AFM force spectroscopy and surface plasmon resonance study of single stranded DNA binding (SSB) protein complexes with circular ssDNA, short oligonucleotide and LNA

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Introduction

Characterization of the DNA-protein interactions should improve the information about abnormal cells and it provides a better understanding of tumour growth, its prevention and medical treatment in the future. The interaction between ssDNA M13mp18 (7249 bp) and the ssDNA-binding protein from E. coli was analyzed using atomic force microscopy (Dimension FastScan) providing images of the formed complexes on mica surface. These data were compared with the interaction of short oligonucleotide (SH-C6-TAGTAGCATT)-protein interaction. Furthermore, this was complemented by determination of binding forces using force spectroscopy with modified cantilever tips with different conditions for immobilization of ssDNA, oligonucleotide and LNA. Study of force spectroscopy was supplemented by hybridization of artificial nucleotide: locked nucleic acid (LNA SH-C6-AATGCTACTA) with complementary oligonucleotide. This artificial nucleotide plays important role in genetic and cytogenetic analyses. The interaction was also characterized using the surface plasmon resonance based real-time bioanalysis (Biacore) providing reference data on kinetics. The direct label-free approach was conveniently employed and the disturbing labelling was not required.

AFM imaging

Immobilization of the circular ssDNA M13mp18 (7249 bases) was complicated as ssDNA preferentially created complex secondary structures. Ss-ssDNA complex successfully deposited on the polylysine-modified mica and it was imaged in the tapping mode. Fig. 1.a demonstrates that SSB prevents formation of the secondary structure of M13mp18 ssDNA, which appears in the native circular form.

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

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 FastScan Dimension (Bruker) operating in the force mapping mode.

SSB protein (1.0 µg/ml) was electrostatically immobilized on mica and oligonucleotide was bound on the mica surface treated with poly-L-lysine. AFM cantilever tip (SCANASYST-Fluid+) was treated with silanization, PEG linker and 10 mM ssDNA M13mp18 (oligonucleotide or LNA). Interactions were carried out in water. In Fig. 2.b, there are shown average F-d curves in pN from measurements. The representative examples are in Fig. 2.c, which provide forces corresponding to specific interactions, respectively. The set-up of the experiments are given in Fig. 2.a.

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 M13mp18 or oligonucleotide (different concentrations) were 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. 3.

The ssDNA-M13mp18 complex was easily formed (kₐ = 2.3x10⁷ l·mol⁻¹·s⁻¹) and it was very stable, as practically no dissociation was observed (kᵢₗ near 0). In other case SSB-oligonucleotide complex quickly was subject to dissociation (kᵢₗ = 2.1x10³ l·mol⁻¹·s⁻¹, kᵢₗ = 1.3x10³ l·mol⁻¹·s⁻¹).

Electrophoretic mobility shift assay

Fig. 4. a) Electrophoresis in 1% agarose gel (40 V, 90 min) of the complex of ssDNAM13mp18-SSB. The concentration of SSB decreased from A to H (200, 133, 89, 59, 40, 26, 18 and 0 µg/ml) while the concentration of ssDNA was constant (25 µg/ml) b) compare with oligonucleotide-SSB complex (same concentrations).

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Fig. 1. a) AFM pseudo-3D topography of the SSB protein-ssDNA M13mp18 complex and b) crystallography structure of E. coli SSB protein bound to ssDNA (PDB 1EG5).

Fig. 2. a) Representative F-d curves recorded for the specific interactions. b) average forces corresponding to specific interactions. c) Representative F-d curves recorded for the specific interactions.

Fig. 3. a) Binding curves of interactions SSB protein with different concentrations of M13mp18 ssDNA b) and different concentration of oligonucleotide SH-C6-TAGTAGCATT.

Fig. 4. a) Electrophoresis in 1% agarose gel (40 V, 90 min) of the complex of ssDNAM13mp18-SSB. The concentration of SSB decreased from A to H (200, 133, 89, 59, 40, 26, 18 and 0 µg/ml) while the concentration of ssDNA was constant (25 µg/ml) b) compare with oligonucleotide-SSB complex (same concentrations).