Penicillin G Acylase, a biocatalyst and its potential application

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Abstract

The PGA enzyme belongs to the family of N-terminal nucleophile (Ntn) hydrolases. The enzyme Penicillin G Acylase (PGA) mainly produced from *Penicillium chrysogenum*, *E.coli*. The synthesis of PGA enzyme is depends on the physico-chemical parameters like carbon source, pH, temperature and media were optimized for higher production of enzyme. There are different methods of purification of PGA enzyme like as ammonium sulphate precipitation followed by Dialysis and Ion-Exchange Chromatography, and TLC. For the industrial application Expression of *Escherichia coli* PGA is usually modulated by several factors, such as growth temperature, phenylacetic acid, oxygen levels, strain variations, and glucose in both wild-type and recombinant *E. coli* . studies showed that cloning of gene and site directed mutagenesis give high expression and production of PGA enzyme compare to direct extraction and purification of PGA enzyme.PGA enzyme has potential applications like as industrial production of semi-synthetic penicillins and cephalosporins, protection in peptide synthesise and production of Amoxicillin (antibiotic) etc.

Keywords: – Penicillin G Acylase, Thermostable Enzyme, Enzyme analysis, Structucural Study of PGA, Enentioselective.

Introduction:-

Enzymes represent an eco-friendly tool with environment to organic synthesize and a highly alternative to conventional chemical catalysis. Now, they are widely used in the production of food, pharmaceuticals, chemicals and bio fuels.

Penicillin G acylase (PGA; EC 3.5.1.11), discovered in 1960 and observed the most employed industrial biocatalysts. It plays a major role the production of 6-aminopenicillanic acid and 7-aminodeacetoxy cephalosprinic acid from Penicillin G and in turns many semi-synthetic antibiotics ampicillin, amoxicillin, cloxacillin, cephalexin, and cefatoxime [1].

Beta-Amino acids are playing important role to attracting interest due to their biological activities and importance as key compounds in the synthesis of pharmaceutical [2]. Beta-phenylalanine (3-amino-3-phenylpropionic acid, BPA) and its derivatives are important to producing antibiotics and chiral building blocks. For instance, (R)-BPA is a component of astins A–C, the antitumor cyclopentapeptides isolated from the roots of the medicinal plant *Aster tataricus*. [3]. Biocatalyst has higher efficiency than the chemical catalyst, reaction condition is also milder. So, the enzyme catalyzed synthetic method is potentially used to the production of pure BPA and its derivatives. The PGA-catalyzed enantioselective resolution of racemic substances could be an alternative to the existing biocatalytic approaches because of its fast reaction rate, high enantioselectivity, and simple isolation procedures. For example, the acylation of amines catalyzed by PGA from Alcaligenes faecalis (Af-PGA) in an aqueous solution was shown to be surprisingly efficient and highly enantioselective for (R)-amines [4]. The direct condensation of phenylglycinonitrile with phenyl acetic acid catalyzed by PGA from *Escherichia coli* (Ec-PGA) was effective and exclusively enantioselective for (S)-phenylglycinonitrile, which led to nearly stoichiometric acylation [5]. Recent study have been done on Ec-PGA-catalyzed synthesis of enantiomerically pure beta-phenylalanine in an alkaline medium (pH 10) has found to favor Ec-PGA-catalyzed acylation reaction [6]. After the higher study it was observed that it has maximum catalytic activity at pH 11 with a higher thermodynamically stability because presence of disulfide bond (Fig.1). Recently, some researchers indicated that an Af-PGA-catalyzed enantioselective acylation in an aqueous medium resulted in the production of pharmacologically interesting a -D-phenylalanine and its substituted derivatives (Fig.2) [7]. Enzymes recovery, stability and reuse are some of the essential properties to make a process economically viable from an industrial point of view , a great work has been done on the immobilization of this biocatalyst [8]. When PGA is used for the production of semi synthetic antibiotics through the kinetically controlled N-acylation of β-lactam nuclei with esters or amides, the overall yield depends on the ratio of the antibiotic synthesis to the

