Crystal structure of L,D-transpeptidase Ldt_{Mt2} in complex with meropenem reveals the mechanism of carbapenem against *Mycobacterium tuberculosis*

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Dear Editor,

Tuberculosis (TB), an infectious disease caused by the bacillus *Mycobacterium tuberculosis*, leads to substantial mortality worldwide [1]. The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *M. tuberculosis* has challenged conventional anti-TB therapy and threatens global disease control of TB [2, 3]. The development of new anti-TB drugs is urgently required [4]. β -lactams are effective antibiotics widely used to treat bacterial infections; however, so far no effective anti-TB antibiotics have emerged from this class of drugs. Previous studies have indicated that an important reason for the lack of effective β -lactam anti-TB antibiotics is the presence of a chromosomally-encoded class A (Ambler) β -lactamase, BlaC, in *M. tuberculosis* [5].

A recent study by Hugonnet *et al.* [6] has demonstrated that a combination of meropenem-clavulanate was effective against 13 XDR strains of *M. tuberculosis*. Meropenem is a member of the carbapenem class of β -lactams and contains a bicyclic nucleus, a pyrroline ring and a β -lactam ring, and clavulanate is a β -lactamase inhibitor. Both are FDA-approved drugs and could potentially be used to treat TB, providing a novel clinical treatment strategy [6]. However, the bactericidal mechanism of this drug combination in treating TB is unclear. Hugonnet *et al.* [6] suggest that one reason for the efficacy of the meropenem-clavulanate combination against XDR strains is that meropenem is poorly hydrolyzed by BlaC.

Carbapenems have been reported to target L,D-transpeptidases that generate the unusual 3→3 transpeptide linkages that are the predominant transpeptide linkages (80%) in the peptidoglycan layer of nonreplicating *M. tuberculosis* [7, 8]. L,D-transpeptidase type 2 (Ldt_{Mt2}) is the main L,D-transpeptidase in *M. tuberculosis* and is critical for cell wall synthesis, virulence and amoxicillin tolerance of *M. tuberculosis* [9]. To kill *M. tuberculosis*

effectively, the combination of meropenem-clavulanate not only inactivates β -lactamase but also prevents the formation of transpeptide linkages in the peptidoglycan layer [9]. As Ldt_{Mt2} is the enzyme that generates 80% of the transpeptide linkages in *M. tuberculosis*, the inactivation of Ldt_{Mt2} is likely to be the major mechanism underlying the effectiveness of meropenem-clavulanate against *M. tuberculosis*. Here, we report the crystal structures of an N-terminal-truncated Ldt_{Mt2} (Ldt_{Mt2}- Δ N54, residues 55–408), a trypsin-degraded fragment of Ldt_{Mt2} (Ldt_{Mt2}- Δ N139, residues 140–408) and the complex of Ldt_{Mt2}- Δ N139 and meropenem, at 2.5, 1.8 and 1.4 Å resolutions, respectively (Supplementary information, Figure S1 and Table S1). These structures reveal the structural mechanism through which meropenem may act to inhibit Ldt_{Mt2}.

Our apo Ldt_{Mt2}- Δ N54 structure (Figure 1A) shows the linear arrangement of the two N-terminal β -barrel domains (residues 60–148 and 149–250) and the C-terminal YkuD domain (residues 251–408). The two N-terminal β -barrel domains, both of which adopt an IgG-like fold, contain one three-stranded and one four-stranded sheet, respectively. We suggest that these two IgG-like domains act as a spacer arm for the YkuD catalytic domain. The YkuD domain is characterized by a β -sheet structure similar to that in Ldt_{Bs} from *Bacillus subtilis* [10] and Ldt_{fm} from *Enterococcus faecium* [11] (Supplementary information, Figure S1) with a root-mean-square deviation of C α superposition of 1.6 Å and 1.8 Å and sequence identities of 20% and 24%, respectively. As in Ldt_{Bs} and Ldt_{fm} [10, 11], the YkuD domain in Ldt_{Mt2} contains a catalytic triad Cys354-His336-Ser337, and it is likely that residue Cys354 is directly involved in enzyme activity and is the target site for carbapenems. Ldt_{Mt2} also contains two additional segments, A (residues 300–323) and B (residues 379–408), which are not observed in Ldt_{Bs} and Ldt_{fm} (Supplementary information, Figure S1).

Co-crystallization of Ldt_{Mt2}- Δ N54 and meropenem failed, but co-crystallization of Ldt_{Mt2}- Δ N139 and me-

Figure 1 Structures of Ldt_{M12} and the Ldt_{M12} -meropenem complex. **(A)** Ribbon representation of the overall structure of Ldt_{M12} - $\Delta N54$ (segments A and B are labeled in red and magenta, respectively). **(B–E)** Different conformations of meropenem in the Ldt_{M12} - $\Delta N139$ -meropenem complex. A 2Fo-Fc ligand-omitted electron density map (green, contour at 1σ), a Fo-Fc map (red, contour at 3σ), and ball-and-stick models of meropenems in State I (**B**, **D**, green) and II (**C**, **E**, gray) are shown. Hydrogen bonds are shown as blue dashed lines in **D** and **E**. **(F)** Proposed mechanisms of the action of meropenem on Ldt_{M12} (the present study) and BlaC [4].

