Communication

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Catherine Michaux, Paulette Charlier, Jean-Marie Frre, and Johan Wouters

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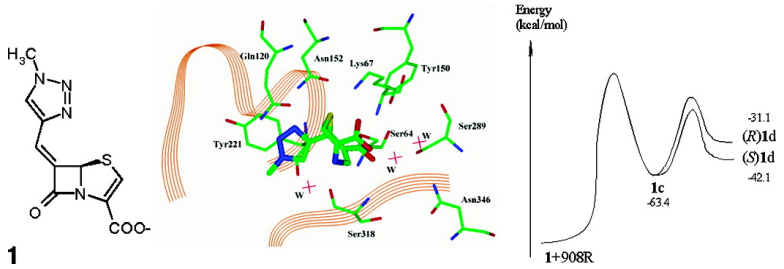

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Crystal Structure of BRL 42715, C6-(*N*1-Methyl-1,2,3-triazolylmethylene)penem, in Complex with *Enterobacter cloacae* 908R β-Lactamase: Evidence for a Stereoselective Mechanism from Docking Studies

Catherine Michaux,[†] Paulette Charlier,[‡] Jean-Marie Frère,[‡] and Johan Wouters*,[†]

Laboratoire de Chimie Biologique Structurale, Université of Namur, 5000 Namur, Belgium, and CIP Université of Liège, 4000 Liège, Belgium

Received December 8, 2004; E-mail: johan.wouters@fundp.ac.be

β-Lactamases are a major cause of bacterial resistance to β-lactam antibiotics such as penicillins, cephalosporins, carbapenems, and monobactams.[1,2] An efficient strategy to overcome the resistance of β-lactamase-containing pathogens to β-lactam antibiotics is co-administration of the enzyme-susceptible β-lactam with a β-lactamase inhibitor.[3] BRL 42715, C6-(N1-methyl-1,2,3-triazolylmethylene)penem, **1** (Scheme 1), is an active-site-directed inactivator of a broad range of bacterial β-lactamases, including the class C enzymes.[4,5] This compound is very active as reflected in the low concentrations of **1** needed to potentiate the antibacterial activity of β-lactamase-susceptible β-lactams.[6−11]

The interactions of β-lactamases of classes A, B, C and D with **1** were investigated kinetically.[12] The mechanism of action of **1** toward class C β-lactamases is here investigated and the crystal structure of *Enterobacter cloacae* 908R β-lactamase in complex with **1** is reported (Table 1).

A stable covalent adduct, a cyclic β-aminoacrylate−enzyme complex, resulting from acylation of the active site serine by the penem followed by intramolecular rearrangement leads to the corresponding dihydrothiazepine (Figure 1). The β-lactamyl carbonyl of the covalent adduct is situated in the oxyanion hole between helix H2 and β-strand B3 where it is H-bonded to Ser318. Orientation of the ligand into the active site is the result of a series of interactions: (i) the methyl triazolyl cycle stacks with Tyr221 and the N3′ atom is involved in H bonding with Asn152 (2.98 Å); (ii) the N4 atom is hydrogen-bonded (2.59 Å) to a water molecule stabilized by two other water molecules; (iii) the carboxylate group, which interacts with another water molecule (3.1 Å) and lies near Asn346 and Ser289, and the triazolyl group are situated on the opposite side of the dihydrothiazepine ring; (iv) the sulfur atom of the dihydrothiazepine cycle lies near Leu119, Asn152, Lys67, and Tyr150.

This structure confirms a mechanism that implies opening of the five-membered thiazole ring system at the C5−S bond upon alcoholysis and rearrangement via a Michael addition to form a seven-membered dihydrothiazepine ring system (Scheme 2).

