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Study of interaction of the PARP family DNA-dependent proteins with nucleosomes by atomic force microscopy

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Motivation and Aim: It is well known that during the life of a cell, numerous damages accumulate in its genetic material. The cellular signal in response to DNA damage is the ADP-ribosylation reaction catalyzed by DNA-dependent proteins of the poly(ADP-ribose)polymerase (PARP) family, PARP1, PARP2, and PARP3. Studies of the activities of these three proteins to the DNA damage response were carried out mainly on the synthetic DNA substrates – free DNA molecules. However, in the cells of the DNA molecule is a part of chromatin – a protein-nucleic acid complex. The first level of chromatin compaction is a nucleosome. The nucleosome is a complex of DNA and a histone octamer, in which the DNA is wounded around a spool of a histone disk like a thread. Today, the effect of DNA-dependent proteins of the PARP family on the structure of the nucleosomal complex has been little studied. In the present work, we investigated this question using an atomic force microscope.
Methods and Algorithms: We utilized the model nucleosome to study the effect of DNA-dependent PARP's family proteins on the stability of the nucleosomal complex. The nucleosome was reconstituted according to the work [1]. For this, DNA containing the "Clone 603" sequence of 147 bp long with linker regions of 79 bp and 120 bp long located at the ends was used. To study the effect of DNA-dependent PARP proteins on the stability of the nucleosomal complex, the dissociation constants of the PARP-nucleosome complexes were determined as described in [2]. The formation of PARP-nucleosome complexes was carried out at 37 °C for 15 minutes appropriate concentrations. Next, the reaction mixture was applied to a mica surface previously modified with APS [3]. The mica surface was scanned on a Scanning Probe Microscope (Multimode 8, Veeco-Brucer, USA) in TappingMode. The scanning area was 2*2 μm with a resolution of 1024 points. The resulting images were processed using the Gwyddion software (v. 2.58). Measurement of various geometric parameters of the structures under study was carried out using the ImageJ software (v. 1.53e).
Results: All three DNA-dependent proteins of the PARP family were found to be able to interact with the substrates used. At the same time, most parts of the protein molecules were localized at the entry–exit site of the linker DNA into the nucleosomal disk (70 % of PARP1 molecules, 77 % of PARP2 molecules, and 86 % of PARP3 molecules). In addition, the stabilization of the nucleosomal complex in the presence of PARP1 and PARP3 proteins was observed: the data scattering of the angles' average value between the entry-exit sites of the linker DNA into the nucleosomal disk in the presence of proteins was smaller. PARP2 had no such significant effect on nucleosome stability. Moreover, it was found that the binding of PARP3 protein to the substrate leads to compaction of the nucleosome: the angle between the entry-exit sites was decreased

from 119 degrees for the native substrate to 103 degrees for PARP3-nucleosome complexes. No such results were found for PARP1/2 proteins.

Conclusion: The results obtained using model substrates disclosed different effects upon the binding of the DNA-dependent PARP proteins with the nucleosome. It can be assumed that the obtained differences are explained by the variation of the proteins' preferences to the chemical and structural nature of nucleosomal substrates. Nevertheless, the obtained results allow us to better understand the regulatory aspects of the biochemical mechanisms in which DNA-dependent PARP family proteins are involved.

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