ESRF	**Experiment title:** Structure of Penicillin-Binding Protein 3 from *Escherichia coli*.	**Experiment number**: LS-1286
Beamline: ID14-EH3	**Date of experiment**: from: 05-06-99 to: 06-06-99	**Date of report**: 30 -Aug- 99
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Names and affiliations of applicants (* indicates experimentalists):

CHARLIER Paulette *
FONZE Eveline *
VERMEIRE Marcel *

Unité de Cristallographie des Macromolécules – CIP
Institut de Physique B5
Université de Liège au Sart Tilman
B4000 Liège
BELGIQUE

Report:

The aim of this project is to determine the three-dimensional structure of a high molecular weight penicillin-binding protein (HMW PBP) involved, with the collaboration of other membrane-bound proteins, in the elongation of the cell wall and the formation of the septum in bacteria.

A very few high molecular weight penicillin binding proteins have been crystallized and only one structure of a HMW PBP, the PBP2x of *S. pneumoniae* has been solved by the group of O.Dideberg .

The **PBP3** from *Escherichia coli* has been crystallized. The crystals obtained so far are quite small and difficult to reproduce. In order to facilitate the crystallization process, we have envisaged to cocrystallize the PBP3 with a Fab fragment of a monoclonal anti-PBP3 antibody. The antibodies 103-12F were chosen on the basis of their dissociation constant ($Kd \sim 5.10^{8}$ M^{-1}). IgG were produced by cell culture and purified on protein G Sepharose. The Fab fragments were prepared by papain digestion and protein A Sepharose purification. The anti-PBP3-**Fab** fragments crystallize as long needles or prisms of 200x200x400 μm^3. Crystals of the **PBP3-Fab complex** grow as large and very thin plates within 24 hours.

During the 3 shifts allocated at ID14-EH3 in June 99, we have tested two types of PBP3 crystals, the PBP3-Fab complex crystals, and we were able to collect a 2.2 Å data set on the Fab crystals.

PBP3

We have tested needles of 10x10x300 μm^3 and bipyramidal prisms of 100x100x400 μm^3, obtained under roughly the same conditions. Several cryprotectants have been tried.

Only two prismatic crystals diffracted to 6-7 Å resolution, with a very weak signal/noise ratio. Partial data sets were collected in order to estimate the space group and the unit cell parameters. They were first analysed with the MOSFLM package, but no unit cell emerged clearly. We have then used the XDS package. We can choose either the best score 'quality of fit' with an orthorhombic space group (oP) and an approximative unit cell of a = 133.2 Å, b = 137.5 Å, c = 515.0 Å, or the highest symmetry with a tetragonal space group (tP) and an approximative unit cell of a = 135 Å, b = 135 Å, c = 515 Å. With a unit cell volume of about 9 432,225 $Å^3$ and with a molecular weight of ~56,000 daltons, the number of molecules per asymmetric unit (N_{au}) can be estimated between 8 to 12.

We may think that the conditions of data collection are correct and that the poor resolution can be attributed to the size and quality of the crystals, correlated with the size of the unit cell. We will try to enhance the size and quality of the prismatic crystals.

Anti-PBP3 Fab

It has already been demonstrated that the crystallization process may be "facilitated" by the help of co-crystallization with antibody fragments. We have applied this method in order to have more control on the crystallization process of PBP3. Fab fragments of antibodies 103-12F against PBP3 have been crystallized easily. The determination of this Fab structure will be the first step in case of the resolution of the PBP3+Fab complex structure by molecular replacement.

The collected data diffracted to about 2.2 Å resolution. Using the XDS package, we have found the space group $P2_12_12_1$ with the unit cell parameters : a = 70.94 Å, b = 105.82 Å, c = 127.74 Å. The total number of reflexions is 21,690 with a completness of 44% at 2.2 Å resolution with an R_{factor} of 6.2%, and 89% of completness at 3.0 Å resolution with an R_{factor} of 5.4%. Assuming about 50% of solvant, the Fab fragment crystallized as a dimere. We are applying molecular replacement in order to solve the structure of the Fab fragment.

PBP3+Fab Complex

The crystals grew from a precipitate within 24 hours and appeared as clusters of large and thin plates.

All the crystals tested gave very bad and/or very weak diffraction patterns. The maximum resolution that we have observed for those crystals was of the order of 8 Å. No data were collected.

Since this is very preliminary results, we are very confident that the quality of those crystals could be improved by controlling the crystal growth speed.