C-159

The crystal structure of the carbapenemase OXA-24 in complex with tazobactam reveals evidences about the interaction with β-lactamase inhibitors and lysine carboxylation.

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Background

The class D (OXA) β-lactamases contains more than 400 members classified in different sub-groups depending on their preferred substrates or kinetic behavior, and genetic origin. A particularly interesting group includes enzymes having increased hydrolytic activity towards carbapenems, the "carbapenem hydrolyzing class D β-lactamases" (CHDL). Within this group, mainly found in *Acinetobacter* spp., the OXA-24/OXA-40 group is one of the most prevalent. Class D enzymes are generally characterized by a poor sensitivity to clavulanic acid, sulbactam and tazobactam, the β-lactamase inhibitors clinically used in combination with a partner β-lactam to conter the β-lactamase-mediated hydrolysis of the latter.

These inhibitors act as very slow substrates of class A β-lactamases, forming a Michaelis-Menten complex long enough so that partner antibiotics are not cleaved before blocking the PBPs.

In this study, we determined the structure of OXA-24 in complex with the mechanism-based inhibitor tazobactam, previously reported as a modest inhibitor by preliminary kinetic studies, and evaluated the role of some class D specific residues in the interaction with this inhibitor, in order to understand the weaker inhibition observed compared to other serine β-lactamases.

Methods

Protein Purification and Crystallization

The expression and purification of the OXA-24 enzyme were performed as described previously (1). Crystals were grown at 20 °C by hanging drop vapor diffusion, 2 µl of 10 mg/mL protein solution (20 mM Tris pH8) were mixed with 2 µl of a 1M ammonium sulfate and 0.1 M bis-tris at pH 6.5 solution. The crystal was soaked for 10 minutes in a 50 mM tazobactam solution.

Data Collection, Structure Determination and Refinement

Data were collected at 100°K on Dectris Pilatus 6M detector at a wavelength of 0.98011 Å on beamline Proxima 1 at the Soleil Synchrotron Facility (Paris, France). X-Ray diffraction experiments were carried out under cryogenic conditions (100°K) after transferring the crystals into a 45% glycerol and 1.8M ammonium sulfate solution. Indexing, integration and scaling were carried out using XDS (2). Refinement to 1.95 Å was carried out using REFMAC5 (3) and Coot (4).

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Results

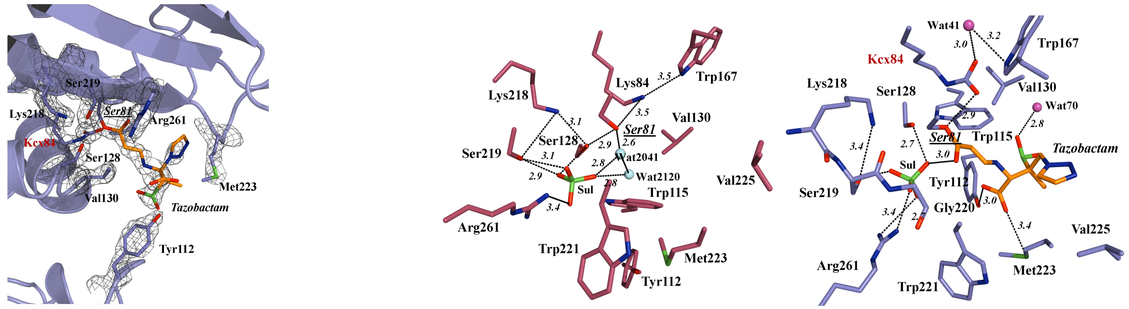

Figure 1. Detailed view of the structure of active site of OXA-24 β-lactamase in complex with tazobactam. Left: $2F_o - F_c$ map contoured at 1.6 σ is shown in grey around tazobactam trans-enamine derivative (in orange). Important amino acid residues are shown in marine blue. Right: Main hydrogen bonds implicated in the stabilization of tazobactam (blue), compared to the apo OXA-24 (PDB: 2JC7; pink), along with the corresponding distances in angstroms (black dashed lines). Kcx: carboxylated lysine in OXA-24/tazobactam. Other color references: oxygen (red), nitrogen (blue), sulfur (green).

