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A New State Ⅰ−plus Observed for the L,D−transpeptidase LdtMt2−ertapenem Adduct*

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Abstract Multidrug-resistant and extensively drug-resistant *Mycobacterium tuberculosis* strains are spreading globally, and thus, new antituberculosis drugs are urgently needed. The *M. tuberculosis* L,D-transpeptidase LdtMt2 is directly involved in peptidoglycan formation, bacterial virulence and β-lactam resistance. This enzyme is a potential antituberculosis target that can be inhibited by carbapenems, FDA-approved drugs that are used in the treatment of tuberculosis. Two different intermediate states, states I and II, have been reported for LdtMt2 interacting with carbapenems, such as ertapenem, imipenem and meropenem. State I was proposed as an initial adduct formation state, whereas state II was proposed as a stable protein-ligand interaction state accompanied by local conformational arrangements of both carbapenem and the protein. Here, we report a new LdMt2-ertapenem interaction state, I-plus, with the same carbapenem conformation as state II and a similar local protein conformation to state I. This new state was proposed as an intermediate state for the transition from state I to state II, in which the ertapenem molecule, but not the protein, undergoes a conformational change. Our work helps elucidate the changes that occur after carbapenem acts on LdtMt2 and, together with the other reported state, demonstrates how the L,D-transpeptidase interacts with carbapenems.

Key words L, D-transpeptidase, LdtMt2, ertapenem, β-lactam
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1 Introduction

Bacterial cell walls are fundamental cellular components that maintain bacterial cell shape, sequester cells from the external environment and are essential for cell survival[1-2]. In bacteria, D, D-transpeptidases, which catalyse the formation of 4→3 transpeptide linkages in the peptidoglycan layer, are responsible for the integrity of bacterial cell walls and have long been proposed as the sole target of traditional β-lactam antibiotics[3-5]. L, D-transpeptidases, nonclassical transpeptidases, have been identified[6-8]. These enzymes maintain the peptidoglycan layer by catalysing the formation of 3→3 transpeptide linkages and are widely distributed in bacteria[4], including difficult-to-treat drug-resistant pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and

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Enterobacter species)[9]. Unlike D, D-transpeptidases, L, D-transpeptidases could not be inhibited by penicillins (penams) or cephalosporins (cephems)[10-11].

Mycobacterium tuberculosis causes tuberculosis, which is a global public health problem[12]. Multidrug-resistant and extensively drug-resistant *M. tuberculosis* strains are spreading globally, indicating the urgent need for new antituberculosis agents. Interestingly, *M. tuberculosis* encodes five L,D-transpeptidases in its genome, and its cell wall is dominated by 3→3 transpeptide linkages, indicating the essential role of L, D-transpeptidase in cell wall formation[13]. Among these transpeptidases, LdtMt2 is directly involved in peptidoglycan formation, bacterial virulence and β-lactam resistance[10]. The deletion of LdtMt2 substantially impaired bacterial virulence, suggesting that LdtMt2 is a potential drug target for anti-tuberculosis treatment. This enzyme could be inhibited by carbapenems along with some other β-lactam antibiotics, *e. g*., faropenem, but not by amoxicillin or cephems. The deletion of LdtMt2 also resulted in bacterial sensitivity to the amoxicillin-clavulanate combination. In contrast, the wild type bacteria are effectively inhibited only by the carbapenem-clavulanate combination or the faropenem-clavulanate combination[14], not by the amoxicillin-clavulanate combination. Thus, LdtMt2 is proposed as one of the main targets of carbapenems and has drawn much attention in anti-infection clinical assays[10,15].

The adduct structures of LdtMt2 with diverse carbapenems and related beta-lactams, including meropenem[15], imipenem, ertapenem[16], faropenem[17], or panipenem (PDB: 6BOI), have been determined, and the interaction between LdtMt2 and carbapenems has been extensively studied. These studies showed diverse interaction modes. For example, faropenem was hydrolysed after covalently binding to the enzyme[17], whereas ertapenem and imipenem were not[16]. Some carbapenems were observed to bind at the inner cavity and are on the same side as a catalytic triad formed by residues H336, S337 and C354[9,17-18], whereas imipenem, ertapenem and meropenem at the outer cavity were located at the opposite side of the catalytic triad[15-16]. Intriguingly, biochemical and structural studies on imipenem, ertapenem and meropenem proposed two sequentially different ligand-binding states for carbapenem binding. State I was proposed as a snapshot of the immediate intermediate state following the acylation step, where residue C354 forms a covalent bond with carbapenems. State II was proposed as the final stable binding state of carbapenems, where the carbapenem molecule undergoes a rotation around the single bond connecting atoms C5 and C6, induces a local protein conformational change characterized by a different rotamer of Y308 and then forms hydrogen bonds with residues Y308 and Y318.

