ESRF	**Experiment title:** Crystallographic studies of Class A and Class D β-lactamases in the apo form and in complex with several inhibitors	**Experiment number**: LS-1622
Beamline: ID14-2	**Date of experiment**: from: 13 may 2000 to: 14 may 2000	**Date of report**: 27 February 2001
Shifts: 3	**Local contact(s)**: Edward MITCHELL	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Guillaume L'Hermite* - Bio-Xtal, 2 route de la Noue, 91193 Gif sur Yvette cedex, France.

Frédéric Frénois* - Bio-Xtal, 2 route de la Noue, 91193 Gif sur Yvette cedex, France.

Wladimir Sougakoff* - L.R.M.A. faculté de Médecine Pitié-Salpêtrière, 91 bvd de l' Hôpital F75634 Paris cedex 13, France.

Lucile Pernot* - L.R.M.A. faculté de Médecine Pitié-Salpêtrière, 91 bvd de l' Hôpital F75634 Paris cedex 13, France.

Jean Delettré - LMCP CNRS URA 09, Université Paris VI, 4 place Jussieu 75252 Paris cedex 05, France.

Report:

The therapeutic problems posed by class D β-lactamases, a family of serine-enzymes hydrolysing β-lactam antibiotics following an acylation-deacylation mechanism, are increased by the very low level of sensitivity of these enzymes to β-lactamase inhibitors. To gain structural and mechanistic insights for the design of new inhibitors, we have undertaken the structural study of the class D β-lactamase OXA-13 from *Pseudomonas aeruginosa* in the apo form and in complex with an antibiotic of the carbapenem family, meropenem, which acts as an inhibitor [1].

We collected X-ray diffraction data sets on the beamline ID14-2 at the ESRF using two distinct crystal forms that we obtained from the same conditions of crystallisation. The two forms corresponded to diamond-shaped crystals (form I) and to medium-sized rods (form II) with well formed faces. Both crystal forms belong to the orthorhombic crystal system with space-group $P2_12_12_1$ and cell parameters a = 50.7 Å, b = 81.7 Å, c = 125.5 Å, $\alpha = \beta = \gamma = 90°$ (form I) and a = 45.3 Å, b= 112.3 Å, c = 125.0 Å, $\alpha = \beta = \gamma = 90°$ (form II). All X-ray diffraction data were recorded on cryo-frozen samples. The crystals were soaked for a few seconds in a cryoprotectant solution containing 22% (w/v) PEG 4000, 0.2 M lithium sulfate, 0.1 M cacodylate sodium buffered at pH 5.5 and 20 % (v/v) PEG 400. Flash-freezing of the crystals was achieved with a nitrogen gas stream at 100 K delivered from an Oxford Cryostream. The data set of the OXA-13 enzyme was collected

from crystals of form I at a maximum resolution of 1.8 Å. The OXA-13:meropenem complex was prepared by soaking crystals belonging to form II into a drop of mother liquor containing 5 mM of meropenem. The crystals were soaked for one hour. The data set of OXA-13 complexed with meropenem was recorded from crystals of form II to 2.0 Å. Each data set was obtained from a single crystal. Raw diffraction images were indexed and integrated with MOSFLM version 6.0 [2]. Data scaling, merging and reduction was carried out with the programs of the CCP4 suite [3]. Relevant statistics are presented in Table 1.

Data set	**OXA-13**	**OXA-13:meropenem**
Resolution limit (Å)	20.0-1.8	30-2.0
Number of measured reflections	167073	280280
Unique reflections	47483	43003
Multiplicity	3.5 (3.0)*	6.5 (2.2)
Completeness (%)	97.3 (32.8)	97.7 (87.8)
$<I/\sigma I>$	16.9 (2.8)	15.5 (1.5)
R_{merge} (%)	5.0 (22)	9.2 (39.9)

Table 1 : Data collection statistics. *The numbers in brackets are the values for the highest resolution shell (1.8-1.9 Å for the OXA-13 data set and 2.0-2.1 Å for OXA-13: meropenem data set).

The crystal structure of OXA-13 was determined by molecular replacement using the program AMoRe [4]. The refined structure of OXA-10 [5] (PDB entry code 1EWZ) at 2.4 Å resolution was used as a search model. After an initial rigid-body refinement applied with the FITING subroutine of AMoRe, two solutions were found with a correlation coefficient and a crystallographic *R*-factor of 67.3 % and 39.7 % respectively. The 2 solutions correponded to 2 molecules in the asymetric unit that are related by a non-crystallographic symmetry defined by a rotation axis which was found to be parallel to the *b* axis, the rotation angle being approximately 180°. The first rounds of refinement of the OXA-13 model were performed with X-PLOR 3.851 and then subsequent cycles of refinement were performed with REFMAC version 4.0 [6,7]. The *R*-factor and *R*-free yielded a value of 19.7 % and 24.7 % respectively. The stereochemistry of the final model was analysed with PROCHECK [8] : 90.4 % of the residues for molecule A and 91.4 % of the residues for molecule B are located in the most favored regions of the Ramachandran plot.

The structure of the OXA-13:meropenem complex was solved by molecular replacement using the model of OXA-13 (without solvent molecules) as a search probe. Two solutions were obtained with the program AMoRe and then refined with X-PLOR 3.851 and with REFMAC at the end of the refinement protocol. The meropenem molecule was modelled covalently bounded to the side chain of Ser67 in the active site of both monomers A and B of OXA-13. The final round of refinement yielded a *R*-factor and *R*-free of 20.4 % and 25.6 % respectively. The stereochemical quality of the final model of OXA-13:meropenem was

analysed with PROCHECK : 91.8 % of the residues for molecule A and 90.4 % of the residues for molecule B are in the most favored regions of the Ramachandran plot.

