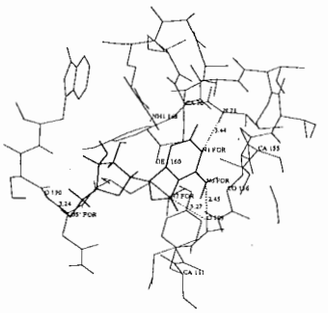
method. Now the refinement of these structures were finished using XPLOR program. The conventional R values of these structures are between 0.18-0.19 and their deviations from the ideal bond length and bond angle are 0.01-0.015Å, 1.0-2.0° . In these complexes we can see the electron density of formycin and the hydrolytic product of ATP. We have analysised the active center geometry of these two proteins in detail. It was found that the base is located in a hydrophobic pocket at the surface of the protein and is recognized by interactions specific toward adenine. These interactions include aromatic stacking, hydrophobic and hydrogen bonds. The sugar of the ligand contacted with side chains of two highly conserved resiudes 160Glu and 163Arg. A depurine mechanism is proposed based upon these structures. The specificity of these RIPs are also discussed.



The complex structure of MMC+Formycin

PS–03.01.10

PURIFICATION AND PRELIMINARY CRYSTALLIZATION EXPERIMENTS ON DNA-BINDING PROTEINS HUMAN TOPOISOMERASE I AND HIV-2 INTEGRASE

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DNA-binding proteins are responsible for many critical biological processes. Their functions can be understood on the molecular level with the promises of three-dimensional structural studies. Since the first crystal structure determination of the E. coli catabolite activator protein (CAP) in 1980, more than 20 DNA-binding protein and protein-DNA complex structures have been reported, thanks to the recent technical advances in molecular genetics, DNA synthesis and protein crystallography. We are currently trying to crystallize a number of DNA binding proteins for crystallographic studies. Two of them are described below.

Topoisomerase I catalyzes the breakage and rejoining of one of the strand of a duplex DNA, thus introduces topological changes of DNA. Eukaryotic topoisomerase I is also found to be the target of the anticancer agent camptothecin. Human topoisomerase I, a 765 amino acid protein, was expressed in insect cells. Large amounts of active, homogeneous and soluble topoisomerase I can be obtained routinely from the cell extracts. Oligonucleotides of different lengths have been designed and purified. Preliminary crystallization attempts have been carried out for the protein itself, the protein-DNA complex and the protein-DNA-camptothecin ternary complex. A series of systematic matrixes, which has been very successful in crystallizing many proteins in this laboratory, is being exploited and the results will be reported.

Integrase is the only protein required for the integration of linear viral DNA into the chromosome of a host cell. Human immunodeficiency virus type 2 (HIV-2) integrase is essential for the replication of the virus and a possible target for effective drugs against AIDS. HIV-2 integrase has a molecular weight of 32 kDa and may contain a zinc finger domain, a catalytic domain and a DNA binding domain. It has been expressed in E. coli and purified to near homogeneity. An apparent obstacle to crystallization is that, even in high glycerol (10 %) and NaCl (0.75 M) concentration, the protein is soluble only to 2 mg/ml. Crystallization experiments carried out in the presence and absence of oligonucleotides of different lengths, using various methods and conditions, will be reported.

03.02 – Viruses

MS–03.02.01 STRUCTURAL STUDIES OF VIRAL CAPSIDS.
by M.G.Rossmann*, Department of Biological Science, Purdue University, West Lafayette, IN 47907, USA

The three–dimensional atomic structure of about a dozen different virus groups have now been determined. These include viruses that encapsidate single–stranded RNA, single–stranded DNA as well as double–stranded DNA. They also include viruses that infect plants, mammals, insects and bacteriophages. In the majority of cases the capsid proteins have an eight–stranded antiparallel β–barrel. The icosahedral organization of the proteins follows the predictions of Caspar & Klug remarkably well. The adaptation to quasi–symmetrical environments is generally produced by different structures of flexible polypeptide ends that regulate the subunit contacts.
The combination of crystallographic and cryo–electron microscopic studies has become an important tool. It has been possible to determine the organization of scaffolding proteins in the assembly of bacteriophages such as φX174. These proteins, like chaperonins, are required for the assembly of empty capsids, but are not present in the mature infectious virions. Other examples of viruses (e.g.parvo– and picornaviruses) with neutralizing antibodies, of viruses complexed with their cellular receptors and in the analysis of reconstituted cores of Sindbis virus.

MS–03.02.02 The Structure of Theiler's Virus **Ming Luo, Center for Macromolecular Crystallography, University of Alabama at Birmingham, Birmingham, Al 35294, USA**

Theiler's virus belongs to the picornavirus family and can be further divided into two subgroups. One is composed of highly virulent strains (e.g. FA and GDVII), which causes acute polioencephalomyelitis in mice, and the other consists of less virulent strains (e.g. BeAn and DA), which causes demyelination in mice after the establishment of a persistent infection. The less virulent strains have be used as an animal model to study human demyelination diseases such as multiple sclerosis. It has been shown that the virulency is related to the capsid protein, probably related to the mode of the virion attachment to the host receptor, host immune response and/or the capsid stability. The three dimensional structure of TMEV at 3.0 Å resolution reported by Luo et al. revealed the structural basis for the neural virulence of TMEV. Potential sites for virus attachment to the host receptor and dominant immunogen determinants distinguishing the two subgroups were mapped on the capsid. The result helps us a great deal in understanding the host receptor recognition, virion stability, and viral pathogenesis of TMEV. We are also in the process of determining the structure of a highly virulent TMEV strain, GDVII. Our group has been successful to crystallize the GDVII virus and the crystals were brought to SSRL to collect X-ray

