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Interaction of DNA replication and DNA repair systems with AP sites adducts with proteins, peptides and low-molecular-weight compounds

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Motivation and Aim: Apurinic/apyrimidinic sites (AP sites) are the most frequent type of spontaneous DNA damage that occurs in the human genome at the steady-state level of ~20,000 per cell under normal conditions, with their number increasing significantly when cells are exposed to genotoxic stress. AP sites arise from the loss of DNA bases through spontaneous hydrolysis of the *N*-glycosidic bond, under the influence of genotoxic factors and as intermediates in DNA repair. AP sites and their derivatives readily react with nucleophilic groups. Since DNA in the cell is always associated with many proteins, this often leads to the formation of DNA-protein cross-links (DPCs). However, the mechanism of interaction of replication machinery with DPCs is not fully understood. The repair of DPCs begins with proteolysis of the protein part to a small peptide by the proteasome or one of dedicated proteases. However, at the moment it is completely unclear what happens with the resulting DNA-peptide cross-links. Moreover, AP sites are surrounded by low-molecular weight nucleophilic compounds such as polyamines. Methoxyamine is another small molecule that specifically inhibits BER by reacting with natural apurinic/AP sites, which prevents their cleavage by APEX1 endonuclease, however its miscoding properties remains unclear.
Methods and Algorithms: Here we used classical biochemical approaches to obtain and to study models of chemically determined AP site conjugated with nucleophilic molecules, namely proteins, peptides, polyamines, and methoxyamine. The nature of the adducted AP sites was confirmed by MALDI mass spectrometry.
Results: We have proposed the mechanism of interaction of DNA polymerases belonging to different families with DPCs in terms of protein surface contacts. We have studied the ability of DNA polymerases to carry out synthesis in the presence of peptide cross-links with AP sites in DNA, polyamine conjugated AP sites, methoxyamine-conjugated AP sites and the ability of nucleases involved in DNA repair to hydrolyze these lesions. *Conclusion*: AP sites readily react with lots of nucleophilic cellular compounds. The adducted AP sites are common phenomenon in the living cell, which necessitates the presence of dedicated pathways for their tolerance and repair.
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