AFM imaging and force-distance curves analysis of single stranded DNA-binding (SSB) protein complex and comparison of kinetic with surface plasmon resonance as reference biosensor method

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Introduction

Formation of specific non-covalent complexes among nucleic acids and proteins seems to be essential for regulation of cellular processes and biochemical pathways. On the molecular level, the interactions between DNA and proteins are playing crucial role in the control of DNA replication, transcription, translation, and also repair of damaged DNA. Thus, detailed characterization of DNA-protein interactions improves the knowledge about abnormal cells and provides a better understanding of tumor growth , its prevention and medical treatment..

The interaction between ssDNA and the ssDNA-binding protein SSB was analyzed using atomic force microscopy (NanoWizzard3) providing images of the formed complexes on mica. Furthermore, this was complemented by determination of binding forces using atomic force spectroscopy with SSB-modified cantilever tip. The interaction was also characterized using the surface plasmon resonance based real-time bioanalysis (Biacore) providing reference data on kinetics of the interaction. In all cases, the direct label-free approach was conveniently employed and the disturbing labelling was not required.

SSB protein

is a stable tetramer in solution (4 identical 18.9 kDa subunits, from *E. coli*) **Fig. 1**. Each monomer binds ssDNA with high affinity and little sequence specificity. SSB protects ssDNA from degradation during replication and prevents restoration of double helix.

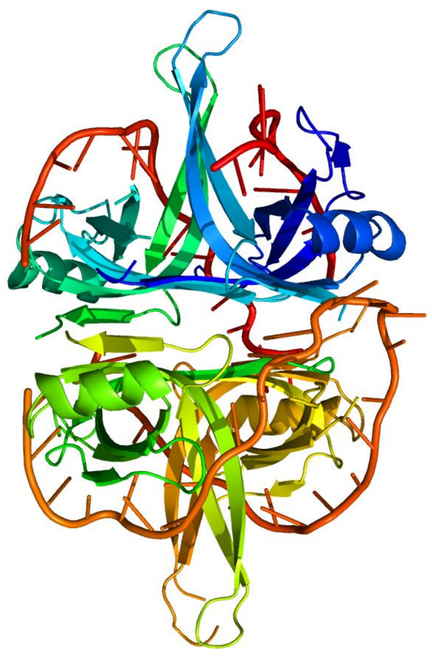


Fig. 1 Crystallography structure of *E. coli* SSB protein bound to ssDNA (PDB 1EYG).

AFM imaging

Immobilization of the circular ssDNA M13mp18 (7249 bases) was complicated as ssDNA preferentially created complex secondary structures. Finally, the SSB-ssDNA complex successfully deposited on the aminopropylsilatrane-modified mica and it was imaged in the tapping mode (NTEGRA-Vita**,** NT-MDT).

Fig. 2 demonstrates that SSB prevents formation of the secondary structure of M13mp18 ssDNA, which appears in the native circular form.

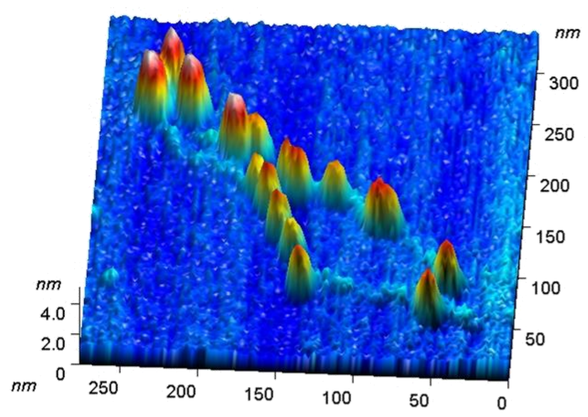


Fig. 2 AFM pseudo-3D topography of the SSB protein-ssDNA complex

Force spectroscopy

AFM force distance curves (*F-d*) allow to measure interaction of the complementary molecules bound on the AFM tip and the surface of mica directly in force units. *F-d* curves were obtained using the NanoWizzard3 (JPK) in operating in the force maping mode.

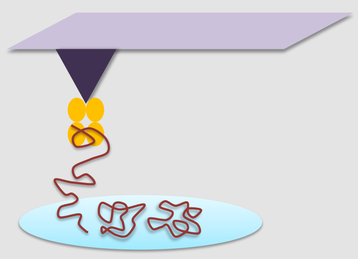


Fig. 3 AFM force spectroscopy - model of interaction between SSB protein (on the tip) and ssDNA M13mp18 (on mica).

