Progress in the structure determination of a complex of human topoisomerase I and double-stranded DNA will be described. Topoisomerases are a class of ubiquitous enzymes that control the level of DNA supercoiling in cells and are vital for transcription, translation and recombination. Topoisomerase I breaks a single strand of duplex DNA, forms a transient protein-DNA complex via a phosphotyrosine bond, and allows relaxation of superhelical strain about the intact DNA strand. Eukaryotic and prokaryotic topoisomerase I enzymes appear to be distinct and to have unique mechanistic characteristics. Human topoisomerase I is a 765 amino acid (91 kD) enzyme made up of several human cancer cell types. We have obtained high-quality crystals of a complex of a recombinantly expressed portion of human topoisomerase I which includes the active site. These crystals belong to a monoclinic space group P2\(_1\) with unit cell dimensions \(a=65.8\), \(b=86.3\), \(c=56.72\) Å, \(\beta=114.09^\circ\) and \(V=206,445\) Å\(^3\). Assuming two molecules per asymmetric unit yields a 44% solvent content. Native diffraction data have been measured to 2.3 Å resolution using flash cooling techniques. In addition, the structure of the Y41H mutant has been determined at 1.7 Å resolution. The basis of the three dimensional structures and the crystal packing interactions of both the wild-type and Y41H GVP has been proposed to explain the cooperative nature of its binding to ssDNA. The model suggests possible involvements of many surface amino acids either in the binding to DNA, or the protein-protein interactions in the GVP-ssDNA complex. In this work, we present the crystal structures of three mutants involving surface amino acids, L32R, K69H and R82C. The hydrophobic L32 residue is converted into a basic arginine, whereas the basic K69 and R82 are converted into somewhat hydrophobic histidine and cysteine respectively. The diffraction data of these three mutants have been collected to 1.9, 2.0 and 2.0 Å, respectively. The structural refinements are in progress and the results will be reported.

The gene V protein (GVP) from the bacteriophage M13 is a single-stranded DNA binding protein which is a homodimer of 87 amino acids. The high resolution structure of the M13 GVP has recently been determined by the multiswavelength anomalous diffraction method. In addition, the structure of the Y41H mutant has been determined at 1.7 Å resolution. On the basis of the three dimensional structures and the crystal packing interactions of both the wild-type and Y41H GVP, a model has been proposed to explain the cooperative nature of its binding to ssDNA. The model suggests possible involvements of many surface amino acids either in the binding to DNA, or the protein-protein interactions in the GVP-ssDNA complex. In this work, we present the crystal structures of three mutants involving surface amino acids, L32R, K69H and R82C. The hydrophobic L32 residue is converted into a basic arginine, whereas the basic K69 and R82 are converted into somewhat hydrophobic histidine and cysteine respectively. The diffraction data of these three mutants have been collected to 1.9, 2.0 and 2.0 Å, respectively. The structural refinements are in progress and the results will be reported.

DNA ligase is a vital enzyme which is required for important cellular processes such as DNA replication, repair of damaged DNA and recombination. The enzyme mediates the formation of phosphodiester bonds between adjacent 3'-OH and 5'-phosphate termini, thereby joining the nicks in the double-stranded DNA. Ligases can be classified into two groups depending on their requirement for ATP or NAD\(^+\) as the cofactor. All eukaryotic enzymes and virally encoded enzymes are ATP-dependent, whereas prokaryotic enzymes require NAD\(^+\) for their activity. DNA ligase from bacteriophage T7 is a monomer with a molecular weight of 41 kDa. Here we report the structure of this enzyme at 2.6 Å resolution.

The protein was crystallized by vapour diffusion method using hanging drops. Crystals belonged to the space group \(P2_12_12\) with unit cell dimensions \(a=65.8\) Å, \(b=86.3\) Å, \(c=78.3\) Å. The structure was solved by MIR using mercury and selenomethionine derivatives. Non-isomorphism between the crystals was a major problem in structure determination. Crystals were found to be grouped around three major forms. The non-isomorphism between the crystal forms was sufficient to allow density averaging between them to improve the electron density maps.

The structure consists of two distinct domains, a larger N-terminal domain (residues 2-240) and a C-terminal domain (residues 241-349). The N-terminal domain is an \(\alpha/\beta\) structure and comprises of three mainly antiparallel \(\beta\)-sheets surrounded by six \(\alpha\)-helices. The ATP-binding site is situated in this domain in a pocket beneath one of the \(\beta\)-sheets. The C-terminal domain con-