	Experiment title: Class D β-lactamases structure.	**Experiment number**: LS 1594
Beamline: BM14 ID14-1	**Date of experiment**: From: 07-06-00 to: 08-06-00 08-06-00 09-06-00	**Date of report**: 12 - Feb - 01
Shifts: **3 + 3**	**Local contact(s)**: **Dr. Gordon Leonard**	*Received at ESRF:*

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Report:

The aim of the project was to elucidate the three-dimensional structure of class D β-lactamases and compare them to their counterparts class A and C β-lactamases, all three being serine-active enzymes which inactivate β-lactam antibiotics by hydrolysing the endogenic β-lactam amide bond. These enzymes are one of the major causes of the increasing phenomenon of bacterial resistance to antibiotics.

Two class D β-lactamases (PSE2 or OXA10 from *Pseudomonas aeruginosa* and OXA2 from *Salmonella typhymurium*) have been expressed, purified and crystallized. Previous attempts of heavy atom derivatives preparation with both proteins having failed, a selenomethionyl form of OXA10 enzyme was then purified and crystallized.

For MAD phasing, three data sets were collected to 1.9 Å from a frozen crystal of SeMet-OXA10 at station BM14. A crystal of native OXA10 was also used to collect a high resolution data set (1.6 Å) at station ID14-1. A crystal of native OXA10 soaked with the β-iodopenicillanate inhibitor was collected to 2.0 Å at station BM14. The same crystal was used for higher resolution data set (1.7 Å) at station ID14-1. A native data set was also collected from one crystal of the second enzyme OXA2 on both stations, with a maximum resolution of 1.45 Å. All data (see the following tables) were integrated using MOSFLM.

Eight Selenium sites were identified with the program SOLVE, in agreement with four methionine residues per monomer and one dimer in the asymmetric unit. The fifth N-terminal methionine residue is disordered.

The experimental phases were improved by density modification with DM software. Automatic interpretation of the electron density maps was performed with The ARP/wARP software. Twelve segments including 382 residues with side chains, correponding to 77% of the protein, were found. The program TURBO-FRODO was used for manual construction of the rest of the protein : 488 residues over the 496 in the sequence were

identified. Refinement proceeded with alterning use of CNS*solve* and TURBO-FRODO, and 514 water molecules were added, leading to a Rfactor of 21.7% and Rfree of 25%, at 2.0 Å resolution. The resulting protein model was used in molecular replacement using the AMORE software to fit the high resolution data collected with the native-OXA10 crystal of different unit-cell. The Rfactor found was 38.4 and the correlation factor 71.3. Refinement with CNS*solve* and addition of water molecules gave a final Rfactor and Rfree of 19.2% and 23.1% respectively for a final resolution of 1.5 Å.

Table statistics for data collection on BM14 beamline

	SeMet-OXA10 λ1	SeMet-OXA10 λ2	SeMet-OXA10 λ3	Native-OXA10+BIP	OXA2
λ (Å)	0,97917	0,97934	0,91184	1	1
Space group	P212121	P212121	P212121	P212121	C2221
Unit cell a(Å)	48,57			48,34	78,98
b(Å)	102,26			94,93	106,9
c(Å)	126,38			125,3	129,2
Max. resolution (Å)	1,91	1,91	1,8	2	1,45
Observations	393,587	392,161	454,95	375,235	
Unique reflections	46,563	46,658	55,652	37,803	86,412
Completeness *overall*	93,9	94,1	94,1	95,1	89,4
outermost resolution shell	73,4	73,7	75,9	86,2	57,1
Average I/σI *overall*	9	8,6	7,7	6,3	10,4
outermost resolution shell	1,1	1,2	0,6	1,8	2
Multiplicity	3,7	3,7	3,6	3,6	5,4
Rsym *overall*	0,054	0,055	0,068	0,093	0,044
outermost resolution shell	0,371	0,362	0,686	0,421	0,297

Table statistics for data collection on ID14-1 beamline

$\lambda = 0{,}934$Å	Native-OXA10 (low)	Native-OXA10 (high)	Native-OXA10+BIP	OXA2
Space group	P212121		P212121	C2221
Unit cell a(Å)	48,68		48,15	78,98
b(Å)	95,44		94,64	106,9
c(Å)	125,74		125,23	129,2
Max. resolution (Å)	2,24	1,6	1,7	1,6
Observations	303,066	750,981	908,356	692,032
Unique reflections	28,874	74,91	60,857	67,534
Completeness *overall*	99,2	97,8	95,5	93,3
outermost resolution shell	95,9	96,2	89,9	91,6
Average I/σI *overall*	14,3	10,3	5	9,7
outermost resolution shell	11,7	6,8	0,6	6,1
Multiplicity	3,9	3,9	4,1	3,5
Rsym *overall*	0,037	0,047	0,096	0,053
outermost resolution shell	0,037	0,097	0,096	0,053

The same procedure as above was used with the 1.7 Å data collected with the native-OXA10 + BIP complex, revealing unfortunately no additional electron density corresponding to a β-iodopenicillanate molecule into the active site.

The OXA2 enzyme shares 36 % sequence identity with OXA10. Its structure was solved by molecular replacement using the AMORE software. The Rfactor found was 50.4 and the correlation factor 49.4.
The automatic construction with The ARP/wARP software identified 433 residues with side chains in 9 separated segments, corresponding to 85% of the protein. Subsequent manual construction with the program TURBO-FRODO allowed the positioning of 482 residues over the 504 in the sequence. Refinement with CNS*solve* and addition of 288 water molecules gave a final Rfactor and Rfree of 20.1% and 21.5% respectively for a final resolution of 1.5 Å.