ABSTRACT

The long and thriving tradition of research on the pathogen *Mycobacterium tuberculosis* in India, particularly at the Indian Institute of Science, and the sequencing of the complete genome of this pathogen have provided an ideal platform for the initiation of a programme on the structural genomics of tuberculosis. As part of the programme, structural studies on single-stranded DNA-binding protein (SSB) and ribosome recycling factor (RRF) from *M. tuberculosis* have been carried out. The novel structural features observed in the case of *M. tuberculosis* SSB (MtSSB) prompted similar studies on the SSB from non-pathogenic *Mycobacterium smegmatis* (MsSSB).

The structures were solved using the well-established techniques of protein X-ray crystallography. The hanging drop vapour diffusion method was used for crystallization in all cases. X-ray intensity data were collected on a MAR Research imaging plate mounted on a Rigaku RU200 X-ray generator except for one data set collected using synchrotron radiation. The data were processed using the HKL program suite. The structure of one of the crystal forms of MtSSB was solved using multiple isomorphous replacement and anomalous scattering methods. The rest of the structures were solved by the molecular replacement method using the program AMoRe. Structure refinements were carried out using the programs CNS and REFMAC. Model building was carried out using FRODO. PROCHECK, ALIGN, INSIGHT, and NACCESS were used for structure validation and analysis of the refined structures.

SSB is an essential accessory protein required during DNA replication, repair and recombination, and various other DNA transactions. The 164 residues long MtSSB was crystallized in two forms, a trigonal form and an orthorhombic form. The structure of the trigonal form was solved using multiple isomorphous replacement and anomalous scattering methods. This structure was used to determine the structure of the other form by molecular replacement. The C-terminal stretch of almost 40 residues could not be modelled because of extremely poor electron density in this region. The N-terminal polypeptide chain in the structure...
exhibits the oligonucleotide-binding (OB) fold. The globular core of the molecule in different subunits in the two forms and those in *Escherichia coli* SSB (EcSSB) and human mitochondrial SSB (HMtSSB) have similar structure, although the three loops exhibit considerable structural variation. However, the tetrameric MtSSB has an as yet unobserved quaternary arrangement and a clamp involving the C-terminal strand that stabilises the dimer. The ellipsoidal shaped quaternary structure with a unique dimeric interface lends the oligomeric protein greater stability, which may be of significance to the functioning of the protein under conditions of stress. Also, as a result of the variation in the quaternary structure the path adopted by the DNA to wrap around MtSSB is expected to be different from that of EcSSB.

The structure of MsSSB has been determined using three data sets collected from related crystals. The structure is similar to MtSSB indicating the clamp arrangement that stabilises the dimer and the ellipsoidal shape of the tetramer, to be characteristic features of mycobacterial SSBs. The central OB-fold is conserved in mycobacterial SSBs as well as those from *E. coli*, *Deinococcus radiodurans* and human mitochondria. However, the quaternary structure in them exhibits considerable variability. The observed plasticity of the subunit is related to this variability. The crystal structures and modelling studies provide a rationale for the variability. A sequence alignment of SSBs from representative bacterial species and mitochondria shows that the strand involved in the clamp mechanism, which leads to higher stability of the tetramer, occurs in all high G+C Gram-positive bacteria. The higher stability is perhaps required by these organisms.

RRF in concert with elongation factor G disassembles the post-termination complex of 70S ribosome bound to transcript mRNA and deacylated tRNA. This process of disassembly is essential for the survival of eubacteria. The crystal structure of MtRRF has been determined and refined against three X-ray diffraction data sets, two collected at room temperature and the other at 100 K. The two room temperature data sets differ in the radiation damage suffered by the crystals before the data used for processing were collected. A comparison between the structures refined against the two data sets indicates the possibility of radiation-induced...
conformational change. The L-shaped molecule is made up of a long three-helix bundle domain (domain I) and a globular domain (domain II) connected by a linker region. The main difference between the room temperature and the low temperature structures is in the rotation of domain II about an axis close to its libration axis. This observation and a detailed comparative study of RRFs of known structures led to an elaboration of the present understanding of the structural variability of RRF. The variability involves a change in the angle between the two arms of the molecule, a rotation of domain II in a plane nearly perpendicular to the axis of the helix bundle and an internal rotation of domain II. Furthermore, the domains and the linker could be delineated into fixed and variable regions in a physically meaningful manner. The relative mobility of the domains of the molecule in the crystal structure appears to be similar to that in the ribosome-RRF complex. That permits a meaningful discussion of the structural features of RRF in terms of ribosome-RRF interactions. The structure also provides insights into the results of inter-species complementation studies. A phylogenetic study employing the structural information indicates that mitochondrial RRF form a monophyletic group distinctly different from the bacterial protein, suggesting RRF to be a good drug target.

In addition to the work on SSB and RRF, the author was involved during the period of studentship in carrying out studies on the structural plasticity of the enzyme uracil DNA glycosylase (UDG), which is described in the Appendix of the thesis. The structures of a new crystal form of free E. coli UDG, containing four molecules in the asymmetric unit, and two forms of its complex with the proteinaceous inhibitor Ugi, containing two and four crystallographically independent complexes, respectively, were determined. A comparison of these structures and the already known crystal structures containing UDG shows that in terms of molecular geometry and mobility, they can be broadly classified into three types: free molecules including those to which small molecules such as glycerol or uracil are bound, those in the UDG-Ugi complex and those bound to DNA. Comparison of the lone DNA bound molecule with the other molecules indicates that the UDG molecule can be considered to be made up of two independently...
moving structural entities or domains. A detailed study of free and DNA bound human enzyme strengthens this conclusion. The domains close upon binding to uracil-containing DNA whereas they do not appear to do so upon binding to Ugi.

A large portion of the work presented in the thesis has been reported in the following publications:


