ESRF	**Experiment title:** **Structure of the *Clostridium thermocellum* X6b xylanase binding domain.**	**Experiment number**: LS-1532
Beamline: ID14-4	**Date of experiment**: from: 9/9/99 to: 11/9/99	**Date of report**: 28/08/00
Shifts:	**Local contact(s)**: S. McSweeney	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Simon J. Charnock*, Johan P. Turkenburg* & Gideon J. Davies

Department of Chemistry, Structural Biology Laboratory, University of York, Heslington, York YO1 5DD, U.K.

Report:

The plant cell wall consists mainly of a complex mixture of polysacccharides; primarily pectins, cellulose and hemicellulose (Brett & Walden, 1990). In most plant material xylan is the major hemicellulose component. This polysaccharide comprises a backbone of β-1,4-linked xylopyranose units, which can be decorated at the 2' and / or 3' carbons with arabinose, 4-methylglucuronic acid or acetate moieties. The glycosidic bonds in the xylan backbone are hydrolysed by endo-β1,4-xylanases (EC 3.2.1.8). It is now widely established that xylanases, in common with the majority of plant cell wall hydrolases, often exhibit a modular structure comprising of catalytic modules linked to one or more non-catalytic modules (NCM). The majority of the characterised NCMs are carbohydrate binding modules (CBMs). The 3D structures of several CBMs are known. A large number of NCMs remain uncharacterised and have been referred to as "X-modules". NCMs from a family designated "X6", are frequently found adjacent to family 10 and 11 xylanase CDs. *Clostridium thermocellum* Xyn10B (formerly xylanase Y) comprises two X6 modules, flanking a family 10 glycoside hydrolase CM, together with a dockerin and a C-terminal family 1 esterase. Crystals of X6b were grown by vapour phase diffusion using the hanging-drop method. The protein concentration was 50 mg/ml in 0.1 M NaAc buffer, pH 4.6, containing 10 mM DTT, 25 % (v/v) glycerol and 12 % (w/v) PEG 8000 as the precipitant. Crystals were grown from microseeds for 1 d. A rayon-fibre loop was used to transfer a single crystal directly into liquid nitrogen. Preliminary X-ray diffraction analysis revealed that they belong to space group $P6_122$ or $P6_522$, with unit-cell dimensions a = b = 90.08 and c = 207.71 Å, and with 1 or 2 molecules in the asymmetric unit.

A three wavelength MAD experiment was conducted on beamline ID14-4 at the European Synchrotron Radiation Facility (ESRF) at Grenoble using an ADSC Quantum-4 CCD detector. A single crystal of X6b was flash-cooled in the home laboratory, and tested for diffraction quality. The crystal was preserved and transported to the ESRF, where it was remounted on the single axis goniometer. Data were collected at the

minimum *f'*, the maximum *f"* and a reference wavelength at an energy above the absorption edge. After indexing an initial diffraction image using the program package HKL2000 (Otwinowski & Minor, 1997), the program STRATEGY (Ravelli *et al.*, 1997) was used to determine the optimal phi range to collect complete anomalous data using a minimal oscillation sweep. A total of 82 images with 1 degree oscillation were collected at each of the three wavelengths. To increase the multiplicity and thus the reliability of the data, a further 40 images were collected at each of the three wavelengths. Data were processed using DENZO/SCALEPACK as part of the HKL2000 suite of programs. The three unmerged datasets were input to SOLVE (Terwilliger & Berendzen, 1999) and scaled and merged internally by this program and 6 Se positions were readily located, corresponding to two molecules of X6b each with three selenomethionine residues in space group $P6_122$. Phases from SOLVE were used as a starting set for phase improvement in DM (Cowtan & Main, 1996). In order to employ non-crystallographic symmetry averaging, the operator relating the two molecules in the asymmetric unit was derived from the positions of the six Se atoms. The electron density map calculated after DM displayed extensive well-defined regions revealing continuous stretches of main chain density, with unambiguous density for carbonyl oxygen atoms and side chains. A model comprising approximately 150 amino acids was built from the initial map using the X-AUTOFIT module in Quanta (Molecular Simulations Inc. San Diego, USA). This model was refined using the CCP4 program REFMAC (Murshudov *et al.*, 1997), with the phases from DM included as experimental restraints. Data to 2.1Å resolution were collected on a Se-Met crystal on beamline BW7B (λ=0.8445Å) at the EMBL Hamburg outstation. The final model of X6b consists of two molecules in the asymmetric unit each consisting of residues 5-160 and a single calcium ion. 304 water molecules, with B values < 65.0Å^2 have also been modelled. The final crystallographic R value is 0.19 with an R_{free} of 0.25.

The structure forms a classical lectin-like β-jelly roll predominantly consisting of four major anti-parallel β-stands on both of each of the two faces. This fold displays most similarity to the *Bacillus thuringiensis* insecticidal toxin (PDB Code 1ciy) and the lectin “wing” domain of the *Vibrio cholerae* neuraminidase (PDB code 1kit). X6b displays a shallow surface groove which runs along the whole of the concave face of the jelly-roll and we would imagine, by analogy with other lectin domains, that this forms the ligand binding site. The X6 modules had previously been described as “thermostabilising domains” on account of their apparent ability to confer stability to the catalytic module. Truncation of Xyl10B leads to a decrease in the stability of the CM. Deletion of X6a is particularly destabilising (30). It is difficult to say, however, that these modules confer true thermostability or whether the truncated proteins are simply less stable due to disruption of their structure and exposure of previously interacting surfaces. We propose that the primary function for many of the X6 modules will be xylan-binding, and that they play a role in the potentiation of catalytic activity on insoluble substrates.

References

Brett, C. and Walden, K. (1990) *Physiology and Biochemistry of Plant Cell Walls (London, Unwin and Hyman)*

Otwinowski, Z., & Minor, W. (1997) in *Methods in Enzymology: Macromolecular Crystallography, part A* (C W Carter, J., & Sweet, R. M., Eds.) pp 307 - 326, Academic Press, London & New York.

Ravelli, R. B. G., Sweet, R. M., Skinner, J. M., Duisenberg, A. J. M., & Kroon, J. (1997) *J Appl Cryst 30*, 551-554.

Terwilliger, T. C., & Berendzen, J. (1999) *Acta Crystallogr. D55*, 849-861.

Cowtan, K. D., & Main, P. (1996) *Acta Crystallogr D49*, 148-157.

Murshudov, G. N., Vagin, A. A., & Dodson, E. J. (1997) *Acta Cryst D 53*, 240-255.