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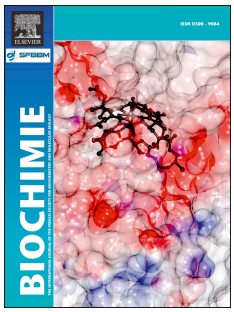

Unusual interaction of human apurinic/apyrimidinic endonuclease 1 (APE1) with abasic sites via the Schiff-base-dependent mechanism

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Abstract

Clustered apurinic/apyrimidinic (AP) sites are more cytotoxic than isolated AP lesions because double strand breaks (DSB) can be formed during repair of closely positioned bistranded AP sites. Formation of DSB due to simultaneous cleavage of bistranded AP sites may be regulated by proteins specifically interacting with this complex lesion. A set of AP DNA duplexes containing AP sites in both strands in different mutual orientation (BS-AP DNAs) was used for search in the extracts of human cells proteins specifically recognizing clustered AP sites. A protein, which formed the Schiff-base-dependent covalent products having an apparent molecular mass of 50 kDa with the subset of BS-AP DNAs, was identified by mass spectrometry as apurinic/apyrimidinic endonuclease 1 (APE1). The identity of trapped protein was confirmed by Western blot analysis with anti-APE1 antibodies. Purified recombinant human APE1 is also capable of forming the 50 kDa-adducts with efficiency of BS-AP DNAs cross-linking to APE1 being dependent on the mutual orientation of AP sites.

In spite of formation of the Schiff-base-dependent intermediate, which is prerequisite for the β-elimination mechanism, APE1 is unable to cleave AP sites. APE1 lacking the first 34 amino acids at the N-terminus, unlike wild type enzyme, is unable to form cross-links with BS-AP DNAs that testifies to the involvement of disordered N-terminal extension, which is enriched in lysine residues, in the interaction with AP sites. The yield of APE1-AP DNA cross-links was found to correlate with the enzyme amount in the extracts estimated by the immunochemical approach; therefore the BS-AP DNA-probes can be useful for comparative analysis of APE1 content in cell extracts.