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Thesis abstract

Defining the molecular details of hSSB1 oligomerisation in response to oxidative DNA damage

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Cells are constantly exposed to sources of oxidative stress. If left unrepaired, the oxidative modification of DNA can result in a loss of genome integrity and may lead to diseases including cancer. The most common form of oxidative DNA damage is the oxidation of the DNA base guanine to the highly mutagenic 8-oxo-7,8-dihydroguanine (8-oxoG). To protect the genome from mutagenesis, the modified base is removed through the process of Base Excision Repair (BER). Single stranded DNA binding proteins (SSBs) are a family of proteins that act to protect the genome from mutagenesis by recognizing and binding to sites of DNA damage, where the DNA is unwound into its single strands. Human Single Stranded DNA binding protein 1 (hSSB1), a novel human SSB, is crucial in the removal of 8-oxoG from the genome through the BER pathway. Previous research has found that the ability of hSSB1 to form dimers, tetramers and higher aggregates (through the formation of disulfide bonds at the C81 and C99 residues) under oxidative conditions is critical in its function in BER. This thesis examines the molecular details of hSSB1 oligomerisation in response to oxidative DNA damage and the mechanism by which the hSSB1 oligomer binds DNA. In this work Nuclear Magnetic Resonance (NMR) spectroscopy and Surface Plasmon Resonance (SPR) experiments are used to determine how oligomeric hSSB1 binds ssDNA and ssDNA incorporating 8-oxoG bases. The findings reveal that binding of non-reduced hSSB1 to 8-oxoG ssDNA is indistinguishable to its binding to unmodified ssDNA, indicating no change in the underlying mechanism. Further, using SPR I show that non-reduced hSSB1 binds more strongly to ssDNA than reduced protein, confirming that hSSB1 oligomers recognise ssDNA with a tighter binding affinity. To determine the structural basis of these oligomeric hSSB1-ssDNA interactions 2D 1H 15N HSQC NMR titrations were carried out with ssDNA oligos of varying length. The data reveal that ssDNA binding takes place via hSSB1 tetramers that are structurally identical to the ones that were previously described in the absence of ssDNA and suggests that hSSB1 binds DNA bi-directionally. Additionally, using 2D 1H-15N HSQC NMR and 2D 1H-1H NOESY NMR experiments, I show that the hSSB1 oligomer unwinds damaged dsDNA and binds to its single strands.

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