MS7-P8 New G-quadruplex DNA structures

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The selection of potential new G-quadruplex DNA sequences has been performed using bioinformatics. The possible formation of G-quadruplex by the selected sequences has been assessed by PCR and fluorescence methods. Attempts for growing suitable single crystals of the selected DNA sequences has been performed in parallel. The results for G-quadruplex formation obtained by the molecular biology method and from crystallization results have been compared in order verify the viability of the methods.

Keywords: Nucleic acid, G-quadruplex, DNA

MS7-P9 Radiation damage in protein-nucleic acid complexes

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Significant progress has been made over recent years in understanding how radiation damage mechanisms affect crystalline protein structure determination. Despite an active field studying the radiation chemistry of nucleic acids interacting with ionising radiation, few MX investigations exist on specific damage manifestations for crystalline DNA/RNA in their complexes with protein. Quantitative controlled comparisons between crystallised protein and nucleic acid damage mechanisms separately remain inherently difficult, but such challenges can be circumvented through investigating naturally forming nucleoprotein complexes. A recent study (1) utilised a model protein-DNA complex C.Esp1396I (2) to quantitatively investigate specific damage mechanisms for protein and DNA in a biologically relevant complex over a large dose range (2.07–44.63 MGy). A computational approach was developed to systematically locate damage sites, identifying typical specific damage sites on the complex. Strikingly the DNA component was determined to be far more resistant to specific damage than the protein for the investigated dose range.

For such complexes, the protein may be simply more susceptible to radiation damage, or may act as an electron/radical scavenger to protect DNA constituents. To address this issue, our previous computational strategy has been extended to statistically investigate damage dynamics in crystals of a large protein-RNA complex: TRAP (tryptophan-binding RNA attenuation protein) bound to 53 base RNA (3). The TRAP-RNA complex naturally crystallises in a 1:1 ratio with its RNA-unbound form, making it an ideal controlled experiment. RNA binding has been observed to stabilise susceptible protein residues, providing direct protection from electron density loss and disorder. Damage-susceptible acidic residues located far from the RNA-binding interface have increased decarboxylation rates upon RNA binding; the direct mechanisms behind this damage heterogeneity, and the implications of scavenging effects within crystalline nucleoprotein complexes are yet to be established.

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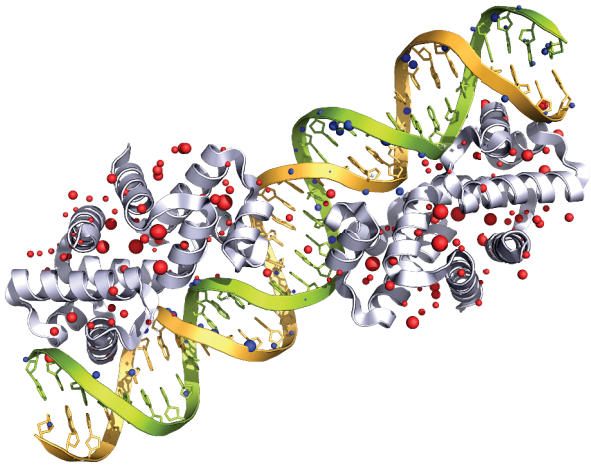

Figure 1. Representation of specific damage distribution throughout the C.Esp1396I complex at 44.63 MGy (1). Specific damage sites are represented as spheres, with radii proportional to electron density loss. Spheres closer/ further than 2 Å to/from the DNA strands are coloured blue/red.

Keywords: macromolecular X-ray crystallography, radiation damage, protein-nucleic acid complexes

MS7-P10 Structural studies on DNA cleavage-and-ligation nucleases of mobile genetic elements involved in spread of antibiotic resistance

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Plasmids and integrative and conjugative elements (ICEs) are major mobile genetic elements (MGEs) that provide routes for rapid acquisition of new genetic information in bacteria and therefore contribute to the spread of antibiotics resistance. Essential for their action are plasmid/ICE-encoded site- and strand-specific one-metal-ion endonucleases called relaxases. Conjugative relaxases cleave a single strand of the DNA substrate by formation of an intermediate covalent adduct with the scissile phosphate of the DNA nic site. After the ssDNA-relaxase molecule is transferred to the recipient cell, relaxases ensure re-ligation of their DNA cargo. Additionally, plasmids and some ICEs encode for DNA replication relaxases, crucial for their maintenance. Understanding plasmid/ICEs conjugal transfer and replication may aid in combating the spread of antibiotics resistance as well as contribute to the development of new tools for DNA delivery into human cells. Structures of replicative and conjugative relaxases RepB, MobM and TrwC that were solved in our lab are compared herein.

Keywords: relaxases, endonucleases, bacterial conjugation, plasmid replication, antibiotic resistance