03-Crystallography of Biological Macromolecules

PS-03.01.07 FIBRE DIFFRACTION STUDIES OF COMPLEXES BETWEEN DNA AND THE RecA PROTEIN OF E.COLI By V.T. Forsyth*, P. Langan, +J. Torbet, +E. DiCapua, Department of Physics, Keele University, England and +European Molecular Biology Laboratory, Grenoble, France.

The recA protein of E.Coli is a relatively small protein of molecular weight 38000 which plays a central role in homologous recombination and in mutagenesis. It is also involved in the control of gene expression during the "SOS" stress reaction. The protein forms filamentous complexes both with single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA). Although the three-dimensional structure of these filaments has been characterised at low resolution by electron microscopy and also by small-angle neutron scattering, there are a number of crucial aspects concerning the structural relationship between the DNA and the protein which are not yet established and which are likely to be important in relating the structure of these complexes to their biological function *in vivo*. Fibre diffraction experiments are being undertaken to study this relationship.

Current work is focussed on the study of complexes between recA and dsDNA. Results from electron microscopy have shown that the recA coats the outside of the DNA so that there are just over 6 recA monomers per turn of the helix which has a pitch of approximately 95A. Within one pitch length of the complex there are believed to be 18 DNA base-pairs corresponding to an average base-pair separation of ~ 5.3A, some 1.9A larger than is the case for normal double-stranded B-DNA. Since this sort of change in the structure of the DNA double helix has been associated with drug-DNA intercalation complexes there has therefore been speculation that an intercalation mechanism is involved in the stabilisation of recA/dsDNA complexes. Fibre diffraction studies are being undertaken on complexes of the protein with double-stranded DNA from calf thymus. Samples of the complex are drawn from concentrated gels and studied in varying conditions of relative humidity. Although initial studies of this complex were undertaken using conventional x-ray equipment these samples deteriorate in the beam and higher quality diffraction patterns have been recorded at the Daresbury Laboratory Synchrotron Radiation Source (SRS) on beamline 7.2. These diffraction patterns are characterised by largely continuous diffraction but also show some sharp reflections. The data are consistent with a roughly six-fold helix having a pitch that varies from 87A to 92A over the humdiity range from 66% upwards. There is also a clear meridional reflection which corresponds to an axial periodicity of 3.7A. Work is now aimed at developing sample preparation procedures so as to improve the quality of these patterns. Magnetic alignment trials are also in progress using the 11T superconducting magnet at Daresbury Laboratory.

PS-03.01.08 THE CRYSTAL STRUCTURE OF FIS MUTANT PRO61-ALA : THE INFLUENCE OF PROLINE RESIDUES ON BENDING OF THE α-HELIX. Hanna S. Yuan*, Institute of Molecular Biology, Academia Sinica, Taiwan. Steven E. Finkel and Reid C. Johnson, Dept. of Biological Chemistry, School of Medicine, UCLA, USA.

Fis is an *E. coli.* site-specific DNA-binding protein with two identical 98-amino acid subunits that functions as a regulator of many different reactions. Fis originally was identified because of its role in stimulation of the site-specific DNA inversion catalyzed by the Hin-family of recombinases.
X-ray crystal structure analysis has been carried out at 1.9 Å resolution for a Fis mutant protein, Pro61-Ala. The structure of Pro61-Ala is almost identical to that of Fis wild type protein, in which each monomer of the Fis dimer has four α-helices (A, B, C and D-helices), and the C-terminal of each subunit comprises a helix-turn-helix DNA binding element. The isomorphous structure of Pro61-Ala explains the mutant's retained activity during stimulation of Hin-mediated DNA inversion reactions. It was found that one of the α-helices, the B-helix, is kinked in the wild type protein by 20 degrees which was assumed previously to be caused solely by the presence of Proline in the center of the helix. However, when we replaced Proline by Alanine in the mutant this does not change the overall bending shape of the B-helix, which was still kinked by 16 degrees. Local peptide backbone movement around residue 57 adjusted the geometry of the helix to accommodate the new main-chain hydrogen bond between the -CO group in Glu57 and the -NH group in Ala61. The kink of the B-helix in Pro61-Ala is obviously not due to the presence of Proline. Other factors, such as hydrophobic packing forces, may be more important determinants for the overall conformation of the α-helix in Fis.

PS-03.01.09 ACTIVE CENTER GEOMETRY AND DEPURINE MECHANISM STUDIES OF COMPLEXES OF TWO RIBOSOME- INACTIVATING-PROTEINS by Huang Qichen*, Liu Shenping, Tang Youqi, Jing Shanwei[a] and Wang You[a], Institute of Physical Chemistry, Peking University, Beijing 100871, China, [a]Shanghai Institute of Organic Chemistry, Academia Sinica, Shanghai 200032, China

Ribosome-Inactivating-Proteins (RIPs) are proteins which enzymatically inhibit protein synthesis of eucaryotic cells by inactivating their Ribosomes. Although they are known as specific N-glycosidases which remove a single adenine base from the conserved sequence of -CGAGAG- within 28S rRNA, and many of them have been sequenced, their detailed molecular mechanisms are unknown.
Trichosanthin (TCS) and Momorcharin (MMC) are two RIPs which are isolated from the root tubes of trichosanthin and seeds of bitter gourd respectively. We have got crystals of these proteins and their complexes with adenosine 5'-triphosphate, formycin (named TCA, TCF for TCS complexes; MCN, MCA and MCF for native MMC and its complexes). Their X-ray reflection data was collected at high resolutions (1.6-2.2A) with different methods. We determined TCA's structure using MIR method according to other's reports and determined MMC's structures by molecular replacement

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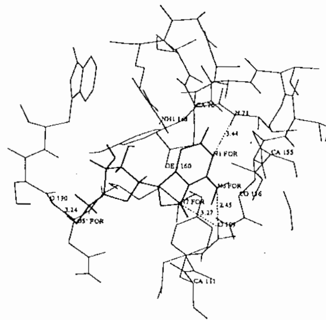
method. Now the refinement of these structures were finished using XPLOR program. The conventional R values of these structures are between 0.18-0.19 and their deviations from the ideal bond length and bond angle are 0.01-0.015A, 1.0-2.0°. In these complexes we can see the electron density of formycin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

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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

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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