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Structural Basis for Progression toward the Carbapenemase Activity in the GES Family of β-Lactamases

Clyde A. Smith,*[,†] Hilary Frase,[‡] Marta Toth,[‡] Malika Kumarasiri,[‡] Kwame Wiafe,[†] Jared Munoz,[†] Shahriar Mobashery,[‡] and Sergei B. Vakulenko*[,‡]

[†]Stanford Synchrotron Radiation Laboratory, Stanford University, Menlo Park, California 94025, United States

[‡]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556, United States

Supporting Information

ABSTRACT: Carbapenem antibiotics have become therapeutics of last resort for the treatment of difficult infections. The emergence of class-A β-lactamases that have the ability to inactivate carbapenems in the past few years is a disconcerting clinical development in light of the diminished options for treatment of infections. A member of the GES-type β-lactamase family, GES-1, turns over imipenem poorly, but the GES-5 β-lactamase is an avid catalyst for turnover of this antibiotic. We report herein high-resolution X-ray structures of the apo GES-5 β-lactamase and the GES-1 and GES-5 β-lactamases in complex with imipenem. The latter are the first structures of native class-A carbapenemases with a clinically used carbapenem antibiotic in the active site. The structural information is supplemented by information from molecular dynamics simulations, which collectively for the first time discloses how the second step of catalysis by these enzymes, namely, hydrolytic deacylation of the acyl-enzyme species, takes place effectively in the case of the GES-5 β-lactamase and significantly less so in GES-1. This information illuminates one evolutionary path that nature has taken in the direction of the inexorable emergence of resistance to carbapenem antibiotics.

β-Lactam antibiotics (penicillins, cephalosporins, monobactams, and carbapenems) constitute the most extensively used class of antibacterial agents for treatment of a wide variety of infections.[1] More than seven decades of use of these antibiotics has resulted in the selection and widespread dissemination of β-lactam-resistant bacteria. Among these, resistance to carbapenems is the most disconcerting, as these broadly resistant organisms are causes of high mortality. The major mechanism of resistance to β-lactam antibiotics in Gram-negative bacteria is the catalytic action of β-lactamases, hydrolytic enzymes that inactivate the antibiotics.[2,3] The enzymes are divided into four major classes: A, B, C, and D.[4] β-Lactamases of classes A, C, and D are active-site serine enzymes, whereas those belonging to class B are zinc-dependent.

The prototypical class-A β-lactamases TEM and SHV were among the first β-lactamases identified in Gram-negative bacteria. Those early resistance enzymes had a narrow-spectrum substrate profile that included primarily penicillins and some early cephalosporins. The progressive introduction of subsequent generations of β-lactam antibiotics has led to the inexorable emergence of novel enzymes with enhanced catalytic competencies.[5] In response to the challenge of organisms with class-A β-lactamases, the first carbapenem, imipenem, was brought to the clinic in the mid-1980s. Shortly thereafter, the newer carbapenems meropenem, doripenem, and ertapenem were introduced. Carbapenems rapidly became antibiotics of last resort because of their exceptional breadth of activity and potency. As a point of departure in the evolution of β-lactamases, the common TEM- and SHV-type enzymes failed to evolve the ability to turn over carbapenems. Indeed, carbapenems serve as inhibitors of these enzymes, as they are able to acylate the active-site serine, but this intermediate does not undergo deacylation to regenerate the catalyst.[6,7] The first insight into this process came from the X-ray structure of the acyl-enzyme species of the TEM-1 β-lactamase with imipenem, which explained the inhibition process.[6] The hydrolytic water molecule is pushed away from the active site by imipenem, and there is an additional H-bond to the substrate that adversely affects its activation. This arrangement imparts longevity to the acyl-enzyme species, resulting in inhibition of the enzyme.

Nonetheless, the extensive use of carbapenems has led to the appearance of specific class-A enzymes with hydrolytic activities against them. These enzymes, which share less than 50% amino acid sequence identity with the TEM- and SHV-type β-lactamases, are found in clinical and environmental strains and include NMCA, IMI, SFC, SME, GES, and KPC carbapenemases.[8] In contrast to the other members of this group of enzymes, the genes for the KPC- and GES-type β-lactamases have been disseminated widely in clinics all over the world.[9] Currently, 12 variants of KPC-type and 22 variants of GES-type enzymes have been reported. Unlike the narrow-spectrum TEM-1 enzyme, KPC- and GES-type β-lactamases are capable of hydrolyzing extended-spectrum cephalosporins.[8] Additionally, all of the KPC-type enzymes that have been studied enhance resistance to carbapenems. Among the GES-type β-lactamases, only variants with N170S substitutions reduce susceptibility to carbapenems.[8] The ability of the KPC enzymes and some GES variants to hydrolyze the majority of available β-lactam antibiotics, including carbapenems, constitutes an immense challenge to our ability to treat life-threatening infections caused by pathogens producing these β-lactamases.[8−11]

