A Polyacrylic Acid as a Carrier for Immobilization of Penicillin Acylase

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ABSTRACT: A copolymer of acrylic acid with divinylbenzene was synthesized by suspension polymerization. This polymer is an effective carrier. Penicillin acylase was immobilized on this carrier to convert benzylpenicillin to 6-aminopenicillanic acid, which may be employed in the manufacture of semisynthetic penicillins. Factors that affect the activity of immobilized penicillin acrylase, such as temperature, pH, and amount of native enzyme, were studied. Under suitable conditions, the activity and activity recovery of the immobilized enzyme were 3100 U/g (dry carrier, *p*-dimethylaminobenzaldehyde method) and 59.7%, respectively. The immobilized penicillin acylase shows a remarkable increase in stability. At 40°C and pH 8.0 the value of the kinetic Michaelis–Menten constant (K_m) of the immobilized enzyme is 2.8×10^{-3} mol/L, and the value of activation energy of enzyme catalysis is 71.5 kJ/mol. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 86: 2067–2069, 2002

Key words: polyacrylic acid; polymeric carriers; penicillin acylase; enzymes; immobilized enzyme

INTRODUCTION

Enzymes immobilized on insoluble polymers are gaining importance in many industrial and biomedical applications. Besides retention of the catalytic activities, it is known that the main advantages of immobilized enzymes are that they are capable of repeated and continuous use and that time is saved in the purification step for removing the enzyme from the product. A wide variety of carriers and methods have been used for the immobilization of various enzymes.

This study describes how a polyacrylic acid as a carrier was synthesized through suspension polymerization of acrylic acid with divinylbenzene in the presence of petroleum ether as poregenerator. The carrier is cheap, nontoxic, hydrophilic, and stable to changes in temperature and pH. It has been found that the polyacrylic acid was an effective carrier for the immobilization of lipase and trypsin, as previously reported.[1,2] We report here the immobilization of penicillin acylase onto this carrier. The immobilized penicillin acylase can be used for conversion of benzylpenicillin to 6-aminopenicillanic acid, which may be employed in the manufacture of semisynthetic penicillins.

The activity of immobilized penicillin acylase in relation to the native enzyme was determined by the PDAB (*p*-dimethylaminobenzaldehyde) method using a potassium salt solution of benzylpenicillin as substrate. Factors such as temperature, pH, and amount of native enzyme, affecting the activity of immobilized penicillin acylase, were studied. The kinetic Michaelis–Menten constant (K_m) of immobilized penicillin acylase and the value of apparent activation energy (E_{app}) of enzyme catalysis were studied.

EXPERIMENTAL

Materials

Penicillin acylase (North China Pharmaceutical Plant), potassium salt of benzylpenicillin (North China Pharmaceutical Plant), *p*-dimethylaminobenzaldehyde (PDAB), and other chemical reagents were chemically pure and of analytical reagent grade.

Preparation of the carrier

Polyacrylic acid beads crosslinked with divinylbenzene were prepared by suspension polymerization. The continuous phase was 70 mL, 34% aqueous solution of calcium chloride. The discontinuous organic phase was composed of monomer and crosslinking agent and poregenerating solvent, petroleum ether (b.p. 90–120°C). The initiator benzoyl peroxide was added to the monomer before suspension. At 70°C polymerization was allowed to proceed for 24 h. The product was washed with excess distilled water, hydrochloric acid, and methanol, then ground and swollen in water.

Preparation of the immobilized enzyme

The 300 U of penicillin acylase was dissolved in 10 mL of 0.05 mol/L phosphate buffer at pH 7.2 and

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TABLE I
Optimum Temperature of Immobilized Enzyme

	Temperature (°C)					
	10	15	20	25	30	40
Activity recovery (%)	41.5	57.8	50.5	44.5	40.8	27.3

this was added to 0.2 g of this carrier (water sorption ~ 85%). The suspension was shaken for 4 h at 15°C and then filtered and washed with excess borate buffer. The immobilized enzyme was obtained and stored wet.

Determination of the activity

The activities of native and immobilized enzyme were determined in the presence of a 6% potassium salt solution of benzylpenicillin in 0.05 mol/L borate buffer, pH 8.0, at 40°C for 15 min. The activity measure was the concentration of 6-aminopenicillanic acid (6-APA), in a complex with *p*-dimethylaminobenzaldehyde.[3] The amount of enzyme that catalyzes formation of 1 μmol of 6-APA from benzylpenicillin in 1 min under experimental conditions was assumed to be one activity unit (U).

RESULTS AND DISCUSSION

Immobilization of enzyme

Immobilization reaction time

The optimum time of reaction for immobilized penicillin acylase was found to be 4 h, as previously reported.[4]

Immobilization temperature

To enable the enzyme to combine effectively with the carrier, and at the same time ensure that the enzyme would not lose activity during the immobilization, a suitable immobilization temperature had to be selected. Some 0.2 g of the carrier was placed in 10 mL, 0.05 mol/L phosphate buffer (pH 7.0) of penicillin acylase (300 U), under different temperatures (10, 15, 20, 25, 30, and 40°C). The suspensions were shaken for 4 h. From Table I it can be seen that a maximum activity recovery of immobilized enzyme was observed at 15°C. Below or above this temperature, either the rate of immobilized reaction decreased with a decrease of temperature or the activity of immobilized enzyme decreased with an increase of temperature.