This structure is in good agreement with spectral properties of the product that are identical to those of the dihydrothiazepine obtained after sodium hydroxide hydrolysis[13] and with mass spectrometry results.[12]

The binding mode and geometry of the covalent adduct of **1**d in class C 908R β-lactamase are quite different from that of a penem analogue, **2** (Scheme 1), in complex with class C extended-spectrum GC1 β-lactamase[14] but consistent with differences observed for a transition-state analogue of cefotaxime in the parental and extended-spectrum enzymes.[15] In particular, the absolute configuration of the cyclic β-aminoacrylate−enzyme complex **1d** is *S* while the other enantiomer of **2** forms upon complexation with GC1 β-lactamase. The absolute configuration at the stereogenic carbon C7 observed in the structure **1d** is consistent with indirect evidences of the literature.[16]

Scheme 1. Chemical Structure of Methylene Penem Inactivators

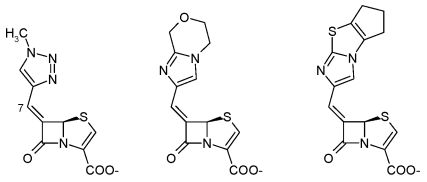


Table 1. Data Collection and Refinement Statistics

space group	$P2_12_12$		
cell index (Å)	$a = 44.158$	completeness (%)[a]	98.5
	$b = 82.042$		(89.6)[a]
	$c = 95.709$	R_{sym}[a,b]	7.1 (21.6)[a]
		I/σ[a]	7.8 (2.4)[a]
wavelength (Å)	0.966	R_{work}/R_{free}[c]	22.7/29.2
resolution (Å)	2.07	rmsd from ideal geometry	
unique reflections	20153	bonds (Å)	0.007
observed reflections	15035	angles (deg)	0.021

[a] Values listed in parentheses are for the resolution shell (2.11−2.07 Å). [b] $R_{sym} = \Sigma|I - \langle I\rangle|/\Sigma$. [c] $R_{factor} = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. R_{free} was calculated with 10% of the reflections set aside randomly throughout the refinement.

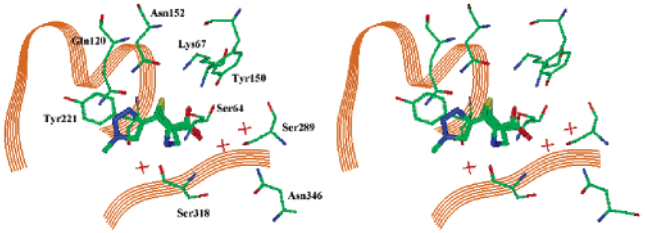
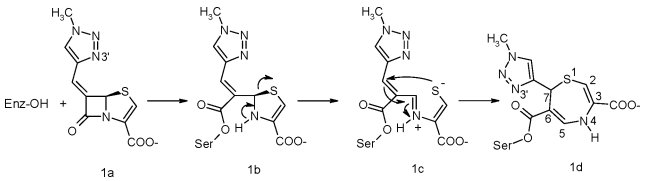


Figure 1. 908R β-lactamase binding site (stereoview) complexed with intermediate **1**d (PDB File 1Y54). Red crosses stand for water molecules.

Scheme 2. Opening of the Five-Membered Thiazole Ring System of **1** (a) Leading to a Cyclic β-Aminoacrylate−Enzyme Complex (d) Covalently Bound to the Enzyme via Ser64



[†] Université of Namur.
[‡] Université of Liège.

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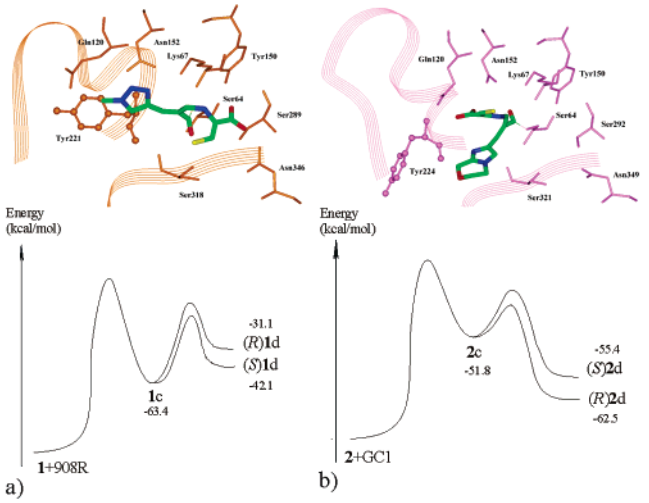

Figure 2. Energy diagrams for (a) the reaction of **1** with a class C β-lactamase in the "close folding"; (b) the reaction of **2** with a class C β-lactamase in the "open folding".

