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Research Article
A Role of Loop 1 in BPU-1: A Class D β-lactamase from Gram-positive Bacteria

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Abstract

Background and Objective: The BPU-1 is a class D β-lactamase conferring antibiotic resistance and first identified from a Gram-positive bacterium, *Bacillus pumilus*. Here we have described an important role on loop 1, which was not investigated previously. **Materials and Methods:** To perform the structure-based alignment of the BPU-1 structure and seven OXA-type crystal structures (OXA-1, OXA-13, OXA-10, OXA-23, OXA-24/40, OXA-48 and OXA-58) from Gram-negative bacteria, 8 three-dimensional structures were superposed. The structure-based alignment revealed that the corresponding region of the loop 1 of BPU-1 was identified in 7 OXA-type β-lactamases. **Results:** It is revealed that the loop 1 of the BPU-1serves an important function equivalent to that of the β5-β6 loop in class D carbapenemases (a kind of β-lactamases causing antibiotic resistance in Gram-negative bacteria). **Conclusion:** The current investigation verified that the short BPU-1 loop 1 could play a potential role in extending the activity of the BPU-1 into carbapenems (imipenem and meropenem: A last-resort β-lactam antibiotic for treating extended-spectrum β-lactamase-producing Gram-negative bacteria).

Key words: Class D β-lactamase, carbapenemase, Gram-positive bacteria

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

If antibiotic-resistant pathogens remain unchecked, it is estimated that by 2050 the global mortality attributed to antibiotic-resistant bacterial infections will soar to 10 million, at a cost of over $100 trillion (http://amr-review.org/). Because new antibiotics are difficult to development, it is crucial that the existing antibiotics are used prudently and that infection control measures are strengthened to prevent the spread of resistant bacteria[1,2]. The β-lactam antibiotics are some of the most successful drugs used for the treatment of bacterial infections and represent roughly 65% of the total world market for antibiotics[3]. Therefore, resistance to β-lactam antibiotics through the acquisition of genes that encode β-lactamases is one of the most serious problems in Gram-negative pathogenic bacteria such as the members of the family Enterobacteriaceae, *Pseudomonas* spp. and *Acinetobacter baumannii*[4,5]. Since the first report observing a β-lactamase was published in 1940[6], more than 1,300 distinct β-lactamase (*bla*) genes have been identified in clinical isolates, showing the remarkable diversity of *bla* genes due to their continuous mutations[5]. They can be separated into the four major Ambler classes, A-D, based on their amino acid sequences[7-10]. As an outcome of this successful evolution, the substrate profiles of modern β-lactamases of Gram-negative bacteria range widely from narrow-to expanded-spectrum, with many enzymes capable of producing resistance to virtually every available β-lactam antibiotic[11].

In Gram-positive bacteria, only β-lactamases of molecular classes A and B have currently been associated with β-lactam resistance[12]. The lack of reports regarding the existence of efficient class C and D β-lactamases in Gram-positive bacteria is puzzling in light of how widespread these enzyme classes are in Gram-negative pathogens[13]. Class D OXA-type β-lactamases of Gram-negative bacteria constitute the largest type of the enzymes, with more than 490 members currently recognized (http://www.lahey.org/studies/other.asp#). Named for their ability to hydrolyze oxacillin, some OXA-type enzymes have evolved to confer resistance to β-lactams of the last-resort carbapenems (imipenem and meropenem)[14,15]. Of note, a recent report described that the discovery of putative class D β-lactamases in the genomes of the *Bacillaceae*, Clostridiaceae and Eubacteriaceae families of Gram-positive bacteria[13]. Furthermore, the report demonstrated that because of the shortening of loop 2 where structural deviations were observed between BPU-1 (class D β-lactamase of *Bacillus pumilus* conferring β-lactam antibiotic resistance) and the OXA-type enzymes (class D β-lactamases of Gram-negative bacteria), BPU-1 employed a unique substrate binding mode different from that of all currently known class A, C and D β-lactamases in Gram-negative bacteria. However, structural deviations were observed in loop 1 as well as loop 2. Here, we describe an important role on loop 1, which was not investigated in this report.