TABLE II
Optimum pH of Immobilized Enzyme

	pH				
	6.8	7.0	7.2	7.5	7.8
Activity recovery (%)	49.8	55.8	59.7	50.2	49.5

Influence of ph value

The penicillin acylase immobilized onto this carrier in the phosphate buffer of different pH values (range from 6.8 to 7.8) but at a constant temperature of 15°C. The activity recovery of immobilized enzyme increased with an increase of pH up to a certain level and then decreased, as seen in Table II. When at pH 7.2, the activity recovery of immobilized enzyme reached a maximum value.

Building capacity of enzyme on carrier

Some 0.2 g of the carrier was treated with different amounts of native enzyme dissolved in phosphate buffer (10 mL) of pH 7.2. The suspensions were shaken for 4 h at 15°C, filtered, and washed with excess borate buffer. The immobilized enzyme was obtained and its activity was determined. From Table III it can be seen that the activity recovery of immobilized enzyme decreased with an increase of the amount of native enzyme. Because this reaction was carried out in a multiphase system, the rate of diffusion of product and substrate decreased with an increase of amount of native enzyme.

Under optimum experimental conditions, the penicillin acylase was immobilized onto this carrier. The activity and activity recovery of immobilized enzyme are 3100 U/g (dry carrier, PDAB method) and 59.7%, respectively.

Thermal stability

The thermal stability of the enzyme can usually be improved through immobilization. The immobilized enzyme was placed in 0.05 mol/L borate buffer of pH 8.0 and the contents were incubated at different temperatures (35, 40, 45, and 50°C) for 1 h. They were then rapidly cooled to room temperature. The activity was determined as mentioned previously. The value obtained at 35°C was assumed to correspond to 100%

TABLE III
Binding Capacity of the Enzyme on this Carrier

	Amount of added enzyme (U/0.2 g carrier)				
	120.4	210.7	301.0	391.3	418.6
Activity recovery (%)	51.0	52.3	59.7	51.9	42.7

TABLE IV
Thermal Stability of Immobilized Enzyme

	Temperature (°C)			
	35	40	45	50
Relative activity (%)	100	100	100	71.5

activity. The results are presented in Table IV. It can be seen that the relative activity of immobilized enzyme decreased with an increase of temperature; the immobilized enzyme retained 71.5% activity even after 50°C for 1 h. Thus, the immobilized enzyme showed good thermal stability.

Storage stability

The immobilized enzyme was stored in 0.05 mol/L borate buffer of pH 8.0 at 35 ± 1°C. The activity of this preparation was determined over the course of time. The result is shown in Table V, where it can be seen that the immobilized enzyme retained 50% activity after being stored for 11 days (264 h). Thus the immobilized enzyme has storage stability.

Determination of kinetic Michaelis–Menten constant

The reaction of enzyme catalysis may be shown as follows[5]:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [ES] \overset{k_0}{\rightarrow} E + P$$

where S is the substrate, E is the native enzyme, $[ES]$ is the enzyme-substrated complex, and P is the reaction product.

If $S \gg E$, then

$$\nu = VS/(K_m + S)$$

that is, $S/\nu = S/V + K_m/V$, in which ν is the instantaneous rate, V is the maximum rate, $K_m = (k_{-1} + k_0)/k_1$ is the Michaelis–Menten constant, and S is the concentration of substrate. The K_m value can be obtained by the Hanes method.[5] In 0.05 mol/L borate buffer of pH 8.0, at 40°C, the value of the kinetic Michaelis–Menten constant of the immobilized penicillin acylase (K_m) is 2.8×10^{-3} mol/L.

TABLE V
Storage Stability of Immobilized Enzyme

	No. of hours					
	0	41	94	142	192	264
Relative activity (%)	100	100	96	75	68	50

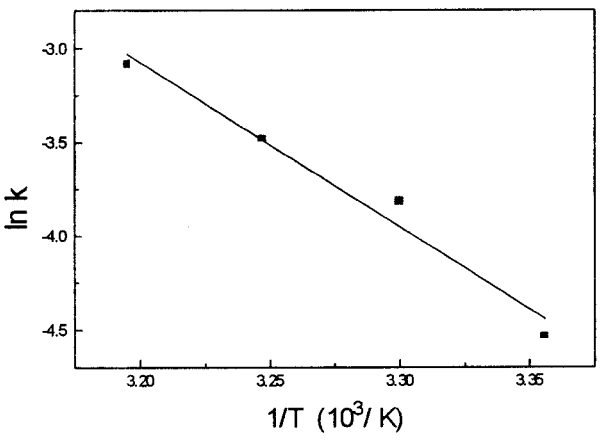

Figure 1 Plot of ln k against $1/T$ at different temperatures.

Determination of activation energy

If $K_m \gg S$, the reaction of enzyme catalysis exhibited a pseudo–first-order dependency on benzylpenicillin in our experiments. Rate constants were determined at different temperatures (25, 30, 35, and 40°C) in 0.05 mol/L borate buffer of pH 8.0. According to the Arrhenius equation,

$$\ln k = -E/RT + C$$

in which k is the rate constant, E is the activation energy, and T is the temperature. The relationship of ln k against the reciprocal of temperature $1/T$ is shown in Figure 1, and the slope of the straight line is $-E/R$. The value of activation energy of enzyme catalysis is 71.5 kJ/mol.

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