




Experiment Report Form

	Experiment title: Structure determination of fructan exohydrolase IIa	Experiment number: MX 179
	Beamline: BM14	Date of experiment: from: 1/12/2003 to: 2/12/2003
Shifts: 3	Local contact(s): Gavin Fox	<i>Received at ESRF:</i>
Names and affiliations of applicants (* indicates experimentalists): *Anja Rabijns, Pharmaceutical Sciences, E. Van Evenstraat 4, B-3000 Leuven, Belgium *Maureen Verhaest, Pharmaceutical Sciences, E. Van Evenstraat 4, B-3000 Leuven, Belgium *Stefaan Sansen, Pharmaceutical Sciences, E. Van Evenstraat 4, B-3000 Leuven, Belgium		

Report:

About 15% of flowering plants use fructans, fructose based oligo- or polysaccharides, as a storage carbohydrate instead of starch or sucrose. Apart from their function as a storage carbohydrate, fructans might also be a stress protectant (drought and cold) [1]. Food industries are interested in fructans for their different health-promoting effects. However, fructan degradation causes an important drawback for industrial harvesting. Fructan exohydrolase (FEH) catalyzes this breakdown. FEH belongs to family 32 of the glycosyl hydrolases, a classification based on general amino acid sequence similarities [2]. At present, no structural information is available for any member of this family. Therefore, elucidating the structure of FEH can not only contribute to a better understanding of its catalytic mechanism, but can also provide a model for the other enzymes of family 32.

Consequently, 1-FEH IIa from Chicory (*Cichorium intybus*) [3] has been crystallized using the hanging drop vapor diffusion method. A 2.35 Å native data set could be collected at the X11 beam line of DESY

(Hamburg, Germany). The crystals are tetragonal, belonging to space group $P4_12_12$, with unit cell parameters $a = 139.83 \text{ \AA}$, $b = 139.83 \text{ \AA}$, $c = 181.94 \text{ \AA}$, $\alpha = \beta = \gamma = 90^\circ$ [4]. A highly redundant SAD data set of a $C_7H_5HgO_3Na$ derivative was measured previously at the ESRF synchrotron (Grenoble, France) at beam line ID14-1 to 3.29 \AA resolution. This dataset enabled us to solve the crystallographic phase problem (AUTOSHARP [5]) and to trace the initial 1-FEHIIa model using the programs ARP/wARP [6] and MAID [7]. Further structure refinement is done by the CNS program (version 1.1) [8].

Now, different soaks with 1-FEHIIa crystals were collected at beamline BM14 at the ESRF synchrotron (Grenoble, France). However, 1-FEHIIa crystals soaked with the substrate 1-kestose did not diffract. 1-FEHIIa crystals soaked with sucrose did not diffract as well. Crystals soaked with inulin (degree of polymerization 5) diffracted to 3.10 \AA and to 3.20 \AA . Statistics are summarized in the table. However, no substrates could be found in the maps.

Table: Data collection and reduction statistics

Values between parentheses indicate data in the highest resolution shell.

	FEHIIa with inulin DP5	FEHIIa with inulin DP5
Space group	$P4_12_12$	$P4_12_12$
Unit-cell parameters (\AA)		
a	139.319	139.958
b	139.319	139.958
c	182.729	182.787
Resolution limit (\AA)	3.10 (3.21-3.10)	3.20 (3.31-3.20)
Total observations	148090	86786
Unique observations	30179 (2025)	29543 (2657)
Completeness (%)	90.3 (62.7)	96.3 (89.0)
Completeness ($I > 2\sigma$) (%)	83.0 (50.9)	65.1 (22.4)
Mean I/σ	13.05 (4.6)	6.36 (2.1)
R_{sym} (%)	11.7 (20.3)	17.0 (37.2)

References

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