DOI 10.18699/SBB-2022-573

Features of the substrate specificity of a novel AP endonuclease from *Pyrococcus furiosus*

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AP endonucleases play one of the key roles in base excision repair (BER) pathway, and present main enzymes of the nucleotide incision repair (NIR). These enzymes catalyze DNA hydrolysis 5' side to the AP site or number of damaged nucleotides. The majority of pro- and eukaryotic AP endonucleases belong to the ExoIII or EndoIV structural families. Nevertheless, a novel endonuclease class of enzymes from archaea and some bacteria, designated endonuclease Q (EndoQ), was identified recently. EndoQ is not structurally related to the known AP endonucleases from ExoIII or EndoIV structural families. However, this enzyme possesses substrate specificity and catalytic activity similar to well-studied AP endonucleases. Present work was aimed to study the kinetic mechanism of damaged nucleotide recognition by EndoQ enzyme from *P. furiosus*. We analyzed kinetics of conformational changes of enzyme and DNA molecules during their interaction. The model DNA substrates containing various damages, including an F-site (the analog of AP site), alpha-adenosine, uridine, hypoxanthine and others, were tested to elucidate the features of lesion recognition. The influence of the nucleotide placed opposite to the lesion on the efficacy of DNA cleavage was analyzed. Strong effect of divalent metal ions on the catalytic stage was shown. The data obtained in current study allowed us to determine kinetic features of specific recognition of various damaged DNA substrates and to evaluate the catalytic activity of EndoQ enzyme in the BER and NIR pathways in archaea.

Acknowledgements: This work was supported by the Russian Foundation for Basic Research grant No. 20-34-90008.