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2D Kinetics and Forced Dissociation of Selectin-ligand Bindings

Mian Long[Δ]

(*National Laboratory of Microgravity, Institute of Mechanics, Chinese Academy of Sciences, Beijing* 100080, *China*)

INTRODUCTIONS Cell adhesion is crucial to many pathophysiological processes, such as inflammatory reaction and tumor metastasis. It is mediated by specific interactions between receptors and ligands, and provides the physical linkages among cells. For example, interactions between selectins and glycoconjugate ligands mediate leukocyte initially tethering to and subsequently rolling on vascular surfaces in sites of inflammation or injury, which is determined by their fast kinetic rates. To mediate cell adhesion, the interacting receptors and ligands must anchor to apposing surfaces of two cells or a cell and the substratum, i.e., the so-called two-dimensional (2D) binding, which differs from interactions in the fluid phase, i.e., the three-dimensional (3D) binding. How structural variations and surface environments of interacting molecules affect their 2D kinetics, and how external forces manipulate their dissociation has little been known quantitatively, and nowadays attracts more and more attentions.

APPROACHES The selectin family of adhesion molecules has three known members: P-, E-, and L-selectin. Their common structure is an N-terminal, calcium-type lectin (Lec) domain, followed by an epidermal growth factor (EGF)-like module, multiple copies of consensus repeat (CR) units characteristic of complement binding proteins, a transmembrane segment, and a short cytoplasmic domain. It binds to the glycoconjugate eptitopes of carbohydrate ligands (e.g., P-selectin glycoprotein ligand 1, PSGL-1) specifically via Lec-EGF domains. As an ideal molecular system, selectin and ligands were employed to perform the following biophysical measurements.

To conduct 2D kinetics measurements of selectin-ligand interactions under external forces, a dual approach is taken that coordinates biological experiments, mechanical measurements, and numerical computation of cell adhesions and molecule binding. The approach comes from three aspects: 1) A theoretical framework was established upon a small system probabilistic model firstly proposed by McQuarrie and then modified by other workers. Coupled by the constitutive equation of force dependence of reverse rate, this framework was employed to predict the kinetics of selectin-mediated cell adhesions under external forces. 2) The state-of-the-art technologies developed includes micropipette aspiration, atomic force microscopy (AFM), dual optical trap, and quantitative rosette assay. Combined with the relevant assays in cellular/molecular biology and immunology, these techniques enable to measure experimentally the 2D binding and force dependence of selectin/ligand dissociation. 3) A numerical simulation of steered molecule dynamics (SMD), based upon a non-equilibrium physical theory from a viewpoint of atomic level, is used to predict the energy landscape of forced dissociation of selecin/ligand interactions.

RESULTS Biological issues focus on the structure-functionality of selectin/ligand interactions under external forces. Here we quantify on the effects of molecular length and orientation, the effects of amino acid mutation of PSGL-1, the rupture force dependence on approaching rate, dislodging velocity, and contact duration, and the bond lifetime dependence on applied forces. While one molecule was coated or captured on one cell/sphere surface or an AFM tip, the counterpart molecule was immobilized onto the other cell/sphere surface or a reconstructed lipid bilayer. Using the techniques as above, the adhesion probability of, the rupture force distribution of, and the force dependence of bond lifetime of selectin/ligand interactions were measured experimentally. Kinetic parameters of reaction rates and binding affinities were obtained by comparing the data to the probabilistic model, while force dependence of selectin/ligand dissociation were predicted using the first-order kinetics of bond dissociation. Experimental data indicated that randomizing the orientation of the molecule or lowering its binding epitope above the cell membrane reduced the forward-rates but not the reverse-rates. These effects manifest only in 2D binding, as Scatchard analysis showed similar 3D affinities of a soluble antibody for cell-bound P-selectin constructs regardless of their orientation and length. Tyrosine replacement of PSGL-1 binding epitope affected the selectin/PSGL-1 bindings. These data also showed that rupture forces of selectin/ligand bonds increased with dislodging rates. Bond lifetimes of selectin/ligand bindings demonstrated a multiphasic transition, i.e., a "catch bond" which the lifetimes increased with external forces, and a "slip bond" which the lifetimes decreased with forces. The force or extension profiles along stretching pathways, predicted from SMD simulations, were correlated to the conformational changes, suggesting that the structural collapses of P-selectin Lec-EGF domains were mainly attributed to the burst of hydrogen bonds within major ? sheet of EGF domain and the disruptions of two hydrophobic cores of Lec domain. These findings further the understandings of cell interactions mediated by adhesive molecules.

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ΔTel. 010-62613540; E-mail: mlong@imech.ac.cn