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A catalase–peroxidase for oxidation of β-lactams to their (*R*)-sulfoxides

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ABSTRACT

In this communication we report for the first time a biocatalytic method for stereoselective oxidation of β-lactams, represented by penicillin-G, penicillin-V and cephalosporin-G to their (*R*)-sulfoxides. The method involves use of a bacterium, identified as *Bacillus pumilis* as biocatalyst. The enzyme responsible for oxidase activity has been purified and characterized as catalase–peroxidase (KatG). KatG of *B. pumilis* is a heme containing protein showing characteristic heme spectra with soret peak at 406 nm and visible peaks at 503 and 635 nm. The major properties that distinguish *B. pumilis* KatG from other bacterial KatGs are (i) it is a monomer and contains one heme per monomer, whereas KatGs of other bacteria are dimers or tetramers and have low heme content of about one per dimer or two per tetramer and (ii) its 12-residue, N-terminal sequence obtained by Edman degradation did not show significant similarity with any of known KatGs.

1. Introduction

β-Lactam is a well-known pharmacophore for antibiotics and is widely used in clinic for the treatment of bacterial infections (von Nussbaum et al., 2006). However, application of these compounds in medicinal chemistry is not limited to their traditional use as antibiotics only. Recent findings have shown that β-lactam derivatives inhibit mammalian serine and cystine proteases, in addition to bacterial transpeptidase. Since, these proteases have been implicated as important targets for the development of inhibitors as potential therapeutic agents, a number of β-lactam derivatives have been synthesized and evaluated for this activity (Doherty et al., 1990; Elliott and Sloane, 1996; Xing et al., 2008; Zhou et al., 2003). In addition, β-lactam derivatives have also been evaluated for the β-lactamase inhibition activity (Drawz and Bonomo, 2010). The activity of β-lactam derivatives correlates strongly with the oxidation state of the sulfur moiety in the lactam ring. Therefore, a systematic study of the biological activity of these molecules requires simultaneous evaluation of the activity of a relevant β-lactam derivative along with its (*S*)-sulfoxide, (*R*)-sulfoxide and sulfone (Aleksanyan et al., 2002). Whereas, (*S*)-sulfoxide and sulfone can be easily prepared by direct oxidation of the parent β-lactam with a variety of reagents, no method, chemical or biological has been reported in literature till date for the direct oxidation of β-lactams, i.e. without involving protection–deprotection steps, which stereoselectively produces (*R*)-sulfoxide. Currently, (*R*)-sulfoxides of β-lactams are prepared by multistep synthetic routes, which are tedious to accomplish.

The preferential formation of (*S*)-sulfoxide during oxidation of penicillins has been attributed to the directing effect of the carboxyamido group, most likely through the hydrogen bonding of the peroxide reagent with the amide proton prior to the delivery of reactive oxygen to sulfur atom of the sulfide (Nieuwenhuis, 1995). We envisaged that the directing influence of amide group is likely to be absent in enzyme catalyzed reactions, since the preliminary reaction of the peroxide occurs at the metal center (Fe^{III}). The delivery of reactive oxygen will then be dictated by the orientation of the substrate within the binding cavity of enzyme or it will occur from sterically less hindered side.

Here, we report isolation of a bacterium identified as *Bacillus pumilis*, which stereoselectively oxidized the parent β-lactams, penicillin G and V and cephalosporin G to their (*R*)-sulfoxides. The enzyme responsible for oxidase activity has been purified and characterized as catalase–peroxidase (KatG). The catalase–peroxidase of *B. pumilis* is similar to other KatG's of bacterial origin in terms of its spectral properties, but differs in terms of oligomeric structure and heme content. Also, 12-residue N-terminal sequence obtained by Edman degradation did not show significant similarity with any of known KatG in the database.

2. Methods

2.1. Source of chemicals and microorganisms

Penicillin G was from USB Corporation, Ohio, USA and cephalosporin G was a gift from Ranbaxy, India. Penicillin V,

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