Measurement of interaction force between RGD-peptide and Hela cell surface by optical tweezers

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Since RGD peptides (R: arginine; G: glycine; D: aspartic acid) are found to promote cell adhesion, they are modified at numerous materials surface for medical applications such as drug delivery and regenerative medicine. Peptide-cell surface interactions play a key role in the above applications. In this letter, we study the adhesion force between the RGD-coated bead and Hela cell surface by optical tweezes. The adhesion is dominated by the binding of $\alpha_5\beta_1$ and RGD-peptide with higher adhesion probability and stronger adhesion strength compared with the adhesion of bare bead and cell surface. The binding force for a single $\alpha_5\beta_1$-GRGDSP pair is determined to be 16.8 pN at a loading rate of 1.5 nN/s. The unstressed off-rate is 1.65×10^{-2} s^{-1} and the distance of transition state for the rigid binding model is 3.0 nm.

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Protein-surface interaction is critical for drug delivery systems. Proteins can be used as adapters, conjugated to liposomes for targeting drug delivery[1]. The interaction between the protein molecules with the drug carriers and the cell surfaces appearing on their way to the target must be studied to assure successful delivery. Protein-surface interactions also play a significant role in biosensors as a diagnostic tool. Proteins such as ligand, are immobilized on the probe surface, and can be used to analyze the corresponding integrin. In general, ligand binding occurs through recognition by the integrin of a short amino acid sequence from the ligand. One of the integrin-binding sites is the RGD (R: arginine; G: glycine; D: aspartic acid) sequence present in fibronectin[2]. Since RGD peptides have been found to mimic the function of adhesion proteins and promote cell adhesion, numerous materials have been RGD functionalized for academic studies or medical applications.

Previous study[3] has reported the strength of $\alpha_5\beta_1$-GRGDSP bond by atomic force microscopy (AFM). However, data, such as the unstressed off-rate, remain scarce. Optical tweezers[4−6], have become a powerful technique to manipulate the microspheres and study the protein-surface interaction mechanics. Compared with AFM with high-force[7], optical tweezers with small stiffness can measure protein interaction when the force is lower than the AFM force precision. This letter aims to measure the total adhesion forces between the Hela cell surface and the bead coated with RGD peptide (GRGDNP) by optical tweezers. We also extract the single-bond properties of $\alpha_5\beta_1$ integrin and GRGDNP from the multiple-bond adhesion force.

In dynamic force spectroscopy, the binding forces must be applied to the bonds in a controlled way. For this purpose, we use optical tweezers. Optical tweezers comprise an optical system that uses laser light to trap and manipulate dielectric particles, such as small beads and cells. External forces applied to the trapped bead can be measured accurately because the displacement of the trapped bead is directly proportional to the lateral force applied to the bead.

Our optical tweezers are based on a modified inverted microscope using a fiber laser (Amonics, Hong Kong) with a wavelength of 1 064 nm and a highest output power of 5 W as trapping source (Fig. 1). The expanded laser beam was focused by lens L1 (focus was 250 mm) to the conjugate point of the microscope objective after the beam was reflected by mirrors M1 and M2. The laser beam was then directed into the microscope, reflected upward by M4, and refocused into a sample chamber after passing through tube lens L2 (focus was 180 mm) and a high NA objective (NA 1.35, Olympus, Japan).

The sample cell consisted of a stainless steel corpus with a volume of 100 μL enclosed by two cover slips one at the botton and one at the top. The sample cell was mounted onto an optical stage that could be moved in three dimensions with nanometer resolution by using piezo-actuators (P-5173CD, PI, Germany). Samples were imaged by bright-field microscopy using a CCD camera. Force detection was carried out by video imaging. A procedure was developed to convert the microscope picture into an intensity profile, allowing

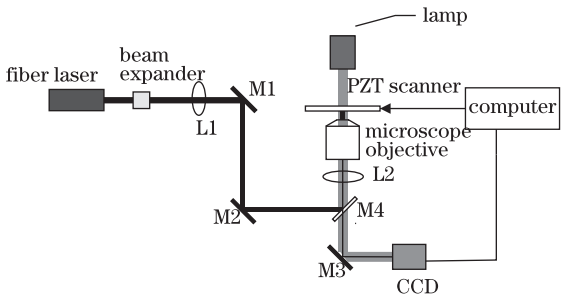

Fig. 1. Schematic of the experimental setup.

determination of both the positions of the beads and their separation from each other. The optical stiffness k was calibrated with viscous drag method.