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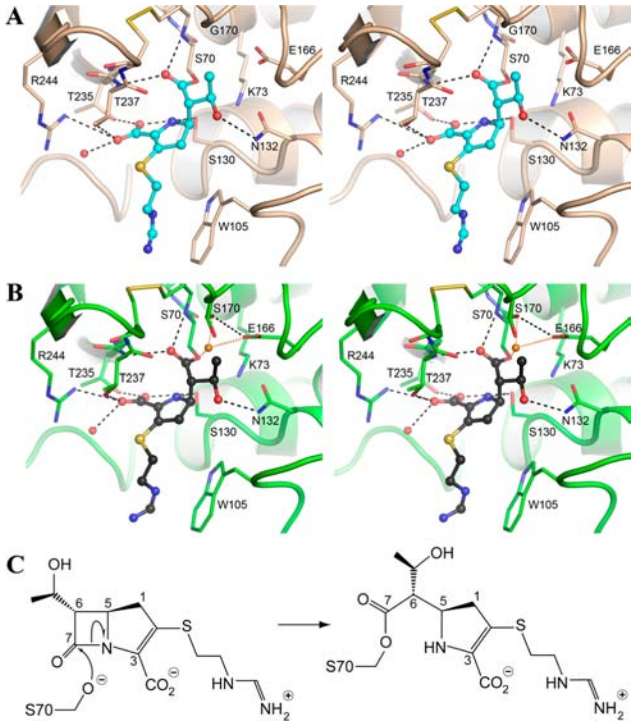

Figure 1. Imipenem acyl-enzyme intermediate complexes. (A) Stereo view of the GES-1 active site with the bound imipenem (cyan). H-bonding interactions with the protein are shown as dashed black lines. (B) Stereo view of the GES-5 active site with the bound imipenem (black). The partially occupied water molecule is shown as an orange sphere, with its H-bonding interactions shown as orange dotted lines. (C) Imipenem (left) acylates Ser70 (right).

Kinetic studies have demonstrated that GES-5 exhibits a 100-fold enhancement in the catalytic efficiency (k_{cat}/K_m) against the carbapenem antibiotic imipenem relative to the marginal activity exhibited by GES-1.[12] The consequence of this is that GES-5 has become a bona fide carbapenemase of clinical importance, whereas the poor activity of GES-1 against carbapenems is on par with that of TEM-1, for which carbapenems serve as covalent inhibitors.[12,13] Hence, the GES-1 and GES-5 β-lactamases provide a unique opportunity to explore the structural means for the broadening of the substrate profile to include carbapenems. We report herein the X-ray structures of the acyl-enzyme complexes of GES-1 and GES-5 carbapenemases with imipenem. By soaking crystals with imipenem for a short duration, followed by flash cooling, we succeeded in generating the acyl-enzyme species for both wild-type GES-1 and GES-5. These are the first enzyme−substrate complexes of native class-A carbapenemases with a clinically important carbapenem antibiotic. These structures, complemented by molecular dynamics (MD) simulations, shed light on how the activity of GES-5 has been enhanced to include carbapenems as substrates.

