<table>
<tr><td> </td><td>Experiment title:
PROTEIN CRYSTALLOGRAPHY AT AFMB-CNRS, MARSEILLE</td><td>Experiment number:
LS1657</td></tr>
<tr><td>Beamline:
ID14-2</td><td>Date of experiment:
from: 10-4-00 to: 11-4-00</td><td rowspan="2">Date of report:
Aug00
Received at ESRF:</td></tr>
<tr><td>Shifts:
3</td><td>Local contact(s):
Mark van RAAIJ</td></tr>
<tr><td colspan="3">Names and affiliations of applicants (* indicates experimentalists):
Marie-Pierre EGLOFF</td></tr>
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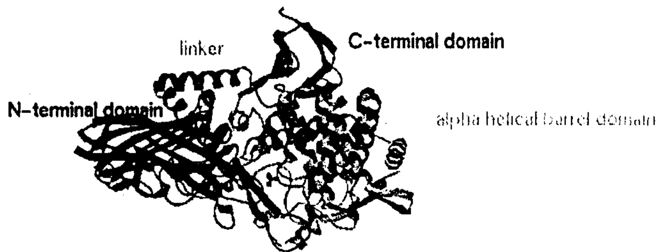
Report:

Maltose Phosphorylase (MP) catalyses the conversion of maltose and inorganic phosphate into β-D-Glucose-1-Phosphate and Glucose. This reaction does not require any co-factors, such as pyridoxal phosphate as it is the case for the well characterized glycogen phosphorylase (which, like MP, also catalyses the phosphorolysis of a glycosidic bond).

MP has been shown to be dimeric in solution, consisting of 2 monomers of 750 amino acids.

We have cloned, expressed purified and crystallized the MP from *Bacillus subtilis*. The crystals were diffracting to 3.2 A resolution at the ESRF (ID14-EH2). We solved the three-dimensional structure of this protein by the MAD method using the selenium as a diffuse scatterer in the selenomethionine-substituted protein crystals.

The protein has an ellipsoid shape (90 A x 60 A x 60 A) and consists of four distinct regions: the N-terminal domain is entirely made up of anti-parallel β-strands, except one short helix, and represents a new protein fold; the linker between the N-terminal domain and the catalytic domain consists of two perpendicular helices; the catalytic domain forms an (α/α)6 barrel which has already been observed in a number of glycosidases and lyases; the C-terminal domain forms a two layered jelly roll motif.



As already mentioned, glycogen phosphorylases catalyze a reaction similar to the one from MP, but with a complete different chemistry : MP does not need any cofactor and proceeds with inversion of configuration. The catalytic site of MP has been compared with that of glycosidases and lyases and this comparison gave some insights in the catalytic mechanism of MP. Moreover, we also get a answer about the structural difference at the active site between maltose phsphorylases and trehalases. To get more information, we have tried to soak some crystals in different substrates or analogues of substrate :

Two data set have been collected on ID14-2 on two crystals soaked with maltose and glucose, respectively. The first data set is 99 % complete between 20 and 2.9 A resolution; its Rsym is 10.1 % and it has a multiplicity of 4.7. The second data set is 95 % complete between 20 and 2.9 A resolution; its Rsym is 8.9 % and it has a multiplicity of 4.0. Unfortunately, none of these substrate are bound. This might be due to the N-terminal poly-histidine tail which partially covers (in the three-dimensional structure) the active site of the protein. Co-crystallization at different pH are being performed, as well as the sub-cloning of the protein in a vector that introduces the poly-histidine tail at the C-terminal end of the protein (opposite to the catalytic site).