

## Hydrolysis of carboxylate ester catalyzed by a new artificial abzyme based on molecularly imprinted polymer

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**Abstract** A new kind of artificial abzyme MIP-3, which contains N-phenyl benzoic amide group and "nano-channel" formed by embedded ZnO nano materials, and is imprinted by a transition-state analogue of *p*-nitrophenyl methyphosphonate in the hydrolysis of *p*-nitrophenyl acetate, was prepared by radical co-polymerization. Hydrolytic kinetics of *p*-nitrophenyl carboxylate catalyzed by MIP-3 was investigated. The results showed that the artificial abzyme exhibited notable substructure selectivity and strong catalytic ability in hydrolysis of *p*-nitrophenyl acetate.

**Keywords:** artificial abzyme, molecularly imprinted polymer, nano-channel, carboxylate, catalytic hydrolysis.

In enzyme catalysis, it has generally been accepted that enzymes recognize and stabilize the transition state of a substrate molecule to get a lower activation barrier. The concept of an abzyme which is 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 abzymes do not seem to be commercially applicable because of the complicated purification processes and high cost. Hence, Ledonhardt et al.<sup>[1]</sup> came out with an idea of generating artificial abzymes by the method of molecularly imprinted polymers (MIPs), of which the recognition, binding and catalytic sites of artificial abzymes are established through molecular imprinting against a transition-state. The simple preparation, high mechanical, thermal and chemical stability, and the needlessness for biologically-based protocols make MIPs "artificial abzymes" interesting alternatives to their biological counterparts.

Beach group<sup>[2]</sup> was successful in the synthesis of MIP that could catalyze the dehydro-fluorination of 4-fluoro-4-(*p*-nitrophenyl)butan-2-one. Matsui et al.<sup>[3]</sup> prepared the catalytically active polymer by molecular imprinting, which was capable of selectively catalyzing an entropically unfavorable C-C bond formation, namely the aldol condensation of acetophenone and benzaldehyde to produce chalcone. Wulff et al.<sup>[4]</sup> developed polymerizable amidines and used them as anchor or catalyst groups to prepare MIPs with a strong acceleration (102-fold) of the hydrolysis of carboxylic esters. However, the maximum acceleration factor 102 reported is not satisfying, especially in contrast to the theoretical calculations we have made<sup>1)</sup>. In this paper, the preparation of a new kind of MIP artificial abzyme,

1) Yu Jianhua, Studies on artificial hydrolytic enzyme based on molecularly imprinted polymers. Postdoctoral research report, January, 2000.

(MIP-3) containing “nano-channel” formed by embedded ZnO materials is reported and the hydrolytic kinetics of carboxylic esters catalyzed by MIP-3 is studied.

### 1 Preparation and characterization of MIP-3

The artificial abzyme MIP-3 was synthesized according to Ref. [4]. The synthetic scheme is shown in Figure 1.

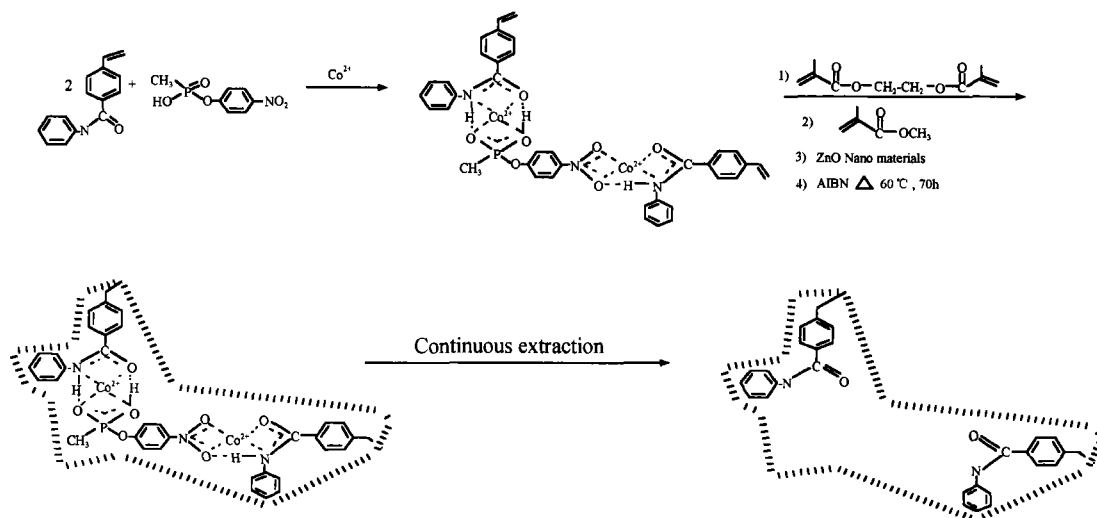


Fig. 1 Synthetic procedure of MIP-3. AIBN: azo-bis-isobutyronitrile.

