Available online at www.sciencedirect.com
ScienceDirect
Enzyme and Microbial Technology 42 (2008) 199–207
ENZYME AND MICROBIAL TECHNOLOGY
www.elsevier.com/locate/emt

Review

The realm of penicillin G acylase in β-lactam antibiotics

Anuj K. Chandel [a,b], L. Venkateswar Rao [b], M. Lakshmi Narasu [a], Om V. Singh [c,*]

[a] *Department of Biotechnology, Jawaharlal Nehru Technological University, Hyderabad 500007, India*
[b] *Department of Microbiology, Osmania University, Hyderabad 500007, India*
[c] *Department of Pediatrics, The Johns Hopkins School of Medicine, Baltimore, MD-21287, USA*

Received 1 September 2007; received in revised form 10 November 2007; accepted 19 November 2007

Abstract

Penicillin G acylase (PGA; EC 3.5.1.11) is a hydrolytic enzyme that acts on the side chains of penicillin G, cephalosporin G and related antibiotics to produce the β-lactam antibiotic intermediates 6-amino penicillanic acid (6-APA) and 7-amino des-acetoxy cephalosporanic acid (7-ADCA), with phenyl acetic acid (PAA) as a common by-product. These antibiotic intermediates are among the potential building blocks of semi-synthetic antibiotics, such as ampicillin, amoxicillin, cloxacillin, cephalexin, and cefatoxime. Currently, β-lactam antibiotics have annual sales of ∼$15 billion and make up 65% of the total antibiotics market; the annual consumption of PGA is estimated to be in the range of 10–30 million tons. The high demand for PGA is being met through a submerged fermentation process that uses genetically manipulated *Escherichia coli* and *Bacillus megaterium* microorganisms. Advancements in biotechnology such as screening of microorganisms, manipulation of novel PGA-encoding traits, site-specific mutagenesis, immobilization techniques, and modifications to the fermentation process could enhance the production of PGA. Commercially, cheaper sources of carbohydrates and modified fermentation conditions could lead to more cost-effective production of PGA. These methodologies would open new markets and create new applications of PGA. This article describes the advancements made in PGA biotechnology and advocates its simulation for production of β-lactam antibiotics.

Keywords: Penicillin G acylase; β-Lactam antibiotics; Fermentation; Immobilization; Down stream recovery

Contents

1. Introduction

Due to the rising interest in sustainable development and environmentally friendly practices, microbial enzyme transformation processes are generally preferred over the conventional chemical conversion process. The former have multiple advan-

* Corresponding author at: 600 N Wolfe Street, CMSC 3-106, The Johns Hopkins School of Medicine, Baltimore, MD-21287, USA.
Tel.: +1 410 614 1804; fax: +1 410 955 1030.
E-mail addresses: osingh1@jhmi.edu, ovs11@yahoo.com (O.V. Singh).

0141-0229/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.enzmictec.2007.11.013

tages, including less chemical load on the environment, higher efficiency, and the ability to dilute multiple downstream transformation attempts while maintaining product yield and recovery. The enzyme penicillin G acylase (PGA; EC 3.5.1.11) is a heterodimeric protein consisting of a small α subunit and a large subunit, which are formed by the processing of a single polypeptide precursor (Fig. 1) [1]. PGA belongs to the structural superfamily of N-terminal nucleophile hydrolases that share a common fold around the active site bearing a catalytic serine, cysteine, or threonine at the N-terminal position [1,2]. Functionally, PGA acts on the side chains of penicillin G, cephalosporin G, and other related antibiotics to produce antibiotic intermediates such as 6-amino penicillanic acid (6-APA) and 7-amino desacetoxy cephalosporanic acid (7-ADCA), leaving behind phenyl acetic acid (PAA) as a common by-product (Fig. 2) [3,4]. These antibiotic intermediates are the building blocks of semi-synthetic penicillins (ampicillin, amoxicillin, cloxacillin, salbactum) and cephalosporins (cephadroxil, cefalexins, etc.) [3,5].

