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IMMOBILIZATION OF AMP-DEAMINASE IN ACRYLAMIDE–GLYCIDYLMETHACRYLATE COPOLYMER

ISAO KARUBE, KEN-ICHI HIRANO,[1] and SHUICHI SUZUKI

Research Laboratory of Resources Utilization
Tokyo Institute of Technology
4259 Nagatsuda-machi, Midori-ku
Yokohama, Japan

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The enzyme AMP-deaminase was entrapped in an acrylamide–glycidylmethacrylate copolymer. No leakage of AMP-deaminase was observed from the copolymer. The optimum pH of native AMP-deaminase was 5.6. The pH was displaced to 5.0 with immobilization in the copolymer. The immobilized AMP-deaminase column maintained more than 95% of initial activity after 10 days of operation.

INTRODUCTION

Many methods are now available for the immobilization of enzymes (1,2). Enzymes can be immobilized by entrapment within the interstitial spaces of cross-linked water-insoluble polymers. The most commonly employed cross-linked polymer for enzyme entrapment is the well-known polyacrylamide gel system (3,4). However, enzymes of low molecular weight often permeate out of the gel matrix. AMP-deaminase (E.C. 3.4.5.6) was immobilized in gel obtained from copolymerization of acrylamide and glycidylmethacrylate. The properties of immobilized AMP-deaminase are described in this paper.

MATERIALS AND METHOD

Materials

AMP-deaminase (*Aspergillus* sp. 110,000 U/g) was obtained from Amano Pharmaceutical Company. Acrylamide and *N,N'*-methylene bisacrylamide were purchased from Wako Pure Chemicals Industries. Glycidylmethacrylate was obtained from Mitsubishi Gas Chemicals Company.

[1] Alternate address: Amano Pharmaceutical Company, Nishiharumachi, Nishikasugaigun, Aichi-ken.

Other solvents and reagents were commercially available analytical reagents or laboratory grade materials. Deionized water was used in all procedures.

Entrapment of AMP Deaminase

Various amounts of acrylamide, *N,N'*-methylenebisacrylamide, and glycidylmethacrylate were suspended in 10 ml of enzyme solution (1580 U/ml.* The total volume of reaction mixture was adjusted to 20 ml with 0.1 M phosphate buffer (pH 5.6). The solution was then saturated with nitrogen gas for 15 min; the polymerization was initiated with 0.25 ml of 10% dimethylaminopropionitrile and 0.25 ml of 5% potassium persulfate and allowed to proceed for 20 min at 20°C. The gel obtained was crushed with a homogenizer and washed with 100 ml of 0.1 M phosphate buffer solution (pH 5.6). The enzyme entrapped was calculated indirectly from the amount of the enzyme eluted in phosphate buffer solution.

Enzyme Assay

Unless otherwise noted, standard assays of the native AMP-deaminase and immobilized AMP-deaminase were carried out as follows: 50 μg of AMP-deaminase or 100–200 mg of immobilized AMP-deaminase was added to 20 ml of 0.05 M acetate buffer (pH 5.6) containing 25 mg of 5'-adenylic acid and incubated for 30 min at 37°C with shaking. A total of 0.5 ml of 2% perchloric acid solution was added to 0.5 ml of reaction mixture. The reaction mixture was sampled before the start of the reaction and after 30 min of reaction. The reaction mixture was diluted and finally adjusted to 50 ml, and the absorbance at 265 nm was determined spectrophotometrically (5).

Column Reaction

The apparatus used for experiments consists of a water-jacketed reactor (diameter 1.2 cm, height 5.0 cm, volume 5.7 cm^3), a peristaltic pump (Mitsumi Science Company, Model SJ-1210), and a fluid reservoir. A total of 5.2 g of immobilized AMP-deaminase (2960 U/g dry gel) was placed in the reactor and 0.1% adenylic acid in 0.03 M acetate buffer (pH 5.0) was employed at SV 3 h^{-1}.

* One unit of activity is defined as the production of 1 μmol of inosinic acid produced per minute at 37°C.

