MECHANICAL REGULATION OF RECEPTOR-LIGAND BINDING BY CONTROLLABLE TRANSPORT

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Summary Binding and unbinding of receptor-ligand bonds in cell adhesion are commonly characterized by measuring association and dissociation kinetic rate constants. The force dependence of bond dissociation has been extensively studied at single-molecule level, while quantification of bond association kinetics is much scattered and its detailed regulation mechanisms remain to be fully understood. Here we theoretically and experimentally investigate the interplay of association kinetics between surface-bound receptors/ligands and transport of protein carrier via diffusion process in a biomembrane force probe (BFP). Dependent of mechanical aspects that control carrier transport, the mean waiting time for receptor-ligand binding to occur augments monotonically with increasing gap distance between the functional surfaces. We gain quantitative understanding of how diffusive transport mechanism can alter the apparent binding behavior of molecular interactions, and better interpretation of intrinsic association rate constant through proper modelling the pertinent experiments.

INTRODUCTION

Reversible binding and unbinding between specific receptor and ligand molecules are crucial processes in cell adhesion, which need to be tightly controlled by mechanical and biochemical signaling pathways that are still being elucidated [1]. Binding and unbinding of molecular bonds are commonly characterized by measuring association and dissociation kinetic rate constants. In dissociation measurements, the time scale of single-bond duration is mechanically determined via a force signal [2-4], while the characteristic time for bond formation, i.e. apparent kinetic on-rate, is much more difficult to measure. Recently this situation has changed with the development of a biophysical method that enables pinpointing association events by monitoring the decreased resumption of thermal fluctuations of an ultrasensitive biomembrane force probe (BFP) [5]. Compared to the typical association rate measurement in 3D situations where at least one protein is in solution, measurement of 2D association rate is closer to the physiological scenario of cell adhesion, but more challenging to perform and has been carried out in a small number of studies [5-7]. In these studies, kinetics measurements of molecular association are indirect and the intrinsic association rate constant must be inferred from observations. It is therefore valuable to have a theoretical framework that bridges the apparent waiting time of bond formation in measurements and intrinsic kinetic association rate constant, accounting for the observed discrepancy from different experimental approaches.

Here we present a theoretical model to understand the association events of surface-bound proteins in a BPF setup, which physically mimicks the scenario of a receptor carrier diffusing towards and reacting with a ligand-bound surface in cell adhesion against any type of tethering effects from membrane or cytoskeleton. The quantitative approach is validated experimentally using a biological interaction consisting of the von Willebrand factor A1 domain (VWF-A1) binding with platelet glycocalicin (GC: the extracellular portion of glycoprotein Ibα) [8], an important mediator of platelet rolling on vascular surfaces. We apply the theory to explain the mechanical regulation of bond association by adjusting gap distance between the functional surfaces, as well as other system parameters that can influence the underlying transport process.

MODEL

The essential feature of 2D association process here is made much more complicated by the fact that the adhesion between receptor and ligand molecules requires two essential steps, requiring first close contact between two opposing surfaces by physical transport before chemical reaction can take effect at an intrinsic rate and lead to formation of bond. Thus the measured values in our bond association experiments are really mixed time scales combining both the transport and reaction effects.

Consider a biophysical model describing the transport-reaction process in the BFP experiments [Fig. 1(a)] and assume that the instantaneous motion of the probe bead under thermal forces against the red blood cell (RBC) linkage is essentially one dimensional [Fig. 1(b)]. We derive the following partial differential equation (PDE) describing a large number of nominally identical experiments in terms of probability density function:

$$\frac{\partial \rho(x,t)}{\partial t} - \frac{k_B T}{6\pi\eta r_p}\frac{\partial}{\partial x}\left(\frac{\partial \rho(x,t)}{\partial x} + \frac{\kappa}{k_B T} x\rho(x,t)\right) + \left(H(x-d+l) - H(x-d)\right)\rho(x,t)(l+x-d)\frac{2\pi r_p r_t}{r_p + r_t} m_p m_t k_f = 0$$

where $\rho(x,t)$ is the probability of finding the apex of probe bead in position x at time t, k_BT the unit of thermal energy, η the medium viscosity, κ the spring constant of RBC, d the initial gap between the two apices of opposing surfaces, (r_p, r_t) and (m_p, m_t) are the radius and site density of the probe and target beads, respectively. $H(.)$ is the Heaviside step function

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that helps to define a window for reaction to be possible, and the combined molecular length l and intrinsic association rate constant k_f are receptor-ligand characteristics and should be inferred through matching the numerical solution to above PDE with pertinent experiments.

