ESRF	**Experiment title:** Structural insights into the mechanism and specificity of a novel pectate lyase	**Experiment number:** LS1383
Beamline: BM14	**Date of experiment:** from: 24/02/99 to: 25/02/99	**Date of report:** 28/02/00
Shifts: BAG	**Local contact(s):** Gordon Leonard	*Received at ESRF:*

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Report:

Introduction

The majority of carbon biologically assimilated in the biosphere is in the form of plant cell wall polysaccharide composites. Such materials comprise highly crystalline microfibrils of cellulose embedded in a matrix of predominantly xylan, mannan and pectic substances, the latter of which are composed of a linear component, homogalacturonan, and a highly branched component, rhamnogalacturonan. The α-1,4-glycosidic linkages of the homogalacturonan backbone can be hydrolysed by polygalacturonanases, or cleaved via a β-elimination reaction by pectin and pectate lyases. PelA is a pectate lyase that belongs to a recently discovered novel family of pectate lyases, according to the classification system of Coutinho and Henrissat. Both native and selenomethionyl PelA were produced from *E. coli* and the recombinant proteins purified.

Crystallisation

Crystals of both native and selenomethionyl PelA were obtained by vapour phase diffusion using the hanging-drop method. A rayon-fibre loop was used to transfer a single PelAcm crystal to a cryoprotectant stabilising solution, comprising the growth buffer supplemented with 25 % (v/v) glycerol. The crystals belong to space group $P2_1$, with unit cell dimensions a

= 48.2, b = 91.8, c = 108.6Å and have two molecules in the asymmetric unit. Diffraction of both native and selenomethionyl crystals went beyond 2Å using in-house X-ray facilities.

Data collection and processing

The crystals were preserved and transported to the ESRF, where they were remounted on the single axis goniometer. A single native data set was collected to 1.5Å on beamline ID14-4 using an ADSC CCD detector and a three wavelength MAD experiment was conducted on beamline BM14 using a MAR CCD detector. The wavelengths for the MAD experiment were chosen by scanning through the absorption edge of the Se-PelAcm crystal. Datasets were then collected at the minimum f', the maximum f'' and a reference wavelength at an energy above the absorption edge, Table 1. After indexing an initial diffraction image using the program package HKL2000, the program STRATEGY was used to determine the optimal phi range to collect complete anomalous data using a minimal oscillation sweep. A total of 180 images with 1 degree oscillation were collected at each of the three wavelengths. MAD data collected at the ESRF were processed using DENZO/SCALEPACK as part of the HKL2000 suite of programs. Data for each wavelength were scaled in SCALEPACK, but multiple observations were not merged at this stage.

Location of Se positions and Phasing

The three unmerged datasets were input to SOLVE and scaled and merged internally by this program. Automated Patterson searching, using data to 2.0Å, readily located six Se positions, corresponding to two molecules of PelAcm each with three selenomethionine residues in space group $P2_1$. The Se positions were refined and phases calculated in SOLVE. These phases were used as a starting set for phase improvement in DM. In order to employ non-crystallographic symmetry (NCS) averaging, the operator relating the two molecules in the asymmetric unit was derived from the positions of the six Se atoms. Two sets of three atoms, each forming nearly identical, irregular triangles could be identified. A least-squares fit of one set onto the other revealed the rotational and translational operators. These were then input to DM for NCS averaging with automatic mask determination.

Model building and refinement

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 300 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, with the phases from DM included as experimental restraints. Refinement converged with an R_{cryst}/R_{free} of 0.15/0.20 and a manuscript is currently in preparation and will be submitted by mid 2000.