

Experiment title: Structure of a novel family PL10 pectate	Experiment
lyase from <i>Pseudomonas cellulosa</i>	number:

LS-1532

Beamline:	Date of experiment:	Date of report:
BM14	from: 07/12/99 to: 09/12/99	
Shifts:	Local contact(s):	Received at ESRF:
3	Vivian Stojanoff	

Names and affiliations of applicants (* indicates experimentalists):

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Report:

The crystal structure of a novel family PL 10 pectate lyase, Pel10A from *Pseudomonas cellulosa* was determined using a three wavelength MAD experiment on beamline BM14. The 3-D complex of an inactive mutant of PL10 was colleted on beamline ID29 in the presence of its substrate trigalacturonic acid. The Michaelis complex, with saccharide units spanning the site of bond cleavage, bares a striking resemblance to the tetragalacturonic acid complex observed for PelC, a genetically and structurally totally unrelated enzyme from family PL 1. The neutralisation of the -1 and +1 subsite saccharide uronic acid groups by a protein liganded Ca^{2+} ion, positioning of an arginine catalytic base relative to the C5 hydrogen of the +1 subsite galacturonic acid residue and other conserved enzyme/substrate interactions, considered in light of much mutagenesis data for both families, have led to the identification of a common catalytic mechanism. The notable absence of a proton donor in proximity of the scissile glycosidic bond in both complexes reveals that these enzymes may utilise a stereotypical trans-elimination mechanism, commencing with proton abstraction from the β -carbon performed by an arginine residue. The orientation of the +1 subsite sugar uronic acid substituent serves to facilitate maximal inductive and conjugative effects promoting the formation of the 4,5-unsaturated product.

References

S. J. Charnock, I. E. Brown, J. P. Turkenburg, G. W. Black and G. J. Davies (2001). Convergent evolution provides evidence for a common trans-elimination mechanism in family 1 and 10 polysaccharide lyases. Submitted to *Proc Natl Acad Sci (USA)*.

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