ESRF	**Experiment title:** The structure of the feruloyl esterase module of xylanase 10B from *Clostridium thermocellum*	**Experiment number**: LS-1942
Beamline: ID29	**Date of experiment**: from: 03/03/01 to: 05/03/01	**Date of report**: 30/08/01
Shifts: 1	**Local contact(s)**: Andrew Thompson	*Received at ESRF:*

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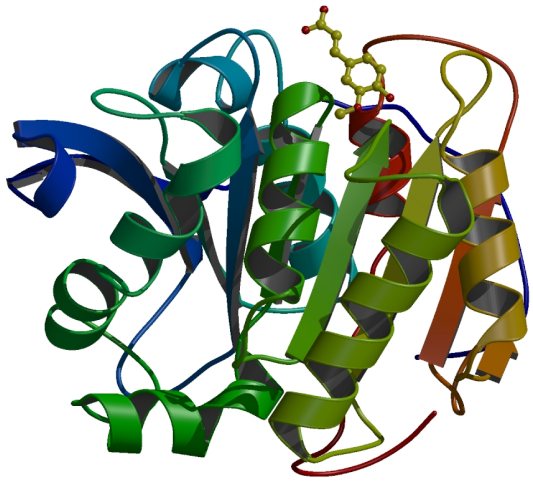
Report:

The degradation of the plant cell wall requires the synergistic action of a large consortium of predominantly-modular enzymes. In Clostridiae these biocatalysts are further organised in a supramolecular multi-enzyme complex, termed the cellulosome, which possesses numerous enzymatic activities. In addition to its well-described cellulolytic activity, it has recently been shown that the cellulosome possesses apparatus specific for the degradation of xylans. Cinnamic acid esterases, which are vested in modules of previously unknown function located within cellulosomal xylanases, hydrolyse the ferulate groups that cross-link arabinoxylans and to lignin, releasing ferulic acid. They play a key role in the degradation of the plant cell-wall and have many promising industrial and medical applications; ferulic acid is an anti-carcinogen, an anti-oxidant and an anti-inflammatory with many implications for human health, such as in the treatment of colonic cancer, Alzhemer's disease and heart related illnesses.

The feruloyl esterase module from a five-domain xylanase, Xyn10B from *Clostridium thermocellum*, has been cloned and over-expressed. The native structure has been solved at 1.6Å resolution using seleno-methionine multiple-wavelength anomalous dispersion data collected on beamline ID29 at the ESRF and refined to a final R_{cryst} of 15.4 % (R_{free} = 17.8 %). The structure of a hydrolytically inactive mutant, S954A, of the enzyme in complex with the reaction product ferulic acid has also been determined and refined at a resolution of 1.4Å (R_{cryst} 14.3 %, R_{free} 16.0 %).

The *C. thermocellum* Xyn10B ferulic acid esterase displays the α/β hydrolase fold and possesses a classical Ser-His-Asp catalytic triad. Ferulate esterases are characterised by their specificity, in contrast to many multi-functional esterases, and the active-centre reveals the binding site for ferulic acid and related compounds. It possesses specificity determinants for both the substrate methoxy and hydroxy ring substituents. The binding site for ferulic acid is located in a small surface depression suggesting that this enzyme is unlikely to be capable of hydrolysing ferulate cross-bridges in ligno-xylan complexes. In contrast to the specific binding of

ferulate, there is an apparent lack of specificity for both the xylan backbone and the arabinosyl moiety presumably reflecting the large potential combination of linkages between the various components that occur in the natural substrate.



Structure of the feruloyl esterase module of xylanase 10B from *Clostridium thermocellum*.

References

J.A.M. Prates, N. Tarbouriech, S. J. Charnock, C. M. G. A. Fontes, L.M. A. Ferreira and G. J. Davies (2001). The structure of the feruloyl esterase module of xylanase 10B from *Clostridium thermocellum* provides insights into substrate recognition. Submitted to *Structure*. PDB codes: 1GKK and 1GKL.