

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 reseeded methods. Two crystal forms of this complex have been obtained. The crystal data for both crystal forms are:  $a=b=122.3\text{\AA}$ ,  $c=113.4\text{\AA}$ , tetragonal I422;  $a=b=62.4\text{\AA}$ ,  $c=160.1\text{\AA}$ ,  $\gamma=120^\circ$ , trigonal P3<sub>1</sub>21. Both crystal forms contain one complex molecule per asymmetric unit. An X-ray structure determination has been undertaken of the I422 crystal form. The 4 $\text{\AA}$  resolution data set for the tetragonal crystal form include a total of 4663 reflections of which 3227 were unique ( $R_{\text{mer}}=0.082$ ). 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  $\text{\AA}$  resolution. The maps showed the molecular outline and orientation in the unit cell. The MBI moiety roughly resembles an elongated ellipsoid with approximate dimensions  $15 \times 4.5 \times 18\text{\AA}$  and the major axis is oriented along the [110] direction. The polypeptide folding could be traced in the map and most of the  $C_\alpha$  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  $\text{\AA}$  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  $\alpha$ -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  $\text{\AA}$  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 1-1.5 $\text{\AA}$  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.

The pairing of VL and VH domains in the Fab fragment of the antibody is outside the range of structures observed in other studies on uncomplexed Fab fragments. We interpret this observation to imply that antigen can induce a fit in the combining site of an antibody by a sliding of VL and VH domains at their interface, generating shifts in the position of the complementarity determining residues of up to 4Å.

Studies on a complex of neuraminidase with a second Fab fragment are well advanced and should further address the question of conformational changes in antigen-antibody interactions.

constrained-restrained crystallographic refinement (CORELS). The current R-factor is 37.2% for 8592 reflections. The resulting 2Fo-Fc map was of sufficient quality to permit a preliminary visualization of the inhibitor bound to the pepsin active site. Several putative differences were observed between the conformations of native and inhibited enzyme, including about a 1.5Å rms main chain movement of the flap residues (75-77) downward, towards the inhibitor. Probable interactions between pepsin and A63218 included a hydrogen bond between one of the key active site carboxylates, asp32, and the 3-OH of the inhibitor. Further refinement of both the apoenzyme and the complex crystal structures should elucidate the bioactive conformation of A63218 as well as the detailed intermolecular contacts and any additional structural changes for the inhibited enzyme.

02.4-6 THE 2.5Å CRYSTAL STRUCTURE OF A POTENT HUMAN RENIN INHIBITOR(A63218) BOUND TO PORCINE PEPSIN. J. Erickson, C. Abad-Zapatero, T.J. Rydel, and J. Luly, Pharmaceutical Discovery Division, Abbott Laboratories, USA.

Human renin is an aspartyl protease that is responsible for the humoral conversion of angiotensinogen to angiotensin I. This process is the first, and rate-limiting, step in the production of the peptide hormone angiotensin II, which exerts a powerful effect on both blood pressure and electrolyte balance. Thus, renin has been the target for the synthesis of a novel class of anti-hypertensive peptidomimetics. A number of potent(sub-nanomolar) renin inhibitors were found to also inhibit porcine pepsin, an aspartyl protease which exhibits nearly 40% sequence homology to human renin. In our attempts to design effective active site blockers for renin and to rationalize existing structure-activity data for Abbott peptidomimetics, several of these inhibitors were co-crystallized with pepsin at pH 2.0 in 20% ethanol. The crystal structure of the (2S,3R,4S)-2-(Etoc-p-I-phe-leu-amino)-1-cyclohexyl-3,4-dihydroxy-6-methyl-heptane(A63218) compound complexed with pepsin was solved at 2.5Å resolution using the partially-refined (R=33%, 10.0-3.0Å data) crystal structure of porcine pepsin in 20% ethanol (Andreeva, et al., 1984; Abad-Zapatero, et al., in preparation). The complex was crystallized in space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>, with cell constants a=123.9Å, b=64.9Å, c=36.2Å, and with one complex in the asymmetric unit. The position and orientation of pepsin in the orthorhombic crystal cell were deduced from cross-rotation function searches and from packing considerations based on a comparison with the monoclinic pepsin crystal. After a six-parameter, rigid body R-factor minimization, the enzyme portion of the complex was refined against 10.0-2.5Å data using the method of

02.4-7 STRUCTURAL STUDIES ON THE LENS  $\beta$ ,  $\gamma$  CRYSTALLINS. By B.Bax, M.Bibby, T.Blundell, H.Driessen, P.Lindley, L.Miller, D.Moss, S.Mylvaganam, S.Najmudin, C.Slingsby, H.White, Department of Crystallography, Birkbeck College, University of London, England.

The monomeric  $\gamma$ -crystallins and the oligomeric  $\beta$ -crystallins are thought to have evolved from a single ancestral gene. These two families of eye lens proteins account for a large proportion of protein in the mammalian lens. A high concentration of protein in the lens is necessary to maintain a high refractive index. We are studying the supramolecular organization of the lens by studying both the monomeric  $\gamma$ -crystallins and the oligomeric  $\beta$ -crystallins. A high resolution (1.5Å) study of bovine  $\gamma$ II crystallin, shows details of protein water interactions. Lower resolution studies of  $\gamma$ IIIb (2.6Å) and  $\gamma$ IVa (2.3Å) show subtle differences between these highly homologous structures (homology about 80%). We have determined the structure of bovine  $\gamma$ IVa using molecular replacement. The study of intermolecular contacts in  $\gamma$ II,  $\gamma$ IIIb and  $\gamma$ IVa crystals gives useful information about possible intermolecular contacts in the lens. Both bovine  $\gamma$ IIIb and  $\beta$ B2 form crystals showing pseudo-symmetry. The structure determination of  $\beta$ B2 at 3.4Å, using M.I.R., yields information about the quaternary interactions in the  $\beta$ -crystallin aggregates.  $\beta$ B2 crystallises with two dimers in the asymmetric unit (Mr 92kDa). Problems in structure determination have been encountered due to poorly diffracting crystals and pseudosymmetry. Progress is being made with a new tetrameric methane derivative. Model building studies have been carried out on the  $\beta$ -crystallins, based on their homology with the  $\gamma$ -crystallins (about 30%). These have revealed highly conserved surface regions on some orthologous  $\beta$ -crystallins from different species.