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Structure and Dynamics of CTX-M Enzymes Reveal Insights into Substrate Accommodation by Extended-spectrum β-Lactamases

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Oxyimino-cephalosporin antibiotics, such as ceftazidime, escape the hydrolytic activity of most bacterial β-lactamases. Their widespread use prompted the emergence of the extended-spectrum β-lactamases CTX-Ms, which have become highly prevalent. The C7 β-amino thiazol-oxyimino-amide side chain of ceftazidime has a protective effect against most CTX-M β-lactamases. However, Asp240Gly CTX-M derivatives demonstrate enhanced hydrolytic activity against this compound. In this work, we present the crystallographic structures of Asp240Gly-harboring enzyme CTX-M-16 in complex with ceftazidime-like glycylboronic acid (resolution 1.80 Å) and molecular dynamics simulations of the corresponding acyl–enzyme complex. These experiments revealed breathing motions of CTX-M enzymes and the role of the substitution Asp240Gly in the accommodation of ceftazidime. The substitution Asp240Gly resulted in insertion of the C7β side chain of ceftazidime deep in the catalytic pocket and orchestrated motions of the active serine Ser70, the β3 strand and the omega loop, which favored the key interactions of the residues 237 and 235 with ceftazidime.

Keywords: β-lactamases; ceftazidime; CTX-M; spectrum; structure

Introduction

The production of β-lactamases is the predominant cause of resistance to β-lactam antibiotics in Gram-negative bacteria. Oxyimino-cephalosporins such as ceftazidime and cefotaxime escape the hydrolytic activity of most β-lactamases. These β-lactams harbor bulky C7β aminothiazol-oxyimino-amide side chains that make them inherently less susceptible to β-lactamases. The methyl function of the cefotaxime oxyimino group is replaced by a carboxypropyl function in ceftazidime (Fig. 1a). Unfortunately, their widespread use prompted the emergence of extended-spectrum β-lactamases (ESBLs). These enzymes confer resistance to oxyimino-cephalosporins and lead to poor clinical outcomes. Before 2000, most ESBLs were derived from penicillinases, such as TEM-1 or SHV-1, by point substitutions. In these ESBLs, the substitutions displace two major walls of catalytic pocket, the β3 strand or the omega loop, and lead to an enlarged active site, which is able to recognize the large aminothiazol-oxyimino-cephalosporins.[1,2]

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Abbreviations used: ESBL, extended-spectrum β-lactamase; MDS, molecular dynamics simulation.