In the experiments, we used the Hela cell, which mainly expresses the $\alpha_5\beta_1$ integrin as the GRGDNP receptors[8]. The Hela cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% bovine serum albumin. Twelve hours before manipulation, the cells were detached in a petri dish with a trypsin-EDTA solution and plated on a glass coverslip coated with 5-μg/mL fibronectin (Roche). The optical manipulation experiment of each sample was completed in 25 min.

Carboxylated polystyrene (PS) beads (2.0-μm diameter, Tianjin BaseLine, China) were coated with a polypeptide containing the RGD sequence (PepTide 2000, Telios Pharmaceuticals, USA) according to two-step protocol. Briefly, 200-μL 5%(w/v) beads were washed in deionized water and MES buffer. After the second wash, the pellet was resuspended in 1 mL of activation buffer containing 12.5-mg NHS, 7.8-μmol EDC, and 20-μmol MES. The beads were incubated at room temperature for 2 h after being well suspended. They were then washed in deionized water to remove the unreacted NHS and EDC. Finally, the beads were resuspended in 1-mL quench buffer (10-mmol/L Tris, 50-mmol/L KCl, 20-mmol/L EDTA) to block the uncoupled sites of the beads. This activity ensured specific binding to the integrin receptors. The beads were incubated in the sample cells at 37 °C for 5 min before manipulation.

In order to measure the binding strength between the bead and cell surface, the following experimental procedure was carried out. Firstly, the cells were fixed at the coverslip. Subsequently a trapped bead was brought in contact with the cell. The diagram of the experimental procedure is depicted in Fig. 2(a). The separation and contact between the bead and the cell then were repeated by controlling the waveform of the piezostage. The position of the trapped bead was oscillated in a trapezoid waveform at 0.5 Hz with peak-to-peak amplitude of 5 μm. The distance between the trapped bead and the cover-slip was fixed, and the hydrodynamic wall effect was corrected by Faxen's Law[9].

A typical force-distance curve used to determine the rupture forces is presented in Fig. 2(b). An immobilized cell was moved toward a trapped bead by the computer-controlled piezo-actuated stage. After contact (1), the cell was retracted at a selected speed to apply a loading rate of 1.5 nN/s. If adhesion between bead and cell occurred, the bead was pulled away from the trapping center (2), thereby increasing the load on the adhesion

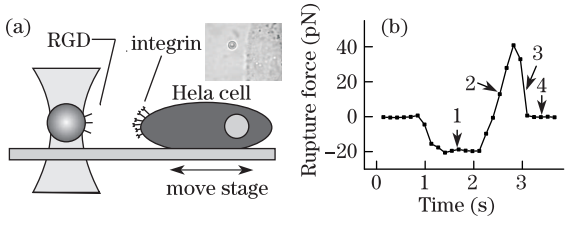

Fig. 2. (a) Schematic drawing and microscopic images (inset) for measuring the rupture force between RGD-coated bead and Hela cell by optical tweezers. (b) Typical force measurement.

site until the bond rupture (3). The bead immediately returned to its equilibrium position in the trap (4). Calibration of the optical stiffness was performed before the experiment. The adhesion forces were evaluated from the maximum bead displacement in the trap before bond rupture and the optical stiffness. The adhesion probabilities were also recorded. For the bead-cell surface adhesion, the contact area is important for the measurement force. In the experiment, we moved the bead perpendicularly to the cell surface, as shown in the inset of Fig. 2(a) to weaken the influence of the contact area.

The force histogram of nonspecific and specific interactions between the PS beads and cells at loading rate 1.5 nN/s is shown in Fig. 3. The specific and nonspecific forces were the rupture forces between the cells and the RGD-coated beads, and the carboxylated PS beads, respectively. The nonspecific bead-to-surface interaction force was weaker than the specific receptor-ligand interaction. The nonspecific protein-protein interactions may have physiological significance in cell adhesion in cooperation with the specific interactions.

