02.1-30 THE STRUCTURE OF YEAST ENOLASE. By L. Lebioda, B. Stec, Department of Chemistry, University of South Carolina, U.S.A. and J. M. Brewer, Department of Biochemistry, University of Georgia, U.S.A.

Following crystallization of yeast enolase A, we have collected native data to 2.25Å resolution. Useful data for three heavy-atom derivatives Sm, Hg and Pt were collected to 2.8Å, 3.2Å, and 3.5Å resolution respectively. MIR refinement provided phases with an average f.o.m. = 0.63 and 2.8Å resolution. Phase refinement carried out using the solvent flattening method increased the f.o.m. to 0.80. The mini-map calculated with the refined phases allowed tracing of 80% of the main chain and alignment of 20% of the sequence. Model building using interactive computer graphics and attempts to improve the map by the phase combination method are in progress.

The molecule of enolase is a dimer with the subunits related by a two-fold crystallographic axis. Each subunit is composed of two domains. The subunit is dome-shaped with the diameter of about 60Å at the flat base and height of about 40Å. The flat bases are in the subunits interface and the dimeric molecule has the shape of an ellipsoid 80x60x60Å.

Each subunit is composed of two domains. There is a deep cleft between the domains in which the Sm^{3+} replaces Mg^{2+} in the structural cation binding site. We assume that the active site is located in the cleft.

Enolase is an αβ protein with about 10% of the residues in β-sheets and 40% in helices. In contrast to the other glycolytic enzymes apparently there is no large parallel β-sheet but only two small three-stranded sheets. The one for which we have presently the sequence aligned is antiparallel.

02.1-31 TERTIARY STRUCTURAL SIMILARITY BETWEEN A A CLASS A β-LACTAMASE AND A PENICILLIN-SENSITIVE D-ALANYL CARBOXYPEPTIDASE-TRANSPEPTIDASE. By R.J. Todd, B.J. Sutton, P.J. Artymiuk, C.B. Baguley and D.C. Phillips, Laboratory of Molecular Biophysics, Rex Richards Building, South Parks Road, Oxford OX1 3QU, UK.

β-lactam antibiotics act by inhibiting enzymes that catalyse the final stage of the synthesis of bacterial cell walls. However it is mainly through the production of β-lactamases that bacteria can mount an effective defence against these antibiotics. It has been suggested that the β-lactamases and β-lactam target enzymes may be evolutionarily related based on the observation that there is some similarity of amino-acid sequence around a common active-site serine residue.

The 3-dimensional structure of β-lactamase I from Bacillus cereus (a class A enzyme) has been solved to a resolution of 2.5Å. The present model derived through the iterative process of map interpretation, restrained-least-squares refinement, phase and map recalculation has a reliability index of 0.30. The molecule comprises a 5-stranded β-pleated sheet with a group of three α-helices on one side of it and eight helices on the other. This arrangement of secondary-structure elements in β-lactamase I shows a striking resemblance to the structure of the penicillin-sensitive D-ala D-ala carboxy-peptidase-transpeptidase from Streptomyces R61 (J.A. Kelly et al., J. Biol. Chem. , 1985, 260, 6449-6458). This conservation of the tertiary structure supports the evolutionary hypothesis, however it also raises questions about the previously held view that separate classes of β-lactamase enzymes evolved independently.

Binding studies with the mechanism-based inhibitor 6-β-bromopenicillanic acid have been performed and a low resolution map calculated. The interpretation of these results will be discussed.

02.1-32 SMALL ANGLE X-RAY SCATTERING FROM ANNELID HEMOGLOBIN IN SOLUTION. C.F. de Souza and I.L. Torriani, Instituto de Física, Universidade Estadual de Campinas. C.F.S. Bonafé and N.C. Meirelles, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, S.P., Brazil.

Hemoglobin from the annelid species Glossoscolex paulistus is one of the giant respiratory proteins (erythrocruorins) with molecular weight of the order of 4.10^6 D. Electron microscopic studies revealed the same hexagonal structure reported for erythrocruorins from other worm species. The minimum molecular weight was estimated to be 20/30,000 D. Detailed information about the number of subunits, their composition and the number of heme groups per molecule is still unknown. Small angle scattering curves were obtained for several concentrations of the protein in solution. The apparent radius of gyration was determined by extrapolation to infinite dilution, using for each concentration the lower angle linear part of the Guinier plots obtained from the smeared scattering data. The value obtained for this parameter was R_g=114 A. This value is similar to the radius of gyration reported for other annelid hemoglobins (J.Pilz et al., Int.J.Biol.Macromol.,1980,2,279). The radius of gyration and maximum dimension were also calculated using the inverse transformation method (O.Glatter,Acta Phys.Austriaca,1977,47, 83).The values obtained were R_g=115 A and D_{max}=270 A.

02.1-33 PRELIMINARY STUDIES ON TUMOR NECROSIS FACTOR (TNF). By A. Bentley, G. Bricogne, R. Fourme, T. Prangé, P. Vachette, LURE Bât.209d, Université PARIS-SUD, 91405 ORSAY cedex, France; W. Fiers, J. Tavernier Biogent, Jozef Plateaustraat 22 and Laboratory of Molecular Biology, State University of Ghent, Belgium.

Tumor Necrosis Factor (TNF) is a protein involved in the regression of tumors in mice. It was first isolated from serum of animals treated with BCG. Human TNF produced by macrophages has now been cloned by several groups.
TNF is a small protein about 17Kdaltons. Crystals were obtained by vapor diffusion at pH 7.9-8.0 in Tris buffer with ammonium sulfate as precipitating agent. This leads to the formation of hexagonal rods 0.4x0.4x0.8 mm in about 2 weeks.
The crystals are trigonal, space group $P3_121$ with a=b= 165 A and c=92.5 A. A complete diffraction data set was obtained at the Synchrotron Facility at LURE on the 2D multiwire proportional chamber (512x512 pixels), acquisition time 20s/frame; Φ-range: 60°, $\Delta\Phi$ = 0.025°/frame. The life-time of the crystals is about 20 h. The off-line treatment of the frames gave a total of 11,250 independent reflections above 3σ . The diffraction extends to 3.5 A in a and b directions but resolution is lower along the c axis.
From small angle X-ray scattering experiments, TNF is found associated as trimers in solution. It is likely that these are preserved in the crystalline state.
The search for heavy atom derivatives is under way .