LS-1796
Marseille BAG

BM14
9-10 September 2000

Project	Responsible	B'line	Date	Method	Space Grp	Cell (A)	MW kDa	mol/au	Resol (A)	Rsym (%)	Comp. (%)	Mult.
phospholipase D	Alain Roussel	BM14	09/09/00	MR	C2	156.422 64.607 89.608 β=111.543	90	1	1.9	8.60	98.60	2.80
crustacyanin	Florence Vincent	BM14	10/09/00	MR	P212121	41.141 79.611 108.955	19	2	1.90	3.20	98.10	4.30
MbraCSP2+C16:OH	Valérie Campanacci	BM14	10/09/00	MR	P422	71.3 71.3 80.2	13	4	2.60	7.00	99.80	4.90

ESRF	**Experiment title:** **Crustacyanin subunit C1**	**Experiment number**: LS1796
Beamline: ID14-2	**Date of experiment**: from: 09.09.00 to: 10.09.00	**Date of report**: Feb 01
Shifts: **3**	**Local contact(s)**: Andy Thompson	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Florence Vincent , AFMB laboratory, CNRS Marseille

Chantal Abergel, IGS laboratory, CNRS Marseille.

Report:

Crustacyanin

The crustacyanin (subunitC1) belongs to the lipocalin family.
Crystals of crustacyaninC1 was frozen to 100 K using 22.7% glycerol as cryoprotectant.
The space group and cell dimensions were confirmed from a preliminary exposure to be P212121, a=41.31Å b=79.91Å , c=109.96 Å.
One data set has been collected at 100 K, with an exposure time of 3 sec per degree.

Data collection

Number of unique reflections	28864
Overall % data > 1 sigma(I) (last shell)	98.1(98.1)
Overall R merge (%) (last shell)	3.1(9.0)
Overall I/sigma(I) (last shell)	12.9(4.1)
Resolution (Å)	23.8-1.89

We tried to solve the 3-D structure of the crustacyanin by molecular replacement, using different lipocalins such as Major Urinary Protein, Retinol Binding Protein… To date we didn't succed in molecular replacement.

ESRF	**Experiment title:** **CSP/cetyl alcohol complex**	**Experiment number**: Ls1796
Beamline: BM14	**Date of experiment**: from: 9 Sep 2000 to: 10 Sep 2000	**Date of report**: Feb 01
Shifts: 3	**Local contact(s)**: Andy THOMPSON	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Valérie CAMPANACCI

Florence VINCENT*

Report:

Crystals of MbraCSP complexed with a pheromone-like compound (cetyl alcohol) were frozen to 100 K with no cryoprotectant.

The space group and cell dimensions were confirmed from a preliminary exposure to be P422, 71.3x71.3x80.2 Å, $\alpha=\beta=\gamma=90°$.

One data set was collected to 100 K, with an exposure time of 60 sec per degree, see table bellow:

Data collection	
Total number of observation	121777
Number of unique reflections	6822
Overall % data > 1 sigma(I) (last shell)	99.8 (99.8)
Overall R merge (%) (last shell)	7.0 (24.1)
Overall I/sigma(I) (last shell)	5.6 (2.3)
Resolution (Å)	40.0 – 2.60

The resolution of this structure involves the obtention of phases by MAD, SAD, MIR or *ab initio* method. The use of these methods for solving the phases will be described elsewhere.

ESRF	**Experiment title:** **Preliminary crystallographic study of a recombinant phospholipase D from Cowpea (*Vigna unguiculata* L.**	**Experiment number**: Ls1796
Beamline: BM14	**Date of experiment**: from: 9 Sep 2000 to: 10 Sep 2000	**Date of report**: Feb 01
Shifts: 3	**Local contact(s)**: Andy THOMPSON	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

The plant phospholipase D (PLD) is considered as a key enzyme involved in various physiological processes such as signal transduction and membrane metabolism. Crystals of the PLD protein from *Vigna unguiculata* have been produced from the recombinant 768 amino-acid long protein. The crystals belong to the monoclinic space group C2, with unit-cell parameters a=157.7Å, b=65.6 Å, c=90.2 Å and β=111.5 There is 1 molecule in the asymmetric unit. Frozen crystals diffract to at least 1.94 Å resolution using synchrotron radiation. We are currently searching for heavy atom derivatives using ytterbium and tungstate in order to solve the 3D structure.

Data Collection and Processing

A single crystal (0.3 x 0.2x 0.1 mm^3)was collected in a Hampton Research 0.5 mm^3 loop, flash frozen to 105K in a cold nitrogen gas stream and subjected to X-ray diffraction. This data set was collected on a MAR CCD at the ESRF radiation synchrotron facility (ID14 EH4) at a wavelength of 0.9465Å. Data collection was carried out with oscillation angles of 1.0° and with a crystal-to-detector distance of 120 mm. The total oscillation range collected was 110°. Space group determination was performed using the autoindexing option in *DENZO* (Otwinowski, 1993). The crystals belong to the monoclinic space group C2 with unit cell parameters a= 157.72Å, b= 65.57 Å c= 90.2 Å, β= 111.5. The packing density for one monomer of rPLDα

(87.157 KDa) in the asymmetric unit of the crystals (volume 867 803Å^3) is 2.49 Å^3 Da^{-1}, a reasonable value for globular proteins and indicating an approximate solvent content of 50.6 % (Matthews, 1968).

The data set was processed using *DENZO* (Otwinowski, 1993) and the *SCALA* program from the *CCP4* package (Collaborative Computational Project, 1994) was used for the scaling and data reduction of the native data set. The crystal diffracted to at least 1.94 Å and 261 944 reflections were measured in the resolution range 24.5-1.94 Å. This was reduced to a data set of 59540 unique reflections with an R_{sym} value of 5.7. It represents a completeness of 94% with a multiplicity of 2.1 and an average I/σ(I) of 7.7. For the highest resolution shell 12199 reflections were measured in the resolution range 2.01-1.94 Å, corresponding to 5866 unique *hkl*, an R_{sym} value of 28.6 and an average I/σ (I) of 1.6, a completeness of 94 % and a multiplicity of 2.8. In order to solve the rPLDα structure, we are currently testing various heavy atoms derivatives using both the phospholipid binding site (tungstate salt) and the two calcium binding sites (ytterbium salt). We determined that a 1 mM sodium tungstate concentration is sufficient to inhibit 40% of the rPLDα activity in 5 minutes (data not shown). This salt will thus be used with the MAD method (Hendrickson, *et al.*, 1990) to solve the structure. The soaking of rPLDα crystals as well as their co-crystallization with both tungstate and ytterbium are currently tested.