Synthesis of a Novel β-Lactam Compound by Penicillin G Acylase

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The enzyme penicillin G acylase (penicillin amidohydrolase, EC 3.5.1.11) catalyzes the reversible hydrolysis of benzylpenicillin to 6-aminopenicillanic acid (6-APA) and phenylacetic acid (PAA). The hydrolysis reaction has optimal pH between 7–9 and the synthetic reaction has an acidic optimal pH. Owing to the booming activities in the introducing of organic solvents into enzyme systems in the last two decades, this enzyme reaction has been done in a water-miscible organic solvent system and a reverse micelle system by several research groups. In this paper, a new acyl donor, methyl 3-phenylpropionate (MPP), which was different from traditional substrates such as phenylacetic acid, was coupled with 6-APA by penicillin G acylase (PGA). Methyl isobutyl ketone (MIBK) was introduced into the reaction medium (a biphasic system), and the advantages of this are discussed.

The enzyme penicillin G acylase (PGA) in immobilized form from Escherichia coli was a gift from Boehringer Mannheim GmbH. The schematic representation of the synthesis reaction from 6-APA and MPP by PGA is shown in Fig. 1. The acyl donor, MPP, binds to PGA first. The phenylpropionyl–PGA intermediate either hydrolyzes to hydrocinnamic acid (HCA) or forms the product by coupling with 6-APA. A set of experiments were designed to test the synthesis results. According to the acyl-enzyme mechanism, the rate-limiting step in the synthesis reaction is the binding of the acyl donor to the enzyme. The first reaction medium contained PGA and MPP only. HCA would be liberated after a period of reaction time only if the acyl donor, MPP, bound to the PGA. The second reaction medium contained MPP and 6-APA without PGA. The third one contained MPP and 6-APA with PGA. All three experiments were done in pH 6.5, 0.05 M phosphate buffer at 36°C. The reactants and products were analyzed by Varian 5000 HPLC with a Varian 2050 UV detector, a Varian CDS 401 integrator, and a Whatman RP-18 column (118 x 4.7 mm). The reactants and products were detected at UV 225 nm (Kasche and Katunsky, 1982). The column was eluted with a gradient of MeOH (HPLC-grade) and 0.067 M KH₂PO₄ solution. The MeOH concentration was increased linearly from 5% to 70% in the first 10 min, kept at 70% MeOH for 5 min, and lowered to 5% MeOH within

![Diagram](image)

**Fig. 1.** Schematic Representation of the Coupling Reaction of 6-APA and Methyl 3-Phenylpropionate.

**Fig. 2.** HPLC Elution Spectra of the Preliminary Coupling Test.

Reaction conditions: 17 mm 6-APA, 50 mm MPP, pH 6.5, 36°C, 150 rpm, 12 h. Elution time (min): 6-APA 2.2, HCA 10.4, MPP 12.8.

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5 min, at a flow rate 1.2 ml/min. For analysis, 10 μl of the properly diluted sample was injected and peaks were identified and measured by standard chemicals. Figure 2 shows the eluting patterns from different reactions after 12 h. When MPP reacted with PGA, HCA was produced (Fig. 2-a). This indicated that the binding of MPP with PGA occurred. When MPP was together with 6-APA without PGA, no reaction took place (Fig. 2-b). This could be told from the fact that no characteristic compound had been detected and both reactants remain at the same concentrations. When MPP was together with 6-APA in the presence of PGA, HCA and a new characteristic peak appeared (Fig. 2-c).

The eluent of the new characteristic peak was collected, concentrated, and identified by fast atom bombardment (FAB) mass spectrometry. In FAB, the sample was analyzed using a VG 7070E high resolution mass spectrometer (V.G. Analytical Limited, Manchester, England) with a "magic bullet" matrix. Typical experimental instrument conditions were as follows: argon atom gun, 8 kV accelerating potential, and 2 mA emission current. The mass spectrum shows that the molecular ion has a molecular weight of 348. The molecular weight 348 was exactly the same as that of the expected coupling product (as shown in Fig. 1). This observation indicates that PGA catalyzed the amide bond formation from MPP and 6-APA.

To study the organic solvent effects on this coupling reaction, MIBK was introduced into the reaction medium (α = 1 / 4). The yield of the coupling product was calculated from the consumption of 6-APA because no standard compound exists. During the first 8 h, the rate of the coupling was a little bit slow in the buffer-MIBK system. After 8 h, the synthetic rate in buffer-MIBK system exceeded that in buffer. At 35 h, the rate in the buffer-MIBK system was 1.7 times higher than that in buffer. The increase of the synthetic rate was due to the pH control. The optimum pH of the coupling reaction was 6.5 and the synthetic rate decreased dramatically when the pH was lower than 6.5. In the buffer system, the HCA which was produced from MPP lowered the pH to 5.5 and decreased the synthetic rate. However, in the buffer-MIBK biphasic system, considerable amounts of HCA partitioned into the MIBK solvent (P_HCA = [HCA]_MIBK/[HCA]_buffer = 2.9) and the pH of the buffer changed only slightly. In conclusion, the advantage of involving MIBK in reaction medium was to maintain the optimum pH in the buffer layer, and the coupling reaction of MPP and 6-APA by PGA proceeded better in the presence of MIBK.

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