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 (NanoWizard3) 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 small sequence specificity. SSB protects ssDNA from degradation during replication and prevents restoration of double helix.

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.

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 CMS 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).

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.