The native form of OXA-13 consisted of a dimer displaying an overall organisation similar to the one found in the closely related enzyme OXA-10 [9]. In the acyl-enzyme complex, the positionning of the antibiotic appeared to be mainly ensured by (i) the covalent acyl bond and (ii) a strong salt bridge involving the carboxylate moiety of the drug. Comparison of the structures of OXA-13 in the apo-form and in complex with meropenem revealed an unsuspected flexiblity in the region of the essential residue serine 115, with possible consequences on the catalytic properties of the enzyme. In the apo form, the Ser115 side chain is oriented outside from the active site, whereas the general base Lys70 adopts a conformation which seems to be not compatible with the activation of the catalytic water molecule required for the deacylation step. In the OXA-13:meropenem complex, a 3.5 Å movement of the backbone of the 114-116 loop towards the side chain of Lys70 was observed, which seems to be driven by a displacement of the neighbouring 91-104 loop and which results in the positionning of the side chain hydroxyl of Ser115 toward the catalytic centre. Concomitantly, the side chain of Lys70 is forced to curve in the direction of the deacylating water molecule which is then strongly bound and activated by this residue. However a distance of ca. 5 Å separates the catalytic water molecule from the acyl carbonyl of meropenem, a structural feature which accounts for the inhibition of OXA-13 by this drug (Figure 1).

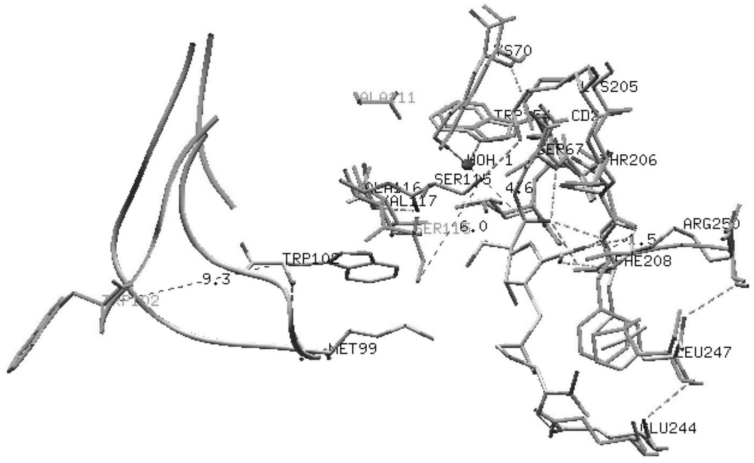

Figure 1 : Detailed view of the active site region in OXA-13. The OXA-13:meropenem acyl-enzyme complex is depicted using CPK colours and is superimposed to the structure of the active site of the apoenzyme (in green). The catalytic water molecule HOH1 is depicted by a red sphere. Hydrogen bonds are represented using green dotted lines. Distances are indicated with black dotted lines.

This work has been submitted for publication to *Journal of Molecular Biology* [10]

References

[1] Mugnier P., Podglajen I., Goldstein F. W & Collatz E. (1998). Carbapenemens as inhibitors of OXA-13, a novel, integron-encoded β-lactamase in *Pseudomonas aeruginosa*. *Microbiology*, **144**, 1021-1031.

[2] Leslie, A. G. (1999). Integration of macromolecular diffraction data. *Acta Crystallogr. Sect. D*, **55**, 1696-1702.

[3] CCP4 (1994). The CCP4 suite programs form protein crystallography. *Acta Crystallogr*. *Sect D*, **50**, 760-764.

[4] Navaza, J. (1994) AMoRe : An automated package for molecular replacement. *Acta Crystallogr*., *sect D*, **50**, 157-163.

[5] Golemi D., Maveyraud L., Vakulenko S., Tranier S., Ishiwata A., Kotra L., Samama J.-P. & Mobashery S. (2000). The first structural and mechanistic insights for class D β-lactamases : evidence for a novel catalytic process for turnover of β-lactam antibiotics. *J. Am. Chem. Soc*. **122**, 6132-6133.

[6] Brünger A.T. (1992) X-PLOR, A System for X-ray Crystallography and NMR, version 3.1 Yale University press, New Haven and London.

[7] Murshudov G.N., Lebedev A. Vagin A.A. & Dodson E.J. (1997). Refinement of maromolecular structures by the maimum-likelihood method. *Acta Crystallogr. Sect. D*, **53**, 240-255.

[8] Laskowski R.A., MacArthur M.W., Moss D.S. & Thornton J.M. (1993) PROCHECK : A program to check the stereochemistry of protein structures. *J. Appl. Crystallogr*. **26**, 283-291.

[9] Paetzel M., Danel F., de Castro L. Mosimann S.C. Page M.G. & Strynadka N.C. (2000). Crystal structure of he class D β-lactamase OXA-10. *Nat. Struct. Biol*. **7**, 918-925.

[10] Pernot L., Frénois F., Rybkine T., L'Hermite G., Petrella S., Delettré J., Jarlier V., Collatz E. & Sougakoff W. (2001). Crystal structures of the class D β-lactamase OXA-13 in the native form and in complex with meropenem. Submitted for publication in *Journal of Molecular Biology*.