Here, we reported a new ertapenem-binding state, which was referred to as state I-plus and proposed this state as a snapshot of the ligand binding intermediate between states I and II. This new state, together with the previously reported states I and II, provides us with a structural basis for designing drugs against L, D-transpeptidases and a chance to investigate how the enzyme interacts with carbapenems step by step.

2 Material and Methods

2.1 Macromolecule production

The full-length *M. tuberculosis* LdtMt2 (Rv2518c) encodes 408 amino acid residues. A truncated mutation, referred to as LdtMt2-*Δ*N140, was constructed *via* inserting residues 140−408 (from residue 140 to the natural C-terminus, residue 408) into a pMCSG7 vector, which contained an N-terminal TEV protease site and 6×His-tag. The protein was overexpressed and purified using the same protocol as previously reported [15]. In brief, the protein was overexpressed in *Escherichia coli* BL21 (DE3) and purified using a Ni-NTA affinity chromatographic column. The N-terminal His-tag was removed by overnight digestion with TEV protease. The protein was then purified using a Superdex G75 10/600 column (GE Healthcare). The purified protein was concentrated to 20 g/L by ultrafiltration and stored at －80℃. The macromolecule production information is summarized in Table 1.

2.2 Crystallization

Crystallization was performed using the vapor diffusion hanging-drop method at 25℃. The protein was incubated with 10 mmol/L ertapenem at 4℃ for 2 h. The protein solution was mixed with the reservoir solution containing 0.1 mol/L Bis-Tris pH 6.0, 0.2 mol/L NaCl and 25% PEG3350 in a 1∶1 ratio. Crystals appeared within 2 to 3 days. The crystallization information is summarized in Table 2.

Table 1　Macromolecule production information

Source organism	*Mycobacterium tuberculosis*
DNA source	genome
Forward primer	AGCTACATATGCACCATCATCATCATCATTCT
Reverse primer	ATATGGATCCTTACGCCTTGGCGTTACCGGC
Cloning vector	pMCSG7 vector
Expression vector	pMCSG7 vector
Expression host	*E. coli* BL21（DE3）
Complete amino acid sequence of the construct produced	snaq ltfqtsspah ltmpyvmpgd gevvgvgepv airfdeniad rgaaekaiki ttnppvegaf ywlnnrevrw rpehfwkpgt avdvavntyg vdlgegmfge dnvqthftig deviataddn tkiltvrvng evvksmptsm gkdstptang iyivgsrykh iimdsstygv pvnspngyrt dvdwatqisy sgvfvhsapw svgaqghtnt shgclnvsps naqwfydhvk rgdivevvnt vggtlp-gidg lgdwnipwdq wragnaka

The primers indicate any restriction sites，cleavage sites or introduction of additional residues，*e.g.* His6-tag，as well as modifications，*e.g.* Se-Met instead of Met.

Table 2　Crystallization

Method	Hanging-drop vapor diffusion
Plate type	24-well plates
Temperature（℃）	25
Protein concentration	20 g/L
Buffer composition of protein solution	20 mmol/L Tris-HCl pH 8.0，150 mmol/L NaCl
Composition of reservoir solution	0.1 mol/L Bis-Tris pH 6.0，0.2 mol/L NaCl and 25% PEG3350
Volume and ratio of drop	2 μl；1 ∶ 1 ratio
Volume of reservoir	450 μl

2.3　Data collection and processing

The crystal was harvested in liquid nitrogen. The X-ray diffraction data were collected on beamline BL19U at the Shanghai Synchrotron Radiation Facility (SSRF) with a wavelength of 0.98 Å. The data were processed by XDS[19] and scaled by Aimless[20]. The statistics of data collection and processing are summarized in Table 3.

