STUDIES OF STREPT(AVIDIN) – BIOTIN INTERACTIONS USING ATOMIC FORCE MICROSCOPY

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Atomic force microscopy (AFM) can be applied to study ligand – receptor interactions. This method allows to measure attractive or repulsive forces between the cantilever tip and the sample surface, elucidating local chemical and mechanical properties like adhesion and elasticity. Based on this techniques we have prepared avidin or streptavidin functionalized tips called biosensors, which could interact with the sample coated by biotin and form highly specific bonds. This approach makes possible investigations of bond-rupture forces on the level of single pairs of molecules.

We have compared intermolecular interactions in two model complexes well-known for creating one of the strongest non-covalent bonds among biological molecules. For each system the unbinding bond force of single pairs of proteins was determined from the center of Gaussian distribution fitted to the force histogram. Under our experimental conditions we have observed that rupture force of the avidin-biotin complex was stronger than of the streptavidin-biotin complex by the same value of loading rate. Some differences in molecular structure of streptavidin and avidin are responsible for their various affinity for biotin binding.

Furthermore, we present loading rate dependence of the rupture force of a single bond in these two systems in a range of loading rate between 300 and 9600 pN/s. Using Bell model we have been able to calculate a dissociation rate and subsequently to estimate an activation energy barrier of the interaction between biotin and streptavidin or avidin.

These results confirm the theoretically predicted disparities in biotin binding sites in avidin and streptavidin.

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