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Single-Molecule Interactions of a Monoclonal Anti-DNA Antibody with DNA

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Abstract

© 2016, Springer Science+Business Media New York.Interactions of DNA with proteins are essential for key biological processes and have both a fundamental and practical significance. In particular, DNA binding to anti-DNA antibodies is a pathogenic mechanism in autoimmune pathology, such as systemic lupus erythematosus. Here we measured at the single-molecule level binding and forced unbinding of surface-attached DNA and a monoclonal anti-DNA antibody MRL4 from a lupus erythematosus mouse. In optical trap-based force spectroscopy, a microscopic antibody-coated latex bead is trapped by a focused laser beam and repeatedly brought into contact with a DNA-coated surface. After careful discrimination of non-specific interactions, we showed that the DNA-antibody rupture force spectra had two regimes, reflecting formation of weaker (20–40 pN) and stronger (>40 pN) immune complexes that implies the existence of at least two bound states with different mechanical stability. The two-dimensional force-free off-rate for the DNA-antibody complexes was ~2.2 × 10−3 s−1, the transition state distance was ~0.94 nm, the apparent on-rate was ~5.26 s−1, and the stiffness of the DNA-antibody complex was characterized by a spring constant of 0.0021 pN/nm, suggesting that the DNA-antibody complex is a relatively stable, but soft and deformable macromolecular structure. The stretching elasticity of the DNA molecules was characteristic of single-stranded DNA, suggesting preferential binding of the MRL4 antibody to one strand of DNA. Collectively, the results provide fundamental characteristics of formation and forced dissociation of DNA-antibody complexes that help to understand principles of DNA-protein interactions and shed light on the molecular basis of autoimmune diseases accompanied by formation of anti-DNA antibodies.

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Keywords

Anti-DNA antibody, DNA, Nanomechanics, Optical trap, Single-molecule force spectroscopy, Two-dimensional kinetics

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