Molecular Interaction of European Lyme Disease Spirochetes probed by AFM

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Abstract
Lyme disease is the most common vector borne disease in the Northern hemisphere. Adherence of its causative agent, i.e., bacteria belonging to the Borrelia genus, to cells or the extracellular matrix (ECM) of tissues is a key step to promote dissemination and colonization leading to further development of the illness. Bacterial adhesins are essential factors for the interaction between the bacteria and ECM components. Despite its high relevance, many details remain to be elucidated regarding their physiological functions, such as characterizing the main molecular interactions between bacterial adhesins and ECM during the initial adhesion stage. In this study, the interaction forces between borrelial surface proteins from infectious European Borrelia genotypes and different ECM proteins are probed. Using single molecule force spectroscopy (SMFS), binding activity and strength for the interactions are determined. Interaction specificities are validated by blocking experiments from adding adhesins into the measurement solution. Dynamic Force Spectroscopy (DFS) is used to further characterize the energy landscape of the interaction, determining binding modes and kinetic binding constants. Our results elucidate the complex adhesive properties of borrelial surface proteins to ECM in pathogen adherence during the infection process.

Expression of Borreliad Adhesins
Recombinant borreliad adhesins were expressed in E. coli cells and purified by affinity column chromatography. Successful purification was determined by SDS-page electrophoresis and Coomasie Blue Staining of the gel. Protein concentration was determined using the Bradford assay.

Tip/Surface Functionalization
AFM tips (M5CT, Bruker, USA) and silicon surfaces were functionalized with amino groups via APTES coating in gas phase. Furthermore, different types of PEG linkers were used to fix the purified proteins on the AFM tips and surfaces, PEG-maleimide linker and NHS-NTA were used for the borreliad adhesions and PEG-acetal or PEG-thiol for coupling the ligand residues of the ECM components to the tip or surface.

Atomic Force Microscopy

Force-Distance Curve

Probability Density Function
For each tip and each loading rate approximately 1000 force-distance curves are recorded and the unbinding events in each curve are determined. The probability density function is derived from histograms of the different recorded unbinding force values.

Bell Evans Model

BBK32 from B. afzelii
Probability of BBK32 binding to human laminin (hLFN)

Ensemble vs Single molecule measurements
Complementation of DFS with Surface Plasmon Resonance

Results
BBK32 from B. afzelii
- Specific interaction to hLFN as shown by the block experiment (binding probability decreases significantly upon addition of free hLFN to experimental chamber)
- Dynamic Force Spectroscopy: dissociation force increases with the loading rate, use Bell-Evans model

DbpA from B. bavariensis
- Significantly higher binding probability to decorin (10%) than to hLFN (1.3%)

Borreliad
- Causative agent of Lyme disease
- Transmitted by hemaphagous arthropods
- Main vector in Europe: Ixodes ricinus
- 60 000 cases per year

Figure 1: Topography of Borrelia recorded by AFM

Figure 2: Preparation of borreliad adhesins

Figure 3: Tip/Surface Functionalization

Figure 4: Bell Evans model for the interaction between BBK32 and hLFN

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