<table>
<tr><td> </td><td>Experiment title:
Structure/function properties of glucan synthesizing glycosyltransferases</td><td>Experiment number:
ls-1604</td></tr>
<tr><td>Beamline:
ID14 1</td><td>Date of experiment:
from: 03 April 2000 to: 04 April 2000</td><td rowspan="2">Date of report:
February 26, 2001
Received at ESRF:</td></tr>
<tr><td>Shifts:
3</td><td>Local contact(s):
Hassan BELRHALI</td></tr>
<tr><td colspan="3">Names and affiliations of applicants (* indicates experimentalists):
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</table>

Report:

The beam time allocated was used productively and successfully. A wide range of saccharide (including the natural substrate sucrose) complexes of the glucan synthesizing enzyme amylosucrase (or an inactive mutant) was studied. The following new data sets were obtained:

Crystals of amylosucrase soaked with 14 mM sucrose for 1 h and for 2 h, respectively. Data in both cases to ca. 2.3 Å
Crystal of amylosucrase soaked with thiosucrose. Data to 2.4 Å.
Crystals of the E328Q mutant co-crystallized with 14 mM maltoheptaose and then soaked further with maltoheptaose. Two data sets to ca. 2.1 Å.

Furthermore, an improved diffraction data set on native amylosucrase was collected. With this crystal diffraction to 1.4 Å could be obtained and in order to get maximal completion (both in the high and in the low resolution regions) three data sets were collected. The refined structure is included in the first publication on the amylosucrase structure (ref. 1) and the coordinates have been deposited at the PDB (1g5a).

All of the structures from the soaking/co-crystallization experiments have been analysed using molecular replacement techniques. In the structures involving sucrose and thiosucrose it was found that tris (from the native crystals) was still bound in the active site. However, a sucrose molecule was identified on the surface of the enzyme.

In crystal of the E328Q mutant co-crystallized/soaked with maltoheptaose it turned out that maltoheptaose is bound both in the active site and on several locations on the surface of amylosucrase (Fig. 1).

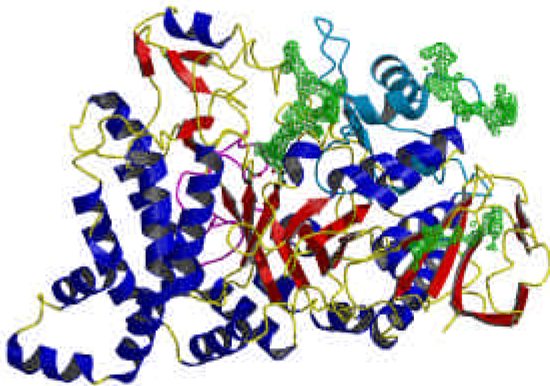

Fig. 1 Electron density from maltoheptaose found in the active site and on remote surface sites.

This new structural information is very important for the understanding of the biological function (chain elongation of glycogen using sucrose as glucosyl source) of amylosucrase.

References:

1. Skov *et al.*, submitted to *J. Biol. Chem*.