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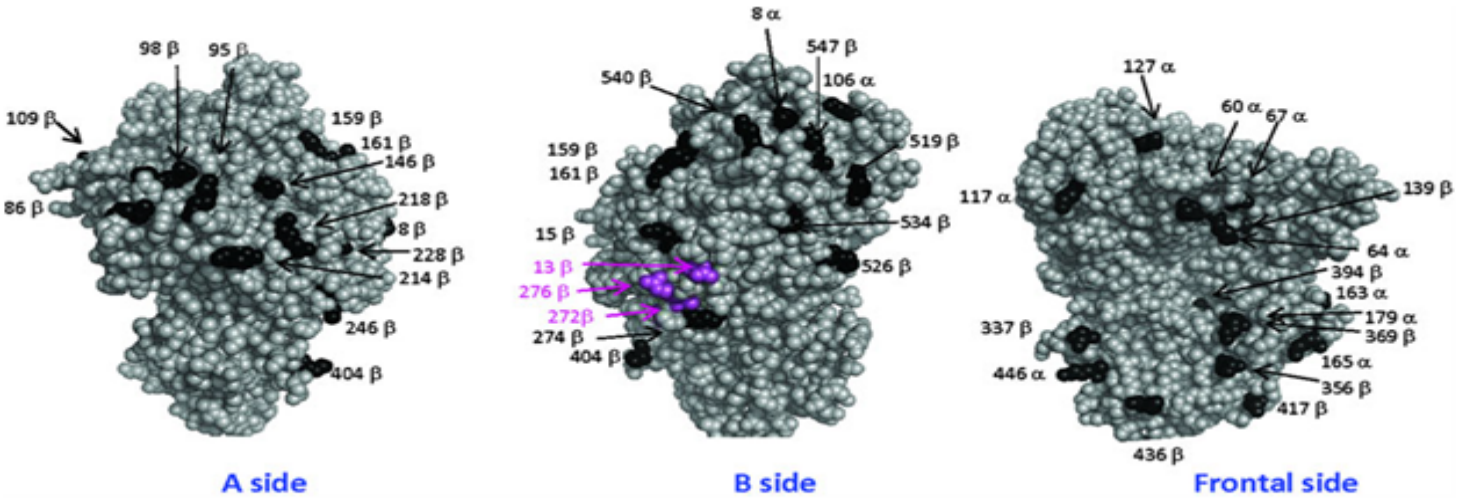
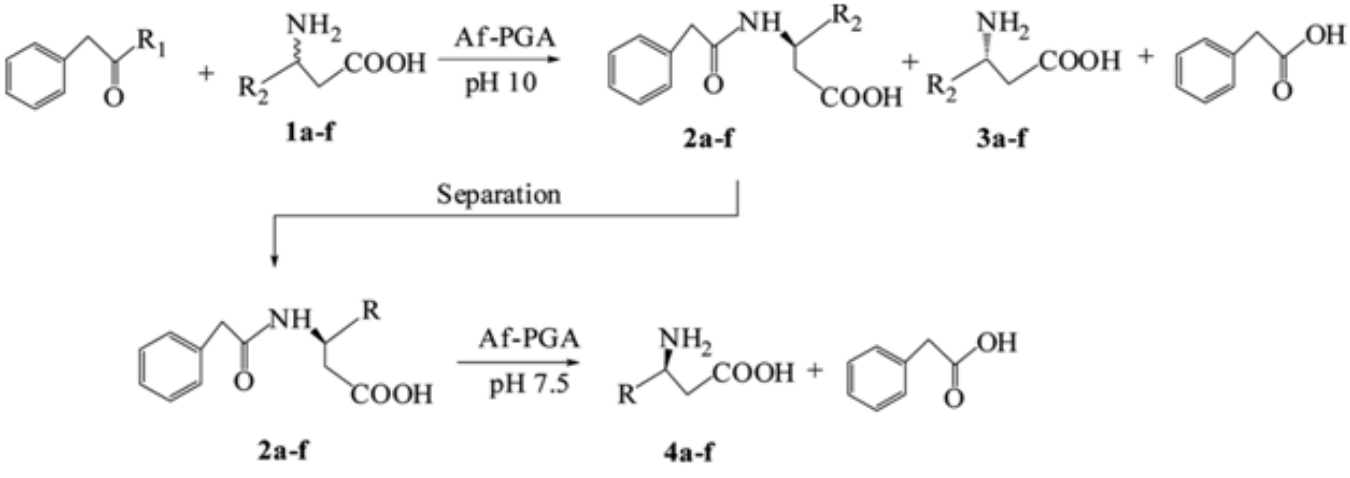


