ELSEVIER

S0032-3861(96)00198-X

Polymer Vol. 37 No. 17, pp. 3993–3995, 1996

0032-3861/96/$15.00+0.00

Catalytic activity of a novel water-soluble cross-linked polymer imprinted by a transition-state analogue for the stereoselective hydrolysis of enantiomeric amino acid esters

Katsutoshi Ohkubo*, Yoshio Funakoshi and Takashi Sagawa
Department of Applied Chemistry and Biochemistry, Faculty of Engineering, Kumamoto University, Kumamoto 860, Japan
(Received 20 December 1995)

A novel water-soluble cross-linked polymer catalyst containing L-histidine and quarternary trimethylammonium groups, which was imprinted by a *racemic* transition-state analogue of phenyl 1-benzyloxycarbonyl-3-methylpentylphosphonate for the hydrolysis of *p*-nitrophenyl *N*-(benzyloxycarbonyl)-L (or D)-leucinate (Z-L (or D)-Leu-PNP), exhibited the notable substrate stereospecificity for Z-L-Leu-PNP in the hydrolyses of enantiomeric amino acid *p*-nitrophenyl esters in 10 vol% DMSO (or MeCN)–Tris buffer pH 7.15) at 303 K. Copyright © 1996 Elsevier Science Ltd.

(Keywords: transition state analogue; imprinted polymer; stereoselective hydrolysis)

Although the polymer catalysts imprinted by transition-state analogues (TSAs) have recently received considerable attention as plastic enzymes or artificial antibodies[1], the substrate-stereospecific catalysis of water-soluble cross-linked polymers recorded by TSA has hitherto been the subject of only limited investigation. Among the TSA-imprinted cross-linked polymers reported previously as esterase-like catalysts for the hydrolyses of amino acid esters[2–5], poly(ethylene imine)[2] is only one water soluble polymer catalyst for the substrate-specific hydrolysis of amino acid esters.

This report demonstrates that a novel water-soluble cross-linked polymer containing a L-histidyl group as a catalytic site, which was imprinted by *racemic* phenyl 1-benzyloxycarbonylamino-3-methylpentylphosphonate (*rac*-TSA) for the hydrolysis of *p*-nitrophenyl *N*-(benzyloxycarbonyl)-L (or D)-leucinate (Z-L (or D)-Leu-PNP), exhibited the catalytic monoclonal antibody-like substrate-stereospecificity for Z-L-Leu-PNP in the hydrolysis of enantiomeric amino acid *p*-nitrophenyl esters (*Scheme 1*).

Phenyl 1-benzyloxycarbonylamino-3-methylpentylphosphonate (*rac*-TSA) was obtained as described previously[2]. A water-soluble cross-linked polymer (TSSP) was prepared by radical polymerization according to *Scheme 2*.

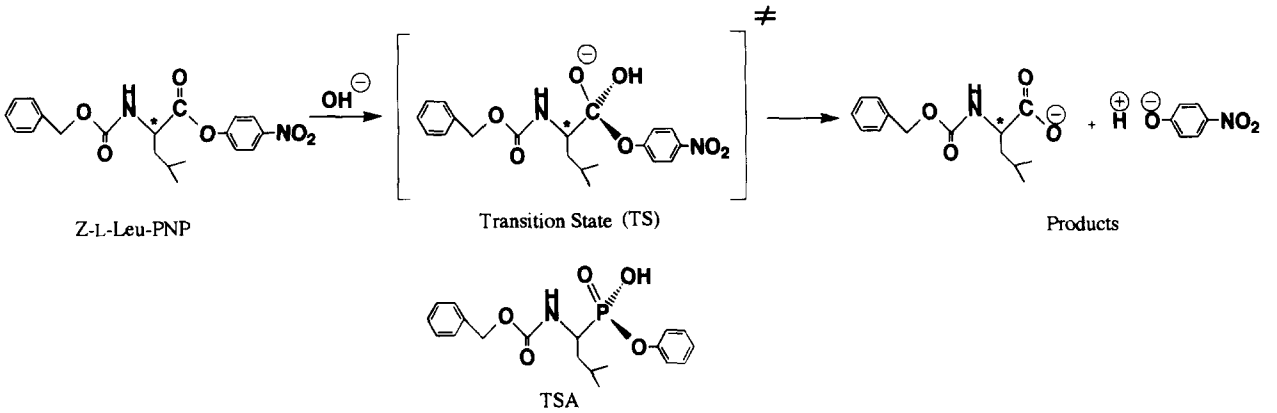
Equivalent amounts (0.26 mmol) of methyl *N*-acroyl-L-histidinate (L-His monomer) and *rac*-TSA were mixed in DMSO (12 cm^3) for 1 h at room temperature in N_2 for making some interaction between L-His monomer and TSA before the polymerization†, followed by the addition of acrylamide (2.6 mmol), *N*-(3-trimetylaminopropyl)acrylamide chloride (2.34 mmol), a cross-linker of *N,N*-ethylenebis(2-propeneamide) (0.52 mmol) and AIBN (0.08 mmol) into the DMSO solution, and then polymerized at 60°C to produce a polymer possessing cross-linker content of 8.6%. The complete removal of TSA from the polymer was performed with 5 vol% Et_3 N–MeOH at first and then with MeOH. The polymer TSSP possessing the randomly distributed quarternary trimethylammonium group through its framework was very soluble in water.

