ESRF	**Experiment title:** Understanding Cephalosporin Biosynthesis: Structural Studies on Deacetoxycephalosporin C Synthase	**Experiment number:** LS-483
Beamline: BL19	**Date of experiment:** from: 23-24 Nov 1996 to: 29-30 Jan 1997	**Date of report:** 26 Febr. 1997
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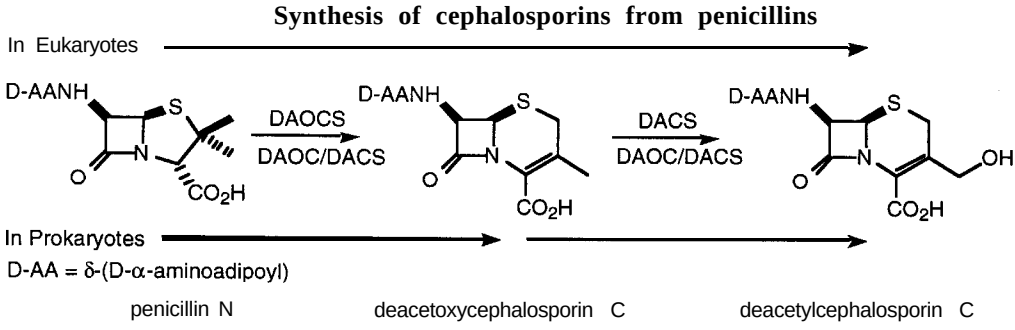
Report:

The penicillin and cephalosporin antibiotics are amongst the most important therapeutic agents in use today. All cephalosporin antibiotics are produced by complex and expensive synthetic modifications of fermented products. The objective of this project is to develop our understanding of the structure and mechanism of the enzyme which catalyses the key step in cephalosporin biosynthesis (deacetoxycephalosporin C synthase, DAOCS) to allow rational engineering of its specificity. Subsequently, the modified enzyme will be introduced into microorganisms so that cephalosporins of choice may be directly fermented without the need for synthetic modification, with concomitant reductions in production costs and toxic byproducts. As a first step toward this goal we are trying to determine the structure of DAOCS at high resolution.

DAOCS catalyses the expansion of the penicillin nucleus by inserting a carbon atom into the five-membered thiazolidine ring of the core (for a review see Baldwin & Schofield, 1993) and provides the link between penicillin and cephalosporin biosynthesis.



DAOCS and the other oxygenases of cephalosporin biosynthetic enzymes use iron and molecular oxygen, but the stoichiometry of their dioxygen utilisation is different to that of the penicillin pathway: only a two electron oxidation of the substrate is achieved for each molecule of dioxygen consumed. In addition, DAOCS requires α-ketoglutarate for catalysis. In each step, one molecule of α-ketoglutarate is transformed into carbon dioxide and succinate. There are so far no structures reported for α-ketoglutarate-dependent enzymes.

DAOCS *from Streptomyces clavuligerus* has been over-produced in *E. coli.* Since the enzyme is highly labile and the crystals are oxygen sensitive we were forced to develop very strict routines both for purification and crystallisation. Numerous crystallisation trials finally produced good quality crystals that diffract to 1.6 Å resolution (spacegroup R3, a=b=106.6 A and c= 70.4 A, one molecule in the asymmetric unit).

We have collected a number of datasets on beamline 19 under various conditions; a native data set to 2.1 Å resolution and several derivative data sets. The native data set contains 16 980 reflections to 2.1 Å resolution with an Rmerge of 0.038 and 99 % complete in the highest resolution bin (further details will be presented later). Due to a large number of free cysteines it is rather difficult to find good derivatives. The heavy atom search so far resulted in 4-5 possible derivatives sharing one single site. We are presently trying to incorporate data from anomalous and multi-wavelength anomalous diffraction (MAD) experiments to improve phasing but most derivatives are poor anomalous scatterers. We are also investigating the possibility to use a selenomethionine derivative for MAD phasing.

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