Fig. 1 Three-dimensional view of the back (A side and B side) and frontal surfaces of mutant penicillin G acylase (PGA) containing three additional Lys residues on the surface opposite the active site (3 KPGA). Existing superficial Lys residues are in black and those introduced by mutagenesis (13β, 272β, 276β) are in pink. The positions in the alpha or beta subunit are specified. Enantioselective Acylation of beta-phenylalanine Acid.

hydrolyses, both of the acyl donor and of the product. It is revealed that acyl donor is predominant at the beginning of the reaction (when the concentration of the acyl donor is high), whereas product prevails at the end of the bioconversion (when the concentration of the antibiotic is high). It was shown that higher the ratio of synthesis of two hydrolyses (S/H), the more viable process. For the checking efficiency of the biocatalyst, ratio of synthesis/hydrolysis vs/vh1 were routinely observed and the result was shown that the affinity of the β-lactam nucleus for the enzyme catalytic pocket: a high value of vs/vh1 indicates that the acyl moiety is transferred to the nucleus very efficiently [9,10].

From *Escherichia coli*, it was shown that PGA is naturally characterized by a high ratio of the rate of synthesis to the rate of hydrolysis of acyl donor (vs/vh1). But, immobilization often negatively affects the synthetic performances of PGA because of problems relating to diffusion of the β-lactam nucleus into the active site that result into a decrease of the S/H ratio [11, 12]. In a recent study for the characterization of the immobilized wild-type PGA by digesting it with soluble trypsin some of the authors of this work developed a new modified "bottom-up" proteomic approach [13]. To increase the immobilization efficiency and stability different types of genetic modification were done and shown that In the case of PGA different mutants also have been designed and successfully immobilized on solid



R1 = -NH_2, -OCH_3;

R2 = C_6H_5- (a), 2-Cl-C_6H_4- (b), 3-Cl-C_6H_4- (c), 4-Cl-C_6H_4- (d), 4-F-C_6H_4- (e), 4-MeO-C_6H_4- (f).

FIG 2 The scheme of Af-PGA-catalyzed enantioselective acylation of BPA and its derivatives.

(Diagram taken from [University of Dammam]

carriers: genetic modifications of the protein surface were shown to improve

the immobilization efficiency and stability of the immobilized preparation. This immobilization protocol preferentially involved those areas of the protein surface richest in Lys; in fact this procedure relies on the formation of Schiff bases between the enzyme amino groups (which are mostly surface Lys groups) and the support aldehydes [14].

On the basis of solid support and Immobilization method on glyoxyl agarose was compared with that on a less hydrophilic carrier (aldehyde-activated Sepabeads EC-HG) to highlight the role played by the nature of the support, carrier activation being equal. The kinetic properties of the immobilized preparations were compared with those from the reference reaction of N-acylation of 7-ACA with mandelic acid methyl ester 1 considering the S/H ratio in terms of vs/vh1. Furthermore, the performances of the biocatalysts in the preparative synthesis of cefazolin were also investigated. In the synthesis of this cephalosporin, the acylation product is not hydrolyzed [15, 16] and, consequently, the yields are strictly related to the vs/vh1 ratio according to the catalytic properties which characterize each biocatalyst.

