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Research review paper

Perspectives and industrial potential of PGA selectivity and promiscuity

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ABSTRACT

Penicillin G acylases (PGAs) are robust industrial catalysts used for biotransformation of β-lactams into key intermediates for chemical production of semi-synthetic β-lactam antibiotics by hydrolysis of natural penicillins. They are used also in reverse, kinetically controlled synthetic reactions for large-scale productions of these antibiotics from corresponding beta-lactam nuclei and activated acyl donors. Further biocatalytic applications of PGAs have recently been described: catalysis of peptide syntheses and the resolutions of racemic mixtures for the production of enantiopure active pharmaceutical ingredients that are based on enantioselective acylation or chiral hydrolysis. Moreover, PGAs rank among promiscuous enzymes because they also catalyze reactions such as trans-esterification, Markovnikov addition or Henry reaction. This particular biocatalytic versatility represents a driving force for the discovery of novel members of this enzyme family and further research into the catalytic potential of PGAs. This review deals with biocatalytic applications exploiting enantioselectivity and promiscuity of prokaryotic PGAs that have been recently reported. Biocatalytic applications are discussed and presented with reaction substrates converted into active compounds useful for the pharmaceutical industry.

Contents

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1. Introduction

Penicillin acylases belong to the N-terminal nucleophile (Ntn) serine hydrolase superfamily of proteins where they represent three distinct families: penicillin G acylases (PGA), penicillin V acylases and cephalosporin acylases (or ampicillin acylases) (Oh et al., 2004). Although their native role in microorganisms (i.e. bacteria and fungi) has not been so far unambiguously elucidated, they are thought to be involved in the catabolism of natural compounds bearing phenylacetyl moiety (Galan et al., 2004).

Penicillin G acylases (EC 3.5.1.11; PGA, penicillin amidohydrolase) are robust industrial catalysts that have been routinely used for several decades for production of 6-aminopenicillanic acid (6-APA) by hydrolysis of natural penicillin G (Arroyo et al., 2003; Chandel et al., 2007; Elander, 2003; Sio and Quax, 2004). In addition to the large-scale production of the key intermediates 6-APA or 7-amino-3-deacetoxycephalosporanic acid (7-ADCA) (Chandel et al., 2007) for chemical syntheses of semi-synthetic β-lactam antibiotics (Chandel et al., 2007), PGAs have been recently applied by pharmaceutical companies (DSM Fine Chemicals, Fermenta Biotech Ltd) also in reverse, synthetic reactions for the large-scale production of β-lactam antibiotics from corresponding β-lactam nuclei and activated acyl donors. While PGAs generally catalyze the hydrolytic reactions at alkaline pH, at acidic or neutral pH they promote acylations. An excellent review (Volpato et al., 2010) deals with the hydrolytic production of β-lactam antibiotic nuclei and kinetically controlled syntheses of β-lactam antibiotics, topics that are beyond the scope of this review.

Thorough research into the structure of PGA and understanding of substrate–enzyme interactions revealed that PGAs are versatile enzymes catalyzing reactions over a wide range of compounds and exhibiting stereoselectivity.

Enzyme enantioselectivity, in general, is a phenomenon with great potential for the pharmaceutical industry. Chiral molecular entities called enantiomers exist always in pairs of which usually only one enantiomer is biologically active or can serve as an intermediate of such compound. Research into enantioselectivity of PGA started in the 60's of the last century and dealt with resolutions of racemic mixtures of α-amino acids and their derivatives (Cole, 1969 Lucente et al., 1965), α-amino alcohols and α-amino nitriles (Romeo et al., 1971). Resolution experiments were later extended to α-substituted α-amino acids (Rossi and Calcagni, 1985), racemic mixtures of β, γ, δ, ε-amino acids (Margolin, 1993; Rossi et al., 1977), aliphatic amines (Rossi et al., 1978), β-aryl-β-amino acids (Soloshonok et al., 1995), β-alkyl-β-amino acids (Soloshonok et al., 1994), phosphonic and phosphonous analogs of alanine (Solodenko et al., 1993), primary or substituted carbinols (Fuganti et al., 1988; Waldmann, 1989), β-amino ketones (Cainelli et al., 1997) and the dipeptide aspartame (Fuganti and Grasselli, 1986).

