Reconsideration of Site-selective Dynamic Force Spectroscopy Analysis of Streptavidin-Biotin Interactions

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To understand and design molecular functions on the basis of molecular recognition processes, the microscopic probing of the energy landscapes of individual interactions in a molecular complex and their dependence on the surrounding conditions is of great importance. Dynamic force spectroscopy (DFS) is a technique that enables us to study the interaction between molecules at the single-molecule level. However, the obtained results differ among previous studies, which is considered to be caused by the differences in the measurement conditions. We have developed an atomic force microscopy technique that enables the precise analysis of molecular interactions on the basis of DFS [1,2]. After verifying the performance of this technique, we carried out measurements to determine the landscapes of streptavidin-biotin interactions.

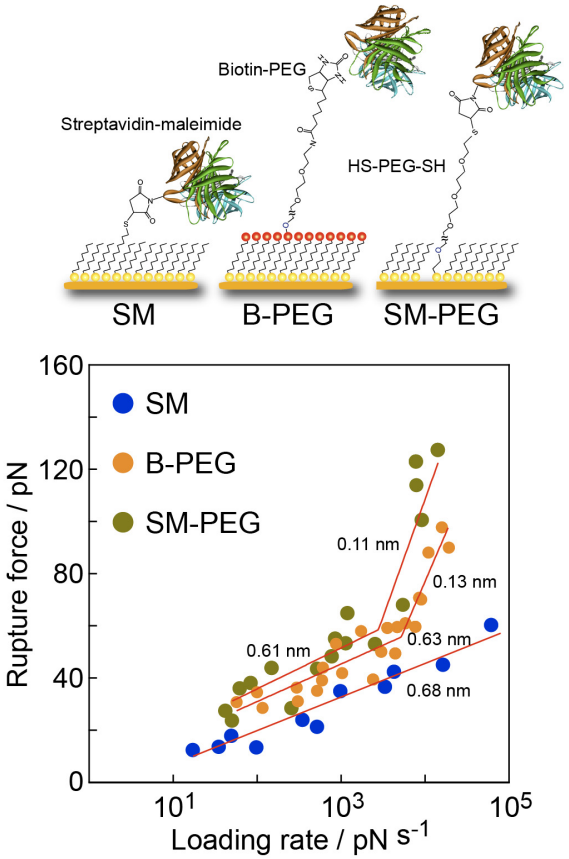

Figure 1 Relationship between the modal rupture force and the logarithm of the loading rate obtained for streptavidin-biotin complex.

Figure 1 shows the relationships between the modal rupture force and the logarithm of the loading rate obtained in 0.01 M PBS (pH 7.4) for the (a) Streptavidin-maleimide (SM), (b) Biotin-PEG (B-PEG) and (c) SM-PEG conditions. For the B-PEG and SM-PEG conditions, the gradient of the slopes exhibits a marked increase at 2×10^3 pN/s. The potential barrier positions were estimated from the two slopes to be 0.13 nm and 0.63 nm for the B-PEG condition and 0.11 nm and 0.61 nm for the SM-PEG condition. For the SM condition, in contrast, only one slope was observed and the potential barrier position estimated from this slope was 0.68 nm. Namely, only the bridged bonding at the middle reaction sites was observed.

The obtained results showed good agreement with theoretical predictions. Lifetimes were also well analyzed. Using a combination of cross-linkers and the atomic force microscope that we developed, site-selective measurement was carried out, and the steps involved in bonding due to microscopic interactions are discussed using the results obtained by site-selective analysis.

Details will be discussed at the conference.

References

[1] A. Taninaka et al., *Appl. Phys. Express* **2009**, 2, 085002.
[2] O. Takeuchi, T. Miyakoshi, A. Taninaka, K Tanaka, D. Cho, M. Fujita, S. Yasuda, S. P. Javis, H. Shigekawa *J. Appl. Phys.* **2006**, 100, 074315.