using the hanging drop technique. The precipitant conditions are 12% PEG8K, 75mM MnSO₄, 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 P2₁. The cell dimensions are a=77.6Å, b=125.7Å, c=81.5Å and β=100.1° 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 K₂Pt(CN)₄. Further screening for heavy atom derivatives is in progress.

References:


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We present here the structure of N-acetylneuraminic acid 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 of the enzyme. The crystal structure of pyruvate covalently bound to the active site residue of N-acetylneuraminic acid lyase has been determined by the generalized molecular replacement method using the hanging drop technique. The precipitant conditions are 12% PEG8K, 75mM MnSO₄, 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 P2₁. The cell dimensions are a=77.6Å, b=125.7Å, c=81.5Å and β=100.1° 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 K₂Pt(CN)₄. Further screening for heavy atom derivatives is in progress.

References:

PS04.01.92 CRYSTAL STRUCTURE OF PECTIN LYASE B DETERMINED BY MOLECULAR REPLACEMENT. Jacqueline Vitali, Brian M. Schick, Harry Kester, Jaap Visser, - Institute of Chemistry, University of York, York, UK.

To understand chemical and structural events associated with the catalysis by tyrosinase 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 β-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 CO₂ and phenol hydroxyl. TPL requires K⁺ for activity and in addition to physiological reaction catalyses β-elimination of a number of β-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 model for structure solution of different TPL complexes. The monomeric 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 holoenzyme, obtained by co-crystalisation 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 + 5'-hydroxypyrindine. The last two complexes were prepared by soaking the holoenzyme crystals and thought to represent the amino acid 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.