Секция «Биоинженерия и биоинформатика»

NANOSCALE DETECTION OF DNA MOLECULE TOPOLOGICAL STRUCTURE BY ATOMIC FORCE MICROSCOPY

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DNA parameters become important in studying of cancerogenic processes during cell vital activity. DNA molecule can get about one million lesions daily. In most cases of DNA damage occurs chemical modifications of DNA bases, which generates change in DNA molecule configurations. By the types of DNA damage generation we can separate Spontaneous, Endogenous and Exogenous processes. In all of these processes we get the same changing in DNA molecule. Produced by Radical induced DNA damage there are only few types of DNA damage: Base changing, Single strand break, Double strand break and Cross Links. As a results of effects like: cross-links, strand break, also we can receive DNA strands unwound (It may depends of connecting particles, which produced after double strands braking process, to the ends of single break molecules). Study of DNA damage can give as information about some of the DNA damaging processes and cancerogenic processes as a result.

Researching of DNA damage request well-known data about mechanical parameters of DNA molecule. By the mechanical parameters we presume mechanical conformation of DNA (in vitro) as length, flexibility and strength. The DNA mechanical parameters detection technique combining high resolution visualizations with non-destructive analysis requirements, which can be satisfied by Atomic Force Microscopy methods.

Materials and methods.

In study of DNA parameters was used pBR322 supercoiled plasmid extracted. Sample preparation covers selection of concentration of DNA solution, surface modifications and relaxation of DNA molecules on surface. In concentration selection we were fitting system of molecules on a surface with non-interaction between molecules and enough to observe of high number of molecules on surface. By the selection we got system with single observable DNA molecules on surface.

Conformation of DNA molecules on surface characterize needed mechanical parameters. Prepared solution of DNA in concentration 0,00025 mkg/mkl was adsorbed from liquid on modified mica surface. Modified surface became positive charged, which gives comfortable conditions to DNA adsorption. DNA can adsorb on surface easy and keeps flexibility of molecules in liquid. After water extraction we have system of DNA molecules fixed on surface.

Samples were scanned in non-contact AFM mode under resonant frequency 140-390kHz.

Result and discussions

In study of exogenous DNA damage processes, initiator dose dependency can be useful. Application of AFM analysis gives directly distribution of damage effect on DNA and damage detection. For example, it can be Ionizing radiation damage to DNA or cancerogenic effects of chemical treatment.

By received data as: length, height distribution and molecule profile height distribution, were characterized normal length, molecule diameter and persistent length as a parameter

of strengthens.

As we can see DNA molecules conformations give as information about DNA mechanical parameters. It gives as possibilities to study DNA damage on nano-scale mechanical level. High number of damages makes DNA molecules more compact and less flexible. In ratio of damage initiator it is possible to calculate dependency between initiator range and produced damage.