In this review, we will focus on biocatalytic applications exploiting enantioselectivity and promiscuity of prokaryotic PGAs, two properties that were discovered or profoundly studied during the last decade. We have focused on biocatalyses providing active compounds useful for the pharmaceutical industry (APIs), or their precursors.

1.1. Sources of penicillin G acylases

Bacterial PGAs belong to the best characterized industrial enzymes. Their traits and biocatalytic potential have been discussed in detail elsewhere (Arroyo et al., 2003; Chandel et al., 2007; Sio and Quax, 2004; van Langen et al., 2000). PAs were reported in bacteria belonging to genera *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Escherichia*, *Kluyvera*, *Providencia*, *Shigella*, *Xanthomonas*, and *Xylella* (Parmar et al., 2000; Shewale and Sivaraman, 1989; Škrob et al., 2003; Sudhakaran et al., 1992). Nature biodiversity seems to be always a major source of novel PGAs with interesting characteristics. The novel bacterial producers that were isolated and added on the list during the last decade are shown in Table 1.

The knowledge of nucleotide sequences of the *pga* (or *pac*) structural genes encoding PGAs and their regulatory regions was exploited for improvement of the enzyme parameters, construction of recombinant strains expressing wild-type or genetically-engineered genes or mining environmental genes encoding PGAs (Gabor et al., 2004). Some of the recombinant strains are used in industry as "tailor-made" strains for overproduction of PGAs (Kallenberg et al., 2005) and consequent production of robust biocatalysts.

Examples of expression systems for PGAs based on different bacterial or yeast hosts are listed in Table 2. Although about 20 different *pga* genes were cloned and at least 14 structural genes were sequenced, only four PGAs were studied as regards enantioselectivity and promiscuity (see further chapters). Unlike bacterial hosts, the yeast expression systems have so far not been reported as industrial production strains for PGAs. Obviously, extended fermentation time (up to 120 h) might be one of the reasons for preferential use of bacterial systems although the yeast host *Pichia pastoris* offers the choice for cytoplasmic or extracellular expression.

1.2. Penicillin G acylase-based catalyst

Enzyme stabilization is required for industrial success of the biocatalyst because enzymes are naturally evolved proteins catalyzing reactions under physiological conditions. Once stabilized, the enzymes retain their capabilities to catalyze reactions under mild conditions and, furthermore, may acquire traits that render them exploitable in organic syntheses. In the latter case, enzyme immobilization eliminates the problem of protein unfolding caused by organic solvents that are frequently present in reaction mixtures. The effect of solvents on enzyme activity and stereoselectivity was reported elsewhere (Illanes et al., 2012). Expected outcomes of enzyme immobilization are: increased enzyme activity in organic solvents, increased temperature stability (Polizzi et al., 2006), long-term operational stability, and catalyst recovery for its repeated usage (Miletic et al., 2012).

Table 1
Recently isolated microorganisms producing PGA.

Microorganism	Enzyme localization	Reference
Bacillus subtilis BAC4	Extracellular	Supartono et al. (2008)
Achromobacter sp. CCM 4824	Periplasmic	Škrob et al. (2003)
Achromobacter xylosoxidans strain 650	Periplasmic	Cai et al. (2004)
Bacillus badius PGS10 (*Bacillus* sp. PGS10)	Intracellular	Rajendhran et al. (2002) (2003)
Shigella boydii (clinical isolate)	Periplasmic	Montazam et al. (2009)
Bacillus sp. MARC-0103	Extracellular	Tahir et al. (2009)
Thermus thermophilus HB27	Membrane fraction	Torres et al. (2012)

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