Characterization of the Dps protein, DNA fragments and nucleoprotein complexes by the atomic-force microscopy

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Creation of the new materials using biological macromolecules is one of the priority sphere of the modern biotechnology. Nowadays the main interest is towards the biopolymers that could create self-assembled structure, and ferritin proteins are one of such biopolymers. It have 24 or 12 identical or nearly identical subunits, forming a large hollow protein roughly spherical in shape. These proteins play a very important role in regulation all metabolic processes of the iron. The hollow center is a crucial part of the protein since it provides a cavity for the accumulation of several hundred of iron ions. This internal iron core has its own magnetic moment providing a chance to use ferritins as a building block with calibrated ferromagnetic property. But creation of such high-tech materials implies the deep knowledge about features of this biological molecule.

During our research we became interested in bacterioferritin Dps. This protein is one of the main factors that condense the nucleotide of *E.coli* on the stationary growth phase and upon different stress conditions [1]. The molecular weight of it's monomer is 18.695 kDa. The functional unit of Dps is a dodekamer consisting of 12 identical subunits. The N-terminal non-structed fragments have 3 aminoacid residues of lysine and are exposed on the surface of the spherical protein. These tails can interact with DNA without any sequence specificity [2]. Dps, compared with other ferritins, has unique properties such as: higher affinity to iron ions, smaller size, sensitivity to low intensity microwave eradiation [3] and possibility to make stable complexes with DNA. The ability to bind DNA enables researchers to create new materials and structures on the basis of well-ordered self-assembled nucleoprotein complexes [4]. But the exact mechanism of DNA-binding by Dps is still unknown. It is considered that Dps interacts with DNA nonspecifically, but it contradicts with recently discovered ability of this protein to affect gene expression in a specific manner. So, what molecular mechanism underlies this binding? Three models on Dps-DNA interaction are suggested so far. The first one has been proposed 20 years ago on the basis of electron microscopic images. It assumes formation of two connected rings around the DNA double helix. Each ring consists of six monomers and is 9 nm in diameter [5]. If so, the spherical shape of the protein should be destroyed and the hydrophobic internal part should be turned out. Otherwise N-terminal tails of Dps will not be able to contact the DNA. It seems to be unlikely. The second model puts DNA between three adjacent dodecamers fixing it strongly [6]. But free N-terminal tails sticking on the surface of these dodecamers theoretically may interact with multiple sites along the DNA, condensing it in unpredictable manner, which also seems to be biologically unrelevant. The central role in the third model is given to the salt bridges formed by magnesium ions between negatively charged spots on the protein surface and DNA backbone [7]. Its flaw is inability to explain the clear dependence of DNA-binding activity on the N-terminal fragments carrying the excessive positive rather than negative charge. Since the applied value of Dps strongly depends on the way of its interaction with different in length

and structural organization DNA, we are trying to study the mechanism of this binding using atomic force microscopy.

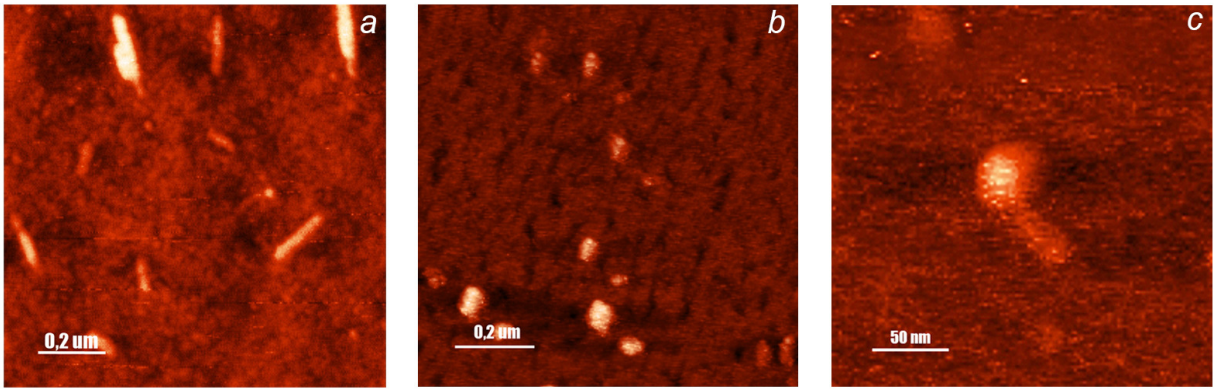

Figure 1. a) Sample: 256 bp DNA-fragment (88,4 ng/mkl in 2 мМ MgCl2); b) Sample: Dps of E.coli (2 mkg/mkl), buffer: 50мМ Tris-HCl (pH 7,5), 10 mM NaCl; c) Sample: Binary complex of Dps with 256 bp DNA-fragment.

An atomic force microscope Integra Vita (Russia) was used for visualization. It was calibrated using purified DNAs of the phage l (48502 bp) and the pGEM plasmid (8896 bp). Estimated length and height of these molecules corresponded to the expected values. However the apparent length of the shorter fragments was larger than expected, indicating significant error in the planar measurements.

The same deviation from expected planar dimensions was observed for free dodecamers of Dps, while in the vertical projection the diameter exactly fits to the expected value. The Dps complexes with different DNA fragments were formed at RT in standard buffer with for 30 min. Samples were loaded on the split of the mica and absorbation was allowed for 5 min. Washed and dried samples were visualized and the images were analyzed by the software Nova (NT-MDT, Russia).

In most cases the protein mainly interacted with one of the DNA ends. We have not found neither triplets of dodecamers, nor embracing DNA hexameric rings, which were proposed by existing models. Basically this interaction with partially unwound DNA ends is in line with our previous conclusion that Dps prefers to interact with structurally unstable AT-rich DNA regions. These findings confirm our data that purified Dps of *E.coli* forms stable dodecamer complexes and binds DNA with some sequence or structure specificity.

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