ESRF	**Experiment title:** The Crystal Structure of a Family 27 Carbohydrate Binding Module Complexed with Cellohexaose and Mannohexaose	**Experiment number**: LS-1811
Beamline: ID14-4	**Date of experiment**: from: 11/11/00 to: 13/11/00	**Date of report**: 29/08/01
Shifts: 2	**Local contact(s)**: Sean McSweeney	*Received at ESRF:*

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Report:

Anaerobic fungi are the most effective plant cell wall degrading organisms described to date; these microbial eukaryotes synthesise a high molecular weight cellulase/hemicellulase multienzyme complex called the cellulosome. Recently, a non-catalytic component of such a complex from *Piromyces equi*, CelC, was shown to posses two carbohydrate binding modules (CBMs) exhibiting 33 % sequence identity to one another. These CBMs, which comprise a new family, CBM27, bind to soluble glucomannan, galactomannan and hydroxyethylcellulose (HEC) and also exhibit affinity for insoluble forms of cellulose and mannan. The structure of the C-terminal CBM was determined in native and ligand complexed forms.

Selenomethionyl and native CBM27-2 crystals were obtained from 100 mM Na/Hepes buffer, pH 7.5, containing 150 mM KSCN, 20 % (v/v) ethyleneglycol and 16-21 % (w/v) PEG3350. These crystals belong to space group $P4_32_12$ (a=b=93.3 Å, c=82.9 Å) and contain two molecules in the asymmetric unit. Multi-wavelength data, 400 images of 0.4^o, were collected on beamline ID14-4 at the ESRF, Grenoble (λ_1=0.9794 Å, λ_2=0.9790 Å, λ_3=0.9322 Å). Selenium sites were located using Solve. CBM27-2 exhibits a typical β-jelly roll structure with a cleft containing several conserved aromatic residues on its surface.

Crystals of CBM22-2 in complex with cellohexaose grew from 20 % (w/v) PEG3350, containing 0.2 M Li_2SO_4 and 10mM ligand. This crystal form belongs to space group C2 (a=107.7 Å, b=43.0 Å, c=35.5 Å, $\beta=105.4^o$). The structure was solved by molecular replacement using the program AmoRe and a single molecule of CBM27-2 from the refined $P4_32_12$ structure as the search model. When the drops comprised 3.0 M ammonium sulphate, in addition to 10 mM mannohexaose, crystals belonging to space group C2 (a=51.1 Å, b=42.7 Å, c=60.1 Å, $\beta=93.7^o$) were obtained. This structure was solved by molecular replacement using the program AMoRe and the structure of CBM27-2 in complex with cellohexaose.

Both ligands were found to occupy the same binding site that comprises 5 subsites. At each subsite, the protein can accommodate both an axial and equatorial C2-OH group, as was expected from isothermal titration calorimetry data that suggested CBM27-2 has a high affinity for mixed glucose/mannose polymers.