2.4　Structure solution and refinement

The structure was determined using molecular replacement by Phaser[21]. The previously reported structure of LdtMt2-meropenem adduct (PDB ID: 3VYP) was chosen as the initial model. The structure was refined with Phenix. refine[22]. The manual modelling was conducted with COOT [23]. The structure fact and coordinate files were deposited in Protein Data Bank with accession ID of 6LQB. The statistics of refinement are summarized in Table 4.

Table 3　Data collection and processing

Diffraction source	SSRF BL19U1
Wavelength（Å）	0.9777
Temperature（℃）	−173
Detector	Pilatus3 6M
Crystal-detector distance（mm）	250
Rotation range per image（°）	1
Total rotation range（°）	360
Exposure time per image（s）	1
Space group	$P2_12_12_1$
a, *b*, *c*（Å）	68.181, 73.207, 103.803
α, β, γ（°）	90, 90, 90
Mosaicity（°）	0.4
Resolution range（Å）	44.97−1.70（1.73−1.70）
Total No. of reflections	760265（39891）
No. of unique reflections	57876（2990）
Completeness（%）	100（99.9）
Redundancy	13.1（13.3）
$<I/\sigma(I)>$	20.3（3.1）
$R_{r.i.m.}$	0.087（0.963）
Overall *B* factor from Wilson plot（Å^2）	14.2

Values for the outer shell are given in parentheses.

Table 4 Structure solution and refinement

Resolution range（Å）	44.97−1.70
Completeness（%）	99.95
σ cutoff	0
No. of reflections, working set	57794
No. of reflections, test set	2003
Final R_{cryst}	0.181
Final R_{free}	0.209
Cruickshank DPI	0.168
No. of non-H atoms	
Protein	4084
Ligand	129
Water	402
Total	4615
R.m.s. deviations	
Bonds（Å）	0.008
Angles（°）	1.172
Average *B* factors（Å^2）	27.17
Protein	25.81
Ligand	42.53
Water	36.14
Ramachandran plot	
Most favoured（%）	96.58
Allowed（%）	3.42

Values for the outer shell are given in parentheses.

2.5 Mass spectrometric assay

The crystal was harvested and then assayed on a quadrupole-time of flight (Q-TOF) mass spectrometer (Agilent, USA). The mass spectrometric data were processed using the Mass Hunter software (Agilent, USA).

3 Results and Discussion

M. tuberculosis LdtMt2 contains an N-terminal transmembrane helix, two IgG-like domains (residues 55−140 and 141−250) and a C-terminal YkuD catalytic domain (residues 251−408) arranged in the order of amino acid sequence. LdtMt2- ΔN140 (residues 141−408) is a truncated mutant that consists of the second IgG-like domain and the C-terminal YkuD domain. Previously, we reported the LdtMt2-*Δ*N140-ertapenem adduct structure[16], where two protein molecules in an asymmetric unit and their respective covalently bound ligands were proposed as two different intermediate states, states I and II, for the enzyme-drug interaction. In brief, state I represents an intermediate state after ertapenem covalently bound to residue C354 of LdtMt2, and state II shows a stable ertapenem-binding state after the flipping of the pyrrolidine ring of ertapenem and the conformational change of a -hairpin (formed by residues 300−323).

Here, we report a new LdtMt2- ΔN140-ertapenem adduct structure at 1.70 Å resolution with R and R free values of 0.181 and 0.209, respectively. The crystal belongs to space group P212121. Two LdtMt2 molecules are observed in an asymmetric unit, referred to as chains A and B. The structure showed that ertapenem formed a covalent bond with the side chain of residue C354, and the core structure of the bound ertapenem, the opened β-lactam four-membered ring and the pyrrolidine ring, was well defined in the electron density map (Figure 1a). The other moiety of ertapenem is of less clear electron density than the opened β-lactam four-membered ring and the pyrrolidine ring. To evaluate whether the bound ertapenem was hydrolyzed or degraded, the crystal was harvested and assayed with a mass spectrometer. The mass spectrum assay revealed that the sample is dominantly consisted of a species with a molecular weight (30.0 ku) that exactly corresponds to the LdtMt2-ertapenem adduct (LdtMt2 29.5 ku, ertapenem 475.51 u), supporting that ertapenem would not be hydrolyzed or degraded (Figure 2). Meanwhile, all atoms of the ertapenem bound in chain B are well defined, also supporting a full ertapenem molecule in crystal (Figure 1b). Chain A presents a similar conformation to that in state I with a C r.m.s.d of 0.46 Å and a different conformation of residues 308−318 involved in carbapenem binding from that in state II (C r. m. s. d of 0.97 Å). Consistent with this observation, residue Y308 displays the same rotamer as that in state I but a different one from that in state II (Figure 3). Interestingly, its covalently bound ligand ertapenem is in a similar conformation to that in state II (Figure 3b) but not to that in state I (Figure 3a). Ertapenem in state II has been reported to form several hydrogen bonds with the side chains of Y308 and Y318 *via* its carboxyl group on the pyrrolidine ring (Figure 4). However, in this new state, the carboxyl group on the pyrrolidine ring only formed a hydrogen bond with residue Y318, not with residue Y308. In contrast, chain B and its ligand are in the same conformations as those in state II.

