Probability of Receptor Unbinding During Ligand Assisted Tether Elongation from the Red Cell Membrane*

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When a system having a series of non-covalently bonded multiple components is pulled to opposite directions from its two ends, sequential and/or simultaneous bond breakages take place. The probability of breakage of individual bonds depends on their intrinsic mechanical properties and on the experimental conditions. We are particularly interested in the pulling mechanics observed when a lectin functional AFM probe was used to pull out the membrane protein, glycophorin A of the red blood cell, together with lipid tether formation. Here our concern is which of the non-covalent interactions, i.e., lectin-glycophorin A or lipid-lipid interactions in the tether is disrupted when the probe is detached from the biomembrane surface. In this paper we conclude that, during the process of lipid tether elongation with a constant tensile force of $\sim 70 - 80$ pN, unbinding of the lectin from glycophorin A takes place with a finite probability in addition to tether breakages that includes complete or incomplete delipidation of glycophorin A. [DOI: 10.1380/ejsnt.2006.273]

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I. INTRODUCTION

When the force mode of the atomic force microscope is used to probe for the location of specific kinds of membrane proteins on a live cell, a new method of mechanical mapping becomes a feasible possibility [1–3]. In this method, an AFM probe is chemically functionalized for the covalent immobilization of ligand molecules with a specific affinity towards a particular kind of receptor protein, and the probe is brought into contact with the surface of live cell membrane. If receptor proteins are within the reach of immobilized ligand molecules on the AFM probe, non-covalent interaction is often established which can be detected as the appearance of an extra adhesive force between the probe and the cell surface. A particularly interesting observation when this method is applied to the lipid bilayer membrane is the formation of lipid tethers behind the ligand-receptor complex formed on the AFM probe. The tether can be stretched up to a few or sometimes a few tens of micrometers before it is detached from the AFM probe [4–6]. A tether is formed with or without an initial increase of the tensile force and followed by a long stretch of a constant plateau force of 30 - 100 pN depending on the choice of the experimental systems. A common observation is that the force of tether extension is almost constant for several micrometer until the plateau is abruptly terminated by a jump of the cantilever to its free position. When the AFM probe is released from the cell membrane by the jump, it may be due to an abrupt breakage of the tether and/or to unbinding of the immobilized lectin on the tip from the cell surface glycoprotein because both interactions require similar ranges of force for breakage. It is also possible that the target membrane protein is denuded of the surrounding lipids and extracted

on the AFM tip as a complex with lectin molecules. It is, however, a remote possibility that the covalent crosslinking system is broken, since the force we observed in tether pulling event was roughly 10 to 20 times smaller than the force to break a covalent bond. In our experiments, we chose wheat germ agglutinin (WGA) as the lectin part and glycophorin A on the red blood cell (to be abbreviated as RBC) as the target glycoprotein in the membrane. It has been shown that these two proteins interact with an dissociation constant of 147 nM [7].

II. EXPERIMENTAL

An example of the retraction part of the force-distance curve obtained on the RBC surface using a modified cantilever with WGA is given in Figure 1 [8]. (Experimental details will be reported elsewhere.) The curve is characterized by a clear rise in force under the dotted arrow signifying the formation of at least one tether, and an ensuing long plateau of a constant force until the cantilever was freed to its free position after two stepwise jumps. In this instance, we think each jump after a plateau represented an elongation and detachment process of a single tether formed behind a single molecular pair of glycophorin A and WGA.

The rupture force is about 70 - 80 pN in each step in this particular instance with an average of 75 pN ($N=50$). The observed plateau force and the following jump events have the characteristics of tether elongation and breaks as described in the literature cited above but without the initial force peak (between 100 to 500 pN) which is sometimes claimed as a necessary process for tether formation [9]. In our case here, the tether forming force is not observed probably because, 1) glycophorin A was not associated with the cytoskeleton, and/or 2) the unbinding force of glycophorin A from cytoskeleton at the time of tether formation was less than the force to extend the tether. Our preliminary result from a separate experiment on the unbinding force of WGA from immobilized glycophorin A

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FIG. 1: An example of force-distance curve obtained on RBC using an AFM probe modified with wheat germ agglutinin with a specific affinity to the membrane protein, glycoprotein A. Under the dotted arrow on the left, a tether is formed and, under the two solid arrows to the right, the cantilever was sequentially freed from the cell surface. The ordinate and the abscissa, respectively, represents force in nN and extension (nm) as well as approximate time of extension (s).

on a gold coated mica surface gave an estimated force of approximately 210 pN (pulling speed 400 nm/s. To be published elsewhere.). The question to be addressed below is whether tether break and/or WGA unbinding from glycoprotein A is responsible for the observed jumps of the cantilever with release force values of 70 - 80 pN.

