



Experiment title: Structural Allergology	Experiment number: LS-1458	
Beamline: ID14 1	Date of experiment: from: 08 December 99 to: 09 December 99	Date of report: 22 February 01 <i>Received at ESRF:</i>
Shifts: 3	Local contact(s): Hassan Belrhali	
Names and affiliations of applicants (* indicates experimentalists): Anette Henriksen, Carlsberg Laboratory Michael Gajhede*, University of Copenhagen, Department of Chemistry Michael D. Spangfort, ALK-Abello Kåre Meno*, University of Copenhagen, Department of Chemistry Osman Mirza*, University of Copenhagen, Department of Chemistry		

Report:

Several crystal-forms of the grass pollen allergen phl 5 were tested. However, none of them yielded sufficient diffraction. It is likely that the poor diffraction relates to cryo-conditions. It is impossible to do experiments with our in-house equipment, due to the size of the crystals. A large number of experiments did not lead to the optimal conditions.

To make optimal use of the allocated beam time measurements on crystals from other projects (see TC-66 and LS-1604) were undertaken. Three data sets involving soaking experiments (glucose, fructose and maltotriose) with crystals of the glucan synthesizing enzyme amylosucrase were obtained. The structure of amylosucrase (ref. 1) was solved recently using data from a MAD experiment done at ESRF (LS-1450)

and the structures from the soaking experiments could be solved using molecular replacement. The glucose complex diffracted to 1.6 Å resolution and showed a glucose molecule bound in the active site (Fig. 1).

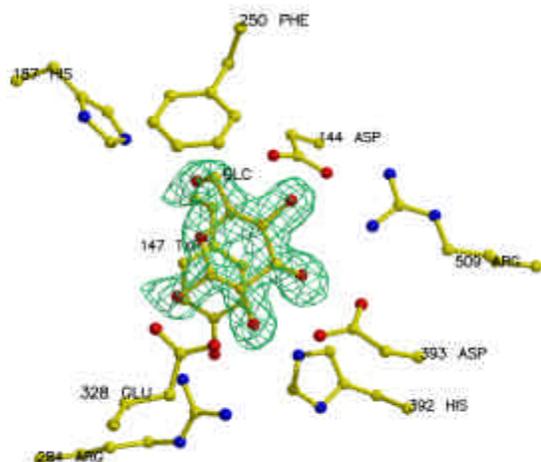


Fig. 1 β -glucose bound in the active site of amylosucrase.

When the enzyme crystals were soaked with fructose no incorporation could be detected (data to 2.2 Å). From the maltotriose experiment (data to 2.3 Å) a binding site on the surface of amylosucrase was identified.

The new data sets, particularly the glucose complex has given important information on the location of the active site (and the remote saccharide binding site) and about the binding of the glucosyl-enzyme intermediate (ref. 2).

Lastly, data on peroxidases were obtained. Soaking experiments on mutants of horseradish peroxidase *c* were performed and data collected.

References:

1. Skov *et al.*, submitted to *J. Biol. Chem.*
2. Mirza *et al.*, to be submitted to *Biochemistry*.