The crystal structures of apo GES-5 and the imipenem complexes of GES-1 and GES-5 were determined to 1.10, 1.15, and 1.25 Å resolution, respectively (Figure 1A,B; Tables S1 and S2 and Figure S1). The crystals were grown under the same conditions, and all three structures contain two molecules in the asymmetric unit that are related by a noncrystallographic twofold rotation. The enzyme−substrate complexes were generated by soaking GES-1 and GES-5 crystals in 30 mM imipenem for times varying from 30 s to 10 min. Although the presence of imipenem in the active sites of the two enzymes could be observed in data from crystals soaked for as little as 30 s, 2 min soaking experiments were used for the detailed comparison of the incorporation of the drug. The initial electron densities for the GES-1 and GES-5 imipenem complexes are shown in Figure S2. In both cases, there was no visible density for the side chain attached to the C_2 sulfur, as it points to the milieu and should be mobile. The occupancies of imipenem were refined for both enzyme molecules in each crystal, and values of 0.94 and 0.96 for GES-1 and 0.63 and 0.67 for GES-5 were obtained. As indicated earlier, imipenem is a good substrate for GES-5, so the lower occupancy of the substrate is indicative of partial deacylation. This was supported by an analysis of the substrate occupancies in GES-5 for different soaking times. The occupancies after 30 s (0.53/0.54) were only slightly lower than those at 2 min, and those after 5 min (0.67/0.68) were essentially the same as those at 2 min, suggesting that the competing acylation and deacylation steps rapidly reach an equilibrium and plateau at about two-thirds occupancy. In GES-1, full occupancy was seen after 1 min, supporting the premise that this enzyme is covalently inhibited by carbapenems. Almost all of the active-site residues that have been identified as playing critical roles in the class-A β-lactamases are conserved in GES-1 and GES-5, including Ser70, Lys73, Ser130, Asn132, Glu166, and Thr237 (Ambler numbering scheme[14]). The only exception is residue 170. This residue is asparagine in most class-A enzymes, whereas in GES-1 and GES-5 it is a glycine and a serine, respectively. In the GES-5−imipenem complex, two rotamers of the serine were observed in one of the molecules (Figure 1B) and only one in the second. In both complexes, the β-lactam ring of imipenem is opened, and a covalent bond to the Ser70 side chain has formed (Figure 1C). The ester carbonyl of the acyl-enzyme intermediate is housed in the oxyanion hole formed by the main-chain N atoms of Ser70 and Thr237. The C_3 carboxylate is directed toward a pocket formed by the side chains of residues Ser130, Thr235, Thr237, and Arg244, and the hydroxyethyl moiety of imipenem points toward the Glu166 and Asn132 side chains. In both GES-1 and GES-5, imipenem makes a total of seven H-bonds with the surrounding protein molecule and additional H-bonds to well-ordered water molecules (Figure 1A,B). In both complexes, the hydroxyethyl moiety is oriented in such a way that the hydroxyl group points away from Ser70 and forms a H-bond with the $N_{\delta 2}$ atom of the conserved Asn132 (Figure 2). The hydroxyethyl methyl group points in the direction of residue 170. The formation of the GES-1 acyl-enzyme intermediate displaces the deacylating water molecule observed in the apo GES-1 structure.[15]

Superimposition of the two GES−imipenem complexes gives an almost perfect overlap of the two structures [root-mean-square deviation (rmsd) of 0.1 Å for all 268 Cα atoms]. The presence of the Ser170 side chain has a profound effect on the conformation of the GES-5 active site, as the Glu166 side chain, the general base that promotes a water molecule for deacylation,[16] moves toward the active-site Ser70 by ~1 Å compared with GES-1 (Figures 1B, 2A, and S3). Glu166 now makes a new H-bonding interaction with one of the conformations of the Ser170 side chain, which was also observed in the apo GES-5 structure (Figure S4). The side chain of Glu166 is much closer to the catalytic serine residue (Ser70), allowing the formation of a H-bond between the $O_{\varepsilon 1}$ atom of Glu166 and the O_γ of Ser70 (3.2 Å), and this proximity closes the pocket typically occupied by the hydrolytic water molecule (Figure 2A). Such a H-bond between the Glu166 and Ser70 side chains is rare in the class-A β-lactamases and has been observed only once in the

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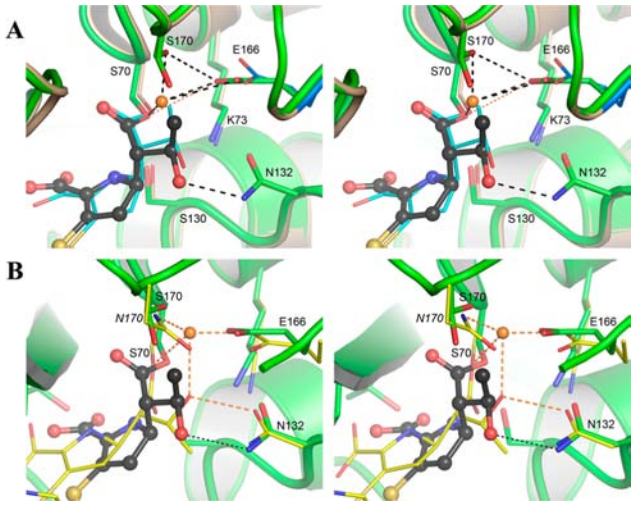

Figure 2. Stereo views of superimpositions of the active sites of the imipenem complexes of GES-1, GES-5 and TEM-1. (A) Superimposition of GES-1 (pink) and GES-5 (green), showing the two alternate conformations of Ser170 and the closer approach of Glu166 to Ser70 in GES-5. The position of Glu166 in GES-1 is shown in blue. The imipenem is shown in black ball-and-stick for GES-5 and thin cyan sticks for GES-1. The H-bonds with the partially occupied water molecule (orange) are shown as black dashed lines, and the H-bond between Glu166 and Ser70 is shown as a dotted orange line. (B) Superimposition of GES-5 (green) and TEM-1 (yellow). The imipenem is shown in thin yellow sticks for TEM-1 and black ball-and-stick for GES-5. The deacylating water in TEM-1 is shown as an orange sphere with four H-bonding interactions depicted as dashed orange lines.

