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IMP Synthesis Using Immobilized Adenosine (Phosphate) Deaminase

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ABSTRACT

Inosinic acid (IMP) has been prepared by the deamination of adenosine monophosphate (AMP) with an immobilized adenosine (phosphate) deaminase extracted from the snail *Biomphalaria glabrata*. The enzyme has been immobilized in polyacrylamide beads. The preparation and characterization of this system are described.

Index Entries: IMP; immobilized adenosine(phosphate)deaminase.

INTRODUCTION

Inosinic acid (IMP) has been industrially obtained by several procedures using as raw materials meat extract, dried sardines, enzymatic deamination of muscle adenylic acid or hydrolysis of inosine triphosphate (ITP) (*1–5*). Kinoshita et al. have described a mutant strain of *Micrococcus glutamicus* that is capable of producing IMP by microbial fermentation (*5*).

We propose a method using adenosine (phosphate) deaminase (E.C. 3.5.4.17.) obtained from snail muscle (*Biomphalaria glabrata*) and entrapped in polyacrylamide gel beads. IMP is synthesized from adenosine-5′-monophosphoric acid (AMP).

This process offers possible commercial advantages because the IMP is about ten times more expensive than AMP.

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MATERIALS AND METHODS

All the reagents used in this work were analytical grade and were purchased from Merck.

Extraction of Enzyme

Snail muscle (6g) was homogenized in 0.06 *M* phosphate buffer (8.0 mL), pH 7.2, using a Potter homogenizer. The homogenate was centrifuged at 13,000 xg for 10 min. The enzyme was precipitated from the supernatant in several steps using 40–80% saturated ammonium sulfate. All procedures were carried out at 0–4°C. The partially purified enzyme (2.0 U/mg of protein) was used throughout this work.

Immobilization of Enzyme

A solution (2.5 mL), prepared by dissolving 30g of acrylamide and 1.0 g of N,N'-methylenebisacrylamide in 123 mL of water, was kept at 4°C. After addition of the catalyst reagents, consisting of N,N,N',N'-tetramethylethylenediamine 0.28% v/v (1.25 mL) and ammonium persulfate 0.14% w/v (5.0 mL), an enzyme preparation with about 2 IU/mg of protein (1.25 ml) and sodium lauryl sulphate (10 mg) was introduced into the mixture. Then this material (c.a. 10 mL) was incubated in a solvent mixture (90 mL) composed by chloroform/toluene 2:1 (v/v) under N_2, at 25°C with continuous stirring (about 200 rpm). After approx 20 min of incubation, the gel, now in the form of beads, was thoroughly washed with deionized water followed by 0.06 *M* phosphate buffer, pH 7.2, in order to remove unpolymerized toxic monomers, the catalyst reagents, detergent, and organic solvents. The granules were washed with the buffer solution until no protein was present in the effluent, as determined by the method described by Lowry et al. (*6*).

Synthesis of IMP

A continuous enzymic system using a gradientless recirculation packed-bed reactor was used to produce IMP from AMP. The equipment was arranged according to Fig. 1. In this lab-scale production of IMP, 20 mL of 466 μM AMP, prepared in 0.0005 M phosphate buffer, pH 7.0, were circulated through 3.5 g of entrapped enzyme preparation, containing about 0.7 U, packed into a column measuring 80 × 20 mm at a flowrate of 3.0 mL/min^{-1}. A column containing DEAE-cellulose (80 × 10 mm), previously treated with 0.1 *M* NaOH, deionized water, 0.1 *M* HCl, deionized water, and 0.0005 *M* phosphate buffer, pH 7.0, was introduced into the circuit, as shown in Fig. 1. Under these conditions, the DEAE-cellulose column retained IMP, whereas AMP was recirculated (*7*). Finally, IMP was removed from the column by using 0.05 *M* phosphate buffer, pH 7.0. Under these conditions 93% of initial AMP was consumed in 90 min.

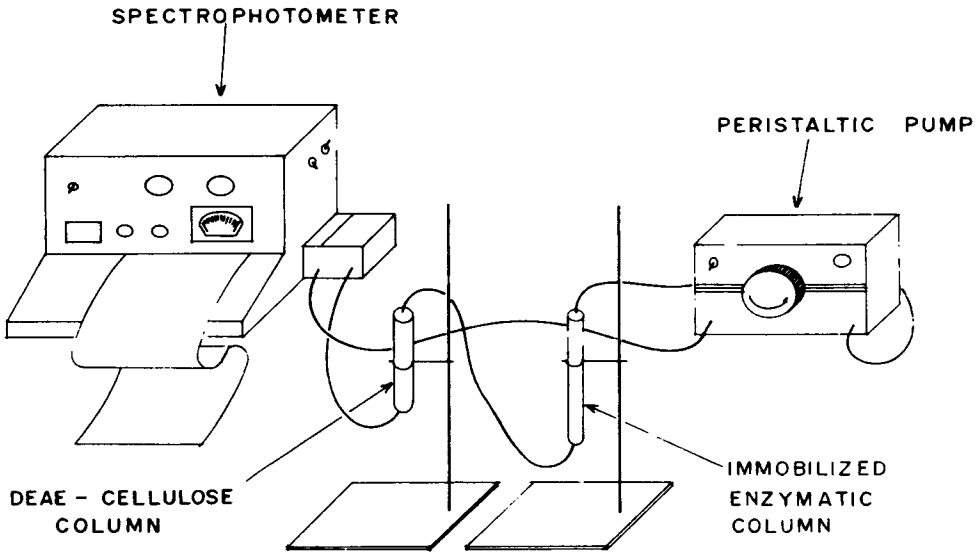

Fig. 1. Scheme of the continuous enzymic system using a gradientless recirculation packed-bed reactor to produce IMP from AMP.

