Site-selective Dynamic Force Spectroscopy of Biotin-Streptavidin/Avidin Interactions

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Dynamic Force Spectroscopy (DFS) enables to investigate the specific interactions between two molecules, such as ligand-recepter pairs. In DFS measurement, the unbinding force applied to a molecular bonding is increased at a constant rate, and the force required to rupture the molecular pair is measured. The energy landscape of the interactions is derived from the relationship between rupture force and loading rate.

However, the constant loading rate required for DFS experiment has not been well realized because the force probe, in general, is retracted from the substrate at a constant velocity instead of constant loading rate, where it is difficult to analyze the landscape in detail. In order to achieve precise measurement, we have developed a system that has a feedback loop to keep the loading rate constant. The unbinding force applied to a molecular pair is detected using Atomic Force Microscopy, and the probe-substrate distance is precisely controlled with the feedback [1,2]. Here, we demonstrate the results of the selective analysis of biotin-streptavidin/avidin interactions obtained using this method.

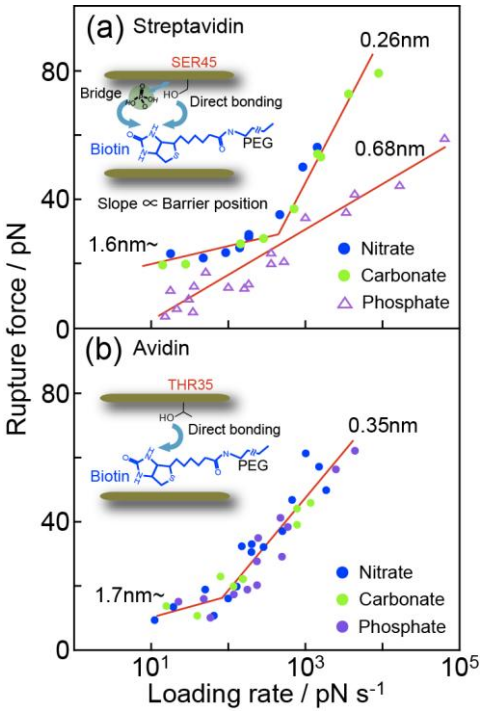

Figure 1 Relationship between modal rupture force and logarithm of loading rate obtained for (a) streptavidin-biotin and (b) avidin-biotin complexes.

Figure 1(a) shows the most frequent rupture forces plotted against the logarithmic form of the loading rate for biotin-streptavidin. For a 0.01 M phosphate (pH 7.4) solution, the potential barrier position was estimated to be 0.68 nm, which is formed by the phosphate molecules in the buffer solution bridging between a streptavidin and a biotin. For a 0.05 M sodium nitrate (pH 7) and a 0.05M carbonate buffer solutions (pH 10), the slopes similarly changed at a loading rate of about 10^2-10^3 pN/s as shown in Fig. 1(a), and the barrier position estimated from the slope was 0.26 nm, which is attributed to the potential barrier formed by the direct bonding between the biotin and streptavidin molecules. In contrast, the slopes for biotin-avidin were almost the same for all solutions as shown in Fig. 1(b), which potential barrier position obtained from the steep slope was estimated to be 0.35 nm, suggesting the formation of a direct bonding in all solutions. Details will be discussed at the conference.

[1] A. Taninaka, O. Takeuchi and H. Shigekawa, *Appl. Phys. Express* **2009**, 2, 085002.

[2] O. Takeuchi et al., *J. Appl. Phys.* **2006**, 100, 074315.