Site-Selective Analysis of Biotin-Streptavidin Interactions using Atomic Force Microscopy

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The variety and selectivity of the interactions between a pair of functional molecules, such as ligand-recepter, play essential roles in biological processes and molecular devices based on molecular recognition properties. However, such interactions include complicated many-body effects arising, for example, from solvent parameters and the manifold structures of functional molecules, which prevent the design of detailed functions in predetermined structures. Therefore, probing the energy landscapes of individual interactions in a molecular complex and their variation depending on the surrounding conditions is of great importance and strongly desired for further advances in biochemistry and its applications.

We demonstrate the first site-selective anatomy of molecular interactions at the single-molecule level in a typical antigen-antibody system, streptavidin-biotin complex, by Dynamic Force Spectroscopy (DFS)[1]. Direct and bridged interactions at each bonding site in a streptavidin molecule, which depend on the solvent conditions, were clearly distinguished and individually analyzed at the single-molecule level.

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 [2].

Figure 1 shows the most frequent rupture forces plotted against the logarithmic form of the loading rate, where streptavidin molecules were fixed on a Au substrate with two different forms as schematically illustrated. The potential-barrier position in the energy landscape is estimated from the slope of the linear relationship between the rupture force and the loading rate. For the case (a), the potential barrier position was estimated to be 0.68 nm, which is attributed to a transient network of water bridges and hydrogen bonds. In contrast, for the case of the type (b), there are two slopes that provide the potential barriers at 0.16 nm and 0.63 nm. Namely, an inner barrier due to direct hydrogen bond between biotin and amino acid in streptavidin was additionally probed. Such a detail analysis has become possible because the precise measurement, particularly at high loading rates, was achieved by the new method.

Details will be discussed at the conference.

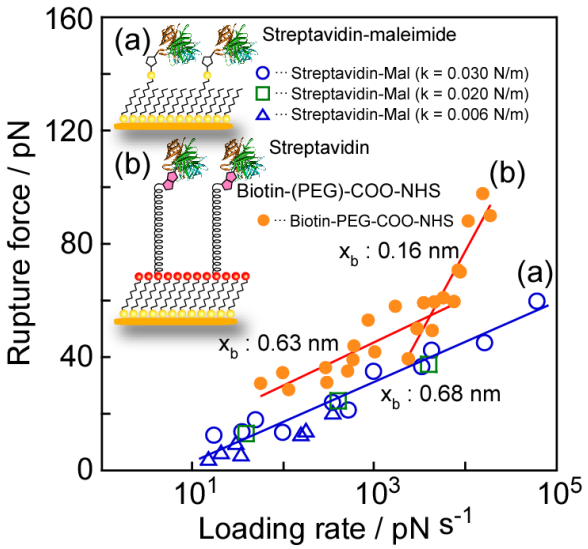

Figure 1 Potential barrier positions obtained for different experimental conditions

[1] R. Merkel, et al., *Nature* 397, (1999), 50.

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