The hydrolyses of 20.0 μmol dm^{-3} Z-L (or D)-Leu-PNP or *p*-nitrophenyl *N*-acetyl-L-leucinate (or phenylalanate) {C_2-L-Leu (or Phe)-PNP} by the soluble polymer catalyst TSSP {methyl L-histidinate (His) unit concentration = 0.23 mmol dm^{-3}} or His (0.10 mmol dm^{-3}) were carried out in 10 vol% DMSO (or MeCN)–Tris buffer (pH 7.15) at 303 K. The pseudo-first-order reaction constants obtained with and without the catalyst (K_{cat} and k_{uncat}, respectively) were determined by monitoring the produced amount of PNP anion spectrophotometrically at 400 nm. The second-order catalytic rate constant k_{cat}^{app} was evaluated by the equation of $k_{cat}^{app} = (k_{cat} - k_{uncat})/[His]$, where [His] denotes the concentration of His unit in the catalyst.

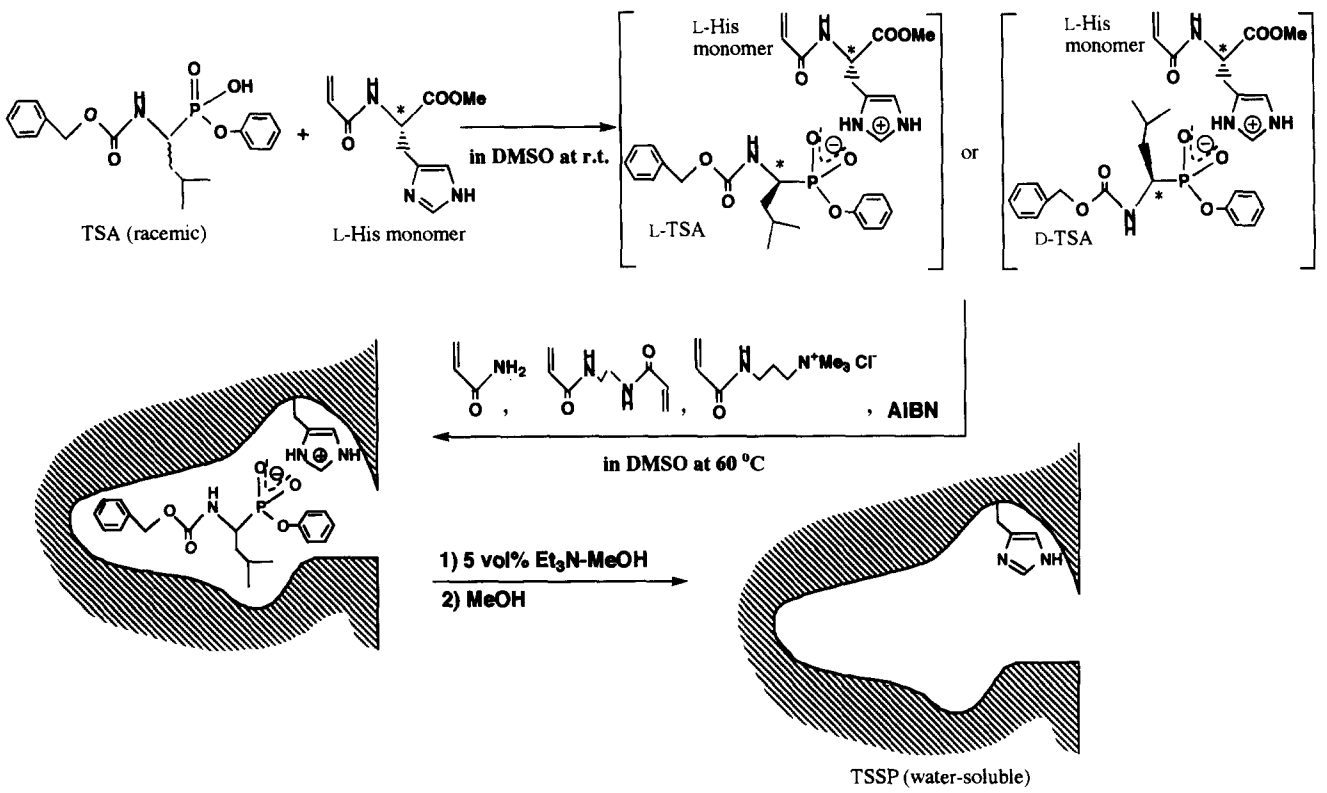
It is notable at first from the kinetic parameters listed in *Table 1* that the soluble polymer catalyst TSSP exhibited a higher esterolytic activity than the catalytic site of methyl L-histidinate (His) *per se* with the k_{cat}^{app}(TSSP)/k_{cat}^{app}(His) ratio = 4.3 (in the Z-L-Leu-PNP hydrolysis). On the other hand, the hydrolysis of Z-D-Leu-PNP with the TSSP catalyst was depressed remarkably and was slower than that of the uncatalytic one in both solvent systems; the apparent stereoselectivity of