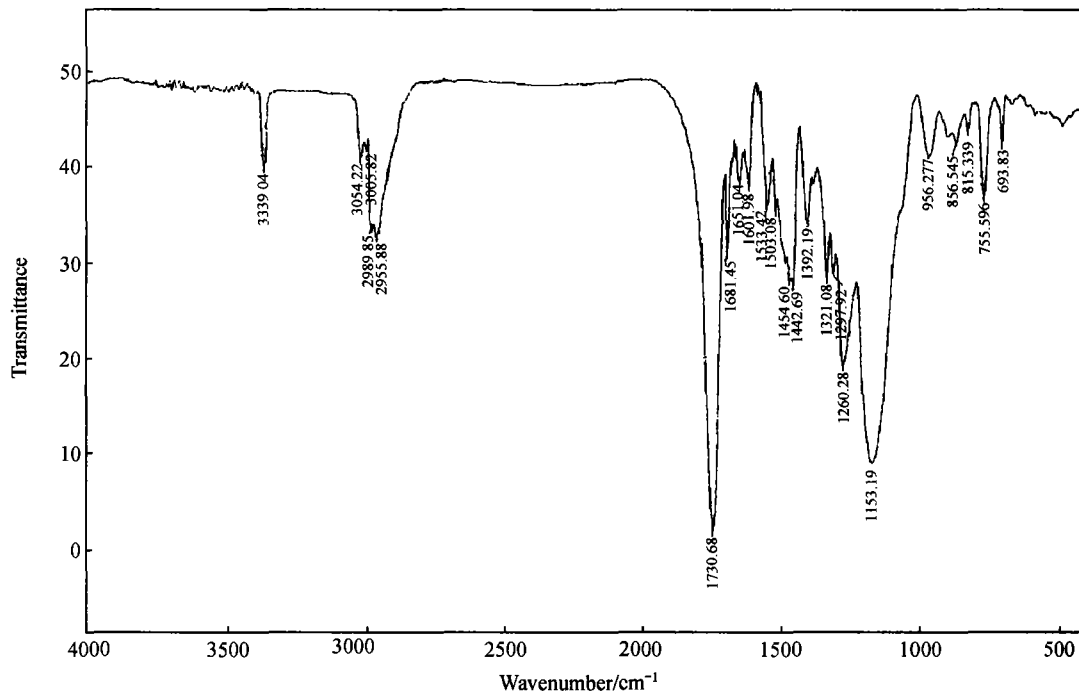


Fig. 2 IR spectra of MIP-3.

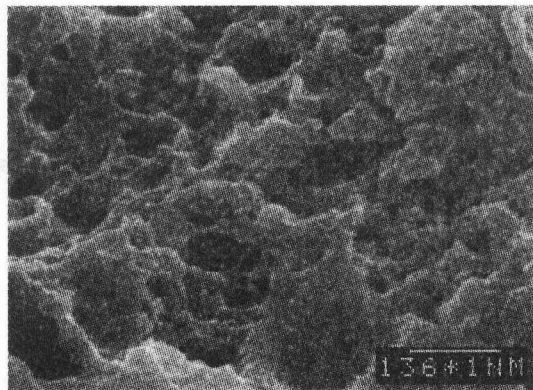


Fig. 3 SEM image of MIP-3.

The IR spectra and image of scanning electron microscopy (SEM) of MIP-3 are shown respectively in figures 2 and 3.

## 2 Catalytic kinetics

The reaction kinetics of catalytic hydrolysis of the carboxylic esters was investigated according to Ref. [5]. When MIP-3 was excessive and the substrate concentration was low, the extinction coefficient of the reaction product was measured at different times and different pH values. The catalytic rate constant was evaluated from  $k_{\text{cat}} = k_{\text{obs}} - k_{\text{uncat}}$ , where  $k_{\text{cat}}$  is catalytically hydrolytic rate constant,  $k_{\text{obs}}$  the apparent hydrolytic rate constant, and  $k_{\text{uncat}}$  the spontaneously hydrolytic rate constant. The study included the following three parts.

