

JOURNAL OF MOLECULAR CATALYSIS A: CHEMICAL

Journal of Molecular Catalysis A: Chemical 101 (1995) L111-L114

Letter

## Catalytic activities of novel L-histidyl group-introduced polymers imprinted by a transition state analogue in the hydrolysis of amino acid esters

Katsutoshi Ohkubo <sup>a,\*</sup>, Yasuo Urata <sup>b</sup>, Shyogo Hirota <sup>b</sup>, Yoshio Funakoshi <sup>b</sup>, Takashi Sagawa <sup>b</sup>, Satoshi Usui <sup>b</sup>, Kohji Yoshinaga <sup>c</sup>

<sup>a</sup> Institute for Fundamental Research of Organic Chemistry, Kyushu University, Fukuoka 812, Japan
<sup>b</sup> Department of Applied Chemistry, Faculty of Engineering, Kumamoto University, Kumamoto 860, Japan
<sup>c</sup> Department of Applied Chemistry, Faculty of Engineering, Kyushu Institute of Technology, Kitakyushu 804, Japan

Received 1 May 1995; accepted 26 May 1995

## Abstract

The catalytic activities of L-histidyl group-introduced, cross-linked polymers imprinted by a transition-state analogue (phenyl 1-benzyloxycarbonylamino-3-methylpentylphosphonate) or a ground-state one (N-(N-benzyloxycarbonyl-L-leucinoyl) anthranilic acid) for the esterolysis of a marked substrate of *p*-nitrophenyl *N*-benzyloxycarbonyl-L-leucinate were examined in the hydrolysis of amino acid esters; a transition-state analogue recorded polymer possessing a low cross-linker content (8.7%) exhibited the highest catalytic activity for the marked substrate hydrolysis with the positively largest activation entropy and the smallest activation free energy.

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

Although the polymers prepared by the 'molecular imprinting' technique for creating a new kind of catalyst, so-called 'plastic enzyme', have recently received the attention of many chemists [1], the transition state analogue (TSA)-recorded polymer catalysts have hitherto been the subject of only limited investigation; there are only three reports on the esterolytic catalysis of the imidazole-or L-histidine-containing cross-linked polymers imprinted with *p*-nitrophenol methylphosphonate (TSA for the hydrolysis of *p*nitrophenol acetate) [2–4]. Therefore, further

investigations of novel TSA-imprinted polymer catalysts seem of interest and significant to design efficient 'plastic enzymes'. This paper deals with the catalytic activities of newly synthesized TSAor GSA (ground state analogue)-recorded crosslinked polymers possessing the catalytic site of L-histidyl group for the selective esterolysis of a marked substrate in the hydrolysis of amino acid esters; Phenyl 1-benzyloxycarbonylamino-3methylpentylphosphonate or N-(N-benzyloxycarbonyl-L-leucinoyl)anthranilic acid was respectively selected as a template molecule of TSA or GSA for the following hydrolysis of a marked substrate, p-nitrophenyl N-benzyloxycarbonyl-L-leucinate (Z-L-Leu-PNP) (Scheme 1).

<sup>\*</sup> Corresponding author. Tel. (+81-92)6512242, fax. (+81-96)6512242.

<sup>1381-1169/95/\$09.50 © 1995</sup> Elsevier Science B.V. All rights reserved SSDI 1381-1169(95)00097-6



The template TSA molecule was synthesized by stirring a mixture of 13.2 mmol triphenylphosphite, 19.8 mmol 3-methyl-1-butanal and 13.2 mmol benzyl carbamate and 2 ml acetic acid at 80°C for 1 h, while GSA was prepared by condensation of 11 mmol N-benzyloxycarbonyl-L-leucine with 10 mmol ethyl anthranilate hydrochloride using 12 mmol N,N'-dicyclohexylcarbodiimide in 40 ml CHCl<sub>3</sub> containing 10 mmol Et<sub>3</sub>N and by successive alkaline hydrolysis with 1 M NaOH. On the other hand, the polymer catalysts were prepared in accordance to Scheme 2.

The prepolymers including the template TSA or GSA molecule in their cavities were at first prepared by the radical copolymerization of 0.27 mmol methyl N-acryloyl-L-histidinate, 2.7 mmol acrylamide, 1.5 mmol N,N'-1,2-ethylenebis(2propenamide) and 0.26 mmol TSA (or GSA) with an initiator of 0.08 mmol  $\alpha, \alpha'$ -azobis(isobutyronitrile) (AIBN) in 12 ml DMSO in  $N_2$  at 60°C for 24 h, and the treatment of the prepolymers with 5.0 vol.% Et<sub>3</sub>N-MeOH to remove the template molecule afforded polymer catalysts (TP-1 and GP-1) indicating a relatively high cross-linker content (33%). Since the content (70%) of the template molecule remaining in TP-1 was very high as compared with that (10%)of GP-1, the same TSA-imprinted polymer catalyst (TP-1'), which has a low cross-linker content (8.7%) in its skeleton and excludes 98% TSA from its prepolymer, was also prepared with the

same monomers mentioned above. In regard to the proximity between the L-histidine imidazole group of the polymer catalyst and the phosphonate part of TSA (or the carboxylate part of GSA) in the prepolymers prepared without such a chelating agent as CoCl<sub>2</sub> [2], the <sup>1</sup>H-NMR spectra of the prepolymers in DMSO-d<sub>6</sub> suggested the interaction between the imidazole NH proton in the former and the phosphonate P=O (or carboxylate C=O) oxygen in the latter (as indicated in Scheme 2) through the change in the chemical shifts of the prepolymers from those of the Lhistidinate monomer and TSA (or GSA); for instance, the shift of the NH proton to the lower magnetic field from 7.40 to 8.47 ppm was observed in the TS-1 prepolymer with such a higher magnetic field shift of the methine proton from 4.00 to 3.75 ppm.

