RESEARCH PAPER

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Production of Cephalexin Through Immobilised *Xanthomonas Compestris* Acylase

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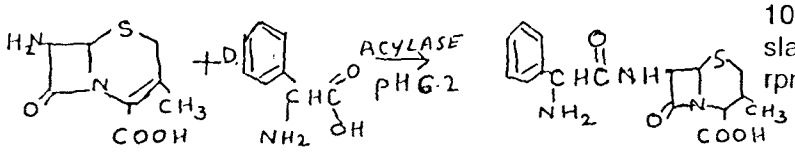
Partially purified acylase from Xanthomonas compestris (X. compestris) catalyzed the condensation of 7-amino des acetoxy cephalosporanic acid (7-ADCA) and D(-) alpha amino phenyl glycine to form cephalexin. At pH 6.2, 46% of 7-ADCA was converted to cephalexin in 45 minutes. Immobilization of this enzyme, by initial adsorption on bentonite followed by entrapment in alginate beads, helped to stablize the enzyme and rendered the biocatalyst reusable.

THERE are many reports on enzymatic synthesis of cephalexins1- 3. Unlike chemical acylation of 7-ADCA, enzymatic process is simple and is of short duration (1hr) **(Scheme I).**



7-ADCA S (-) alpha phenyl glycine Cephalexin, Scheme I

Since cephalexin acylase is a regiospecific catalyst, there is no need to protect the interfering functional groups. Thus, by using enzymic process, cephalexin can be obtained in a single step amidation reaction. Enzymatic processes for cephalexin developed so far have not been successful commercially because of low yield and high cost of the catalyst[4]. However, with the advent of immobilization techniques, biocatlyst can be used economically. In this paper synthesis of cephalexin using partially purified immobilized acylase from **X. compestris** is described.

EXPERIMENTAL

Fermentation

Xanthomonas compestris Pv vesicatoria NCPPB 422, a local strain, was grown in a complex medium consisting of sodium glutamate (0.2%), yeast extract (0.2%), peptone (0.5%), dipotassium phosphate (0.2%), magnesium chloride (0.1%), ferrous sulfate (0.01%) and sucrose (2%). One litre of sterile aerated medium at pH 7 was inoculated with 100ml of seed medium, prepared from 24h old agar slants, and was incubated for 48 h at 30°C and 300 rpm agitation.

Preparation of crude acylase enzyme

The broth was treated with n-butyl acetate (1% v/v), to kill the organisms, centrifuged (9000 rpm, 20 minutes) and washed the cells twice with distilled water. The cells were suspended in 20ml of phosphate buffer (0.1M, pH 6) and were lysed using a French press (10,000 PSI). The resulting suspension was centrifuged (10000 rpm, 45 mins). To the supernatant, ammonium sulfate (60% w/v) was added to precipitate the protein, which was separated by centrifugation (8000 rpm, 10 mins). The active protein was stored at 4°C until further use.

Assay for acylase activity

The crude enzyme was assayed for acylase activity employing cephalexin as substrate by Balasingham method[5].

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Immobilization of crude acylase

To the aqueous solution of enzyme (15ml containg 14 mg of enzyme protein) glutaraldehyde (0.4ml, 25% w/v) was added to cross-link the enzyme and held for 24 h at 4°C. This solution was mixed with bentonite (7.5gm), previously treated with sod. EDTA solution (1mM in 0.1M citrate buffer at pH 6 for 1h). The volume of the suspension was adjusted to 50ml with citrate buffer and was mixed with 50ml orf sodium alginate solution (4.5% w/v) with constant stirring. The suspension was added dropwise into 500ml of calcium chloride solution (3% w/v). The beads, so formed, were gelated for 1 h. with constant stirring.

The beads were assayed for acylase activity as described before[5].

Enzymatic Cephalexin Synthesis

A solution of 7-ADCA (3 to 25mM) in phosphate buffer (0.1M, pH 6.2) was mixed with two fold molar excess of D(-) alpha phenyl glycine and enzyme preparation equivalent to 2mg of protein. The mixutre was stirred at 300 rpm for 150 minutes at 37°C and was estimated for cephalexin by spectrophotometric method as described by Smith[6].