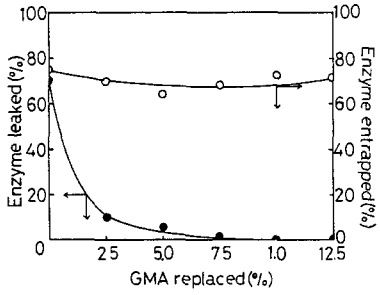

FIG. 1. Effect of replacement of acrylamide with glycidylmethacrylate (GMA) on the leakage of AMP-deaminase. Various AMP-deaminase gels were employed. A total of 100 mg of immobilized AMP-deaminase was suspended in 5 ml of 0.07 M phosphate buffer solution (pH 5.0) and incubated for 42 h at 20°C. The activities of the immobilized AMP-deaminase and the solution were determined under standard conditions. ●, Enzyme leaked; ○, enzyme entrapped.

RESULTS AND DISCUSSION

Preliminary experiments showed that the gel obtained from 17.5 wt/vol% of acrylamide and 2.5% of *N,N'*-methylenebisacrylamide is suitable for the immobilization of AMP-deaminase. However, 70% of the AMP-deminase activity was lost during 42 h incubation at 20°C. This result was caused by the leakage of AMP-deaminase from polyacrylamide gel.

A 20% (wt/vol) gel preparation consisting of 2.5% *N,N'*-methylenebisacrylamide, and various amounts of acrylamide and glycidylmethacrylate was used in the following experiments. Figure 1 shows the effect of the replacement of acrylamide with glycidylmethacrylate on the leakage of AMP-deaminase. No leakage of AMP-deaminase was observed when 10% of acrylamide was replaced with glycidylmethacrylate. Therefore, the gel matrix of the acrylamide–glycidylmethacrylate copolymer may be smaller than that of polyacrylamide. Furthermore, it is assumed that the coupling of the epoxy group of glycidylmethacrylate with free amino groups of AMP-deaminase occurs during copolymerization. In the present work, our experiments did not offer further corroborating evidence for this postulate. Approximately 70% of the added AMP-deaminase was entrapped in the copolymer (Fig. 1). A 20% gel preparation consisting

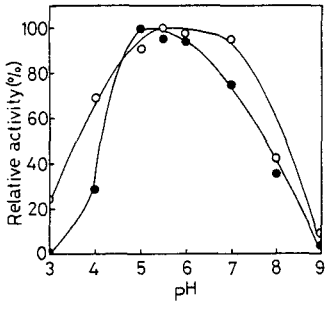

FIG. 2. The pH–activity profiles of native AMP-deaminase and immobilized AMP-deaminase. A total of 100 mg of immobilized AMP-deaminase or 50 μg of AMP-deaminase was used. The enzyme assay was carried out under standard conditions at various pH values; 0.07 M citrate buffer (pH 3.0), 0.07 M acetate buffer (pH 4–5), 0.07 M phosphate buffer (pH 6–7), and 0.07 M veronal buffer (pH 8–9) were employed. ○, Native AMP-deaminase, ●, immobilized AMP-deaminase.

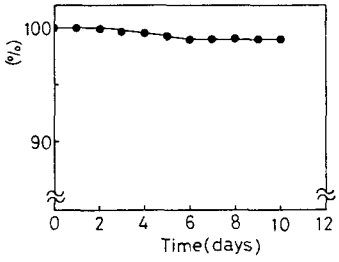

FIG. 3. Stability of the immobilized AMP-deaminase column. The activity of the immobilized AMP-deaminase column was determined under standard conditions.

of 15.7% acrylamide, 2.5% *N,N*-methylenebisacrylamide, and 1.8% glycidylmethacrylate was used thereafter.

Figure 2 shows pH–activity profiles of native AMP-deaminase and immobilized AMP-deaminase. The optimum pH of native AMP-deaminase was 5.6, and it was displaced to 5.0 on immobilization in the copolymer. Immobilized AMP-deaminase was more unstable than native AMP-deaminase under acidic conditions as shown in Fig. 2.

Figure 3 shows the operational stability of the immobilized AMP-deaminase column by continuously passing (downflow) a substrate solution at 37°C. The column maintained more than 95% of initial activity after 10 days of operation, indicating that the column is very stable.

In conclusion, the acrylamide–glycidylmethacrylate copolymer is suitable for entrapment of low molecular weight enzymes.

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