using the hanging drop technique. The precipitant conditions are 12% PEG8K, 75mM $MnSO_4$, 100mM cacodylate pH 5.2. In order to obtain large single crystals, a temperature difference between the nucleation event and the growth event is essential. The space group of cholesterol oxidase is monoclinic $P2_1$. The cell dimensions are a=77.6Å, b=125.7Å, c=81.5Å and ß=109.1° with two molecules per asymmetric unit. X-ray data collection is carried out by flash-cooling the crystals to 115K in a nitrogen stream. One heavy atom derivative has been obtained by soaking the crystals in 0.5mM $K_2Pt(CN)_4$. Further screening for heavy atom derivatives is in progress.

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PS04.01.91 THE STRUCTURE OF N-ACETYLNEURAMINATE LYASE IN COMPLEX WITH INHIBITORS: A NEW SUB-FAMILY OF ALDOLASES. M.C. Lawrence[1], J.A.R.G. Barbosa[1], B.J. Smith[1], N.E. Hall[1], P.A. Pilling[1], H.C. Ooi[2] & S.M. Marcuccio[2]. [1]Biomolecular Research Institute, 343 Royal Parade, Parkville, Victoria 3052, Australia and [2]Biomolecular Research Institute, Private Bag 10, Clayton South MDC, Clayton South, Victora 3169, Australia

We present here the structure of N-acetylneuraminate lyase in complex with a known inhibitor hydroxypyruvate [1] and in complex with pyruvate via borohydride reduction. In both instances pyruvate is revealed covalently bound to the active site residue Lys-165. As the aldol cleavage reaction characteristic of this enzyme involves intermediate Schiff base formation between enzyme and substrate, the bound conformation of pyruvate presumably mimics that of the reaction intermediate state. The structures reveal in particular the interaction between the carboxylate group and the enzyme, the stereochemistry of the Schiff base complex and the role of the conserved residue Tyr-137 in substrate binding.

The structure of N-acetylneuraminate lyase [2] is similar to that of dihydrodipicolinate synthase [3] and the respective *E. coli* enzymes share a 24% sequence identity. Both sequences are similar to a third protein, MosA, of uncertain function and structure. We now show that two further enzymes (5-keto-4-deoxyglucarate dehydratase and trans-O-hydroxy-benzylidene-pyruvate hydratase-aldolase) are also members of this family. These enzymes all share pyruvate as either a product or substrate and we conclude that they share an active site structure and reaction pathway similar to that determined above for N-acetylneuraminate lyase. We predict these latter enzymes also to be $(\alpha/\beta)_8$ barrels and propose that they form a closely related aldolase sub-family.

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PS04.01.92 CRYSTAL STRUCTURE OF PECTIN LYASE B DETERMINED BY MOLECULAR REPLACEMENT. Jacqueline Vitali[1], Brian M. Schick[1], Harry Kester[2], Jaap Visser[2], and Frances A. Jurnak[1], [1]University of California at Riverside, Department of Biochemistry, Riverside, CA 92521 and [2]Wageningen Agricultural University, Department of Genetics, NL-6703 HA Wageningen, The Netherlands

Aspergillus Niger produces four pectin lyases which are involved in the degradation of pectic substances. Pectin lyase B (pelB) is a member of this family. It contains 361 amino acids and is homologous to pectate lyases C and E whose structure has been determined. The overall sequence identity is 18.6% with pelE and 15.8% with pelC. When only the central parallel β– helix motif is considered, the sequence identity is 29.3% with pelE and 24.7% with pelC.

Crystals of pelB are orthorhombic, $P2_12_12$, with cell constants a = 83.70, b=88.80, c= 42.28 Å. Data were measured to 1.7 Å resolution, 33106 unique reflections, Rsym = 0.054. The structure was determined by the generalized molecular replacement method using XPLOR, 7.0 - 3.0 A data with F > 4*sigma, and a 5.0 - 24.0 A shell of integration in the rotation function. The search model consisted of most of the central β-helix motif of pelB (207 amino acids) in which all amino acids were replaced with Ala except for those common in the two proteins (67 amino acids). The solution corresponds to the top peak in both PC refinements and translational searches with signal to noise ratios of 1.33 and 1.22, respectively, the signal to noise ratio being defined as the ratio of the top peak to the top error peak, and has no steric conflicts with symmetry related molecules. After rigid body, Powell, and B refinement, the R and Rfree indices were 0.482 and 0.512, respectively. The electron density map has density for several of the missing side chains and for several of the missing loop regions in the expected positions, indicating the correctness of the solution. We are in the process of diffusing heavy atoms into the crystals in order to improve the phasing. Our progress on the refinement will be reported in the presentation.

PS04.01.93 ELUCIDATING THE MECHANISM OF TYROSINE PHENOL-LYASE Alfred Antson[1], Guy Dodson[1], Keith Wilson[1,2] and Tatyana Demidkina[3], [1]Department of Chemistry, University of York, York Y01 5DD, UK., [2]European Molecular Biology Laboratory (EMBL), c/o DESY, Notkestrasse 85, D-2000 Hamburg 52, Germany, [3]Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 32 Vavilov St., Moscow 117984, Russia

To understand chemical and structural events associated with the catalysis by tyrosine phenol-lyase (TPL) we performed X-ray studies of several complexes of this enzyme with cofactor (pyridoxal 5'-phosphate) and substrate analogues. TPL catalyses reversible ß-elimination of L-tyrosine to produce pyruvate, phenol and ammonia. This reaction goes through a number of intermediate steps which involve two enzyme-associated bases that abstract protons from the substrate Cα and phenol hydroxyl. TPL requires K+ for activity and in addition to physiological reaction catalyses ß-elimination of a number of ß-substituted amino acids and also the racemisation of alanine. In our study we used the X-ray structure of the apo enzyme as a crystallographic matrix for structure solution of different TPL complexes. The monovalent cation binding site has been derived from difference Fourier maps between X-ray data from apo enzyme crystals soaked with Cs+ and K+. The structure! of the holo enzyme, obtained by co-crystallisation with pyridoxal 5'-phosphate, has been refined to a crystallographic R-factor of 17.7 % (Rfree=20.5 %) at 1.9 Å resolution. X-ray data have been collected from freeze-trapped complexes of TPL with L-Alanine and with L-Alanine + 4-hydroxypyridine. The last two complexes were prepared by soaking the holo enzyme crystals and are thought to represent the quinonoid intermediate that forms after the abstraction of the Ca proton. Refinement of these structures is presently underway. Details of the ligand interactions and the possible catalytic mechanism will be presented.