Structural Information -

After the mutant study on the *E.coli* the comparative study of the precursors from four gram negative and two gram positive bacteria were used and it was shown that Ser290 was absolutely conserved and Lys299 was highly conserved between species,

Fig 3: Structure of mature PGA showing secondary structural units by chain (red=Chain A, yellow=Chain B). The active site residue, Ser1:B, is shown in magenta in both structures.

including PGA from *E. coli* W(Fig 3 and Fig.4.) [17]. Lys299 and Ser 290 are critical residues for the autocatalytic processing of the PGA precursor. Due to the close proximity of Lys299 to Ser290, it has been determined to be the most probable candidate responsible for the pH dependent activation of the autocatalytic processing events in the periplasm [17].

In nature, PGA is initially produced in E.coli as a single-chain precursor in the and after removal of several polypeptides; the enzyme reaches a mature state in the periplasm [18]. The mature enzyme is a heterodimer of a small a-unit (209 residues) and a large ß-unit (557 residues) [19, 20]. PGA is characterized as an N-terminal-nucleophile (Ntn) hydrolase; the Ntn superfamily is comprised of enzymes that share a common fold around the active site and at the N-terminal position that

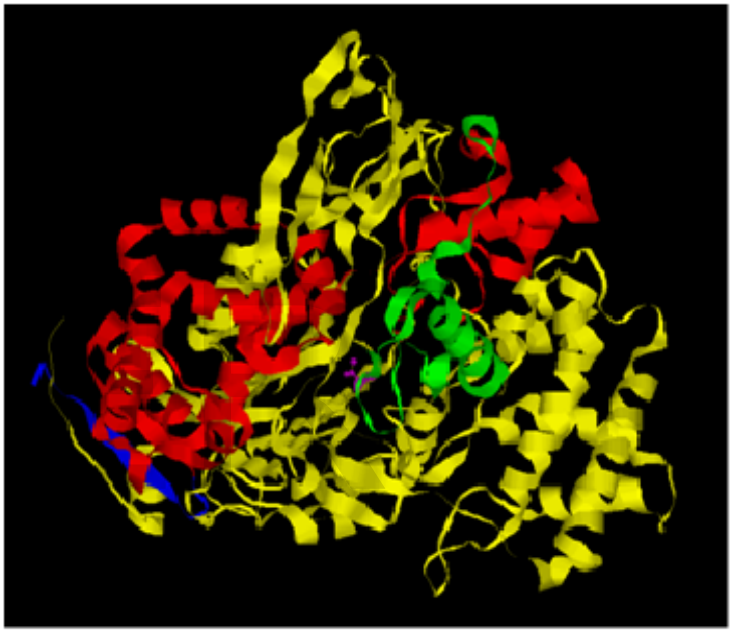


Fig 4: The secondary structure of the precursor to PGA. The signal sequence is shown in blue, linker sequence in green (blocking the active site), and the sequences of amino acids that will form the A and B chains after processing are shown (red and yellow, respectively).

contain a catalytic serine, cysteine, or threonine [21, 22]. The hydrophobic active site resides inside the pyramidal shape structure created by the two chains. The specificity of PGA towards phenylacetyl group of PG in due to presence of several hydrophobic amino acid residues present at active site of PGA [8]. Numerous site specific, mutational studies of PGA have aided in clarification of the structure and mechanism of PGA. Due to wide spread use in biotechnological and pharmacological products and research there is requirement of mechanistic and structural understanding of PGA.

With the help of protein engineering to improve the selectivity of PGA with respect to the amino group on the alpha carbon

(Ca) in the synthesis of ampicillin from the acyl side chain donor rac-phenylglycine methyl ester (rac-PGME) and the beta-lactam nucleus 6-aminopenicillanic acid (6-APA) so that less expensive racemic acyl donors can be used in the synthesis of b-lactam antibiotics.