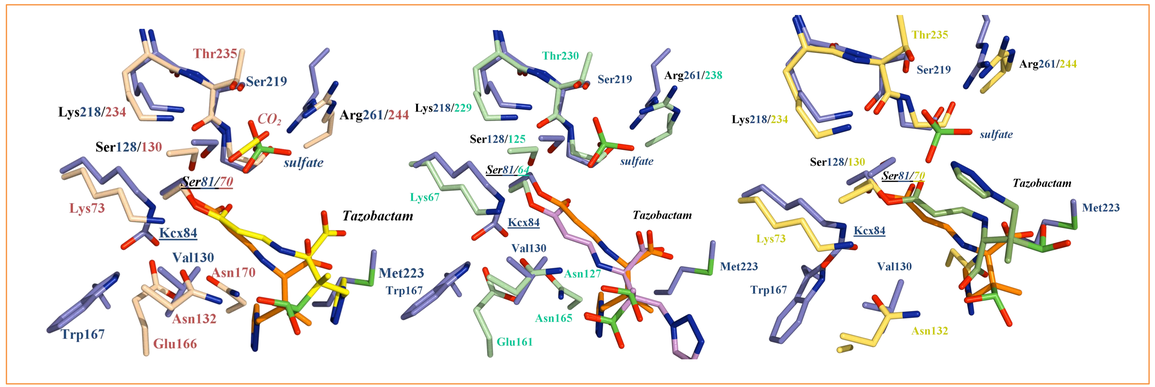

Figure 2. Comparison of OXA-24 in complex with tazobactam with different class A β-lactamases. Left: *Bacillus licheniformis* BS3 (PDB: 4A5R; salmon); center: GES-2 (PDB: 3NIA; aquamarine); right: SHV-1 (PDB: 1RCJ; yellow). Residues numbering is in agreement with Ambler's scheme, or according to the information retrieved from the PDB entries. Other color references: oxygen (red), nitrogen (blue), sulfur (green).

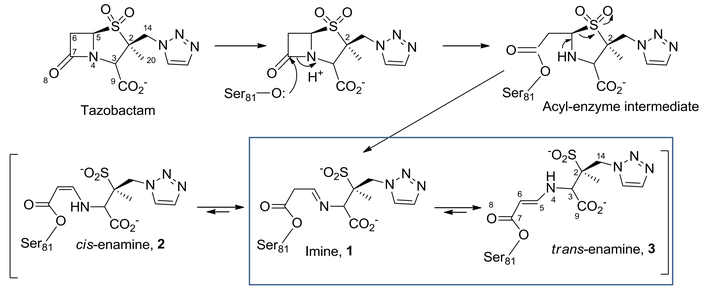

Figure 3. Postulated mechanism for the association of OXA-24 with tazobactam:

1. Upon nucleophilic attack of Ser81, tazobactam rings are opened so that an imine or enamine form of tazobactam are formed, probably co-existing in equilibrium. In the case of the 3NZT structure, hydrolyzed tazobactam is observed as the trans-enamine form.
2. .....

Table 1: X-Ray data collection and refinement statistics for OXA-24 β-lactamase in complex with tazobactam

Crystal	OXA-24 / tazobactam
PDB code	3znt
Data Collection:	
Space group	P 41 21 2
Cell parameters (Å)	a = b = 102.9, c = 86.3, α = β = γ = 90
Resolution range (Å)[a]	46.02 – 1.95 (2.06 – 1.95)
Total number observations	604768
No. of unique reflections	34412
R_{merge} (%)[a,b]	7.0 (51.4)
Redundancy [a]	17.6 (17.9)
Completeness (%)[a]	100 (100)
<I>/<σI> [a]	25.5 (7.0)
Refinement:	
Resolution range	46.0 – 1.95
No. of protein atoms	2053
Number of water molecules	78
R cryst (%)	18.1
R free (%)	20.9
RMS deviations from ideal stereochemistry	
bond lengths (Å)	0.012
bond angles (°)	1.43
Mean B factor (all atoms) (Å²)	32.5
Ramachandran plot:	
favoured region (%)	97.9
allowed regions (%)	2.1
outlier regions (%)	0.0

[a] Statistics for the highest resolution shell are given in parentheses.
[b] Rmerge = $\sum | I_i - I_m | / \sum I_i$ where I_i is the intensity of the measured reflection and I_m is the mean intensity of all symmetry-related reflections.

Conclusions

The presence of bulky hydrophobic residues (Tyr112, Trp115, Trp221 and Met225), as well as modifications in the hydrogen network within the active site of OXA-24 could reduce the affinity towards inhibitors like tazobactam and/or prevent the reorganization of the molecule required for an efficient inactivation.

The presence of Met223 and Val130 (instead of Asn in the second conserved motif), contribute to a modified hydrogen bond network that could alter the acyl-enzyme complex, partially reflected by the opposite orientation of the tazobactam's thiazolidine ring.