roperenem was successful. The asymmetric unit of Ldt_{M12} - $\Delta N139$ -meropenem co-crystals contains two Ldt_{M12} -meropenem complexes. Meropenem adopts different

conformations in the two complexes, hereafter referred to as State I (Figure 1B and 1D) and II (Figure 1C and 1E). In both states, meropenem is covalently linked to

the catalytic residue Cys354 via thioester formation. The carbapenem nucleus of meropenem appears clearly, displays well-defined electron densities and shows the same conformation in both State I and II (Figure 1B and 1D). The planar arrangement of atoms C2, C3, N4, C5, and C31 of meropenem in complex with Ldt_{M12} results in the appearance of a double bond between C3 and N4 (Figure 1F), replacing the C2/C3 double bond observed in meropenem [12, 13]. The C3/N4 double bond was also observed in meropenem covalently linked to BlaC [6] and other β -lactamases [13]. This tautomerism of the carbapenem nucleus is accompanied by the covalent linking of meropenem to Ldt_{M12}. As tautomerization of the carbapenem nucleus has been reported to cause slow turnover in carbapenem hydrolyzation by BlaC [6] and other β -lactamases [13], we propose that tautomerization helps to stabilize the Ldt_{M12}-meropenem adduct.

Despite these similarities, the two states are distinct from each other. In State I, atom C7 of meropenem (Figure 1F and Supplementary information, Figure S2) is close to atom N4 at a distance of 2.87 Å. This conformation is very similar to that reported previously for meropenem in complex with BlaC [6], and likely represents the initial step during the action of meropenem on Ldt_{M12}. In State II, the carbapenem nucleus has flipped approximately 180 degrees around the single bond connecting atoms C5 and C6 (Supplementary information, Figure S3), resulting in a larger C7-N4 distance of 3.21 Å.

Interestingly, by flipping the carbapenem nucleus in State II, residue Tyr308 of Ldt_{M12} adopts a different rotamer conformation by rotating approximately 90 degrees around the C α /C β bond (Supplementary information, Figure S3). We suggest that rotating of the Tyr308 side chain in Ldt_{M12} and flipping of the carbapenem nucleus in meropenem are the second step following the β -lactam ring opening in the first step during the action of meropenem on Ldt_{M12}, and result in three additional hydrogen bonds (^{Tyr318}OH-^{Mer}OH62, ^{Tyr318}OH-^{Mer}O32, and ^{Tyr308}OH-^{Mer}O32) between the enzyme and meropenem in State II (Figure 1E and Supplementary information, Figure S3). In contrast, only one hydrogen bond (^{Gly353}N-^{Mer}O7) is observed in State I (Figure 1D). Residues Tyr308 and Tyr318 that form hydrogen bonds with meropenem are conserved in Ldt_{M12} and its homologs, but not in other L,D-transpeptidases (Supplementary information, Figure S1). Mutant protein Y318F or Y318A hydrolyzes meropenem at a faster rate than the wild-type protein (Supplementary information, Figure S4), supporting that the additional hydrogen bonds are important for stabilization of the enzyme-drug adduct and indicating that the flipping of meropenem and the resulting additional hydrogen bonds are involved in the mechanism underlying

the inhibition of Ldt_{M12} by meropenem (Figure 1F).

As a main peptidoglycan-crosslinking L,D-transpeptidase, Ldt_{M12} is an important target in the development of drugs against XDR *M. tuberculosis* [9, 14]. Our analyses of the Ldt_{M12}-meropenem complex structure reveal a two-step mechanism of drug actions (Figure 1F top). The first step involves opening the β -lactam ring of meropenem and formation of a thioester between meropenem and Ldt_{M12}, accompanied by the simultaneous tautomerization of the C2/C3 double bond to C3/N4 double bond (State I). These events of the first step are also observed in the complex of meropenem and BlaC [6] (Figure 1F bottom). During the second step, the C5/C6 single bond of meropenem rotates approximately 180 degrees, and this rotation induces localized structural changes in segment A (including residues Tyr308 and Tyr318) of Ldt_{M12}, leading to the formation of several hydrogen bonds (State II), stabilizing the structure of the Ldt_{M12}-meropenem adduct, and resulting in a slow turnover in drug hydrolysis. This mechanism may be shared by carbapenems, such as meropenem and imipenem, which have been reported to inhibit Ldt_{M12}.

Furthermore, we explored the role of tautomerization in stabilizing the complex of Ldt_{M12} and antibiotics. When we performed mass spectrometry assays with cepheids such as cephalotin and cefuroxime, a subclass of β -lactams that do not inhibit Ldt_{M12}, we found that cepheids also form covalent adducts with Ldt_{M12} (Supplementary information, Table S2 and Figure S6). We thus propose that the mechanism of Ldt_{M12} action on cepheids is the same as that of Ldt_{M12} action on cepheids [14] and involves a tautomerization of the cepheid nucleus (a β -lactam ring and a dihydrothiazine ring) (Supplementary information, Figure S5A). As cepheids cannot inhibit Ldt_{M12}, we suggest that tautomerization alone is not sufficient to stabilize the adduct of Ldt_{M12} and cepheids. To stabilize the adduct of Ldt_{M12} and carbapenem (or any other potential inhibitor of Ldt_{M12}), tautomerization is necessary but not sufficient, and the conformational change in the second step likely also plays an important role. The results reported here, together with the reported structure of the BlaC-meropenem complex [15], reveal the structural basis for the stabilization of enzyme-carbapenem adducts and may suggest new strategies for the design of antibiotics derived from β -lactams to fight against XDR *M. tuberculosis*.

Accession codes

Coordinates and structure factor files of Ldt_{M12}- Δ N54, Ldt_{M12}- Δ N139, and the Ldt_{M12}- Δ N139-meropenem complex have been deposited in the Protein Data Bank with accession codes 3 VYN, 3 VYO and 3 VYP.

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(Supplementary information is linked to the online version of the paper on the *Cell Research* website.)