RESULTS

The crude acylase enzyme showed activity of 0.8033 units per mg of protein. This activity was marginally reduced after immobilization (0.79 units/mg of protein). The rate of enzymic reaction in presence of various 7-ADCA concentration is shown in **Table 1.**

The reaction velocity was highest at 15 mM concentration of 7-ADCA. The rate of reaction is marginally higher with immobilized enzyme.

A double reciprocal plot of 7-ADCA concentration versus initial reaction velocity reveals increased Michael is-Menten constant, Km, for immobilized enzyme. The process of immobilization has, thus, reduced the affinity between 7-ADCA and the enzyme slightly.

The maximum reaction velocity was slightly higher in case of immobilized enzyme **(Fig.1)**. In case of free enzyme, at 5 mM 7- ADCA concentration, maximum cephalexin formation was found after 45 minutes. Thereafter, the level of cephalexin was reduced due to reaction taking place in the reverse direction. Immobilized acylase produced cephalexin uniformly upto 120 minutes **(Table 2)**.

The immobilized biocatalyst retained its 100% activity upto 8 reuses at 48 h. interval.

DISCUSSION

The cephalexin acylase enzyme present in **x. compestris** has significant activity, which is higher than that shown by **Xanthomonas oryzae** IFO 3995[7]. The acylase activity found during our investigation is comparable with that of **Paeudomonas maltophilia IFO 12690** and **Acetobacter xylinus IFO 3194**[8]. The enzyme has high affinity towards 7-ADCA as depicted by low Km values. The immobilized enzyme is reusable and can be employed in presence of high 7-ADCA concentrations for a longer duration, so that cephalexin can be synthesized with an increased productivity.

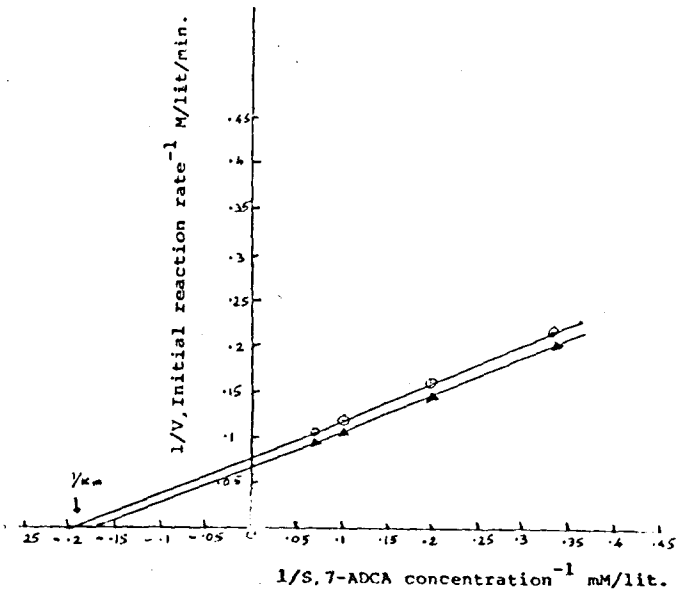

Fig. 1: Double reciprocal plot for the effect of 7-ADCA concentration on initial reaction rate.
▲ For immobilize enzyme. ⊖ for free enzyme

Table 1: Cephalexin formation in presence of free and immobbilized acylase

7-ADCA concentration mM/lit.(S)	Initial reaction velocity (v) M/lit./min.	
	For free enzyme	For immobilized enzyme
3	0.36	0.358
5	0.56	0.5
10	0.38	0.958
15	1.036	1.07
20	0.414	0.706
25	0.248	0.03

Table 2: Time course during cephalexin formation

Time, in minutes	% conversion of 7-ADCA to cephalexin by	
	Free enzyme	Immobilized enzyme
1	13.8	12
15	29	22
30	38	34
45	46	37
60	30	40
90	20	33
120	9	24
150	3	20

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