Bioforces play significant role in most biological processes from cellular mobility to replication of DNA and other affinity recognition processes.

SSB protein (1.0 µg/ml) was electrostatically immobilized on the AFM cantilever tip (JPK PPP-NCHAuD) and ssDNA (0.5 µg/ml) was bound on the mica surface treated with aminopropylsilatrane, interactions were carried out in 10 mM HEPES and 2 mM $NiCl_2$. The *F-d* curves were generated automatically (24 hours, ~ 25 000 curves) and sorted to extract the those indicating the expected biointeraction. The representative examples are in **Fig. 4** and **Fig. 5**, which provide forces corresponding to nonspecific (0.198 nN) and specific (5.47 nN) interactions, respectively. The set-up of the experiment is given in **Fig. 3**.

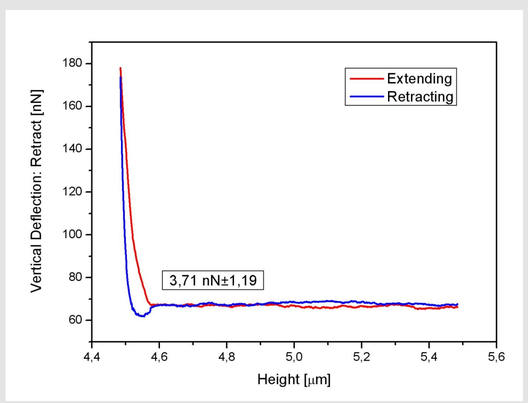


Fig. 4 Force vs. distance (height) curves recorded for bare tip interacting with ssDNA on mica (blank, only non-specific interaction).

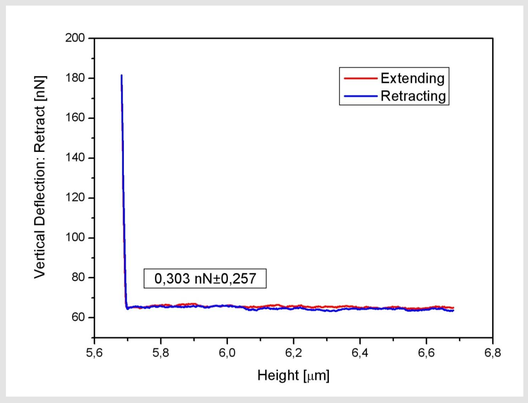
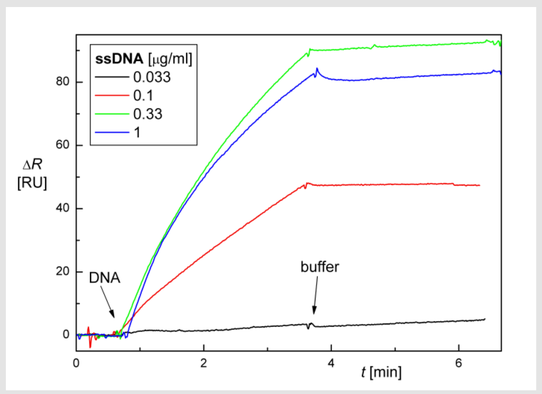


Fig. 5 Typical *F-d* curve recorded for the specific interaction between the SSB-modified tip and ssDNA immobilized on mica.

Surface Plasmon Resonance

SPR is a label-free technique for real-time kinetic studies of affinity interactions. Biacore 3000 SPR biosensor was used, the SSB protein was covalently immobilized on the CM5 chip (carboxymethylated dextran layer, classic EDC/NHS activation, 3.6 µg/ml SSB in pH 4.5).

The second partner - ssDNA (different concentrations) was dissolved in the HBS-P buffer and allowed to interact with the chip. The obtained association and dissociation curves (sensorgrams, relative signal measured in Resonance Units RU, non-specific binding subtracted) are shown in **Fig. 6**.



The SSB-ssDNA complex was quickly formed (ka = $2.3x10^7$) and it was very stable, as practically no dissociation was observed (kd near 0).

Fig. 6 Binding curves of interactions SSB protein with different concentrations of M13mp18 ssDNA.

Conclusions

The specific interaction between SSB protein and ssDNA was studied using SPR providing kinetics of the complex in solution. Furthermore, at the single-molecular level, AFM imaging and spectroscopy proved existence of SSB-ssDNA complex and allowed to directly measures the binding force, respectively. Both techniques provided valuable complementary results characterizing interactions of DNA-specific proteins.

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