ESRF	**Experiment title:** Structure of high molecular penicillin-resistant Penicillin-Binding Proteins 5 from *Enterococcus hirae* and *Enterococcus faecium.*	**Experiment number**: LS 1593
Beamline: ID14-EH1	**Date of experiment**: from: 08-06-00 to: 09-06-00	**Date of report** : 31- Aug-00
Shifts: 3	**Local contact(s):** Dr. Gordon Leonard	*Received at ESRF:*

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Report:

The aim of this project is to determine the three-dimensional structure of a multimodular low-affinity penicillin-binding protein (PBP) which is implicated in the enterococcal peptidoglycan synthesis and β-lactam resistance. These proteins, anchored to the membrane by their N-termini, are essentially constructed of two modules exposed on the outer face of the cytoplasmic membrane. The transpeptidase activity is located in the C-terminal module, and the N-terminal domain is possibly interacting with other proteins of a multienzyme complex. To date, only one structure of a PBP is available in the Protein Data Bank, the PBP2x from *Streptococcus pneumoniae* (1PMD, 1QME, 1QMF), but even at a resolution of 2.4 Å, the refined structure contains only 558 residues over the 701 residues crystallized protein. In the model, more than 100 residues are missing in the N-terminal module.

We were able to obtain soluble forms of *Enterococcus hirae* PBP5 (**PBP5Eh**), *Enterococcus faecium* PBP5 (**PBP5Efm**) and a selenomethionyl-PBP5Efm derivative by removing the 31 N-terminal amino acids hydrophobic anchoring peptide. The three soluble proteins were overproduced in *E. coli*, purified in large scale and crystallized. Small crystals are obtained for the three native proteins but also in presence of different β-lactam antibiotics.

Selenomethionyl-PBP5Efm

PBP5Efm contains 12 methionines for 645 residues. The best crystals have been obtained in the presence of penicillin. After several assays with those crystals on BM14 and ID14-3 beamlines (projects IN-96, IN-120, IN-121, in collaboration with Dr. B. Schoot from HMR (Aventis)-Romainville-France), a full and useful MAD data set was finally collected on the ID14-4 beamline (IN-134). Two crystals have been measured at three wavelengths (peak, inflection, remote), the best one diffracting to 2.4 Å. At each wavelength, 540 frames have been collected with an exposure time of 0.5 s. and an oscillation of one third of degree. The crystal to detector distance was 230 mm. The data have been processed with *Mosflm* and are complete to 2.4 Å. The space group is monoclinic C2 with a = 79.3 Å , b = 128.8 Å , c = 236.1 Å and β = 93.92°. Relevant with 3 molecules per asymmetric unit, 30 selenium sites have been found with *Shake and Bake* (*Solve* has been tried without any success). The sites were refined with the *MLPHARE* program from *CCP4* suite. The MAD map calculated after density modification was suitable to initiate the protein construction. We were unable to find the Cα trace with the *ARP-WARP* program. So, the building process has been continued with classical construction/refinement cycles. About 70% of the **PBP5Efm** protein(~71 kDa., 645 amino acids) has now been determined.

PBP5Efm

A second crystal form obtained with the native **PBP5Efm** in presence of oxacillin has been measured on the ID14-1 beamline. 100 frames have been collected with an exposure time of 45 s. and an oscillation angle of 1°. The crystal to detector distance was 200 mm. The data have been processed with *Mosflm* and are complete to 3.0 Å. The space group is orthorhombic $C222_1$ with a = 68.4 Å , b = 132.7 Å , c = 162.5 Å. With the partial **PBP5Efm** model obtained so far, a molecular replacement solution has been found, showing a good packing in the unit-cell and a reasonnable electronic density map. These data will provide complementary informations for finalizing the establishement of the three-dimensional structure of the multimodular low-affinity PBP5 from *E. faecium*.

PBP5Eh

A few diffraction tests have been performed on ID14-1. The crystals tested so far were twinned or poorly diffracting.