Abstract

Maintenance of the genomic integrity of the cell is crucial for the survival and successful propagation of an organism. However, this integrity is under continuous threat from DNA-damaging agents. In addition, errors in replication and transcription, resulting in the incorporation of inappropriate bases and hence mutations, disrupt the genomic integrity. Therefore, cells have developed a number of DNA-repair and error avoidance mechanisms to maintain the genomic integrity. The genome of pathogenic mycobacteria, including *Mycobacterium tuberculosis*, is more prone to damage, as they are constantly exposed to DNA-damaging agents produced by the host macrophage which it inhabits. In addition, mycobacterial genomes, being rich in G+C content, are more susceptible to cytosine deamination and guanine oxidation. Therefore, various DNA-repair and error avoidance mechanisms for maintaining the genomic integrity are extremely important for mycobacteria. This laboratory has been actively contributing towards the national and international structural biology efforts on mycobacterial proteins involved in maintenance of the genomic integrity. The author's contribution to this effort has been concerned with the base excision repair enzyme, uracil-DNA N-glycosylase (Ung), and nucleotide pool sanitization enzyme, MutT1 from mycobacteria. A brief overview of the available literature on the structural and biochemical studies of these two proteins is provided in the introductory chapter. Uracil in DNA can occur either due to deamination of cytosine within DNA or due to misincorporation of dUTP during replication. Uracil-DNA glycosylase is one of the important enzymes involved in the base excision repair (BER) pathway, which removes uracil form both single- and double-stranded DNA and hence avoids consequent mutations. 8-oxo-dGTP and 8-oxo-GTP are formed in the nucleotide pool as a consequence of oxidation of guanosine nucleotides. MutT proteins, sanitize the nucleotide pool and hence avoid the error due to incorporation of 8-oxo-dGTP and 8-oxo-GTP, during replication and transcription, respectively. Structural studies on uracil-DNA glycosylases from various sources including mycobacteria have been extensive, while those on MutT proteins have been less extensive.

Uracil-DNA glycosylase is the first enzyme of the base excision repair pathway that removes uracil from both single- and double-stranded DNA by cleaving the bond between uracil and deoxyribose. UNG/Ung proteins are inhibited by a well known proteinaceous inhibitor, uracil-DNA glycosylase inhibitor (Ugi), which is encoded by the phages PBS-I and PBS-II as part of their defense mechanism against host Ung. Ugi has been well characterized biochemically and structurally and it has been extensively used in structural studies of UNG/Ung as it mimics the DNA bound to the enzyme. Apart from Ugi, UNG/Ungs are also inhibited to various extents by uracil, one of the products of the enzymatic reaction, and some of its analogs and derivatives. Although inhibition by these small molecules has been well studied biochemically, the modes of their binding and interactions with the enzyme have not been extensively explored. Structures of free UNG/Ung from many sources and their complexes with Ugi have been reported. The structure of the complex with oligonucleotides, however, is known only for the human, *E. coli* and Herpes simplex virus 1 (HSV1) enzymes. Of these, the oligonucleotide bound to HSV1 does not contain uracil. The structures of complexes of UNG/Ung with uracil, uracil analogs and a few of its derivatives have also been reported. Earlier analyses of the relevant structures revealed

concerted conformational changes in the enzyme, leading to closing of the active-site cleft consequent to the binding of DNA containing uracil. Subsequently, it was demonstrated that Ung is a two-domain enzyme and that the domains close in on the bound DNA containing uracil. The mere presence of free uracil in the active site of the enzyme does not lead to the closure of the active site. The native structure of *M. tuberculosis* Ung (*Mt*Ung) with a citrate molecule bound in the active site and the structure of its complex with Ugi have previously been reported from this laboratory. The near atomic resolution structures of and thermodynamic data on complexes of *Mt*Ung with uracil and its derivatives and new crystal forms of the free enzyme, are reported here. A detailed structural examination of these high-resolution structures and the thermodynamic data have, among other things, led to striking insights into conformational selection on DNA binding and the modes of *Mt*Ung-ligand interactions.