SME-1 β-lactamase.[17] In this robust carbapenemase, there is also no deacylating water molecule, which suggests that in SME-1 and GES-5, a transient water molecule from the milieu could serve this role. In molecule B of the GES-5–imipenem complex, the Ser170 side chain is orientated in such a way that there is no H-bond to Glu166, and the latter residue adopts two distinctly different conformations, one similar to that observed in molecule A of this complex and the other shifted away from Ser70 in a configuration reminiscent of GES-1 (Figure 2A). These rotamer conformations observed in the GES-5 structure were sampled in our MD simulations (described below), and the presence of a H-bonding interaction between Ser170 and Glu166 was found to be vitally important for carbapenemase activity (also discussed below).

The superimposition of GES-1 and GES-5 shows that the conformations of imipenem in the two enzyme complexes are essentially identical (Figure 2A), with an rmsd of only 0.4 Å when just the two imipenem molecules are superimposed. Binding of imipenem does not lead to any significant conformational changes in the active sites of these enzymes relative to the apo structures. Superimposition of the imipenem complexes of GES-1 and GES-5 onto the apo forms of the enzymes gave rmsd values of 0.32 and 0.20, respectively, based upon all of the atoms in the segments of the structure containing the important active-site residues (positions 70–73, 129–133, 165–171, and 234–240) (Figure S5). However, in contrast to apo GES-5, a water molecule was observed in the GES-5–imipenem complex (Figures 1B and 2A) that occupies a site similar to that assigned to the deacylating water in GES-1.[15] The electron density for this water molecule appeared later in the refinement and had refined occupancies of 0.30 and 0.35 in the two independent GES-5 molecules. It is anchored by H-bonding interactions to Ser70 and Glu166, with an additional H-bond to one of the Ser170 conformations in molecule A (Figure 2A), and it is displaced toward the acyl intermediate by ~1 Å relative to the position observed in apo GES-1. It would appear that in this location the water molecule would make two very short contacts to the bound imipenem, 2.5 Å to the ester carbonyl carbon and 1.3 Å to the methyl group of the hydroxyethyl moiety. These distances would preclude simultaneous occupation of the active site by imipenem and the water molecule. The low occupancy of the water molecule (0.30–0.35) and the corresponding higher occupancy of the imipenem (0.63–0.67) indicate that when imipenem is present in the active site of GES-5, the water molecule is absent. A question then remains as to why acylation of the active-site serine in the prototypic class-A β-lactamase TEM-1 and in GES-1 inhibits the enzyme activity, whereas in GES-5 there is sufficient progress toward improved catalysis that this enzyme could be called a bona fide carbapenemase. This puzzle is tantalizing, especially with the availability of X-ray crystal structures for all three enzymes acylated by imipenem and the fact that the imipenem complexes of GES-1 and GES-5 presented here appear to be nearly identical except for the different amino acids found at position 170 (Gly and Ser, respectively). This last observation indicated to us that the dynamics of the proteins might have a role to play in manifesting the mechanistic outcomes.

An analysis of the TEM-1 β-lactamase–imipenem complex indicates that the orientation of the hydroxyethyl group of the imipenem is a determining factor in the longevity of the acyl-enzyme species.[6] The hydroxyl group of the hydroxyethyl substituent forms H-bonds with the $O_{\delta 1}$ atom of Asn132 and the hydrolytic water molecule (Figure 2B). This water molecule is involved in four H-bonds. The extensive solvation of the water molecule might diminish its nucleophilicity.[6,7] The hydroxyl group of the hydroxyethyl moiety actually occupies the position of the hydrolytic water molecule, which is displaced to the position seen in the structure of the complex. In this arrangement, the hydroxyethyl moiety impedes the movement of the hydrolytic water molecule to the acyl carbonyl, resulting in the stability of the complex.

Comparison of the structures of the imipenem complexes of GES-1 and GES-5 with that of the TEM-1[6] shows that in the TEM-1 enzyme, the entire imipenem molecule has moved across the active site by ~1.7 Å in the direction away from Asn132 and Glu166 and toward Arg244 (Figure 2B). Importantly, the hydroxyethyl group in the GES complexes is rotated ~120° relative to that in the TEM-1 complex, preventing the formation of a H-bond with the hydrolytic water molecule. As a result, the hydroxyethyl rotamer observed in the GES-1 and GES-5 complexes would not be expected to interfere with the effective activation of the hydrolytic water molecule. This is one factor, but the dynamics of the protein appear to play a role in the catalysis as well, as described below.