The hydrolysis of  $2.0 \times 10^{-5}$  mol dm<sup>-3</sup> amino acid esters, such as Z-L-Leu–PNP and *p*-nitrophenyl *N*-benzyloxycarbonyl-L-alaninate (or phenylalaninate), Z-L-Ala (or Phe)–PNP, by the polymer catalyst (65.1–193  $\mu$ mol of the L-histidine unit) was carried out at 293 K in 10% v/v DMSO/Tris-buffer (pH 7.15), and the hydrolysis rate was followed spectrophotometrically by monitoring the absorption of the produced *p*-nitrophenolate anion at 400 nm. The catalytic activity of TP-1, GP-1, or TP-1' was evaluated from the apparent second-order rate constant,  $k_{cat}^{app}$ , given by the equation of  $k_{cat}^{app} = (k_{cat} - k_{uncat})/[His]$ , where  $k_{cat}$  and  $k_{uncat}$  are pseudo-first-order rate





constants obtained with and without the polymer catalyst, respectively, and [His] denotes the catalytically active L-histidyl group concentration (determined by the <sup>1</sup>H-NMR spectra) in the polymer catalyst.

In Table 1 are listed the rate constant ratio  $(k_{cat}/k_{uncat})$  and  $k_{cat}^{app}$  values for the hydrolysis of Z-L-Leu-PNP by *N*-acryloyl-L-histidinate

monomer (His), GP-1, TP-1, and TP-1' at 303 K. As the  $k_{cat}/k_{uncat}$  and  $k_{cat}^{app}$  values in Table 1 indicate, the catalytic activities of the templateimprinted polymers followed the order of (None) < (His) < GP-1 < TP-1 < < TP-1'. Thus, the catalytic activities of the TSA-imprinted polymers (TP-1 and TP-1') were appreciably higher than that of the GSA-recorded one (GP-1). TP-

Table 1

Kinetic parameters for hydrolysis of Z-L-Leu-PNP by TSA-or GSA-imprinted polymer catalysts at 303 K \*

Catalyst	$\frac{10^3 k_{\text{cat}}}{\text{min}^{-1}}$	$k_{\rm cat}/k_{\rm uncat}$	$\frac{10^3 k_{cat}^{app}}{mol^{-1} dm^3 min^{-1}}$	<i>ΔH</i> <sup>≠</sup> kcal mol <sup>−1</sup>	$\Delta S^{\neq}$ cal mol <sup>-1</sup> K <sup>-1</sup>	$\Delta G^{\star}$ kcal mol <sup>-1</sup>
GP-1	4.78	2.0	12.3	21.8	10.6	18.6
TP-1	5.22	2.2	66.8	19.2	5.3	17.6
TP-1′	8.79	3.7	98.2	31.2	45.2	17.5
His	3.44	1.4	10.4	4.5	-47.1	18.8
None	2.40			22.6	-4.35	23.9

<sup>a</sup> Reaction conditions are given in the text.

1' possessing a larger cavity compared with that of TP-1 was found most effective among the polymer catalysts tested. The catalytic activities of these imprinted polymers are also reflected in the activation parameters determined by the temperature dependency of  $k_{cat}^{app}$  values. The increased values of activation enthalpy  $(\Delta H^{\neq})$  for GP-1, TP-2 and TP-1' compared with that for His imply that the reaction of the polymer catalysts with the substrate in their template-shape recorded cavities is energetically impaired by the somewhat restricted proximity between the catalytically active L-histidine imidazole group of the polymers and the susceptible carbonyl group of the substrate. However, the positively enhanced values of activation entropy  $(\Delta S^{\neq})$  for GP-1, TP-2 and TP-1', which probably demonstrates the efficient substrate inclusion by the polymer catalysts through their hydrophobic interaction with the substrate, decreased the values of the activation free energy  $(\Delta G^{\neq})$  in the same activity order of None < His < GP-1 < TP-1  $\le$  TP-1'. Therefore, the entropic factor rather than the enthalpic one played an important role in the esterolytic catalysis of the present template-imprinted polymer catalysts. Thus, the TP-1' polymer catalyst having the low-cross linker content (8.7%) exhibited the highest activity with the rate constant ratio of  $k_{cat}/k_{uncat}$  = 3.7 for the Z-L-Leu–PNP hydrolysis, but it indicated the lower activity for the hydrolysis of other amino acid *p*-nitrophenyl esters such as Z-L-Phe–PNP possessing a more hydrophobic but sterically-hindered side chain and Z-L-Ala–PNP having a more sterically facilitated side chain; the observed  $k_{cat}/k_{uncat}$  ratios were 1.8 for Z-L-Phe– PNP and 2.2 for Z-L-Ala–PNP. Hence, the TP-1' catalyst was also capable of exhibiting an efficient molecular recognition ability in its esterolytic catalysis.

The further investigation of the TSA-imprinted polymer catalysts improved by changing their frameworks, TSA, and so on for the enhancement of their catalytic abilities are now in progress.

## References

- [1] F. Flame, Science, 263 (1994) 1221.
- [2] D.K. Robinson and K. Mosbach, J. Chem. Soc., Chem. Commun., (1989) 969.
- [3] K. Ohkubo, Y. Urata, S. Hirota, Y. Honda and T. Sagawa, J. Mol. Catal., 87 (1994) L21.
- [4] K. Ohkubo, Y. Urata, S. Hirota, Y. Honda, Y. Fujishita and T. Sagawa, J. Mol. Catal., 93 (1994) 189.