**02.1–38** LINEAR DICHROISM MEASUREMENTS ON SINGLE CRYSTALS OF THE PYRIDOXAL PHOSPHATE DEPENDENT ENZYME MITOCHONDRIAL ASPARTATE AMINOTRANSFERASE. By M.G. Vincent, D. Picot, G. Eichele and J.N. Jansonius, Biozentrum, University of Basel, Switzerland and H. Kirsten and P. Christen, Biochemisches Institut, University of Zürich, Switzerland.

Aspartate aminotransferase (mAspAT) is an $\alpha_2$ dimeric enzyme of $M_r$ ~ 90000. Each subunit has a copy of the coenzyme pyridoxal phosphate, which is reversibly modified into pyridoxamine phosphate when the dicarboxylic amino acid substrate is transformed into oxo-acid product. The free forms of the enzyme and the catalytic intermediates each have a specific electronic absorption spectrum. The transition dipole moments corresponding to the ($\pi$-$\pi^*$) absorption bands are restricted to the coenzyme ring plane.
Polarized absorption spectra have been measured on triclinic and orthorhombic single crystals of derivatives of mAspAT mimicking various functional states of the enzyme. The data from the orthorhombic crystals have been used to determine the orientations of the transition dipole moments (TDM's) of the coenzyme in these derivatives with respect to the crystallographic axes. Correlation with X-ray crystallographic data has, moreover, allowed determination of the TDM directions in the coenzyme pyridine ring plane. Calculations based on these results have correctly predicted the observed polarization directions in the triclinic crystals.

Although most of the MIR map was clear, there were several disordered regions which made it impossible to trace more than 75% of the two protein chains by inspection. To aid in interpreting various portions of the protein, a program was undertaken to identify side chains by chemical modification and difference Fourier analysis.

In addition, the partial model structure was improved by a refinement method similiar to that used by Huber and his coworkers on citrate synthase (Remington et. al., J. Mol. Biol.,258,111-152(1982)). That is, 2Fo-Fc and Fo-Fc maps using combined MIR and calculated phases were used to adjust the partial structures in cycles of refinement.

The ricin model building is nearly complete and tentative backbone tracings have been made for each chain. Crystallographic refinement using 2.5A data is now in progress to confirm this tentative structure.

**02.1–39** STRUCTURAL INVESTIGATIONS OF THE GLYCOLYTIC ENZYMES OF TRYPANOSOMA BRUCEI. By R.K. Wierenga*, O. Misset, F.R. Opperdoes, and W.G.J. Hol*, International Institute of Cellular and Molecular Pathology, Avenue Hippocrate 74, B-1200 Brussels, Belgium and *State University Groningen, Nyenborgh 16, 9747 AG Groningen, Holland.

Our ultimate goal is the development of new drugs against sleeping sickness, a tropical infectious disease caused by protozoan haemoflagellates of the Trypanosoma brucei complex (T. rhodesiense and T. gambiense). The glycolytic enzymes of these organisms are located within a separate organelle (glycosome) (Opperdoes, F.R. and Borst, P. (1977), FEBS Lett., 80, 360-364) in which they are probably associated with each other as a multienzyme complex. This situation is unique and not found in other eukaryotic cells. For this reason the trypanosomal glycolytic enzymes may serve as an excellent target for non-empirical drug design.
For a comparison of the three-dimensional structure of trypanosomal enzymes and mammalian counterparts we have started with the isolation and crystallization of the glycolytic enzymes from T. brucei.
A procedure has been worked out for the simultaneous purification of large quantities of four enzymes : hexokinase (HK 4 mg), triosephosphate isomerase (TIM 1 mg), aldolase (ALDO 15 mg) and phosphoglycerate kinase (PGK 2 mg) in high yield starting from 40 g wet weight trypanosomes obtained from the blood of 80 infected rats. These four pure enzymes have been subjected to a plethora of crystallization experiments on a microscale which has led so far to crystals of ALDO, TIM and HK. Currently we are investigating the X-ray quality of the crystals obtained.

Subseqent analysis revealed that these domains arose by gene duplication (Villafranca and Robertus, J.Biol. Chem.,256,554-556(1981)).

**02.1–40** THE CRYSTAL STRUCTURE OF TROPONIN C FROM TURKEY SKELETAL MUSCLE AT 2.8Å RESOLUTION. By O. Herzberg and M.N.G. James, MRC Group in Protein Structure and Function, Department of Biochemistry, University of Alberta, Edmonton, Alberta, T6G 2H7.

Troponin C is one of three proteins that comprise the troponin complex. It binds $Ca^{2+}$ ions and via large conformational changes triggers the process that leads to muscle contraction.

Crystals of troponin C from turkey skeletal muscle (MW=18,000) suitable for high resolution X-ray studies have been grown from a $Ca^{2+}$ containing solution. Their space group is $P3_221$ with cell dimensions: a=b=66.6Å, c=61.0Å, $\alpha=\beta=90°$, $\gamma=120°$. The crystal structure was determined by the MIR method. Native data were collected to 2.2Å resolution and eight heavy atom derivative data sets to 2.8Å resolution. The heavy atom sites were interpreted by a combination of difference Patterson and difference Fourier maps, and the sites parameters refined using a phase refinement computer program. A centroid phase set was obtained at 2.8Å resolution with an overall figure of merit of 0.80. The electron density map, calculated with these phases was plotted on transparent sheets, revealing stretches of continuous density of a rather globular shaped molecule including a substantial amount of $\alpha$-helix. $C_\alpha$ atom positions were assigned on this map, and their coordinates as well as the electron density map, transferred to a MMSX graphics system on which the fitting of a polyalanine chain to the map was carried out. The amino acid composition of troponin C from turkey skeletal muscle differs only by two residues from that of chicken. The sequence of the latter is known, and based on it the assignment of the side chains to the polyalanine model is in progress.

(Research supported by the Medical Research Council of Canada and the Alberta Heritage Foundation for Medical Research.)