To understand the favorable turnover of imipenem by GES-5, we used the X-ray structures of the acyl-enzyme species of GES-5 and TEM-1 with imipenem for MD simulations. During an 11 ns simulation, we noted that the side chains of Glu166 and Ser170 of the GES-5 enzyme remained in contact with one another for the duration (Figure 3A,B). However, this was not the case for Glu166 and Asn170 of the TEM-1 structure, as only ~15% of the snapshots exhibited H-bonding between the two residues (data not shown). As we will explain, this interaction is important for the carbapenemase activity.

In representative snapshots of the simulations of GES-5, Glu166 makes three H-bonds, one to Ser170 (Figure 3B), one to

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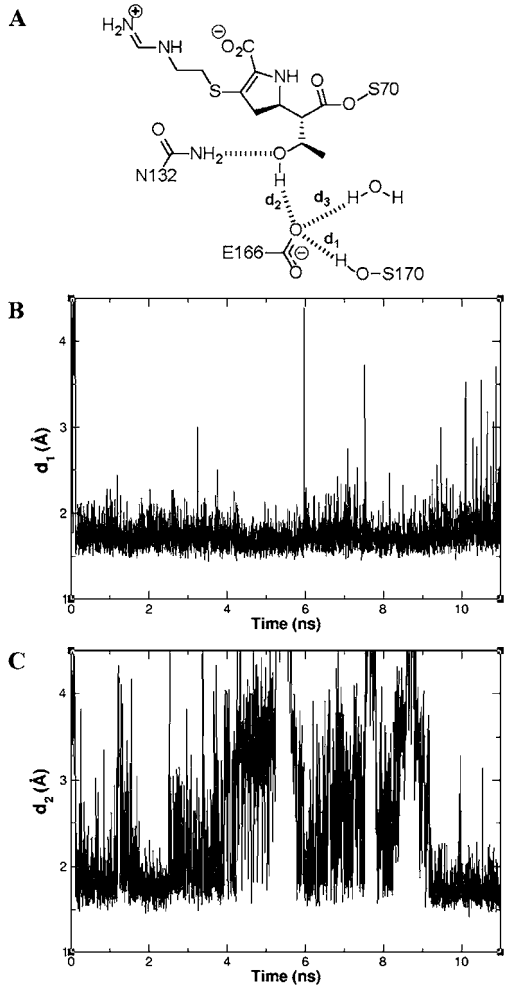

Figure 3. MD simulations of GES-5 acylated by imipenem. (A) Cartoon of a representative snapshot with the distances d_1, d_2, and d_3 indicated. H-bonds are shown as dashed lines. (B, C) Fluctuations of (B) d_1 and (C) d_2 as functions of time over the 11 ns MD simulation.

the imipenem hydroxyl group (Figure 3C), and one to the hydrolytic water (Figure S6). This brings about two effects: First, it tethers the hydroxyethyl group away from the trajectory of the hydrolytic water, assisted by the H-bond between Asn132 and the hydroxyethyl hydroxyl group (Figure 3A). Second, it allows Glu166 to be in position to activate the hydrolytic water. The implication of the stable H-bond between Glu166 and Ser170 for the GES-5 enzyme is that the side chain of Glu166 remains in position at all times to interact with the hydrolytic water and promote it for the deacylation step.

In TEM-1, and by extension in the GES-1 enzyme as well, the situation is different. The presence of Asn at position 170 of TEM-1 disrupts this arrangement. First, Asn170 has a longer side chain that sterically does not allow a large enough opening for a water molecule to come close to the ester carbonyl of the imipenem complex. More importantly, however, MD snapshots of TEM-1 sampled Asn170 and Glu166 in H-bonding arrangements only ~15% of the time (Figure S7). This lack of tethering of Glu166 allows a larger sampling of the side-chain rotamers in the complex with imipenem. The interaction of the hydrolytic water molecule and the side chain of Glu166 is lost in TEM-1, and therefore, the water molecule is not properly positioned within the active site to perform deacylation. This effect would dominate in GES-1 with Gly at position 170, which obviously cannot interact with Glu166 at all. Thus, the higher turnover efficiency of imipenem observed for GES-5 is due to the special role played by Ser170, which must have been a force in its selection for the carbapenemase activity.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, characterization data, and tables and figures as described in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

csmith@slac.stanford.edu; svakulen@nd.edu

Notes

The authors declare no competing financial interest.

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