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Report:

The penicillin and cephalosporin antibiotics are amongst the most important therapeutic agents in use today. These antibiotics are produced from fermentation derived materials as their chemical synthesis is not commercially viable. The objective of this project is to develop our understanding of the structure and mechanism of the enzyme which catalyses the key steps in cephalosporin biosynthesis (deacetoxycephalosporin C synthase, DAOCS) to allow rational engineering of its specificity towards substrates and products. Subsequently, as a long time goal, the modified enzyme will be introduced into micro-organisms so that cephalosporins of choice may be directly fermented without the need for synthetic modification, with concomitant reductions in production costs and toxic by-products. As a first step towards this end we have recently determined the high resolution structure of DAOCS using data collected on BM14, ESRF (Valegård et al., in preparation). The structure was solved from merohedrally twinned crystals by the MIR method. This is the first structure of a 2-oxoacid dependent oxidase/dioxygenase to be reported.

DAOCS catalyses the expansion of the five-membered thiazolidine ring of the penicillin nucleus into the six-membered dihydrothiazine ring in the core of the cephalosporins thus providing the link between penicillin and cephalosporin biosynthesis (for a review, see Baldwin & Schofield, 1993). 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.

DAOCS from ***Streptomyces clavuligerus*** has been over-produced in E. ***coli*** (Lloyd et al., in preparation). Numerous crystallisation trials (Morgan et al., 1994; Lloyd et al., in preparation) finally produced good quality crystals that diffract to beyond 1.3 Å resolution (spacegroup R3, a=b=106.4 Å and c= 71.2 Å, one molecule in the asymmetric unit). During the last year we have collected a number of data sets on these crystals (native as well as derivative soaks) at BM14, ESRF.

Structure solution

By analysing the observed distribution of intensities with those predicted theoretically it became obvious that the DAOCS crystals were merohedrally twinned. This specific type of crystal twinning is caused by a rotatation involving a symmetry element of the lattice, which causes the reciprocal lattices of the twins to coincide. Extensive trials to obtain untwinned crystals of DAOCS or to solve the structure by molecular replacement using the IPNS model (Roach et al., 1995) all failed. We developed a detwinning procedure (Terwisscha van Scheltinga et al., in preparation) based on a modification of the method of Fisher & Sweet (1980). The detwinned data were used to solve the structure by multiple isomorphous replacement using three derivatives. To obtain starting phases, two data sets at different wavelength of a selenomethionine delivative crystal were collected at BM14. This ensured that the twinning fraction was the same for both data sets. It is thus clear that the excellent beam characteristics and the possibility to tune the wavelength was cruicial for the successful structure determination in this case. Full details of the procedures will be published elsewhere (Terwisscha van Scheltinga et al., in preparation). A full model has been built into an electron density map and refinement of the model is in its final stages.

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