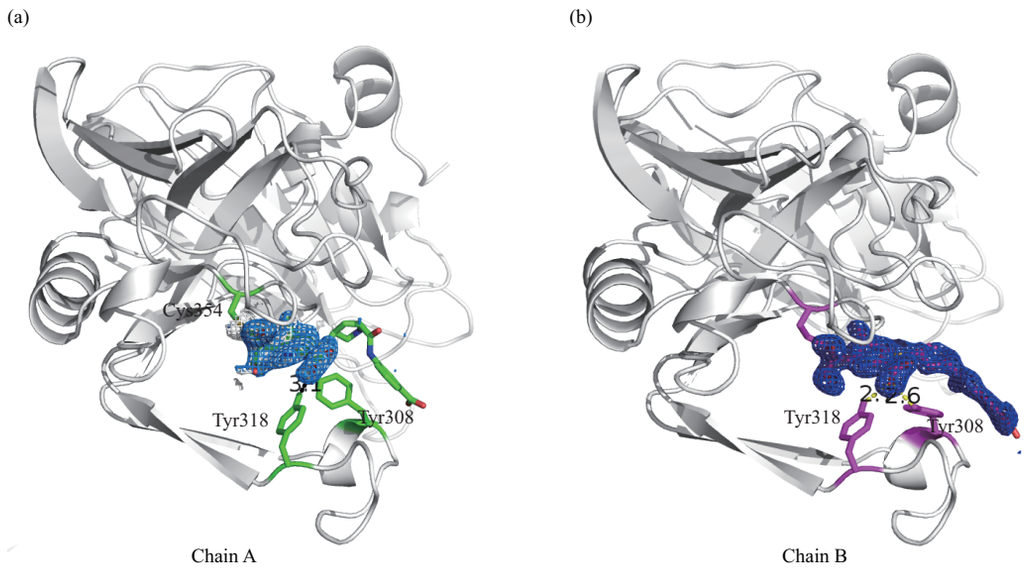

Fig. 1　The state I-plus of LdtMt2-carbapenem adduct

State I-plus (a) and II (b) are observed in a same crystal structure. The bound ertapenem is shown in stick and surrounded by 1fofc ligand-omitted electron density map contoured at 3 δ.

