Extractive Deacylation of Benzylpenicillin in Aqueous Two-Phase Systems *

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Penicillin acylase (PA) (EC 3.5.1.11) catalyzes the deacylation of benzylpenicillin (BP) to 6-aminopenicillanic acid (6-APA) and phenylacetic acid. The reaction has a pH dependent equlibrium, where high pH favours the deacylation reaction.

The interesting product from the deacylation step is 6-APA, key intermediate for the production of semisynthetic penicillins. PA immobilized to solid matrixes is used for industrial production of 6-APA. Several studies describe the binding of this enzyme to different matrix materials.[1-4]

Aqueous two-phase systems have lately become of increased interest in biotechnology. The purification of intracellular enzymes in large scale has been most actively studied and has been recently reviewed.[5-6] Aqueous two-phase systems have also been applied to biological analysis[7] and for the regeneration of coenzymes.[8] The utilization of aqueous two-phase systems in bioconversions has only recently been observed.[9] This communication investigates the use of aqueous two-phase systems for bioconversions using the deacylation of BP with PA as a model system.

PA was partitioned in different aqueous two-phase systems in order to obtain a very low partition coefficient where K=activity (top phase)/activity (bottom phase). For best results, the enzyme should be almost completely partitioned to the bottom phase. Phase systems consisting of poly(ethylene glycol) (PEG) and dextran only resulted in $K>0.03$, which was not considered satisfactory. However, phase systems consisting of PEG and salts gave K values <0.01.

The enzyme stability in these phase systems was determined from measurements of the activity[10] (Figure 1). The stability was very poor in the phase system containing magnesium sulfate. In the phase system composed of PEG-potassium phosphate, the enzyme even seems to be stabilized. This system was chosen for further studies. The partition coefficient in this phase system for substrate and products were: $K_{BP}\sim 8.0$, $K_{6\text{-APA}}=1.35$ and $K_{\text{phenylacetic acid}}=1.7$.

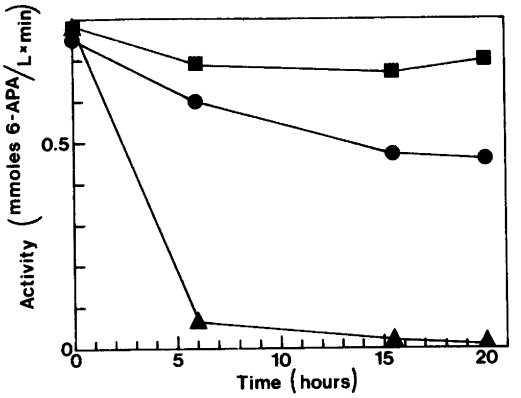

Fig. 1. The stability of PA. Aqueous two-phase systems: (■) PEG 20000, 8.9 % (w/w)/potassium phosphate, 7.6 % (w/w) and (▲) PEG 3350, 12 % (w/w)/magnesium sulfate, 10 % (w/w). (●) 0.05 M sodium phosphate buffer. pH was 7.8 and the temperature 37 °C.

The conversion of BP to 6-APA was then studied in a repeated batch conversion (Figure 2). The study was performed as follows: when the 6-APA formation in the stirred tank ceased, the stirring was stopped. When the phases had separated, the top phase was removed, followed by addition of new top phase with fresh BP. The stirring was than started again. pH control was not used. This was shown to affect the reaction velocity(decrease). Thus, before the fourth conversion, the pH was increased by titration, which increased the reaction velocity again. The specific productivity for the conversions measured during the first hour of each conversion was 0.89–3.16 μmol 6-APA/mg protein×min. The specific productivity for PA immobilized to solid matrices has been reported to be between 0.51–4.77 μmol 6-APA/mg protein×min.[11]

Advantages achieved by using aqueous two-phase systems for biotechnological conversions are the following: the enzyme can be reused; good mass transfer is achieved between the phases, which will prevent accumulation of inhibitory products; high enzyme concentrations can be used; the activity can easily be reestablished when it is falling; no activity is lost due to the immobilization procedure. One disadvantage is that the product stream is polluted with PEG. In conclusion, aqueous two-phase systems can be

* Communication at the Meeting of the Swedish Biochemical Society in Lund, 9–10th June, 1983.

0302-4369/83 $2.50

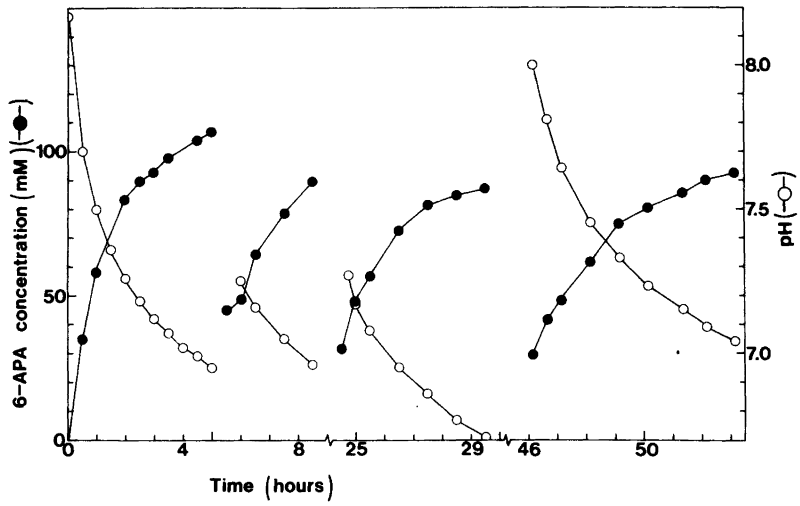

Fig. 2. Repeated batch conversion of BP (100 mg/ml phase system) to 6-APA with PA (0.3 mg protein/ml phase system, 105 U/mg protein) at 37 °C. The volume of the phase system was 50 ml. Aqueous two-phase system: PEG 20000, 8.9 % (w/w)/potassium phosphate, 7.6 % (w/w). Composition of added top phase: PEG 20000, 17 % (w/w)/potassium phosphate, 4.5 % (w/w). 6-APA concentration (●) and pH (○) are indicated.

an interesting alternative to immobilization to solid matrices.

Acknowledgement. This study was supported by the National Swedish Board for Technical Development.

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Received July 7, 1983.