Characterizing Association Kinetics of Selectin-PSGL-1 Binding Using a Thermal Fluctuation Assay*

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Receptor-ligand interactions are crucial to many biological processes such as inflammatory cascade and tumor metastasis. Two-dimensional (2D) forward rate of surface-bound receptor-ligand interactions governs the bond formations as well as cell adhesions. While the dissociation kinetics of receptor-ligand interactions has been extensively investigated, the association kinetics, however, has been poorly characterized. Here we developed a novel thermal fluctuation assay using optical tweezers technique to visualize the bond formation of surface-bound receptor-ligand interactions. L- or P-selectin construct (sLs, sPs, or PLE) and their ligand (P-selectin glycoprotein ligand 1, PSGL-1) were respectively captured onto the surface of silica microbeads, and the time course of sequential association-dissociation events was monitored. Upon the first-order association kinetics, 2D forward rate of sLs-PSGL-1 binding was predicted to be 2.5×10^{-8} μ m^2/s, while it was ~4.0-fold higher and ~8.0 fold lower for sPs-PSGL-1 and PLE-PSGL-1 binding, respectively, suggesting that the association kinetics of selectin-PSGL-1 interactions is identity-specific and molecular length-dependent. It was also found that the association was affected by such the biophysical factors as the initial distance between two microbeads, the diffusion of molecule-bearing microbead, as well as force transducer stiffness. These results further the understanding in the fast kinetics of selectin-ligand bindings and provide the new insights in the biophysical bases of surface-bound receptor-ligand interactions.

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