ESRF	**Experiment title:** Structural allergology/ From sucrose to specific glucan polymers.	**Experiment number:** LS-1752 LS-1753
Beamline: ID14-3 ID29	**Date of experiment:** from: 08 December 2000 to: 09 December 2000 from: 17 February 2001 to: 19 February 2001	**Date of report:** August 30, 2001
Shifts: 3+6	**Local contact(s):** Steffi Arzt, Andrew Thompson	*Received at ESRF:*

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General about the report.

Applications LS-1752 and LS-1753 were given a block allocation of 6 shifts. The first beam time schedulled (3 shifts) did not give any data due to a failed dewar transport. Replacement time was given in connection with the second schedulled beam time and altogether 6 shifts were used on February 17-19, 2001. Anette Henriksen from the Carlsberg Research Center joined the February measurements. We here give a joined report for the two applications.

Structural allergology.

Phl p 5.

Many different crystals of the grass pollen allergen Phl p 5 were tested, but most of them diffracted very poorly (>5 Å). A complete three wavelength MAD data set was collected on a crystal soaked in $HgCl_2$, for which diffraction spots were seen to 3.5 Å. Data processing unfortunately showed that there was only useful data to 4.2 Å. It has not been possible to solve the structure from this data set. Furthermore, a native data set was collected, which after data processing showed useful diffraction to 3.5 Å. This is the highest resolution on Phl p 5 crystals that we have ever collected. Two other native data sets were collected but both to a resolution no better than 4 Å. Finally, several other potential heavy atom derivative crystals were tested. One of them showed decent diffraction and absorption edge scanning confirmed the presence of WO_4^{2-}. A complete data set to 4.2 Å was collected. It has not been possible to solve the structure on the basis of these measurements.

Some very small crystals of a complex between a Fab fragment from a specific antibody and Phl p 5 were tested. They diffracted only enough to confirm that it was protein crystals, but far to weakly for data collection.

Bet v 1.

Data was collected to 1.5 Å on a crystal grown from a drop containing a specific Fab fragment and the birch pollen allergen Bet v 1. Unfortunately, the crystals turned out to contain only Bet v 1 with no Fab bound.

From sucrose to specific glucan polymers.

Amylosucrase.

The structure of amylosucrase (AS) was solved recently with data from a MAD experiment performed at ESRF [1]. We have also published a paper about the structure of an AS:glucose complex and the structure of an E328Q mutant in complex with the natural substrate sucrose [2]. Many experiments with AS complexes have already been performed (see also report for LS-1604) and from the February beam time we obtained three data sets with the E328Q mutant:

Crystals co-crystallized with maltoheptaose soaked with 20 mM maltoheptaose. Data to 2.0 Å.

Crystals co-crystallized with sucrose soaked with 20 mM maltotetraose. Data to 2.2 Å.

Crystals co-crystallized with maltoheptaose soaked with 20 mM fructose. Data to 2.2 Å.

Glucosyltransferase-I from *Streptococcus downei.*

A new crystal form of the catalytic core (GFT-Ic) was tested. Only poor diffraction to ca. 5 Å was seen.

Other projects (Antte Henriksen, Carlsberg Research Center).

***E. coli* b-ketoacyl [acyl carrier protein] synthase I (KAS I)**

A data set has been collected at cryogenic temperatures (100K) from a decarboxylation deficient mutant (Lys328Ala) of *E. coli* KAS I soaked in dodecanyl-CoA. The space group was determined to be $P2_12_12_1$ with a = 59.12 Å, b = 139.47 Å and c = 211.66 Å. The data was collected using oscillations of 0.3°. Applying cryo-annealing lowered the mosaicity of the crystal from 0.5° to 0.1° and increased the resolution of the initial frames from 2.0 Å to 1.5 Å. In spite of the low temperature used for the experiment severe radiation damage was observed and it was necessary to translate the crystal three times in order to obtain a complete data set. The radiation damage also resulted in a very low completeness of the high-resolution data (2.0 - 1.5 Å) and this data was omitted in the final scaling. The structure of the dodecanyl:KAS I (Lys328Ala) complex have complemented the structural data already collected on this mutant (experiment LS-1757), have supplied us with the structural background for the decarboxylation deficiency and elucidated the catalytic role of the active site residues: Lys328, His333 and His298.

Reference:

1. Skov, L.K., Mirza, O., Henriksen, A., Potocki De Montalk, G., Remaud-Simeon, M., Sarcabal, P., Willemot, R.M., Monsan, P. and Gajhede, M. (2001) *J. Biol. Chem.* **276**, 25273-25278.

2. Mirza, O., Skov, L.K., Remaud-Simeon, M., Potocki de Montalk, G., Albenne, C., Monsan, P. and Gajhede, M. (2001) *Biochemistry* **40**, 9032-9039.