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[2] and Wang You[2], Institute of Physical Chemistry, Peking University, Beijing 100871, China, [2]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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