Abstract

Avibactam is a non-beta-lactam beta-lactamase inhibitor in phase III clinical development that is active against class A, C, and some class D enzymes. Its spectrum of activity is wider than the beta-lactamase inhibitors ticarcillin, sulbactam and clavulanic acid. About 400 class D beta-lactamases have been identified so far. These display a great variety of sequence and substrate specificity including carbapenemase activity for enzymes such as OXA-24/48 and so which are respectively expressed by clinically important A. baumannii and K. pneumoniae strains. The efficacy of avibactam on class D beta-lactamases shows large fluctuations that cannot be explained only by the lack of structural data.

In this study, we measured the inhibition of the carbapenemase OXA-24 and the cephalosporinase OXA-163 by avibactam, showing a significantly higher susceptibility for the latter. In addition, we determined the structures of the OXA-24/avibactam complex by x-ray crystallography at a 3.1Å resolution. The ring-opened avibactam molecule is covalently bound to the active Ser81 of OXA-24. The corresponding 2Fo-Fc electron density map is shown at a 1σ level and dashed lines denote hydrogen bonds between avibactam and the protein. The first motif (Lys65) is only 6% defined.

The bond between the sulfate part of avibactam and the rest of the molecule seems to be cleared as proposed by Ehmann et al. but stays bound to Arg261 and to Lys218 and Ser219 from the third conserved motif.

From the available data, the lower susceptibility of OXA-24 to avibactam compared to OXA-48 and -163 could not be attributed to a single structural feature. Several factors are therefore likely responsible.

Introduction

Penicillin Binding Proteins (PBPs) are the target of the widely used antibiotics of the beta-lactam family. They belong to 2 groups, the high molecular mass PBPs which are multidomain and involved in the peptidoglycan polymerization (class A and B) and the low molecular mass PBPs (class C) involved in peptidoglycan maturation and degradation.

One of the most efficient ways developed by bacteria to confer resistance to antibiotics is the expression of proteins (beta-lactamases) capable of hydrolyzing them. These enzymes belong to four classes (A-D) depending on their sequence similarity and catalytic mechanisms.

Inhibiting both families of enzymes simultaneously is a very efficient antibacterial strategy. However, resistance mechanisms arise more and more represent a growing threat for patients in hospitals, emphasizing the continuous need for new efficient molecules.

Avibactam is a non-beta-lactam beta-lactamase inhibitor in phase III clinical development that is active against class A, C and some class D enzymes.

In this work, we present the structure of the class D OXA-24/avibactam and the class C PBP with DD-peptidase activity from Actinobacillus R39 (R39 DD-peptidase) in complex with avibactam, The acylation rates of OXA-48, OXA-163 and the R39 DD-peptidase for this compound were also determined.

Methods

Protein purification and crystallization.

The OXA-24 beta-lactamase and the R39 DD-peptidase were expressed and purified as described previously [2, 3]. Crystals were grown at 30 °C by hanging drop vapor diffusion. For OXA-24, 2 μL of 10 mg/ml protein solution (20 mM Tris pH 8) were mixed with 2 μL of a 1 mM ammonium sulfate and 0.1 M bis-tris at pH 6.1 solution. The crystal was soaked for 25 minutes by adding 0.5μl of a 0.5M avibactam solution. For the R39 DD-peptidase, 2.5 μL of 25 mg/ml protein solution (also containing 5 mM MgCl2 and 20 mM Tris, pH 9) were mixed with 2 μL of well solution (2 mM ammonium sulfate and 0.1 M MES, pH 9) and 0.5 μL of 0.1 M CoCl2 solution. Crystals were soaked in a solution containing 0.6 μL of 3 M ammonium sulfate and 0.1MM MES, pH 6, and 0.4 μL of 0.1 M CoCl2 solution.

Structure determination.

Data were collected on Proxima 1 beamline of the Soleil Synchrotron (Saint Aubin, France). X-rays were monochromated, integrated and scaled using XDS. Refinement and building were carried out using REFMAC5 (4) and COOT (8). Data and refinement statistics are summarized in Table 1.

Structural dynamics.

The acylation rates of OXA-24, OXA-163 and the R39 DD-peptidase for avibactam were determined from racemization experiments using the reporter substrates ristocetin, cephalosporin and Thiolleucine S26.

Acknowledgment

We are grateful to the staff of the Proxima 1 beamline of the Soleil Synchrotron (Saint Aubin, France) for excellent technical assistance. This research was funded in part by AstaZeneca (Walldorf, USA,).