Applications -

1. Penicillin G acylases are involved mainly in the industrial production of semi-synthetic penicillins and cephalosporins, which remain the most widely used group of antibiotics.

2. Penicillin acylases are useful as biocatalysts in many potentially valuable reactions such as protection of amino and hydroxyl groups in peptide synthesis, as well as in the resolution of racemic mixtures of chiral compounds.

3. Penicillin V shows higher stability in aqueous solutions at lower Ph during extraction from the fermented broth, which could lead to a higher yield of 6-APA.

4.15% of all manufactured 6-APA worldwide is produced from penicillin V

5. Annual worldwide production of 9,000 tons 6-APA is produced enzymatically from penicillin G and V.

6. Penicillin acylases can be used for the protection and deprotection of the amino groups of amino acids by direct enzymatic synthesis and acyl group transfer reaction.

7. The key intermediates 6-APA and 7-ADCA are obtained by the enzymatic deacylation of PenicillinG Potassium (PenGK) and CephalosporinG (CephG) respectively.

8. The enzymatic conversion is brought about by the cleavage of a side chain of the molecule, in a highly specific manner:

9. Important for the production of Amoxicillin (antibiotic)

Novel Thermostable penicillin G Acylase from Achromobacter xylosoxidans

Using gene that encodes for novel penicillin G acylase (PGA) designated as pgaW in *Achromobacter xylosoxidans* was cloned and overexpressed in Escherichia coli. The genes openreading fram is composedof 2,586 nucleotides. The protein sequence that was translated, about 50% amino acid identical to several characterized PGAs including those of *Providencia rettgeri*, *Kluyvera cryocrescens*, and *Escherichia coli*. Biochemical studies showed that the half-life of inactivation ($t_{1/2}$) was 55 min at 55°C and 8 min at 60°C, compared to the 15 min at 55°C for PGA from *A. faecalis*, i.e. it has four times longer heat stability than PGA from *A. faecalis*. To our knowledge, this is the most thermo stable PGA ever characterized [23].

This conclusion was deduced by the molecular study of PGA650, homology structural modeling and amino acid composition analyses. The results suggested that the increased number of buried ion pair networks, lower N and Q contents, excessive arginine residues, and remarkably high content of proline residues in the structure of PGA650 could contribute to its high thermostability, which provides significant advantages over other well-characterized penicillin acylases in β-lactam conversions for its potential application in industry.

Advantages -

- **Reduced reaction time**- increased operating efficiency.
- **Specificity** - The production of unwanted by-products is avoided and there is no need to extensively refine and purify the desired product.
- **Cost saving** - enzymes can be immobilized and therefore re-used several times, providing valuable cost savings.
- **Environmentally friendly** - enzymes are non corrosive and fully biodegradable.

Conclusion -

Biotechnological applications of penicillin G acylase has emerged as a serious alternative to traditional chemical procedures for the manufacture of b-lactam antibiotics, small peptides and pure isomers from racemic mixtures. However, few successful examples of penicillin acylase catalyzed processes have actually replaced the industrial production of such compounds on the bases of productivity, spite of their environmental and economic benefits. Different modern developments in enzyme technology such as immobilization, non-aqueous biocatalysis and site-directed mutagenesis are used to high production of PGA. Enzyme engineering, immobilized enzyme, non aqueous biocatalysis, delination of enzyme –solvent interaction are the areas which need to be defined precisely to ensure further success in biocatalysis .The expression level of the enzymes give high results compare to traditional method. It has observed that The specific activity of the different crude extracts of PGA activity is strongly affected by mutation. Production of PGA on industrial ways important because of its potential application in Amoxicillin (antibiotic) production, enzymatic conversion, protection and deprotection of the amino groups of amino acids etc.

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