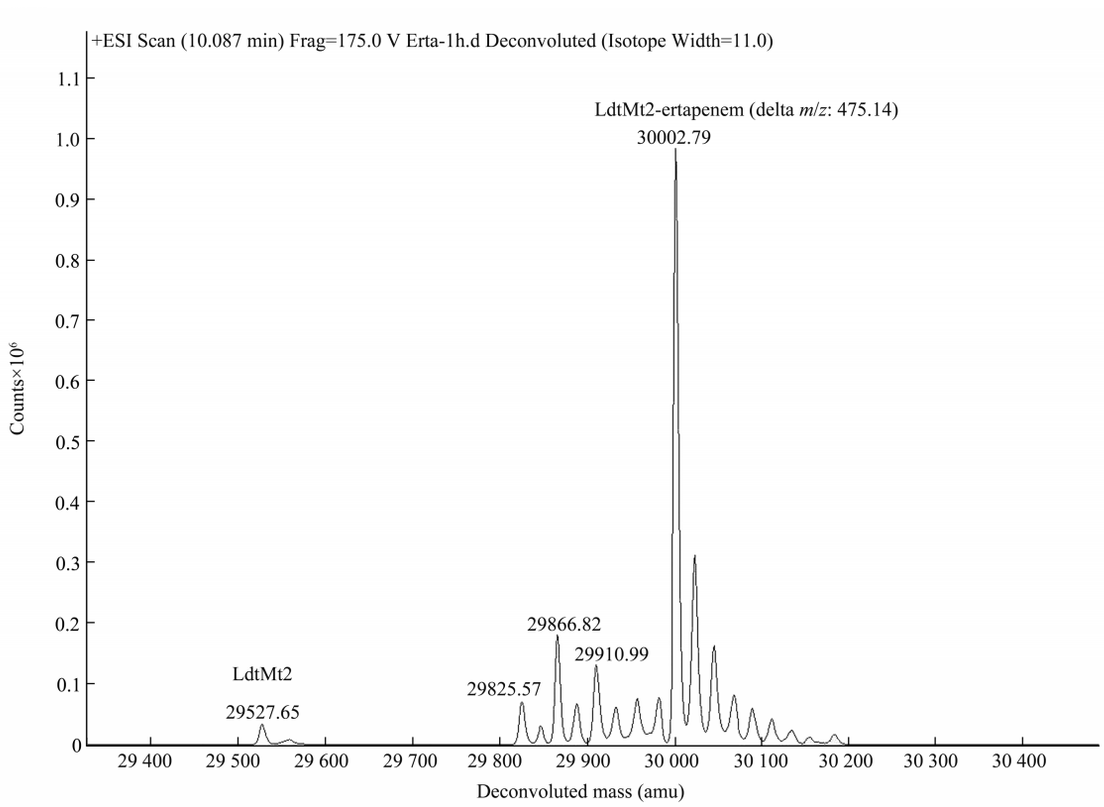

Fig. 2　The mass spectrometric assay of protein crystal

The species with molecular weight of ertapenem-free and -bound LdtMt2 were indicated by LdtMt2 and LdtMt2-ertapenem.

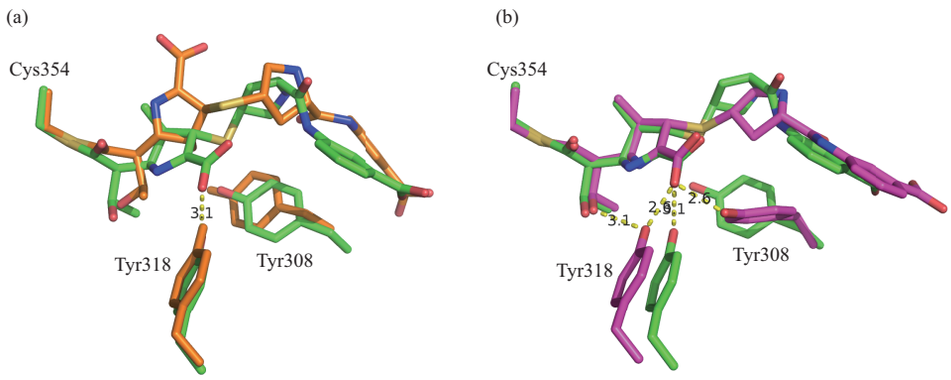

Fig. 3　Local comparison of the state I−plus with state I（a）（PDB ID：6IYV chain A）and state II（b）（PDB ID：6IYV chain B）