* To whom correspondence should be addressed

† In the 400 MHz ^{1}H n.m.r. spectra of the DMSO-d_6 solution including L-His monomer and *rac*-TSA, the chemical shift of the imidazolyl NH proton (in L-His monomer) from 6.60 to 7.20 ppm and that of the amide C(=O)NH proton (in TSA) from 7.53 to 6.90 ppm suggested hydrogen bond formation or electrostatic interaction between them



Scheme 1 Transition-state (TS) and a transition-state analogue (TSA) for the esterolysis of Z-L-Leu-PNP

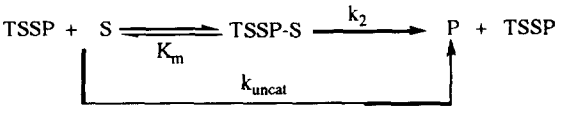


Scheme 2 Preparation of the water-soluble polymer TSSP

TSSP is given by the enantiomer rate ratio k_{cat}(L/D) = 3.0 (10% v/v DMSO–H_2O) – 7.2 (10% v/v MeCN–H_2O). Since TSSP did not hydrolyse C_2-L-Leu (or Phe)-PNP efficiently, the soluble TSSP catalyst can recognize both the skeletons of the *N*-benzyloxy (Z) group and the L-Leu side chain of the substrates with the substrate-stereospecificity.

In this respect, the hydrolysis of Z-L-Leu-PNP with TSSP in the presence of *rac*-TSA decreased the reaction rate monotonously and stopped the catalytic reaction completely under the condition of [*rac*-TSA]/[TSSP] = 2.0 (*Figure 1*). Therefore, the soluble TSSP polymer has the reaction cavity which recognizes the skeleton of *rac*-TSA, especially that of L-TSA. This implies that the complex formation of L-His monomer with L-TSA in DMSO through the hydrogen bonding or electrostatic interaction was predominant rather than with D-TSA in the preliminary mixed system of L-His monomer and *rac*-TSA for the synthesis of TSSP polymer (see *Scheme 2*).

In regard to the facilitated incorporation and hydrolysis of Z-L-Leu-PNP in the cavity of TSSP, the kinetic parameters of K_m and k_2 were obtained from the linear relation of $1/(k_{cat} - k_{uncat}) = K_m/(k_2 - k_{uncat})[TSSP] + 1/(k_2 - k_{uncat})$ in the following simplified process for the esterolysis of Z-L-Leu-PNP with TSSP ([TSSP] = 0.12–0.50 mmol dm^{-3}) in 10% v/v DMSO–H_2O in Tris buffer (pH 7.15) at 303 K.