MATERIALS AND METHODS

Because we focused on β-lactamases with carbapenemase activity in Gram-positive bacteria, we searched the structures of BPU-1 and the OXA-type enzymes with (or without) carbapenemase activity in the Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). The crystal structures of BPU-1 (Protein Data Bank identification number [PDB ID] 5CTM), non-carbapenemases (OXA-1 [PDB ID 3ISG] and OXA-13 [PDB ID 1H8Y]), OXA-10 (PDB ID 1EWZ) with low carbapenemase activity and carbapenemases (OXA-23 [PDB ID 4JF4], OXA-24/40 [PDB ID 3G4P], OXA-48 [PDB ID 3HBR] and OXA-58 [PDB ID 4OH0]) were found.

To perform the structure-based alignment of the BPU-1 structure and 7 OXA-type crystal structures (OXA-1, OXA-13, OXA-10, OXA-23, OXA-24/40, OXA-48 and OXA-58), 8 three-dimensional structures were superposed by Coot program (SSM superpose)[16]. The structural superposition showed noticeable conformational differences in eight crystal structures.

Structure-based alignments of 8 three-dimensional structures were performed by the PROMALS3D program (http://prodata.swmed.edu/promals3d/promals3d.php). Using this structure-based alignment, the corresponding region of the loop 1 of BPU-1 was identified in 7 OXA-type β-lactamases.

RESULTS AND DISCUSSION

According to the structure-based alignment of the BPU-1 structure and 7 OXA-type crystal structures (Fig. 1), β8 and β9-strands of BPU-1 is almost equivalent to β5 and β6-strands in Gram-negative class D β-lactamases (OXA-1, OXA-10, OXA-13, OXA-23, OXA-24/40 and OXA-58). Therefore, loop 1 (connecting β8 and β9-strands) and the β5-β6 loop (connecting β5 and β6-strands) are in the same position. Both loops comprise maximally nine residues. According to our recent report[17], the overall structure of

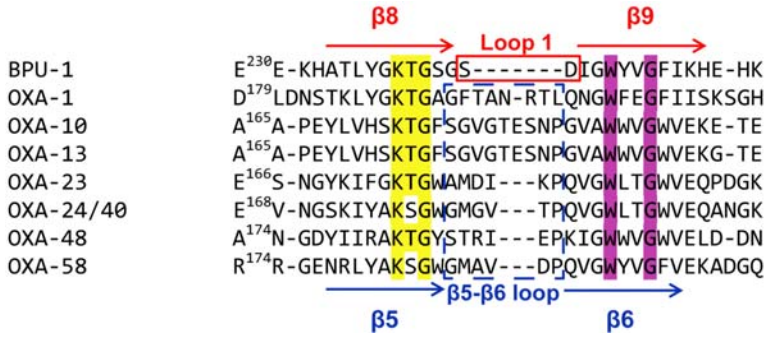

Fig. 1: Structure-based sequence alignment (in the region covering β5-β6 loop or loop 1) of a class D β-lactamase (BPU-1) of Gram-positive bacteria and 7 class D β-lactamases (OXA-type enzymes) of Gram-negative bacteria. Loop 1 and β5-β6 loop are indicated in a red box and a blue broken box, respectively. The KT/SG (yellow) and WxxG (magenta) motifs (the key regions important to the catalytic activity) were also shown. The secondary structure assignment for BPU-1 and seven OXA-type β-lactamases is indicated at the top and bottom, respectively. Numbering is in accordance with the numbering system of each enzyme. Structure-based sequence alignment was performed using the PROMALS3D program (http://prodata.swmed.edu/promals3d/promals3d.php)