The specific protein-protein adhesion strength may reach the value of several tens of piconewtons, as shown in Fig. 3. The origin of interaction can mainly be attributed to the following interactions: the electrostatic interactions, van der Waals forces, steric forces, hydrophobic interaction, and hydrogen bonding[10]. The specific interaction involves numerous short range contacts within the binding pocket; thus there should be higher energy barriers in phase space. In contrast, nonspecific interactions are likely to be more long range in nature and are not likely to involve a binding pocket, and thus have weaker binding strength.

The binding of the integrin and the ligand is ensured by noncovalent bonds that exhibit the various mechanical and dynamic properties[11], and quantitative characterization of the forces at the level of the ligand-receptor bonds is helpful for understanding their functions and properties. A theoretical model for understanding how force can affect the adhesion complex was proposed by Bell[12], and was later expanded on by other researchers[13]. The single-bond properties can be obtained from the multiple-bond data. In the method in Ref. [14], the adhesion force was assumed to be independent of the number of linked bonds and shared equally between them. The probability density for the unbinding force f of initial number of linked bonds N_t can be written as

$$D_{N_t}(f) = k_0 m^{-1} \exp\left(\frac{f\Delta x}{k_{\mathrm{B}}T}\right) \cdot \exp\left\{k_0 m^{-1} \frac{k_{\mathrm{B}}T}{\Delta x}\left[1 - \exp\left(\frac{f\Delta x}{k_{\mathrm{B}}T}\right)\right]\right\}, \quad (1)$$

where m is force loading rate, k_{B} is Boltzmann constant, T is absolute temperature, Δx is the distance between bound state and transition state along the reaction coordinate, and k_0 is the intrinsic dissociation rate constant ($k_0 = 1/t_{\mathrm{off}}$, where t_{off} is the natural lifetime of the bond). Experimental histograms are linear combinations of the distributions $D_{N_t}(f)$ for initial number of linked bonds N_t, and the coefficients of the distribution is determined by $p_\alpha(N_t) = \alpha_{N_t}/(\alpha_1 + \alpha_2 + \cdots + \alpha_{N_t}^m)$, where

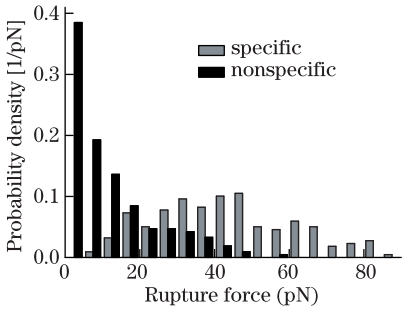

Fig. 3. Distribution of detachment forces for specific (grey) and nonspecific (black) interaction after a contact time of 1 s at the loading rate 1.5 nN/s, respectively.

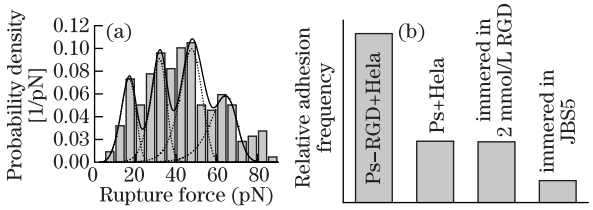

Fig. 4. (a) Rupture force histograms for loading rates 1.5 nN/s which include 220 attachment events. The shaded bars show experimental results which are collected in bins of approximately 5 pN width. The histograms are normalized in respect to the total number of events and the bin width, so that they show the probability density of events. The solid lines are the simulation results fitted simultaneously to the complete data set. (b) Specificity test of the $\alpha_5\beta_1$ integrin and GRGDNP interaction.

N_t^m is the maximal number of initial bonds considered. The distribution of the rupture force is

$$D(f) = \sum_{N_t=1}^{N_t^m} p_\alpha(N_t) D_{N_t}(f). \tag{2}$$

For given values of k_0 and Δx, distributions $D_{N_t}(f)$ of rupture force f are calculated for $N_t = 1, \cdots, N_t^m$ at loading rate m. The distributions $D(f)$ are superimposed via $p_\alpha(N_t)$ and the relative weights are determined by fit of $D(f)$ to the complete set of experimental data. The fitting for the values of k_0 and Δx stops when the deviations between simulations and experimental histograms are minimized. The binding force for a single bond at loading rate m can be calculated from Eq. (1).