Scheme 3 A simplified reaction process of the ester (S) hydrolysis with TSSP

Table 1 Catalytic activity and stereoselectivity of the soluble polymer catalyst TSSP for the hydrolyses of Z-L (or D)-Leu-PNP and C_2-L-Leu (or Phe)-PNP in 10% v/v DMSO-H_2O (A) or 10% v/v MeCN-H_2O (B) in Tris buffer (pH 7.15) at 303 K[a]

	Z-L-Leu-PNP		Z-D-Leu-PNP		C_2-L-Leu-PNP	C_2-L-Phe-PNP
Parameter	In solvent A	In solvent B	In solvent A	In solvent B	In solvent B	In solvent B
10^5K_{cat} (s^{-1})	11.6 (5.50)	8.03	3.83	1.11	6.56	10.3
10^5k_{uncat} (s^{-1})	4.82	3.97	4.82	3.97	7.24	9.51
k_{cat}/k_{uncat}	2.4 (1.2)	2.0	1/1.3	1/3.6	1/1.1	1.08
$10^2\ k_{cat}^{app}$ (mol dm^{-3})	29.5 (6.8)	17.6	0<	0<	0<	3.4

[a] [His unit is TSSP] = 0.23 mmol dm^{-3} and [substrate] = 20.0 μmol dm^{-3}. Values in parentheses were obtained with 0.10 mmol dm^{-3} methyl L-histidinate (His)

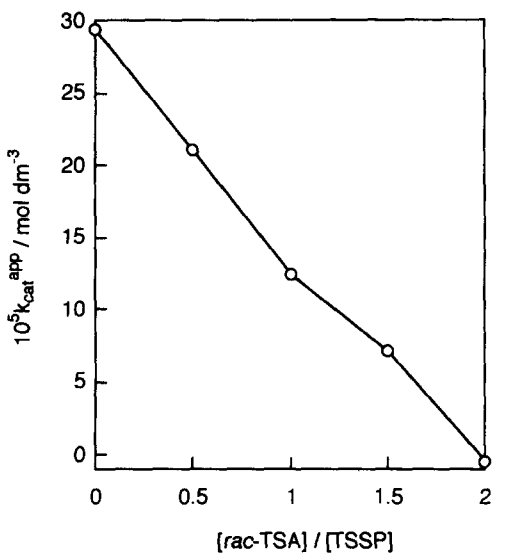


Figure 1 Inhibition of the Z-L-Leu-PNP (20.0 μmol dm^{-3}) esterolysis with TSSP ([His unit] = 0.23 mmol dm^{-3}) by TSA (0–40.0 μmol dm^{-3}) in 10% v/v DMSO-H_2O in Tris buffer (pH 7.15) at 303 K

It is also worth emphasizing that the soluble TSSP catalyst actually incorporates Z-L-Leu-PNP to form the TSSP–substrate complex with $K_m = 2.24 \times 10^{-4}$ mol^{-1} dm^3 and hydrolyses Z-L-Leu-PNP efficiently with the reaction rate ratio $k_2/k_{uncat} = 16.2$ ($10^5k_2 = 64.8\ s^{-1}$).

Thus, the present soluble TSSP catalyst, the reaction cavity of which seems to be predominantly recorded by the shape of L-TSA, was found to exhibit the efficient substrate-stereospecific hydrolysis of Z-L-Leu-PNP through the substrate incorporation into its reaction cavity.

REFERENCES

1 Wulff, G. *Angew. Chem. Int. Edn Engl.* 1995, **34**, 1812 (and literature cited therein)
2 Ohkubo, K., Urata, Y., Honda, Y., Nakashima, Y. and Yoshinaga, K. *Polym. Commun.* 1994, **35**, 5372
3 Robinson, D. K. and Mosbach, K. *J. Chem. Soc., Chem. Commun.* 1989, 969
4 Ohkubo, K., Urata, Y., Hirota, S., Honda, Y. and Sagawa T. *J. Mol. Cat.* 1994, **87**, L21
5 Ohkubo, K., Urata, Y., Hirota, S., Honda, Y., Fujishita, Y. and Sagawa, T. *J. Mol. Cat.* 1994, **93**, 189