ESRF	Experiment title: *Clostridium thermocellum* Xyn10B Carbohydrate-Binding Module 22b: The Role of Conserved Amino Acids in Ligand Binding	**Experiment number**: LS-1673
Beamline: ID14-1	**Date of experiment**: from: 19/07/00 to: 22/07/01	**Date of report**: 30/08/01
Shifts: 1	**Local contact(s)**: Hassan Belrhali	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Hefang Xie,[1] David N. Bolam,[1] Simon J. Charnock,[2] Gideon J. Davies,[2] Michael P. Williamson,[3] Peter J. Simpson,[3] S. Raghothama,[3] Carlos M. G. A. Fontes,[4] Fernando M.V. Dias,[4] Luis M. A. Ferreira,[4] and Harry J. Gilbert[1]

1. Department of Biological and Nutritional Sciences, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, U. K.,
2. Structural Biology Laboratory, Department of Chemistry, University of York, Heslington, York YO10 5DD, U. K.,
3. Department of Molecular Biology and Biotechnology, Krebs Institute, University of Sheffield, Sheffield S10 2TN, U. K., and
4. CIISA-Faculdade de Medicina Veterinária, Rua Prof. Cid dos Santos, 1300-477 Lisboa, Portugal

Report:

The majority of plant cell wall hydrolases are modular enzymes which, in addition to a catalytic module, posses one or more carbohydrate binding modules (CBM). These carbohydrate-active enzymes and their constituent modules have been classified into a number of families based upon amino-acid sequence similarities. The *Clostridium thermocellum* xylanase, Xyn10B, contains two CBMs that belong to family 22 (CBM22). The crystal structure of CBM22b reveals a surface cleft which presents several conserved residues implicated in ligand binding. These amino acids have been substituted and the structure and biochemical properties of the mutants analyzed. Data were collected on ESRF beamline ID14-1. The data show that R25A, W53A, Y103A, Y136A and E138A exhibit very little affinity for xylotetraose. Conversely, mutations Y103F and Y136F have little effect on ligand binding. We show that the cleft of CBM22b does indeed form the ligand-binding site. Trp 53 and Tyr 103 participate in hydrophobic interactions with the ligand, whilst Glu 138 most likely makes important hydrogen bonds with the tetrasaccharide. Although Arg 25 and Tyr 136 are likely to form hydrogen bonds with the ligand, they also play a critical role in maintaining the structural integrity of the binding cleft, since the R25A mutant also perturbs the position of Trp 53 and Tyr 103. Members of CBM family 22 that lack one or more of Arg 25, Tyr 103, Tyr 136 or Glu 138 have been shown not to bind xylan. We therefore propose that CBM family 22 be considered as two distinct sub-families CBM22a and CBM22b based upon whether the modules contain the amino-acids responsible for xylan-binding, as observed in CBM22b but not in CBM22a.

References

Xie, H., Bolam, D.N., Charnock, S.J., Davies, G.J., Williamson, M.P., Simpson, P., Fontes, C.M.G.A., Ragothama, S., Ferreira, L.M.A. & Gilbert, H.J. (2001). The Clostridium thermocellum Xyn10B carbohydrate-binding module CBM22b : The Role of Conserved Amino Acids in Ligand Binding. *Biochemistry* **40**, pp. 9167-9176.