III. ANALYSIS

To have an approximate contribution of lectin unbinding reaction to the 75 pN step in the force curve as described above, we will consider the unbinding reaction of lectin (A) and glycoprotein (B) as assisted by an externally applied tensile force. The reaction is formulated as follows where p_u and k_u represent, respectively, the fraction of unbound lectin ($0 \leq p_u \leq 1$), and the rate constant of unbinding under the condition where binding reaction is negligible.

$$\frac{dp_u}{dt} = k_u(1 - p_u)$$

Under the applied tensile force of F , the fraction of bound lectin that reaches the transition state will be assisted for the forward reaction by F as, $k_u = k_u^0 \exp(F\Delta x/k_B T)$, where k_u^0 , k_B and T are respectively, the unbinding rate constant in the absence of F , the Boltzmann constant, and temperature. Δx is the difference between the equilibrium length of the bond to be broken and its value in the activated state for bond breakage [10, 11]. In the case of lectin binding to glycoprotein A, the values of k_u^0 have been determined to be $2.42 - 6.12 \times 10^{-4} \text{ s}^{-1}$ for WGA (personal communication from Dr H. Krotkiewski), corresponding to the half-life, $t_{1/2}$, of 2000 ± 860 s.

As stated above, application of a tensile force to the bond assists its dissociation by a factor of

$\exp(F\Delta x/k_B T)$. The value of Δx for the unbinding of ligand-protein complexes is in the range of 0.2 - 0.3 nm [5]. Since, in the case of AFM experiments where WGA was used as the ligand for glycoprotein A, the unbinding of the two took place with a force of $F = 210$ pN where this factor is $10^4 - 10^6$, the half-life of the lectin-glycoprotein A bond is significantly reduced to the millisecond range. When $F = 70 - 80$ pN, it is in the range of 50 - 130 and the half-life of the ligand-receptor bond is reduced to 3 - 80 s. When the force curve measurement was performed with the pulling speed of $1 \mu\text{m/s}$, many of the rupture events were observed within 1 second from the start of retraction phase of the force curve. Therefore, if the half-life of the WGA-glycoprotein bond under the applied force of 70 - 80 pN is 3 s as the shortest case in the above estimate, a significant fraction (up to approximately 30%) of the rupture events must be attributed to the unbinding of ligand-receptor pairs in addition to the rupture event of lipid tethers.

The rupture events with 70 - 80 pN break force were also observed when membrane proteins were pulled out from the cell surface after forming covalent bonds with the probe. In this case, with an estimated value of $\Delta x = 0.1$ nm, the reduction of half-life of the covalent bond is only about 1/10, and the probability of covalent bond break within a few seconds of tether extension is infinitely small. In this case, therefore, the rupture force of $\sim 70 - 80$ pN represents the cut-off event of the lipid tether including the case of completely or incompletely denuding glycoprotein A of its boundary lipids.

To make a reliable estimate of the half-life of a bond in question, accurate knowledge of the dissociation rate constant and the value of Δx is required, especially the error in the latter within about 20% may lead to the error in the estimated half-life in about 100%, especially when Δx is relatively large, due to the exponential dependence of $t_{1/2}$ on $F\Delta x$.

IV. CONCLUSION

When a plateau of about 70 - 80 pN in the force curve obtained in the pulling process of glycoprotein A from the RBC surface is terminated, unbinding of the specific lectin, WGA, from glycoprotein A takes place with a finite probability (up to 30% of the total rupture events), although the unbinding force, when separately determined, was almost three times larger.

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- [1] M. Grandbois, W. Dettmann, M. Benoit, H. E. Gaub, J. Histochem. Cytochem. **48**, 719 (2000).
 - [2] A. Ikai, R. Afrin, H. Sekiguchi, T. Okajima, M. T. Alam, S. Nishida, Curr. Protein Pept. Sci. **4**, 181 (2003).
 - [3] H. Kim, H. Arakawa, T. Osada, A. Ikai, Ultramicroscopy **97**, 359 (2003).
 - [4] R. M. Hochmuth and W. D. Marcus, Biophys. J. **82**, 2964 (2002).
 - [5] E. Evans, V. Heinrich, A. Leung, K. Kinoshita, Biophys. J. **88**, 2288 (2005).
 - [6] J. Y. Shao and R. M. Hochmuth, Biophys. J. **71**, 2892 (1996).
 - [7] B. Krotkiewska, M. Pasek and H. Krotkiewski, Acta Biochim. Pol. **49**, 481(2002).
 - [8] R. Afrin, ISSS-4 Abstract (Omiya, Japan, 2005).
 - [9] Z. Li, B. Anvari, M. Takashima, P. Brecht, J. H. Torres, W. E. Brownell, Biophys J. **82**, 1386 (2002).
 - [10] G. I. Bell GI, Science, **200**, 618 (1978).
 - [11] R. Merkel, P. Nassoy, A. Leung, K. Ritchie, E. Evans, Nature **397**, 50 (1999).