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Analysis of critical amino acid residues in the active center of a novel DNA glycosylase Fpg-like protein 1 *Streptomyces coelicolor* superfamily "helix-2-turn-helix" DNA glycosylase

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Motivation and Aim: There is DNA in living organisms under the constant action of genotoxic agents. These agents could be internalized in cell (radiation or UV radiation) or appear like cell's reactive metabolites (for example reactive oxygen species). To maintain the integrity of the genome, cells have repair systems. One of these systems is the base excision repair system, which is responsible for removing the most common DNA damage, oxidative damage to nucleobases. The major DNA glycosylase of the bacterial cell is formamidopyrimidine-DNA glycosylase (Fpg), which recognizes and removes 8-oxoguanine from DNA, a marker of oxidative stress. Today, DNA–glycosylases are divided into several superfamilies on the basis of the enzymes structural homology. Fpg belongs to the helix-2-turn-helix superfamily of DNA–glycosylases. Another key member of this superfamily DNA-glycosylase Nei – recognizes and removes oxidized pyrimidines.

Recent full genome sequencing of a number of bacteria has shown that there are bacteria with 4 homologs of DNA–glycosylases of the «helix-2-turn-helix» superfamily in their genome, whereas normally there are 1-2 glycosylases of this superfamily in the bacterial cell. Alignment of the aminoacid residues of the new Fpg DNA–glycosylases showed altered aminoacid residues in both the active center of the enzyme and the recognition center of the damaged base. Such mutations may affect not only the substrate specificity of the enzyme but also the quality of the enzyme-DNA interaction. The study of Fpg-like proteins will allow us to understand the contribution of amino acids not only in catalysis but also in the recognition of damaged bases, which may allow us to create a tool for designing enzymes with a given substrate specificity.

The aim of this work is to biochemically characterize the enzyme Fpg-like protein 1 *Streptomyces coelicolor*, and to establish the spatial structure of the free enzyme using protein crystallography to further characterize the mechanism of the enzyme using physicochemical methods.

Methods and Algorithms: We have used molecular cloning and optimized protocol of protein purification to get new Fpg-like proteins. Using biochemical approaches, we have got first understanding of affinity and substrate specificity of these enzymes. By protein crystallography method we have crystals and resolved the structures of new Fpg-like proteins from *Streptomyces coelicolor* and *Bacteroides thetaiotaomicron*.

Results: In the molecule of «helix-2-turn-helix» superfamily belonging proteins there are some structural features that provide the mechanism of recognize, affinity and catalysis. Most “helix-2-turn-helix” glycosylases have high-conservative PELPEVET N-terminal motif and use their N-terminal proline residue as a key catalytic nucleophile. Additionally, «helix-2-turn-helix» proteins have helix-two-turn-helix domain and zinc-finger motif to contribute residue to the active site. However, new Fpg-like proteins have significant diversity of N-terminal motif and residue recognizing damage. We have crystallized Fpg-like protein from *Streptomyces coelicolor* with 1.63 Å resolution and reveal the presence of Zn-finger domain in C-terminal of protein, α-helix rich region in N-terminal and some another Fpg-like features. However, substrate preferences make these proteins similar to Nei.
Conclusion: We have revealed structural and biochemical aspects of new atypical Fpg-like proteins to determine relationships these proteins to known glycosylases families by protein crystallographic approach and also we have investigated aminoacid substitutions in Fpg *E. coli* to estimate contribution distinguished active center aminoacids in catalysis, affinity and recognition Fpg-like proteins with respect to Fpg wild type.
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