Иллюстрации

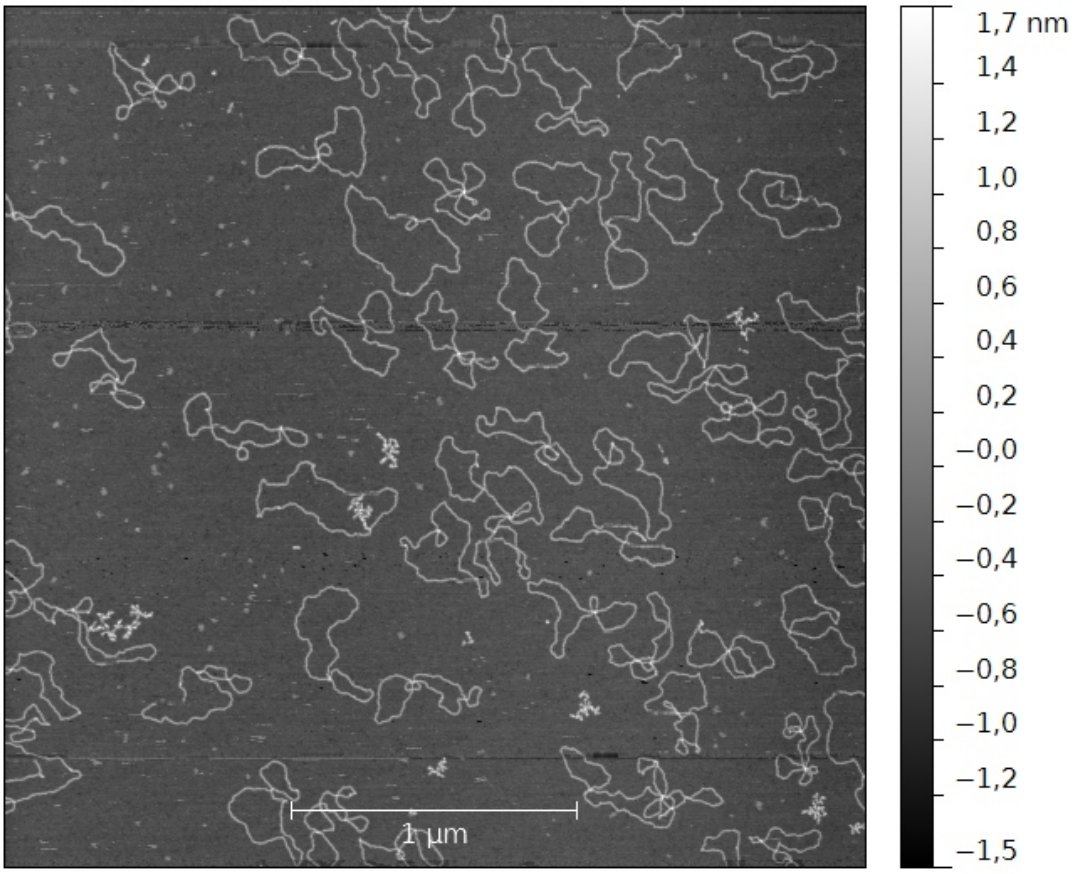

Рис. 1: Images of DNA: conformation of relaxed molecules

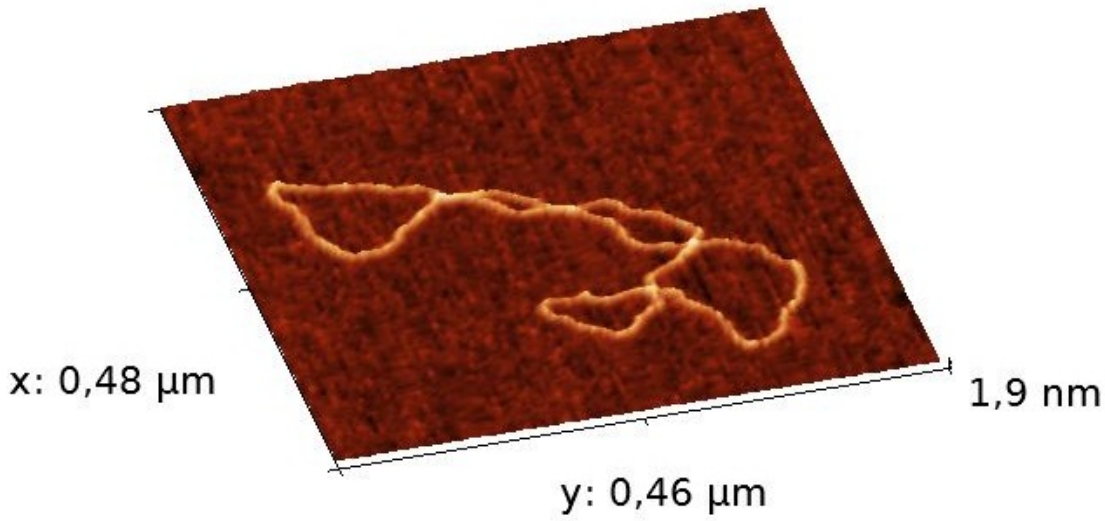

Рис. 2: Images of single DNA: on the left – low damage molecule (Group A)

Molecules	Length, nm	Diameter, nm	Persistent length, nm
Group A (relaxed on surface normally, 3 loops maximum)	~1450 nm	~10 nm	~40 nm
…	…	…	…
Group B (relaxed on surface fractionally, up to 3 loops)	~1300 nm	~15 nm	~25 nm
…	…	…	…
Group C (fragmented or destroyed, branched in chains)	~ 30 - 600	~34	not in range

Рис. 3: Table: Parameters of relaxed rings of plasmid pBR322 DNA measured by AFM