Poster Presentations

[MS5-P05] Crystal structure of galactarolactone cycloisomerase from Agrobacterium tumefaciens.

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D-Galacturonic acid is the main component of pectin, a natural polysaccharide, which is found in primary cell walls of terrestrial plants. Pectin-rich waste materials could be used as a source for production of biochemicals and biofuels, wherein a comprehensive understanding of the metabolic pathway is required. D-Galacturonic acid has two routes of degradation in bacteria: isomerase pathway and the oxidative pathway. [1-3] The oxidative pathway has been proven to be active in *Agrobacterium tumefaciens* [4-5] and *Pseudomonas syringae* [6].

A novel galactarolactone cycloisomerase enzyme (E.C. 5.5.1.-) from A. tumefaciens (At Gci) has been found that converts D-galactaro-1,4-lactone to a linear 3-deoxy-2-keto-hexarate in the oxidative pathway. [7] At Gci belongs to the enolase superfamily. The enolase family enzymes have many similarities in their reaction mechanism, but they catalyse different overall reactions, such as elimination of water [8], interconversion of enantiomers [9], and elimination of ammonia [10]. Each of them uses a common partial reaction in which an active site base abstracts an α-proton of the carboxylic acid containing substrate, which results in an intermediate that is stabilized by coordination to the essential Mg2+ ion.

The enzymes belonging to the enolase superfamily have a bi-domain structure with an α+β capping domain that contains the substrate specificity determining residues, and a (β/α)7β domain. The essential Mg2+ ion and the residues involved in the reactions that the enzymes catalyse are situated in the C-terminal modified TIM-barrel. The enolase superfamily enzymes are divided into subgroups based on different structures of their active sites. At Gci seems to belong to the mandelate racemase subgroup. [11]

The synchrotron source X-ray diffraction data of At Gci was measured at ESRF, Grenoble and obtained at 1.6 Å resolution. The refinement is currently on-going (at the moment Rwork= 15.6 % and Rfree= 17.8 %). The challenge has been in the interface between the flexible loops, in which the active site is located. Our aim is to define also the complex structures of the wild-type enzyme and some site-directed mutants with a substrate or a substrate analogue to elucidate the reaction mechanism and the role of the active site amino acid residues.

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