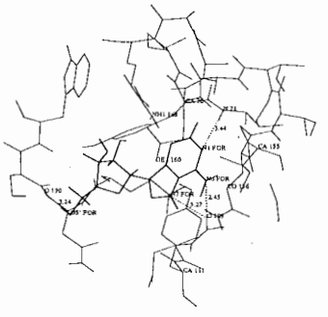
method. Now the refinement of these structures were finished using XPLOR program. The conventional R values of these structures are between 0.18-0.19 and their deviations from the ideal bond length and bond angle are 0.01-0.015Å, 1.0-2.0° . In these complexes we can see the electron density of formycin 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 residues 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

