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Using fluorescently labeled proteins to study the interactions of APE1 with DNA and Polβ during the BER pathway

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The most common forms of DNA damage are the single base lesions which are corrected through the base excision repair (BER) pathway. BER is a multistep process involving several repair enzymes including apurinic/apyrimidinic endonuclease 1 (APE1) and DNA polymerase β (Polβ). During the BER process APE1 recognizes and incises DNA strands containing AP site. Then Polβ adds single nucleotide to the 3'-hydroxyl and removes the dRP group with its intrinsic dRP lyase activity. According to the "passing the baton" model of the BER process, DNA intermediates in BER are processed and then passed from one enzyme to another in a coordinated fashion. The aim of the present study was to create an experimental system based on the fluorescent labeled APE1 that would allow us to analyze the protein-DNA and protein-protein interaction in BER process.

In this work we have prepared Alexa-488 fluorophore Cys-labeled human APE1. The wild type enzyme and several mutant forms containing substitutions of surface Cys residues by Ser were analyzed. It was found that Alexa-488 fluorophore was sensitive to the process of the interaction between labeled APE1 and THF-containing (THF is an analog of AP-site) DNA. The changes in the fluorescence intensity have been attributed to the stages of the enzyme-DNA interaction process. Using the stopped-flow fluorescence technique we have studied the mechanisms of interaction between labeled APE1 and DNA substrates of different length in the presence or absence of Polβ. Obtained data revealed that Polβ stimulates both APE1 binding with THF-containing DNA duplex with formation of the catalytic competent complex and the APE1 dissociation from its product. These findings confirm the "passing the baton" model for APE1 with Polβ coordination in BER and explain Polβ stimulation effect on APE1 activity.

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