RESULTS AND CONCLUSIONS

Both theoretical model and experimental data show the augment in waiting time with increasing surface gap distance [Fig. 1(c)], which suggests an appealing interpretation: the overall rate of bond formation is governed by two individual "resistances" of probe diffusion and intrinsic reaction in series, and d appears as an important parameter governing their relative contributions. When d is smaller than l, the opposing protein pairs are already in close contact so that association can occur even without probe transport; as d goes beyond the certain distance, the receptors and ligands are separated far and probe transport dominates the overall process. Thus, the apparent on-rate (reciprocal waiting time) measured from the thermal fluctuation assay should also depend on the BFP spring constant, BFP probe bead radius as well as the medium viscosity through bead diffusion. We quantify these dependences both theoretically and experimentally in accessible range of involved parameters and the global agreement is satisfactory (results not shown).

In summary, bond formation can be transport-limited or reaction-limited controlled by gap distance and many other mechanical and physical properties such as tethering stiffness, medium viscosity, surface geometry etc. The proposed model can serve as an inverse approach to determine intrinsic kinetic association parameter from 2D apparent waiting time measurements in receptor-ligand binding.

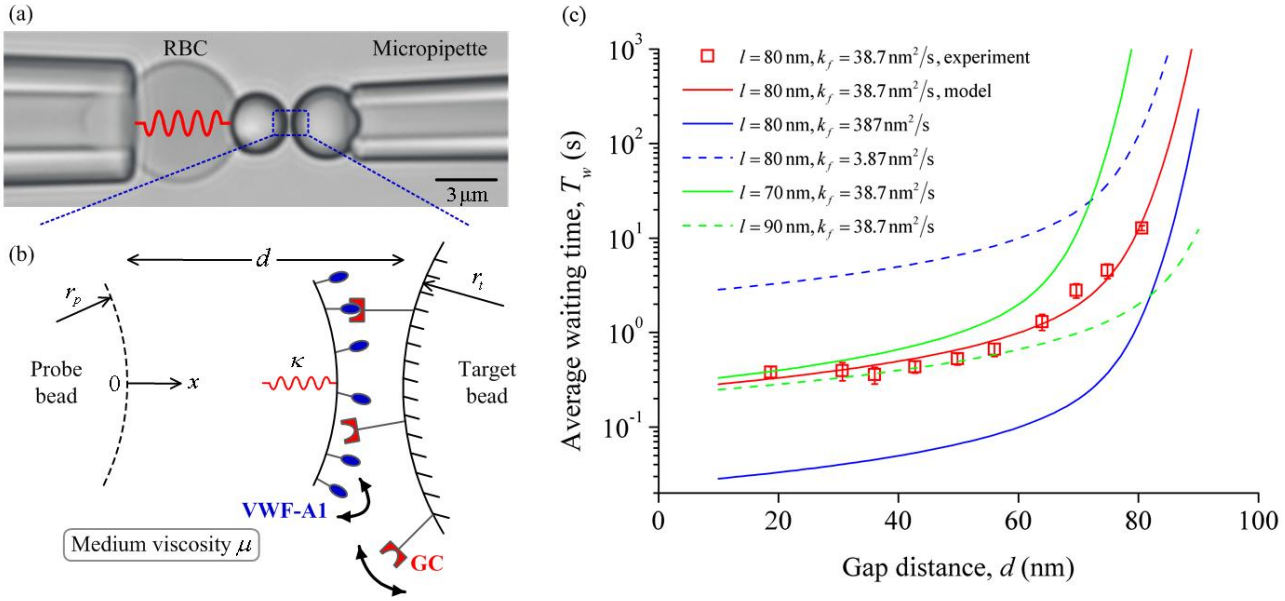

FIG. 1. (a) Setup of BFP experiment for receptor-ligand binding. (b) Fokker-Planck type of model describing the diffusion-reaction process in bond association. (c) The average waiting time T_w as a function of the gap distance d between the opposing surfaces for various values of bond characteristics in shooting for experimental data.

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