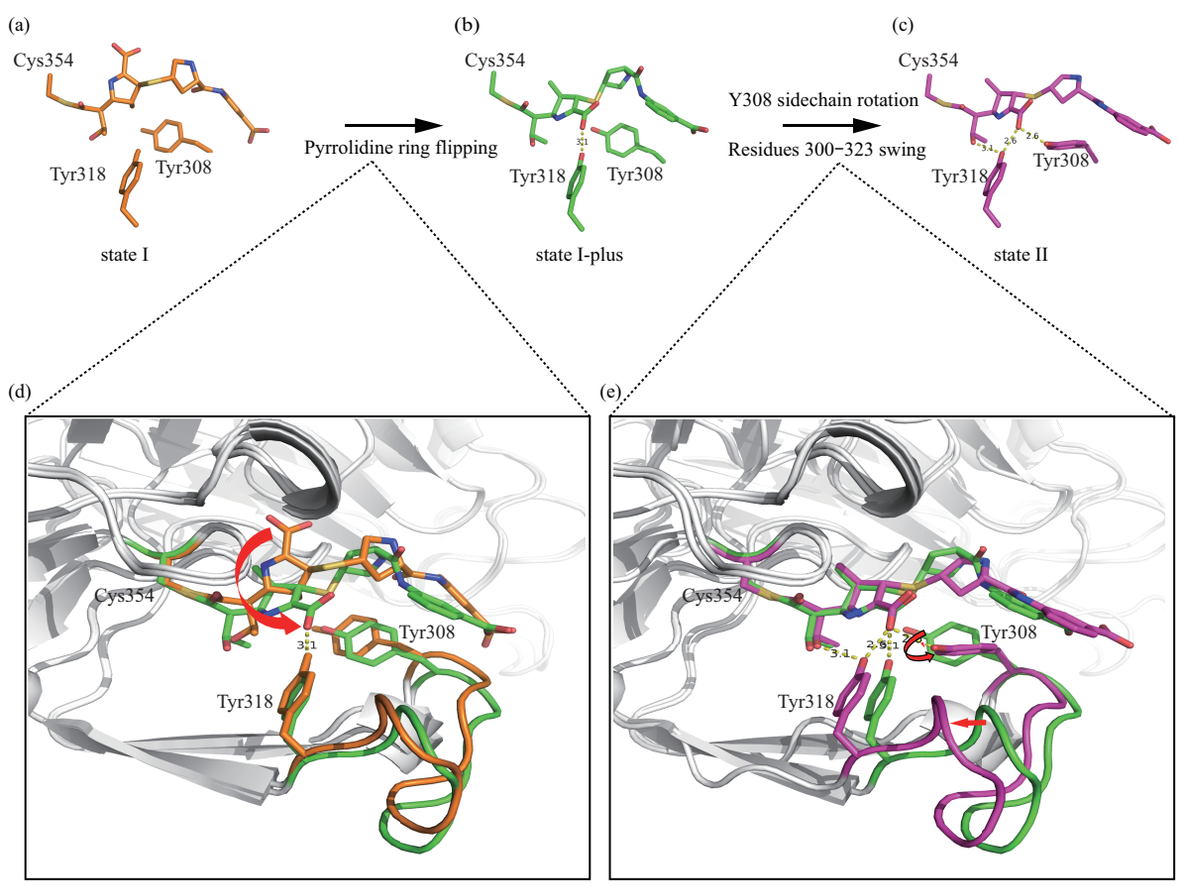

Fig. 4　A proposed model of how LdtMt2−ertapenme adduct transits from State I to State II *via* the State I−plus intermediate

(a−c) State I, I-plus and II are shown in orange (a), green (b) and magenta (c), respectively. (d) The conformational change from state I to state I-plus. The red arrow indicates the flipping of the pyrroliding ring of the carbapenem molecule. (e) The conformational change from state I-plus to state II. The red arrows indicates the rotation of residue 308 sidechain and the swing of residues 300−323.

In a previous study, we observed states I and II for the LdtMt2-carbapenem interaction[15-16]. We have proposed that state I is an immediate intermediate state after enzyme-ligand binding and that state II is a snapshot of the final enzyme-ligand interaction model. We therefore suggested that both the protein and the bound carbapenem would simultaneously undergo conformational changes to form a stable carbapenem-binding state (supplemental movie 1). This hypothesis of the LdtMt2-carbapenem interaction explained why residues Y308 and Y318 are involved in the binding of some carbapenems, such as imipenem, ertapenem and meropenem. However, it did not agree with reports showing that some β-lactams, such as panipenem and faropenem, were observed at a different binding cavity from that bound by imipenem, ertapenem and meropenem, although those β-lactams also formed a covalent bond with residue C354[9,17-18].