The overall structure of the GC1 enzyme is equivalent to that of the 908R enzyme. In the later structure, the so-called Ω loop (189−226) is well defined. The extended specificity of the GC1 enzyme appears to be entirely due to only additional three residues (213-215) after position 210 in this Ω loop.[17,18] These additional amino acids lead to a more flexible binding site and allow an alternative fold ("open folding") in which Tyr224 (equivalent to Tyr221 in 908R) is displaced by ∼6 Å relative to its position in the free enzyme. As a result, the main difference between the two enzymes complexes is the position of this tyrosine residue. This has consequences for the binding mode of the ligand. Indeed, in 908R the triazolyl cycle of **1** is able to stack with Tyr221, unlike the heterocyclic double ring of **2** in GC1. Moreover, the carboxylate moiety of **1** does not interact with Gln120 and Asn152 while it does so for **2** in CG1. This explains why the absolute configuration is reversed.

Docking and energy minimization studies[19] were performed to further understand and quantify differences in conformation and stereochemistry between the complexes observed with the 908R and GC1 class C β-lactamases. Those preliminary calculations open interesting perspectives.

A structure corresponding to an intermediate covalently bound to Ser64 (Scheme 2) of both penems **1** and **2** was obtained by docking simulation (Figure 2; a stereoview of these complexes is available in the Supporting Information). In the case of **1**, the triazolyl moiety is close to Tyr221, and the thiolate group lies along the B3 β-strand. The *S* isomer of the compound is therefore strongly favored.

Indeed, the thiolate group is only able to react with one side of the double bond. In contrast, the heterocyclic ring of **2** binds along the B3 β-strand, as in the crystal complex with intermediate **d**. The thiolate moiety is near Leu119 and Gln120 and is positioned in such a way that only intermediate **2d** with *R* configuration can be produced. The binding energy of the two complexes is −63.4 and −51.8 kcal/mol for **1** and **2**, respectively. As a result, flexibility of Tyr221 resulting from the conformation of the Ω loop would be, in large part, responsible for the position and stereochemistry of the ligand in the binding site. Interaction with this residue was also considered as essential for the binding of ceftazidime and its transition-state analogue to *Citrobacter freundii* GN346 β-lactamase.[15] Furthermore, a recent crystal structure of GC1 in complex with another penem inhibitor (**3**), shows both *R* and *S* configurations with 30% and 70% occupancies, respectively.[20] In this later complex, the Ω loop is in the same position ("close folding") as in 908R, positioning Tyr224 (Tyr221) near the active site.

To test our hypothesis, the reaction product in *R* and *S* configurations for **1** and **2** was docked in the corresponding enzyme and the binding energy of the complex was evaluated. In the case of **1** with the *R* configuration, the carboxylate group points toward the water surface and the triazolyl ring lies near Tyr150, Thr316, and Asn346 (data not shown). Such a complex is less stable than that of **1** with the *S* configuration ($\Delta\Delta E$ = 11.0 kcal/mol) (Figure 2). It is noteworthy that the docked complex of the *S* isomer is close to the observed crystal one but remains slightly different because the constrained cocrystal conformation of **1** cannot be modeled by the docking program. In the case of **2**, the heterocyclic group of the *S* isomer is buried near Gly63, Ser64, Asn152, Gln222, Ala223, Gly225, and Ser321. The carboxylate group interacts with Gln120 and lies close to the solvent surface. The modeled *R* complex, similar to the crystal one, is more stable than the ***S*** complex ($\Delta\Delta E$ = 7.1 kcal/mol).

These preliminary analyses confirm the stereoselective mechanism of action of **1** and of methylidene penem inactivators in general toward class C β-lactamases as illustrated in Figure 2.

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Supporting Information Available: Modeling studies, crystallization, data collection, structure refinement, and stereoviews of Figure 2. This material is available free of charge via the Internet at http://pubs.acs.org.

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