Figure 4 shows the experimentally obtained rupture force histograms for the RGD-cell surface, including 220 rupture events for the loading rates $m = 1.5$ nN/s. The histograms are compared to simulated rupture force distributions. Fitting simulations to measurements yielded the unstressed off-rate $k_0 = 1.65 \times 10^{-2}$ s^{-1} and the $\Delta x = 3.0$ nm, corresponding to intrinsic force $F_0 = k_{\rm B}T/\Delta x = 1.38$ pN. Thus, our results, which are extracted from multiple-bond data, compare very favorably with earlier results from single-bond experiments, which give $k_0 = 1.5 \times 10^{-2}$ s^{-1} and confirm that the adhesion of the RGD-coated bead and the Hela cell is dominated by the $\alpha_5\beta_1$ integrin and GRGDNP.

The specificity of this interaction was verified by three different tests: (i) experiments on cells after the addition of monoclonal antibody against $\alpha_5\beta_1$, JBS5 (CHEMICON, 1/100 dilution), (ii) experiments with beads that were not coated with GRGDNP, and (iii) experiments in a buffer containing 2 mmol/L of the GRGDNP which saturated all binding sites. The adhesion frequencies were significantly reduced in all of these tests, as shown in Fig. 4(b). These experiments demonstrated that the force measurements between the Hela cells and GRGDNP can be mostly attributed to the interaction between the integrin $\alpha_5\beta_1$ and GRGDNP.

In conclusion, by using optical tweezers, we demonstrate a method to measure the binding force between the micro-beads and cell surface. We study the adhesion force between the bead and Hela cell surface. When the bead is coated with GRGDNP, the cell adhesion is dominated by the $\alpha_5\beta_1$ and the GRGDNP with higher adhesion probability and stronger adhesion strength. The unbinding force for a single $\alpha_5\beta_1$-GRGDSP pair is determined to be 16.8 pN at a loading rate of 1.5 nN/s. The unstressed off-rate and the distance of transition state is extracted from multiple bond model as 1.65×10^{-2} s^{-1} and 3.0 nm, respectively. This simple surface modification strategy and force measurement method can be potentially applied to study the nanoscale liposome for drug delivery systems.

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References

1. M. Backer, T. Gaynutdinov, V. Patel, B. Jehning, E. Myshkin, and J. Backer, Bioconjugate Chem. **15,** 1021 (2004).
2. M. D. Pierschbacher and E. Ruoslahti, Nature **309,** 30 (1984).
3. P. Lehenkari and M. Horton, Biochem. Biophys. Res. Commum. **259,** 645 (1999).
4. A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, and S. Chu, Opt. Lett. **11,** 288 (1986).
5. J. Bu, G. Yuan, Y. Sun, S. Zhu, and X. Yuan, Chin. Opt. Lett. **9,** 061202 (2011).
6. X. Yuan, Y. Zhang, R. Cao, X. Zhao, J. Bu, and S. Zhu, Chin. Opt. Lett. **9,** 031201 (2011).
7. K. C. Neuman and A. Nagy, Nat. Methods **5,** 491 (2008).
8. V. Serru, F. Le Naour, M. Billard, D. Azorsa, F. Lanza, C. Boucheix, and E. Rubinstein, Biochem. J. **340,** 103 (1999).
9. J. Happel and H. Brenner, *Low Reynolds Number Hydrodynamics* (Martinus Nijhoff, Hague, 1983).
10. D. Leckband, Annu. Rev. Biophys. Biomol. Struct. **29,** 1 (2000).
11. E. A. Evans and D. A. Calderwood, Science **316,** 1148 (2007).
12. G. Bell, Science **200,** 618 (1978).
13. E. Evans and K. Ritchie, Biophys. J. **72,** 1541 (1997).
14. T. Erdmann, S. Pierrat, P. Nassoy, and U. S. Schwarz, Europhys. Lett. **81,** 48001 (2008).