### 2.1 Measurement of the apparent extinction coefficients of *p*-nitrophenol

The apparent extinction coefficients of *p*-nitrophenol at the concentration of  $2.02 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$  were measured in 10% DMSO/H<sub>2</sub>O (Tris-HCl buffer, pH 7.013 ~ 9.021 and including  $0.1 \text{ mol} \cdot \text{L}^{-1} \text{ KNO}_3$ ) at 298 K on a Shimadzu UV265 spectrophotometer at 400 nm. The results are shown in Table 1.

Table 1 The apparent extinction coefficients of *p*-nitrophenol at 400 nm

pH	7.013	7.502	8.006	9.021
$\epsilon_{\text{obs}} / \text{L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1} \times 10^{-3}$	8.767	10.99	13.01	16.89

### 2.2 Measurement of spontaneously hydrolytic rate constants of *p*-nitrophenyl carboxylic esters

The spontaneous hydrolysis of two different kinds of *p*-nitrophenyl carboxyl esters ( $2.02 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$ ) were carried out in 10% DMSO/H<sub>2</sub>O (Tris-HCl buffer, pH 7.013 ~ 9.021, including  $0.1 \text{ mol} \cdot \text{L}^{-1} \text{ KNO}_3$ ) at 298 K. The reaction rate was monitored by the spectrophotometric determination of *p*-nitrophenyl anion formation. The experimental method and conditions were the same as described in Sec. 2.1. A graph of  $\log(v_{\infty} - v_t)$  against  $t$  was plotted and the rate constants for the spontaneous reaction were obtained. The results are shown in Table 2.

Table 2 Spontaneously hydrolytic rate constants of *p*-nitrophenyl carboxylic esters

pH	<i>p</i> -nitrophenyl acetate				<i>p</i> -nitrophenyl ( <i>p</i> -nitrophenyl)-acetate
	7.013	7.502	8.006	9.021	7.013
$k_{\text{uncat}} / \times 10^5 \text{ min}^{-1}$	4.601	19.81	46.03	162.8	9.272

### 2.3 Measurement of catalytically hydrolytic rate constants of *p*-nitrophenyl carboxylic esters

The MIP-3 catalyzed hydrolysis of two different kinds *p*-nitrophenyl carboxylic ( $2.02 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$ ) was carried out in 10% DMSO/H<sub>2</sub>O (Tris-HCl buffer, pH 7.013 ~ 9.021) at 298

K. The experimental method and conditions were the same as the above-mentioned. The results are shown in Table 3.

Table 3 Catalytically hydrolytic rate constants of *p*-nitrophenyl carboxylic esters

		<i>p</i> -nitrophenyl acetate			<i>p</i> -nitrophenyl ( <i>p</i> -nitrophenyl)-acetate
pH	7.013	7.502	8.006	9.021	7.013
$k_{obs}/\times 10^5 \text{ min}^{-1}$	1162	1828	2302	4605	20.95
$k_{obs}/k_{uncat}$	252.6	92.28	53.00	28.29	2.137
$(K_{obs}-k_{uncat})/k_{uncat}$	251.6	91.28	52.00	27.29	1.250
$k_{cat}/\times 10^5 \text{ min}^{-1}$	1157	1808	2257	4442	11.51

### 3 Discussion

The important conclusions below can be drawn from the present data.

(i) When molecularly imprinting polymer “artificial abzyme” MIP-3 catalyzes the hydrolysis of *p*-nitrophenyl carboxylic esters, it has not only catalytic activity, but also structure selectivity. The catalytic activity comes from the functional groups in imprinted cavities and the selectivity comes from the three-dimensional cavities constructed in the polymer matrix.

(ii) The catalytic activity of MIP-3 is much higher than the ever reported ones<sup>[4~6]</sup> because of the construction of nano-channel. The existence of nano-channel in polymer matrix not only decreases the time needed for substrate to enter the cavities imprinted by template molecules, but also increases the specific surface area of an artificial abzyme.

(iii) The dependence of the catalytic activity of artificial enzyme on pH value is less than that of spontaneous hydrolysis. The more difficultly the substrate is hydrolyzed, the more neutrally pH value approaches, and the stronger the catalytic activity of the mimic enzyme is. The results show that the catalytic activity of the synthesized enzyme mainly comes from the cavities constructed in the polymer matrix by molecularly imprinted technique and from the effect of the functional groups in the cavities with the transition state of reactive substrate.

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