diffraction data. The data collection was performed at Station X7 using X-rays of 1.08 Å wavelength. The diffraction patterns were recorded on the newly installed image plate system made by Mar research. A total of abot 50 useful images were processed and the space group of the crystals was determined as C2, a=575.4Å, b=323.7Å, c=558.4Å, β=108.5°. There is a whole virus particle in the asymmetric unit, which results in a 60 fold nonocrysatllographic averaging in phase determination by the molecular replacement method. About 50% unique data were collected to 3.5Å resolution and the Rmerge was 11.5% for data with $I/\sigma>2.0$. A locked rotation function was calculated using data to 4.0 Å resolution and the virus orientation was clearly identified in the unit cell. Work is now continued on the translation of the virus particles in the unit cell. Once the position of the virus particles is known, phases could be first derived from the BeAn coordinates and improved by electorn density averaging over the 60 fold noncrysatllographic symmetry. The final structure of GDVII will reveal the differences between the highly-viruslent group and the persistent group on the capsid. We shall learn more about the relationship bewteen virus infection and demyelination and persistence infection.

MS-03.02.03 STRUCTURE OF THE VIRION AND EMPTY CAPSIDS OF A22 IRAQ 24/67 FOOT-AND-MOUTH DISEASE VIRUS. By S. Curry*, R. Abu-Ghazaleh, W. Blakemore, E. Fry†, T. Jackson, A. King, S.Lea†, J.Newman, D. Stuart†. AFRC Institute for Animal Health, Pirbright Laboratory, Pirbright, Woking, U.K. †Laboratory of Molecular Biophysics, Oxford University, U.K.

We have crystallized and determined the three-dimensional structure of A22 Iraq 24/64 foot-and-mouth disease virus (FMDV). We now have representative structures of at least one subtype from three of the seven serotypes defined for FMDV. The results of the structural analysis of A22 Iraq confirm and extend the findings from the comparison of O and C type viruses.

A22 Iraq crystallized in space group I222; a data set 70% complete to 3Å was collected. Structure factors were phased using a partially refined model of $A10_{61}$ (Fry, E. *et al.* unpublished) FMDV and a $2|F_O| - |F_C|$ electron density map was calculated at 3Å resolution. This map, averaged over the fifteen protomers in the asymmetric unit, was of high quality and enabled an initial model to be built. Refinement is in progress.

Preliminary analysis of the structure reveals that structural differences between A22 Iraq and other serotypes are confined to external loops. As observed for O1BFS (Acharya, R. *et al.* (1989), *Nature (London)*, **337**, 709-716.) and CS8-c1 (Lea, S. *et al.* manuscript in preparation) FMDV, the longest surface loop which connects strands G and H of the beta-barrel core of VP1 is largely disordered. This GH loop is an important antigenic feature and also implicated in binding to the cellular receptor. The mobility of this feature appears to be strongly conserved in FMDV. The implications of this finding will be discussed. Elsewhere on the surface of the virion there are shifts of several Ångströms in the positions of surface oriented loops. Moreover, there is evidence that structural alterations in one loop due to sequence variation can affect the conformation of adjacent loops. This structural information is being used to interpret the large amount of serological data available for serotype A FMDV.

Empty capsids of FMDV lack the genomic RNA and are reported also to lack the cleavage of the precursor VP0 into VP2 and VP4 which is simultaneous with encapsidation and contributes to the stability of the virion. In order to probe both the mechanism of VP0 cleavage and the RNA-protein interactions on the interior of the capsid, we have determined the structure of empty capsids of A22 Iraq. Curiously, in these capsids VP0 has largely been cleaved into VP2 and VP4. Our investigation into the reasons for this finding will be presented.

Empty capsids crystallized isomorphously with the virus. A difference map revealed that the external surface structures of the virus and empty capsid are identical. However, on the interior surface the n-termini of VP1 and VP2 and the c-terminus of VP4 appear to be disordered in the empty capsid relative to the virus. These termini are all close to the interface between pentameric subunits which are assembly intermediates in FMDV. The presence of RNA in the virus appears to be necessary to stabilize these portions of the capsid polypeptides.

MS-03.02.04 THE STRUCTURE OF CANINE PARVOVIRUS. By Hao Wu*, Jun Tsao, Michael Chapman, Walter Keller, Mavis Agbandje, and Michael Rossmann, Dept. of Biol. Sci., Purdue University, West Lafayette, IN, 47907. *Current address: Dept. of Biochem. and Mol. Biophys., Columbia University, New York, NY 10032.

The structures of canine parvovirus (CPV) full and empty particles have been determined to atomic resolution. The structure of CPV empty capsid has also been refined recently.

Each subunit of the virus possesses an anti-parallel β barrel motif that has been found in most viruses whose structures are known. On the viral surface, there are canyons around the fivefold axes and prominent spikes at the three-fold axes. With analogy to Rhinovirus14, the canyons might be the receptor binding sites. Residues related to the antigenic properties as well as the host range determinants of the virus are found on the threefold spikes. Extensive interactions are observed among the threefold related subunits. Five β hair-pins at each fivefold axis make up a β cylindrical structure. A substantial volume of electron density is shown to be DNA, corresponding to about 13% of the virus genome. Different protein conformations are observed at the region where DNA binds.

MS-03.02.05 STRUCTURAL STUDIES OF TYPE B NEURAMINIDASE. By Clinton L. White*, Musiri N. Janakiraman, W. Graeme Laver, Gillian M. Air and Ming Luo; Center for Macromolecular Crystallography, University of Alabama at Birmingham, Birmingham, Alabama, USA.