MutT proeins, which belong to Nudix hydrolase superfamily, are known to sanitize the nucleotide pool and hence avoid the error due to incorporation of non-canonical nucleotides, specifically 8-oxo-dGTP and 8-oxo-GTP, during replication and transcription, respectively. An antimutator role of MutT proteins have been established from a series of studies involving those from human, *E. coli*, *M. tuberculosis* and others sources. As indicated in the introductory chapter, mycobacterial MutT1 and MutT2 have been shown to have an 8-oxo-guanosine triphosphatase activity. Interestingly, unlike *E. coli* MutT (*Ec*MutT) and human MutT homologue 1 (*Hs*MTH1), *M. tuberculosis* MutT1 (*Mt*MutT1) has been shown to hydrolyse 8-oxo-GTP and 8-oxo-dGTP to corresponding nucleoside diphosphates but not to nucleoside monophosphates at normal substrate and enzyme concentrations. To understand the basis of this novel and unusual activity and substrate specificity, it was desirable to carry out structural studies on *Mt*MutT1. However, on account of problems with the expression of *Mt*MutT1, *Mycobacterium smegmatis* MutT1 (*Ms*MutT1) was chosen for structural studies. Preliminary studies on *Ms*MutT1 suggested the presence of an N-terminal Nudix hydrolase domain (*Ms*MutT1-NTD) corresponding to the single-domain *Ec*MutT and a C-terminal histidine phosphatase domain (*Ms*MutT1-CTD) in the protein. The presence of a phosphatase domain in *Ms*MutT1 in addition to a Nudix hydrolase domain of the type that constitutes *Ec*MutT and *Hs*MTH1, was intriguing. Subsequently, detailed crystallographic studies of *Ms*MutT1 and its complexes, along with complementary biochemical studies were carried out. The protein appears to be an enzyme in which the binding sites are formed primarily by intermolecular interactions of the type that bring the Nudix domain of one molecule and the phosphatase domain of a neighbouring molecule into close proximity. The enzyme is capable of hydrolysing 8-oxo-GTP and 8-oxo-dGTP into the corresponding nucleoside diphosphates and monophosphates, simultaneously, sequentially or both. A detailed examination of the crystal structures leads to a proposal as to how this is achieved.

Diadenosine polyphosphates (Ap_nA, n=2–6), particularly Ap_4A, are involved in several important physiological processes. The substantial sequence identity of the Nudix hydrolase domain (domain 1) of *Ms*MutT1 with a known Ap_4A hydrolase suggested that *Ms*MutT1 could also hydrolyse diadenosine polyphosphates. Biochemical experiments yielded results in conformity with this suggestion, with Ap_4A as the best among the substrates. ATP is a product in all experiments; small amounts of ADP were also observed in the experiments involving Ap_4A and Ap_6A. Hydrolysis was inhibited by fluoride ions in all cases. The mechanism of action and its

inhibition in relation to Ap_nA were explored through the X-ray analysis of the crystals of the *Ms*MutT1 complexes with Ap_5A, Ap_4A, ATP and ATP.MgF_3. The aggregation pattern of molecules in these crystals is similar to that found in a majority of *Ms*MutT1-NTP crystals. Substrate molecules occupy the same site in two of them. ATP occupies this site as well as another site at an intermolecular interface in the third crystal. The protein-ligand interactions observed in these crystal structures lead to an explanation of the molecular mechanism of hydrolysis of Ap_nA by *Ms*MutT1. The crystals of the ATP.MgF_3 complex exhibit a new packing arrangement. The structure of the complex provides an explanation for the fluoride inhibition of the activity of the enzyme. It would thus appear that MutT1 has a major role involving the hydrolysis of diadenosine polyphosphates, which could be elucidated at the molecular level.