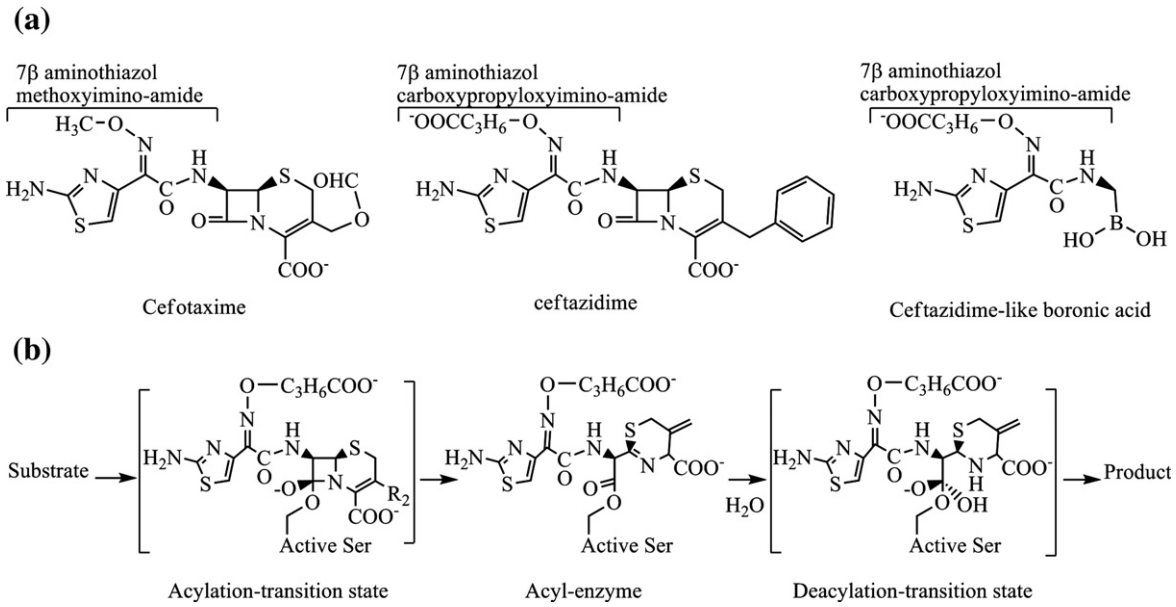

Fig. 1. Characteristics of β-lactams and the reaction cycle of serine β-lactamases. (a) Aminothiazol-oxyimino β-lactam antibiotics and the ceftazidime-like glycylboronic acid. (b) The basic reaction pathway is shown with the acylation and deacylation transition states.

In 1995, CTX-M enzymes, a new group of ESBLs, emerged worldwide and are now the most frequently observed ESBLs.[3] CTX-M enzymes share less than 40% identity with TEM- and SHV-type enzymes. They differ from most TEM and SHV ESBLs by a much greater hydrolytic activity against cefotaxime than against ceftazidime. However, Gly240-harboring CTX-M enzymes exhibit an unusually high activity against ceftazidime. CTX-M-16, which derives from CTX-M-9 by the substitution Asp240Gly, exhibits a 10-fold higher catalytic efficiency against ceftazidime (k_{cat}/K_m, 0.043 *versus* 0.004 μM^{-1} s^{-1}) than CTX-M-9.[3] Unlike the well-studied TEM and SHV enzymes, there have been few structural studies of the CTX-M family.[4–9] The surprising feature of CTX-M structures is that the active site is not enlarged.

ESBLs such as CTX-Ms use a reactive serine (Ser70), a catalytic water molecule and the activator residue Glu166 to hydrolyze the β-lactam ring by an acid–base catalytic mechanism (Fig. 1b).[2,10–14] Glycylboronic acid-based compounds are useful for probing substrate recognition in that they are reversible inhibitors of CTX-M β-lactamases and bind to such β-lactamases as acylation transition-state analogs (step 2, Fig. 1b).[9]

We obtained the X-ray structure of the CTX-M-9 in complex with a ceftazidime-like boronic acid,[9] which bears the C7β aminothiazol-oxyimino-amide side chain of ceftazidime (Fig. 1a). The derivative CTX-M-16 was four-fold more susceptible (K_i, 4 *versus* 15 nM) than CTX-M-9 to this ceftazidime analogue inhibitor.[9] To investigate this behavior, in this present study we determined the X-ray structure of CTX-M-16 in complex with ceftazidime-like boronic acid. The acyl–enzyme structures of CTX-M-9 and CTX-M-16 in complex with ceftazidime were then modeled from these crystallographic structures and analyzed by molecular dynamics simulations (MDSs). The results provided insight into the recognition of aminothiazol-oxyimino cephalosporins and the enzymatic dynamics.

Table 1. Data processing and crystallographic refinement statistics for CTX-M-16 crystal structure in complex with ceftazidime-like boronic acid

Cell constants	a=45.12 Å b=106.66 Å c=47.76 Å β=102.14°
Resolution (Å)	1.80 (1.86–1.80)[a]
Total reflections	112,493
Unique reflections	37,585
R_{merge} (%)	6.6 (29.81)[b]
Completeness (%)	94.3 (87.6)[b]
$[I]/[\sigma(I)]$ (°)	7.9 (2.1)[b]
Resolution range for refinement	10–1.80
No. of protein residues	524
No. of water molecules	897
RMSD	
Bond lengths (Å)	0.010
Angle (deg.)	1.55
R-factor (%)	14.6
R_{free} (%)[c]	20.0
Average B-factor	
Protein	8.45
Compound	18.92
Water molecules	23.01

[a] Value in parentheses is for the highest resolution shell used in refinement.
[b] Values in parentheses are for the highest resolution shell information used in refinement.
[c] R_{free} was calculated with 5% of reflexions set aside randomly.