA before the polymerization; 400 MHz ¹H NMR analysis of the [²H₆]Me₂SO 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% Me₂SO-Tris buffer.

References

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