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Studies on the Inactivation of Class C β-Lactamases by Carbapenems: Doripenem and P99 Cephalosporinases

Nozomi OHUCHI[1], Robert BONOMO[2], Nobutada TANAKA[3], Kazuo T. NAKAMURA[3], Michiyoshi NUKAGA*[1]

[1]Faculty of Pharmaceutical Sciences, Josai International University, Togane-shi, Chiba 283-8555, Japan

[2]Research Service, Veterans Affairs Medical Center, Cleveland, Ohio 44106, USA

[3]School of Pharmaceutical Sciences, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan

Introduction

Class C cephalosporinases possess a wide substrate spectrum, a high catalytic efficiency (*kcat*/Km), and are resistant to β-lactamase inhibitors. These properties result in enzymes that have emerged as a significant clinical threat.

Carbapenem antibiotics possess the broadest antibacterial spectrum of all β-lactams. As intravenous formulations, carbapenems are primarily used in the treatment of serious nosocomially acquired infections caused by penicillin and cephalosporin resistant bacteria. Carbapenem antibiotics are often the "last resort" in the treatment of infections caused by bacteria resistant to penicillins and cephalosporins.

The class C AmpC β-lactamase of *Escherichia coli* inactivated by imipenem forms a covalent complex in the active site. This structure (PDB 1LL5) demonstrates that imipenem is bound to Ser64 and that the carbonyl oxygen of the acylserine bond was not positioned in the oxyanion hole; imipenem was rotated 180°, moved approximately 3 Å away from its expected location and adopts a conformation similar to that seen in the TEM-1-imipenem complex.

In order to understand doripenem (DOR), newer carbapenem, behavior, we studied the reaction of *Enterobacter cloacae* P99 β-lactamase with doripenem and the atomic structure of doripenem inactivated P99 β-lactamase was solved to 1.0 Å resolution.

Methods

P99 β-lactamsae was expressed in *E. coli* AS226-51 and purified by *m*-aminophenyl boronic acid affinity, ion exchange and gel filtration columns, to homogeneity. Kinetics and mass spectrometry were preformed. The vapor diffusion method was applied for ligand-free P99 crystallization at room temperature. The 39.8 kDa P99 β-lactamase crystallizes in space group $P2_12_12$ with one molecule in the asymmetric unit and the following cell dimensions: a =49.6 Å, b = 69.2 Å, and c = 62.0 Å. A crystal was soaked at room temp. for 30 min in a PEG holding solution containing 5 mM DOR before data collection.

Result and Discussion

The Ki^{app} of DOR = 3. 7 ± 0.5 uM; k_{inact} = 0.32 ± 0.02 s^{-1}; k_{inact}/Ki^{app} = 0.09 ± 0.01 $uM^{-1}s^{-1}$; and tn=5. We established that P99 had a charge to mass ratio (m/z) of 39239 ± 4 amu (39235 expected). Reacting DOR (I, inhibitor) with P99 (E, enzyme) in a 5:1 (I/E) ratio we demonstrated two covalently acylated products (m/z= 39660 ±4 and 39616 ± 4). These correspond to the acyl enzyme and a modification of DOR reacted with P99 (-C_2H_4O).

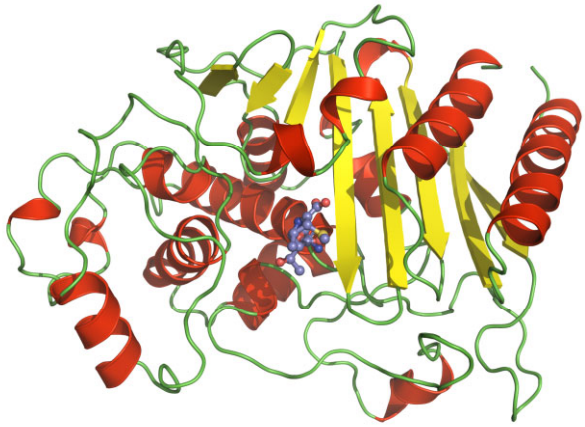

Fig. 1 Ribbon diagram of the P99 β-lactamase complex with doripenem, drawn by Pymol.

The atomic structure of P99 was solved at 1.0 Å resolution [RMSD difference=0.449 for P99 apo PDB: 1XX2 (Cα of res num. 5-355)]. We observed only one conformation is present: the carbonyl oxygen of DOR is positioned in the oxyanion hole (S64 and S318) and the C2 carbon is seen in the sp3 configuration, suggesting there is Δ^1 pyrroline ring (Δ^2-Δ^1 tautomerization). This binding form is quite different from AmpC-imipenem complex.

References

[1] M. Nukaga et al., J Am Chem Soc. 130, 12656 (2008).

* nukaga@jiu.ac.jp