β-Lactam antibiotics, in particular penicillins and cephalosporins, represent one of the world's major biotechnology markets. With annual sales of ∼$15 billion, they make up ∼65% of the total antibiotics market [5]. β-Lactam antibiotics alone constitute most of the world's antibiotic sales: 3×10^7 kg/year out of a total 5×10^7 kg/year produced worldwide [6]. Therefore, the annual consumption of PGA is estimated to be in the range of 10–30 million tons [6]. To meet the requirements for the bulk production of β-lactam antibiotics, significant growth has occurred in the past two decades. Industries that produce β-lactam antibiotics have introduced PGA biocatalysis by replacing multistep conversion processes with cheap and promising enzymatic conversion, which has an efficiency of ∼80–90% [5]. PGA-mediated conversion of β-lactam antibiotics provides a novel direction for antibiotics industries and promotes a safer and cleaner environment [3,5]. Apart from β-lactam hydrolysis, recent developments have resulted in multiple applications of PGA, including peptide synthesis, resolution of racemic mixture,

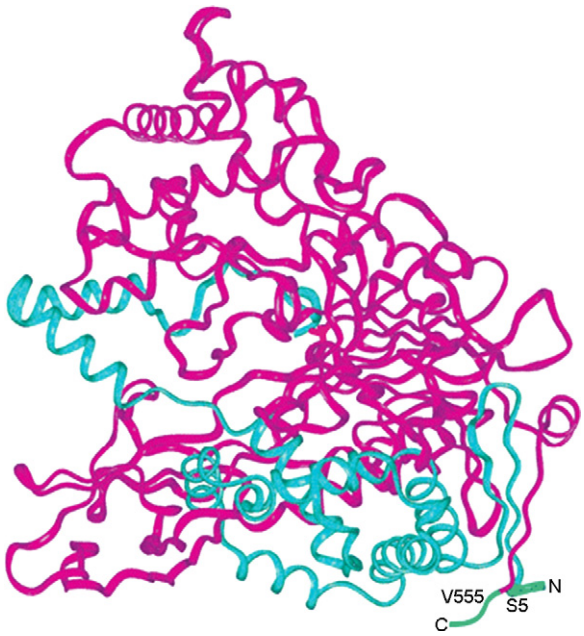

Fig. 1. PGA dimeric structure showing beta-subunit—magenta, A-subunit—blue ribbon. The polypeptide regions trimmed from the N terminus of the A-subunit and from the C terminus of the beta-subunit are indicated in green. The amino acid residues to be connected with the four amino acids linker are labeled. In red, at the center of the molecule, the catalytic serine residue is indicated (Source: Flores et al., 2004, with permission and courtesy of "Protein Science"). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

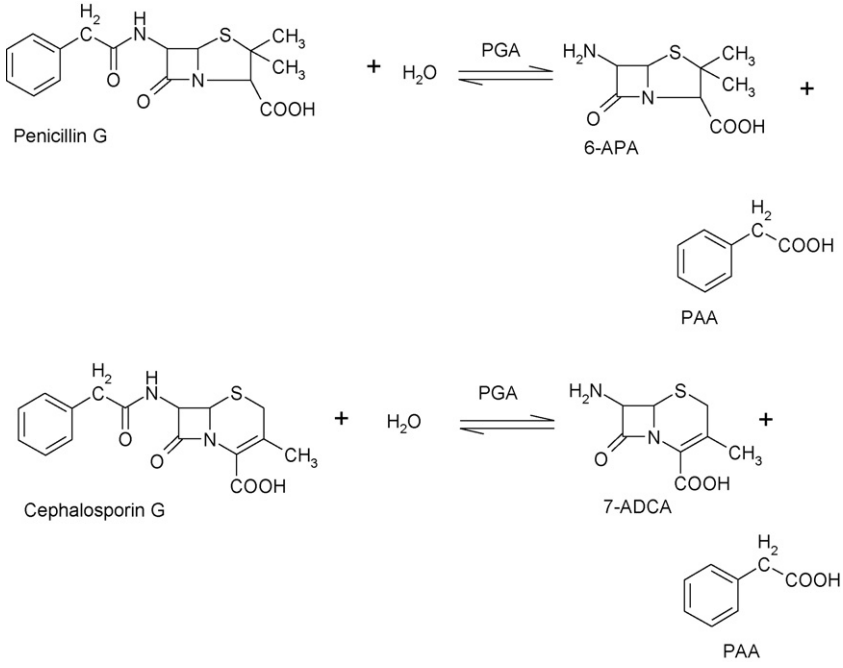

Fig. 2. Enzymatic conversion of penicillin G and cephalosporin G into 6-APA and 7-ADCA leaving phenyl acetic acid as common side product.