DOI:10.16156/j.1004-7220.2013.s1.055

Spring constant regulate selectin-ligand bond dissociation

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Forced dissociation of selectin-ligand complex is crucial to such biological processes as leukocyte recruitment, thrombosis formation, as well as tumor metastasis[1]. Although several assays and techniques, e.g., dynamic force spectroscopy (DFS), have been applied to probe the complex at single-bond level, the discrepancies in the loading rate dependence of bond rupture force were found in the assays, presumably due to the different pathways in energy landscape and binding kinetics of molecular complexes[2]. However, the underlying mechanisms remain unclear.

Here an optical trap (OT) assay was used to quantify the bond rupture at $r_f \leqslant 20$ pN/s with low k(~10-3-10-2 pN/nm) when P-selectin and P-selectin glycoprotein ligand 1 (PSGL-1) were respectively coupled onto two glass microbeads. Our data indicated that the bond rupture force, f, retained the similar values when r_f increased up to 20 pN/s. It was also found that f varied with different combinations of k and v even at same r_f. Most probable force, f^*, was enhanced with spring constant when $k < 47.0 \times 10^{-3}$ pN/nm, indicating that the bond dissociation at low r_f is spring constant-dependent and that bond rupture force depends on both loading rate and mechanical compliance of force transducer[4].

And we further quantified the dissociation lifetime of selectin-ligand bond with low stiffness ranging from 3.5×10^{-3} to 4.7×10^{-2} pN/nm. Our results indicated that bond lifetime yielded distinct distributions with different probe stiffness, implying the stochastic feature of bond dissociation. It was also found that the mean lifetime varied with probe stiffness and that the catch bond nature was visualized at $k \geqslant 3.0 \times 10^{-2}$ pN/nm.

Upon the experimental observations, we proposed that probe stiffness, k, affects the zero-force reverse rate k_r^0 and in turn alters the bond rupture force by regulating the diffusion of bond complex. A Morse potential was applied to define the kinetics of forced dissociation of a bond based on Kramers' rate theory[3] and Brownian dynamics. Theoretical calculations and Monte Carlo simulations were conducted to study the kinetics of forced dissociation. Zero-force off-rate k_r^0 was estimated and probability distribution of bond rupture force P_d and most probable rupture force f^* were predicted at varied probe stiffness, which were compared with those measurements using OT assay. It was found that the probe stiffness dependence of k_r^0 at relatively low stiffness ($k \leqslant 1$ pN/nm) is mainly attributed to the confined Brownian diffusivity when bonded site is connected to a mechanical probe. Moreover, the simulations on dissociation kinetics are

coincident with the aforementioned measurements, which provides the biophysical interpretation on forced dissociation of a receptor-ligand bond.

Acknowledgement: This work was supported by National Natural Science Foundation of China grants 10902117, 31230027, 30730032, and 10332060.

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