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Structural features of substrate recognition by APE1-like endonucleases

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The integrity of DNA in the cell is preserved by a complex repair system that implements the recognition and removal of DNA damage and restoration of normal DNA structure. AP endonuclease hydrolyzes the phosphodiester bond on the 5′ side of the abasic nucleotide in the base excision repair pathway. In the nucleotide incision repair pathway, an enzyme directly recognizes and catalyzes the cleavage of DNA on the 5′ side of various specific nonbulky lesions such as 1,N^6-ethenoadenosine (εA), 5,6-dihydrouridine (DHU), deoxyuridine (dU), the α-anomer of adenosine (αA) and some other. Despite notable efforts to identify the causes of wide substrate specificity of AP endonucleases, the question on how a given nucleotide is accommodated by the active site of the enzyme remains unanswered. The main purpose of this work was to elucidate the structural features of substrate recognition by APE1-like endonucleases. Molecular dynamic (MD) approach was used to build and to analyze complexes of APE1-like endonucleases including human APE1, zAPE1 from *D. rerio,* xAPE1 from *X. laevis* and Rrp1 from *D. melanogaster* with damaged DNA. Obtained MD data for all tested enzymes revealed that damaged nucleotides have steric interactions with a protein loop Asn229-Thr233. It was found that significant reorganization and relocation of this loop are required to accommodate damaged nucleotide in the active site of the enzymes. To verify MD simulation data three mutant forms of APE1 from *D. rerio*, containing substitution N229G, A230G or E236A were obtained by a site-directed mutagenesis. Pre-steady-state kinetic and PAGE analysis of enzyme catalytic activity revealed that E236A substitution significantly improved the enzyme toward NIR-substrates. A comparison of MD simulation data with catalytic efficiency of these enzymes allowed us to gain deep insight into the mechanism of damage recognition. It was concluded that the Asn229-Thr233 loop could control enzyme specificity.

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