Detection of IMP Production

The IMP production was monitored at 263 nm (*8*) in a continuous flow cell. A decease of 1.0 o.d. at 263 nm is equal to 0.25 μmoles of AMP converted to IMP (0.0005 *M* phosphate buffer, pH 7.0).

RESULTS AND DISCUSSION

The enzyme obtained from snails was used in this work, since previous work carried out in this lab had already demonstrated that this animal is a good source of the enzyme (*7*). However, any other source could easily replace it. Cardoso (*7*) has previously shown that at pH 7.0 a phosphatase activity present in the adenosine (phosphatase) deaminase preparation from muscle of snail was inactive. Therefore, no inosine is formed under the conditions of this study. Cardoso (*7*) showed that DEAE-cellulose is able to retain IMP prepared in phosphate buffer with a low ionic strength and at pH 7.0, whereas AMP and inosine are not retained.

Completion of AMP to IMP conversion was determined when no further change at 263 nm was observed in the column effluent (Fig. 2). The DEAE-cellulose column containing IMP was removed from the circuit and separately washed with 0.05 *M* phosphate buffer, pH 7.0. Spectrum of the effluent from the DEAE-cellulose was analyzed and shown to be identical to that reported by Kalckar (*8*) for IMP, with a maximum extinction at 248 nm.

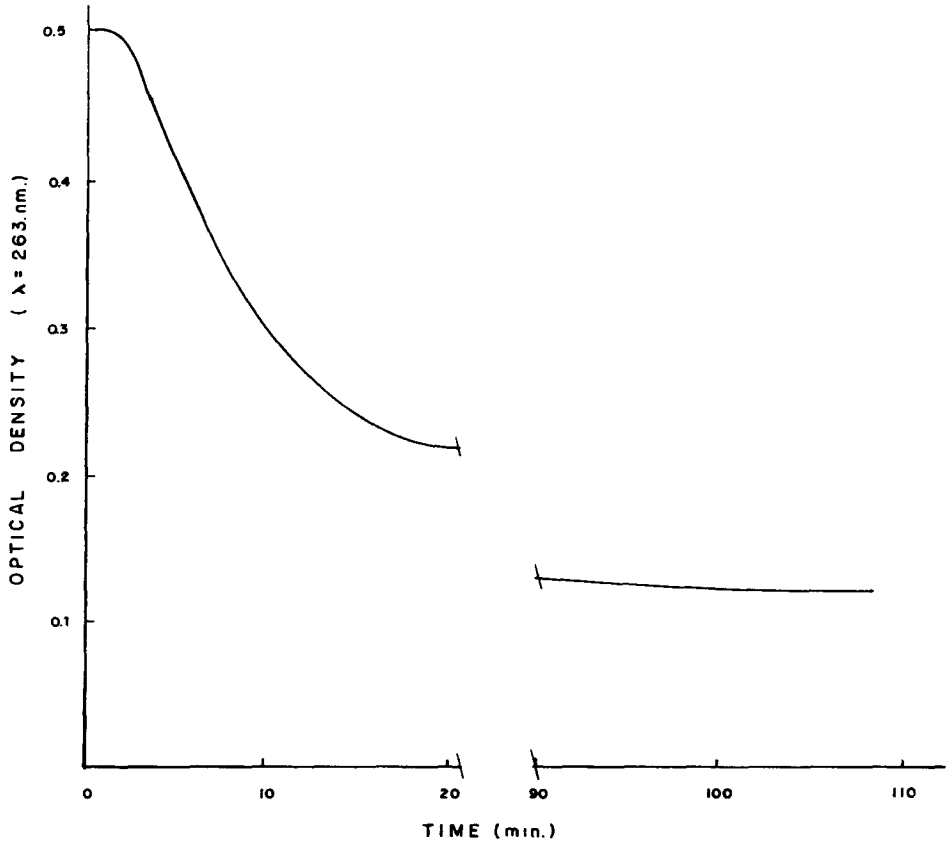

Fig. 2. Time course of AMP to IMP conversion by using immobilized adenosine (phosphate) deaminase. 20 mL of 466 μM AMP was circulated through a column containing immobilized enzymatic preparation and the effluent was monitored at 263 nm in a flow cell. An additional DEAE-cellulose column was introduced in the system to retain IMP and recirculated AMP into the enzymatic column.

This column was used several times over a 3-mo period with no apparent loss of activity. Between uses it was stored at 4°C.

The production of IMP from AMP using immobilized adenosine (phosphate) deaminase can be performed easily by using this system. The addition of a DEAE-cellulose column to retain IMP increases the efficiency of the system and the ease of isolating the purified IMP.

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