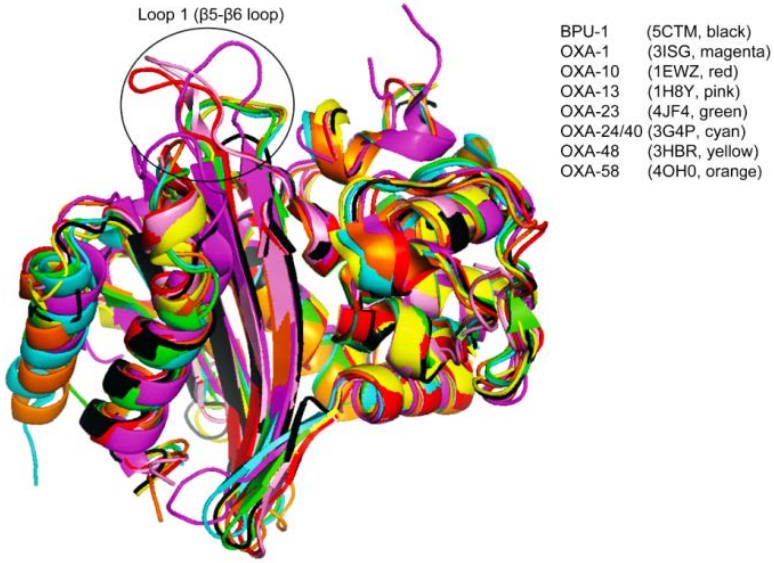

Fig. 2: View of the superposition of a class D β-lactamase (BPU-1) of Gram-positive bacteria and 7 class D β-lactamases (OXA-type enzymes) of Gram-negative bacteria. Structural deviations were shown in the black circle (β5-β6 loop or loop 1). Superpositions were performed using COOT program (SSM superpose) to align the complete chains. Source: PyMOL (http://www.pymol.org)

OXA-48 (a worldwide-spread class D carbapenemase) is very similar to those of OXA-1 (non-carbapenemase), OXA-10 with low carbapenemase activity[11] and OXA-13 (non-carbapenemase) (Fig. 2). However, a structural difference between OXA-48 and OXA-1 (OXA-10 or OXA-13) appeared in β5-β6 loop, which may vary in length and orientation. The length of the β5-β6 loop in OXA-48 is shorter than those of β5-β6 loops (adopting an open confirmation) in OXA-1, OXA-10 and OXA-13 (Fig. 1). This loop extends into the outer portion of the active site in OXA-48. This feature also existed in other carbapenemases (OXA-23, OXA-24/40 and OXA-58) (Fig. 2).

Docquier *et al.*[18] hypothesized that the short-loop connecting β5 and β6-strands plays a potential role in conferring the carbapenemase activity of the OXA-48 enzyme. De Luca *et al.*[19] have performed direct evolution study on the OXA-10 using three OXA-10 loop variants (OXA-10 loop 23, OXA-10 loop 24/40 and OXA-10 loop 48) which substituted the β5-β6 loop of the OXA-10 with the structurally-equivalent loops of three class D carbapenemases (OXA-23, OXA-24/40 and OXA-48). Crystal structures and kinetic data revealed that although OXA-10 loop 24/40 and OXA-10 loop 48 did not show significant changes in the molecular fold of the enzyme except for the β5-β6 loop, these OXA-10 loop variants acquired significant (approximately 10-fold) carbapenemase activity (k_{cat}/K_m) for imipenem. Taken together, these data suggest that the β5-β6 loop in class D carbapenemases play a crucial role in the carbapenemase activity.

Moreover, the catalytic efficiency (k_{cat}/K_m) of BPU-1[13] against carbapenems (imipenem and meropenem) is similar to those of the clinically important OXA-23 (k_{cat}/K_m value 7.4×10^4 M^{-1} sec^{-1} against imipenem) and OXA-48 (k_{cat}/K_m value 6.2×10^3 M^{-1} sec^{-1} against meropenem) class D β-lactamases of Gram-negative pathogens[18,20]. The length of the loop 1 in BPU-1 is shorter than those of β5-β6 loops in OXA-1, OXA-10 and OXA-13 (Fig. 1). The structural conformation of BPU-1 loop 1 does not adopt an open confirmation shown in those of OXA-1, OXA-10 and OXA-13. The loop 1 of BPU-1 extends into the outer portion of its active site, which is shown in the β5-β6 loop of class D carbapenemases (OXA-23, OXA-24/40, OXA-48 and OXA-58) (Fig. 2).

CONCLUSION

Taken together, these data suggest that the BPU-1 loop 1 serves an important function equivalent to that of the β5-β6 loop in class D carbapenemases. In our view, the short BPU-1 loop 1 can play a potential role in extending the activity of the BPU-1into carbapenems (imipenem and meropenem).

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