In this study, the state I-plus represented by chain A and its ligand in our new structure was proposed as a snapshot of an intermediate between state I and state II, where the ligand underwent a significant conformational change but the enzyme did not. This new state suggested that the conformational changes of the protein and the carbapenem are not simultaneous after the protein binds carbapenems. In other words, the conformational change of the bound carbapenem is independent of the protein conformational change. We therefore proposed that different β-lactams first bind to L, D-transpeptidase *via* the same binding cavity as that of ertapenem (the outer cavity), undergo a conformational change without the accompanied protein conformational change and finally bind to a different cavity (the inner cavity for panipenem and faropenem) or remain at the outer cavity (ertapenem, imipenem and meropenem). This new hypothesis for the interaction of L, D-transpeptidase and carbapenems may explain why different β-lactams were observed bound at different cavities, although more evidence is still needed.

For a long time, D,D-transpeptidases and related enzymes have been proposed as the sole target of traditional β-lactam antibiotics. The mechanisms of how these enzymes interact with β-lactams and how β-lactams eliminate bacterial survival have been extensively studied. However, the identification of L, D-transpeptidase and the observation of the unusual 3→3 transpeptide linkages dominating the linkages in the cell walls of some bacteria, such as *M. tuberculosis*, renewed our knowledge about β-lactams and their actual targets *in vivo*[13-14]. In *M. tuberculosis*, approximately 80% of the transpeptide linkages are the 3→3 type, and LdtMt2 is proposed to contribute to this phenomenon. Moreover, LdtMt2 is involved in bacterial virulence and drug resistance and was proposed as a potential antituberculosis target[10]. Considering this enzyme could be inhibited by carbapenems and not by amoxicillin or cephems, the detailed interaction mechanism should help us to further develop these drugs. Therefore, the intermediate states revealed by our current and previous works will help us to understand the detailed mechanism of how L, D-transpeptidase interacts with carbapenems and to design new drugs or develop carbapenems to fight against bacteria harbouring L,D-transpeptidases.

Supplementary material 20200079_Movie1. gif and 20200079_Movie2. gif are available at paper online（http：//www.pibb.ac.cn or http：//www.cnki.net）

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References

[1] Dramsi S, Magnet S, Davison S, *et al*. Covalent attachment of proteins to peptidoglycan. FEMS Microbiol Rev, 2008, **32**(2): 307-320

[2] Yadav A K, Espaillat A, Cava F. Bacterial strategies to preserve cell wall integrity against environmental threats. Front Microbiol, 2018, **9**:2064

[3] Meroueh S O, Minasov G, Lee W, *et al*. Structural aspects for evolution of beta-lactamases from penicillin-binding proteins. J Am Chem Soc, 2003, **125**(32): 9612-9618

[4] Mainardi J L, Fourgeaud M, Hugonnet J E, *et al*. A novel peptidoglycan cross-linking enzyme for a beta-lactam-resistant transpeptidation pathway. J Biol Chem, 2005, **280**(46): 38146-38152

[5] Kumar P, Arora K, Lloyd J R, *et al*. Meropenem inhibits D, D-carboxypeptidase activity in *Mycobacterium tuberculosis*. Mol Microbiol, 2012, **86**(2): 367-381

[6] Mainardi J L, Legrand R, Arthur M, *et al*. Novel mechanism of beta-lactam resistance due to bypass of DD-transpeptidation in Enterococcus faecium. J Biol Chem, 2000, **275**(22): 16490-16496

[7] Lamichhane G. Novel targets in M. tuberculosis: search for new drugs. Trends Mol Med, 2011, **17**(1): 25-33

[8] Correale S, Ruggiero A, Capparelli R, *et al*. Structures of free and inhibited forms of the L, D-transpeptidase LdtMt1 from *Mycobacterium tuberculosis*. Acta Crystallogr D Biol Crystallogr, 2013, **69**(Pt 9): 1697-1706

[9] Kumar P, Kaushik A, Lloyd E P, *et al*. Non-classical transpeptidases yield insight into new antibacterials. Nat Chem Biol, 2017, **13**(1): 54-61

[10] Gupta R, Lavollay M, Mainardi J L, *et al*. The *Mycobacterium tuberculosis* protein LdtMt2 is a nonclassical transpeptidase required for virulence and resistance to amoxicillin. Nat Med, 2010, **16**(4): 466-469

[11] Gokulan K, Khare S, Cerniglia C E, *et al*. Structure and inhibitor specificity of L, D-transpeptidase (LdtMt2) from *Mycobacterium tuberculosis* and antibiotic resistance: calcium binding promotes dimer formation. AAPS J, 2018, **20**(2): 44

