02.4-3 X-RAY CRYSTALLOGRAPHIC STUDIES ON THE COMPLEX OF MUNG BEAN TRYPSIN INHIBITOR WITH PORCINE TRYPSIN. By Li Genpei, Chen Zhongguo, Zeng Jie, Tang Youchi, Institute of Physical Chemistry, Peking University, Beijing; Lin Guangda, Zhang Rongguang, Xuan Jiancheng, Chi Zhengwu, Tsao Tienchin, Institute of Biochemistry, Academic Sinica, Shanghai, China.

The Mung bean trypsin inhibitor (MBI) has been thoroughly characterized and its complete amino acid sequence also has been determined.
The molecular weight is about 8000 daltons with 72 residues. Crystals of the inhibitor with Porcine trypsin (PTRY) complex have been grown suitable for X-ray diffraction studies by combination of vapor diffusion in hanging drops and reseeding methods. Two crystal forms of this complex have been obtained. The crystal data for both crystal forms are: a=b=122.3Å, c=113.4Å, tetragonal I422; a=b=62.4Å, c=160.1Å, γ=120°, trigonal $P3_121$. Both crystal forms contain one complex molecule per asymmetric unit. An X-ray structure determination has been undertaken of the I422 crystal form. The 4Å resolution data set for the tetragonal crystal form include a total of 4663 reflections of which 3227 were unique (R_{mer}=0.082). The phase problem for the structure of MBIPTRY was solved by using the technique of molecular replacement. The computed phases and measured amplitudes were used to calculate Fourier and difference Fourier maps at 4 Å resolution. The maps showed the molecular outline and orientation in the unit cell. The MBI moiety roughly resembles an elongated ellipsoid with approximate dimensions 15x45x18 Å and the major axis is oriented along the [110] direction. The polypeptide folding could be traced in the map and most of the C_α atoms could be accommodated in corresponding electron density. The overall shape shows that the MBI molecule is made up of two globular domains, each approximately 15 Å in diameter. The two domains have large surface areas in close proximity but at this resolution there are only two strands of electron density connecting them. The most striking feature of MBI is that its polypeptide folding does not include any α-helices. The overall shape and size of the MBI, as well as folding pattern have also been checked with the results of the trigonal crystal structure at 3 Å resolution. The future of the MBI-PTRY structure at higher resolution is now in progress.

02.4-4 MODELLING OF ANTIBODIES AND THEIR ANTIGEN COMPLEXES: TOWARDS AN UNDERSTANDING OF ANTIBODY-ANTIGEN INTERACTIONS. By J.C. Cheetham, S. Roberts and A.R. Rees, Laboratory of Molecular Biophysics, Oxford University, England.

The modelling of structures of homologous proteins, on the basis of a crystallographic study of one member of the family, is now well established. Application of similar methods to a series of five monoclonal antibodies (Gloops 1 to 5) produced structural models for each, in which the complementarity determining regions (CDR's) were modelled on the basis of sequence homology and length using a database of known antibody structures (de la Paz et al, EMBO J., 1986,5,415-425). For one of these antibodies, Gloop2, a docked model for the antibody:antigen complex with hen egg-white lysozyme (HEL) has been produced, and energy minimised using GROMOS. An attempt to model the effects of single and double mutations within the antibody CDR's has been made, and the results assessed in the light of experimental data from site directed mutagenesis (SDM) experiments (Roberts et al., unpublished results).
Preliminary modelling of a second antibody:antigen complex using Gloop5, which shows significantly enhanced affinity for HEL relative to Gloop2, has also been undertaken with a view to establishing the origin of the difference between the two antibodies. The results of this study might then allow the design of a mutagenesis experiment in which, by making specific mutations within the combining site region, the binding characteristics exhibited by one antibody towards a specific antigen may be engineered into another.

02.4-5 THE STRUCTURE OF A COMPLEX BETWEEN INFLUENZA VIRUS NEURAMINIDASE AND AN ANTIBODY. By P.M. Colman, J.N. Varghese, A.T. Baker, P.A. Tulloch, W.G. Laver (1), G.M. Air (2) and R.G. Webster (3). CSIRO Division of Protein Chemistry, Parkville 3052, Australia, (1) John Curtin School of Medical Research, Australian National University, Canberra, (2) Department of Microbiology, University of Alabama, Birmingham, Alabama, (3) St. Jude Children's Research Hospital, Memphis, Tennessee.

There are currently few hard data describing the structural details of the interaction of an antibody with an antigen. In the one example described to date, the two components unite without changes in the conformation of either (Amit, A.G. et al. Science 233, 747-753).

X-ray diffraction analysis of crystals of the influenza virus antigen neuraminidase complexed with Fab fragments from an antineuraminidase monoclonal antibody has enabled the identification of an antigenic epitope on the neuraminidase (Colman, P.M. et al. Nature In press). Amino acid sequence changes within the epitope are known to abolish the binding of the antibody to neuraminidase.

Changes in the structure of the antigen accompany the binding of the antibody. Shifts of 1-1.5Å are observed in one of the surface loops of the neuraminidase, and result in a small replacement of one of the active site amino acids, Arg371. This movement might explain the lowered neuraminidase activity of this particular complex to small tri-saccharide substrates. There appears to be no steric hindrance imposed by the antibody on the access of a small substrate to the enzyme active site.