Synthesis of Cephalexin in Aqueous Medium
with Carrier-bound and Carrier-free Penicillin
Acylase Biocatalysts

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Received: 3 March 2008 / Accepted: 18 April 2008 / Published online: 10 July 2008
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Abstract The use of very high substrate concentrations favors the kinetically controlled
synthesis of cephalexin with penicillin acylase (PA) not only by Michaelian considerations,
but also because water activity is depressed, so reducing the rates of the competing
reactions of product and acyl donor hydrolysis. Commercial PGA-450, glyoxyl agarose
immobilized (PAIGA) and carrier-free cross-linked enzyme aggregates of penicillin acylase
(PACLEA) were tested in aqueous media at concentrations close to the solubility of
nucleophile and at previously determined enzyme to nucleophile and acid donor to
nucleophile ratios. The best temperature and pH were determined for each biocatalyst based
on an objective function considering conversion yield, productivity, and enzyme stability as
evaluation parameters. Stability was higher with PAIGA and specific productivity higher
with PACLEA, but best results based on such objective function were obtained with PGA-
450. Yields were stoichiometric and productivities higher than those previously reported in
organic medium, which implies significant savings in terms of costs and environmental
protection. At the optimum conditions for the selected biocatalyst, operational stability was
determined in sequential batch reactor operation. The experimental information gathered is
being used for a technical and economic evaluation of an industrial process for enzymatic
production of cephalexin in aqueous medium.

Keywords Penicillin acylase · Enzyme immobilization · Cephalexin · Cross-linked enzyme
aggregates · Multipoint covalent attachment

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Introduction

Penicillin acylase (penicillin amidohydrolase, E.C. 3.5.1.11) is a flexible enzyme that is able to catalyze several reactions of organic synthesis [1–6]. Among those reactions, the synthesis of semisynthetic penicillins and cephalosporins from the corresponding β-lactam nuclei and suitable acyl donors is of paramount importance to the pharmaceutical industry [7]. Synthesis can be conducted under thermodynamic [8, 9] or kinetic [10, 11] control. Under thermodynamic control, substrate conversion is limited by the equilibrium of the reaction. This is not so in the case of synthesis under kinetic control, where the formation of an acyl-enzyme complex generates a kinetic competition of nucleophilic attack either by the nucleophile substrate or water, which allows, in principle, to obtain substrates conversions well over the equilibrium. The conversion yield is the result of that competition. The drawback of synthesis under kinetic control is the extra cost represented by the use of an activated acyl donor and the precise control of the reaction required to avoid unnecessary product hydrolysis after maximum conversion yield is attained [12]; however, in most cases high conversion yields and productivities are obtained [13-15].

Cephalexin is a pharmacologically relevant semisynthetic cephalosporin produced mainly by chemical synthesis; however, an industrial facility for producing cephalaxin by a totally enzymatic process has entered into operation recently [16], with reduction of 50 to 15 kg waste/kg product being a major factor for success [17]. The kinetically controlled synthesis of cephalaxin requires an activated acyl donor (phenylglycine methyl ester, PGME, or phenylglycine amide) to form an acyl-enzyme complex with the enzyme that is further attacked nucleophilically by the β-lactam nucleus, 7-amino3-desacetoxycephalosporanic acid (7ADCA) to yield the product, according to a well-reported mechanism [18–22]. Penicillin acylase is a moderately expensive enzyme so that stabilization is mandatory to increase the efficiency of utilization and reduce its impact on processing cost. Among the many strategies used for penicillin acylase stabilization [13], immobilization to solid supports [23–27] and autoimmobilization by aggregation are prominent [28–30]. Multipoint covalent attachment to activated agarose gels and cross-linked enzyme aggregates (CLEAs) are particularly promising in terms of enzyme stabilization and have been used successfully to immobilize penicillin acylase [31–36]. We have previously used glyoxyxylagarose immobilized penicillin acylase [37, 38] and CLEAs [39, 40], as well as the commercial biocatalyst PGA-450 [41], in the kinetically controlled synthesis of cephalaxin in organic medium using ethylene glycol as cosolvent with PGME as acyl donor and obtained yields close to stoichiometric and fair productivities, being higher in the case of CLEAs. The presence of organic cosolvents was required to attain high yields at moderate substrates concentrations [37, 42–44], but the effect of organic solvent concentration was not significant when working at very high substrates concentrations [38, 40]. Therefore, the hypothesis was raised that at such conditions concentration yields as high as in organic medium and even higher productivities could be attained in a fully aqueous medium, with the additional advantages of cost savings in solvent and waste treatment and the environmental benefits associated. Very high substrates concentrations should favor the kinetically controlled synthesis of cephalaxin with penicillin acylase (PA) not only by Michaelian considerations, but also because water activity is depressed reducing the rates of the competing reactions of product and acyl donor hydrolysis, and good synthesis to hydrolysis rates may be obtained by keeping the enzyme saturated by the nucleophile [45, 46].

Results are presented on the kinetically controlled synthesis of cephalaxin from 7ADCA and PGME in aqueous medium at very high substrates concentration with three different