DOI 10.18699/SbPCD-2018-35

The influence of pH on damaged DNA processing by human apurinic/apyrimidinic endonuclease 1

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Key words: human APE1, FRET-substrate, stopped-flow kinetics

Motivation and Aim: Human apurinic/apyrimidinic endonuclease 1 (APE1) is a key participant in the cascade of DNA base excision repair reactions. APE1 initiates the search and repair of apurinic/apyrimidinic (AP) sites in DNA, which are not only cytotoxic, but also can lead to mutagenesis, if left unrepaired. It is known from X-ray diffraction data that enzyme amino acid residues interact preferentially with one of the duplex strands to form usually hydrogen bonds and electrostatic contacts. The enzyme active site is formed by a large number of polar amino acids, which provide extensive contact with the phosphate groups of the DNA substrate binding site. Therefore, the aim of this study was to elucidate the influence of pH on the efficiency of formation of the enzyme-substrate complex and catalytic reaction.
Methods and Algorithms: In the present work, we used stopped-flow fluorescence techniques to conduct a comparative kinetic analysis of the conformational changes of the enzyme and DNA substrate molecules during recognition and cleavage of the abasic site in the pH range from 5.5 to 9. DNA-substrate contained FRET pair FAM-BHQ1 and furan residue F as nonreactive analog of AP-site.
Results: We have observed the changes in FAM fluorescence during the interaction of APE1 with the FAM-F substrate lead to a fast decrease in the fluorescence intensity (within 10 ms) followed by the increase phase in the time range 50 ms to 10 s. It was proposed that the initial decrease in the FRET signal reflects the decrease in the distance between FAM and quenching BHQ1 residues due to DNA bending in the complex with APE1. This step was detected only in the case of pH from 5.5 to 7.0. The increase in FAM fluorescence intensity in the next phase most likely reflects a release of the cleaved DNA product from the complex with the enzyme. The kinetic curves were fitted to sum of exponential equations. The rate constants for the formation of the catalytically competent complex and the observed catalytic constant were calculated.
Conclusion: It was shown that the activity of APE1 increases with increasing pH due to acceleration of the rates of catalytic complex formation as well as catalytic reaction.
Acknowledgements: This work was supported by grant from Russian Science Foundation No. 18-14-00135.