[12] Zaman K. Tuberculosis: a global health problem. J Health Popul Nutr, 2010, **28**(2): 111-113

[13] Lavollay M, Fourgeaud M, Herrmann J L, *et al*. The peptidoglycan of *Mycobacterium* abscessus is predominantly cross-linked by L, D-transpeptidases. J Bacteriol, 2011, **193**(3): 778-782

[14] Hugonnet J E, Tremblay L W, Boshoff H I, *et al*. Meropenem-clavulanate is effective against extensively drug-resistant *Mycobacterium tuberculosis*. Science, 2009, **323**(5918): 1215-1218

[15] Li W J, Li D F, Hu Y L, *et al*. Crystal structure of L, D-transpeptidase LdtMt2 in complex with meropenem reveals the mechanism of carbapenem against *Mycobacterium tuberculosis*. Cell Res, 2013, **23**(5): 728-731

[16] Zhao F, Hou Y J, Zhang Y, *et al*. The 1-beta-methyl group confers a lower affinity of l,d-transpeptidase LdtMt2 for ertapenem than for imipenem. Biochem Biophys Res Commun, 2019, **510**(2): 254-260

[17] Steiner E M, Schneider G, Schnell R. Binding and processing of beta-lactam antibiotics by the transpeptidase LdtMt2 from *Mycobacterium tuberculosis*. FEBS J, 2017, **284**(5): 725-741

[18] Bianchet M A, Pan Y H, Basta L a B, *et al*. Structural insight into the inactivation of *Mycobacterium tuberculosis* non-classical transpeptidase LdtMt2 by biapenem and tebipenem. BMC Biochem, 2017, **18**(1): 8

[19] Kabsch W. Xds. Acta crystallogr D biol crystallogr, 2010, **66**(Pt 2): 125-132

[20] Winn M D, Ballard C C, Cowtan K D, *et al*. Overview of the CCP4 suite and current developments. Acta Crystallogr D Biol Crystallogr, 2011, **67**(Pt 4): 235-242

[21] Bunkoczi G, Echols N, Mccoy A J, *et al*. Phaser. MRage: automated molecular replacement. Acta Crystallogr D Biol Crystallogr, 2013, **69**(Pt 11): 2276-2286

[22] Afonine P V, Grosse-Kunstleve R W, Echols N, *et al*. Towards automated crystallographic structure refinement with phenix. refine. Acta Crystallogr D Biol Crystallogr, 2012, **68**(Pt 4): 352-367

[23] Emsley P. Tools for ligand validation in Coot. Acta Crystallogr D Struct Biol, 2017, **73**(Pt 3): 203-210

L,D–转肽酶LdtMt2–厄他培南加合物的新状态Ⅰ–plus结构*

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摘要　具有多重耐药性和广泛耐药性的结核分枝杆菌菌株正在全球范围内传播，因此迫切需要新的抗结核药物. 结核分枝杆菌的L,D-转肽酶LdtMt2直接参与肽聚糖的形成、细菌毒性和β-内酰胺抗性. 该酶是潜在的抗结核靶标，可以被碳青霉烯类抗生素抑制，碳青霉烯类抗生素是FDA批准的用于治疗肺结核的药物. 据报道，LdtMt2与碳青霉烯类抗生素（如厄他培南、亚胺培南和美洛培南）相互作用时，存在两种不同的中间状态，即状态I和II. 状态I被认为是初始加合物形成状态，而状态II被认为是稳定的蛋白质-配体相互作用状态，并伴有碳青霉烯类抗生素和蛋白质的局部构象排列. 本文报告了一个LdMt2-ertapenem新中间状态I-plus，具有与状态II相同的碳青霉烯类抗生素构象和与状态I相似的局部蛋白质构象. 该新状态是从状态I转变为状态II的中间状态，构象变化发生在厄他培南分子而不是蛋白质. 我们的工作有助于阐明碳青霉烯类抗生素对LdtMt2作用后发生的变化，并与其他报道的中间状态一起揭示L,D-转肽酶如何与碳青霉烯类抗生素相互作用.

关键词　L,D-转肽酶，LdtMt2，厄他培南，β-内酰胺
中图分类号　Q5，Q7　　**DOI**：10.16476/j.pibb.2020.0079

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