Electromagnetophoretic Force Measurement of a Single Binding Interaction between Lectin and Yeast Cell Surfaces

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A novel measurement method of the binding force between a micrometer-sized particle and a solid surface in an electrolyte solution has been established by using the electromagnetophoretic buoyancy on the particle. By this method, we investigated the binding force between a yeast cell surface and an oligosaccharide-binding protein, concanavalin A (Con A), fixed on a silica capillary wall. The force measurement was carried out up to 60 pN. In a lower surface concentration of Con A, yeast cells could be desorbed by a force less than 60 pN. However, in a higher surface concentration after treated by 1 mg ml⁻¹ solution, yeast cells were adsorbed with a force stronger than 60 pN. In this case, the addition of 10 mg ml⁻¹ D-mannose solution to the medium reduced the binding force to less than 60 pN. The observed adsorption force of yeast cells ranged within 30–40 pN, regardless of the interfacial amount of Con A. This force was thought to be the single binding force between a mannose group of the cell surface and an active site of Con A. Moreover, the dissociation rate constant of the single binding of yeast cell and Con A complex was determined as $4.6 \times 10^{-3} \text{ s}^{-1}$ and the increment of the binding distance at the transition state as 0.33 nm from the desorption kinetic experiments of yeast cell under the constant pulling conditions of 10, 20 and 30 pN. Such satisfactory results demonstrate the novel advantages of the present method.

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Introduction

Cellular events in biological systems, such as cell aggregation, recognition of reporter molecules and cell migration, are mediated by the specific interaction of cell surface tethered molecules which can form weak non-covalent bonds.^{1,2} The study of these molecular interactions is essential to understand the chemical functions and molecular recognition ability of cell surfaces. The measurement of the binding force between a cell surface and a protein is one useful technique to investigate those interactions.

A number of methods, such as hydrodynamic-flow-based method,³ laser tweezers⁴ and atomic force microscopy (AFM),⁵ have been invented to investigate the interaction force between two different molecules, between a cell surface and a molecule, or between two different cells.

Recently, we proposed a new technique for measurement of the particle-wall interaction force using the electromagnetophoretic (EMP) force on the particles, which was controlled by the current under a homogeneous high magnetic field of 10 T. Innovative advantages of this force measurement method are (1) the high sensitivity of pN, which can detect the physical and chemical interaction forces of micro-particles adsorbed on a substrate surface, and (2) the feasibility in a closed liquid system without any direct contact to the particles as in the case of AFM, which uses a cantilever to be attached by the particle.^{6,7}

In the present study, we focused on the oligosaccharide-protein interaction, which plays a key role in cellular recognition.^{8,9}

Concanavalin A (Con A) is a well characterized lectin, which has oligosaccharide-binding proteins.¹⁰ Con A has the binding specificity to mannose.^{11,12} For the particle which has an oligosaccharide on the surface, we chose yeast cell. It is known that a yeast cell surface is covered by a mannan polysaccharide layer.¹³ Therefore, Con A can bind to the surface of yeast cells by forming a mannose-Con A complex. In the present study, the interaction force between yeast cell surface and Con A functionalized silica surfaces was investigated using the electromagnetophoretic force measurement technique. Measurement of the desorption force of yeast cells from Con A functionalized silica surface can afford the insight in the binding interaction between a mannan polysaccharide on the cell surface and Con A. Furthermore, the new method was applied to the study of the dissociation kinetics of the mannan polysaccharide-Con A complex.

Experimental

Materials

Con A-tetramethylrhodamine conjugate was purchased from Molecular Probes (Carlsbad, CA). We used common baker's yeast clumps whose shape was spherical and whose diameter was 7 μm . Yeast cells were dispersed in 1.0 M KCl aqueous solution (conductivity: 0.112 S cm⁻¹). D-Mannose was purchased from Nacalai Tesque (Kyoto, Japan). Water used in all experiments was purified by Milli-Q system (Millipore, USA).

Preparation of Con A loaded capillary cell

Fixation of Con A molecules on a fused-silica capillary inner

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Scheme 1 A schematic presentation of a bound Con A on a fused-silica capillary inner surface. The Con A molecule was fixed chemically by the bonding of aminosilane and glutaraldehyde.

wall was performed by the procedure shown in Scheme 1. A square fused-silica capillary (Polymicro Technology, Phoenix, AZ), which had a $100 \times 100 \mu\text{m}$ inner section and a $300 \times 300 \mu\text{m}$ outer section, was cleaned by 0.1 M NaOH and Milli-Q water. The cleaned capillary was then rinsed with a 2% acetone solution of *N*-(2-aminoethyl)-3-aminopropyl triethoxysilane for 30 min, following a 10-min air purge and incubation at 45°C overnight.¹⁴ The aminosilanized capillary was incubated at 37°C for 2 h after being filled with a 5% solution of glutaraldehyde (pH 7.4). The capillary was then functionalized by $0.01 - 1 \text{ mg ml}^{-1}$ Con A solution (pH 5.4). Below pH 7, Con A was fixed as a dimer.¹⁰ The fluorescence images of the capillary were observed with a confocal laser scanning microscope system (532 nm diode pumped laser, Compass 315M-100, COHERENT, Santa Clara, CA; confocal scan unit, CSU22, YOKOGAWA, Tokyo, Japan; inverted microscope, Eclipse SE2000-U (objective $20\times$, N.A 0.45), Nikon, Kawasaki, Japan), and the surface Con A concentration was estimated from the fluorescence intensity using Image J.¹⁵

Electromagnetophoretic force measurement apparatus

The magnetic field was generated by a helium-free superconducting magnet (JMTD-10T100HH1, JMT, Japan), which had a room temperature bore of 100 mm diameter, it gave magnetic fields up to 10 T. The capillary cell was set on the cell holder in the homogeneous magnetic field in the bore. Both edges of the fused-silica capillary cell were connected with PTFE tubes equipped with Ag|AgCl electrodes which were made by electrolysis of Ag wire in KCl solution. The sample solution was introduced into the tube by a syringe pump that was controlled out of the bore. A function synthesizer (WF 1915, NF, Tokyo, Japan) and an amplifier (Model 610-C, Trek, Tokyo, Japan) were used to provide the current, designed by a waveform making software (105, NF, Tokyo, Japan), to the sample solution in the capillary cell. The applied current was measured by a digital multimeter (VOAC 7512, IWATSU, Tokyo, Japan). The adsorption-desorption behavior of particles was observed by an optical microscope attached with a CCD camera (ME421, ELMO, Japan) and the CCD image was displayed on a monitor.

Measurement of the yeast cell-Con A binding force

The new force measurement technique had been demonstrated

to measure the adsorption force of a polystyrene particle on a silica capillary cell-wall by desorbing the particle from the surface using the electromagnetophoretic force.^{5,6}

The procedure of the force measurement is shown in Scheme 2. For the electric current, i , and the homogeneous magnetic flux density, B , in the conductive fluid including micro-particles, the electromagnetophoretic force which works on each spherical particle, F_{EMP} , is given by:¹⁶

$$F_{\text{EMP}} = 2 \left(\frac{\sigma_p - \sigma_f}{2\sigma_f + \sigma_p} \right) \frac{iBV}{S} \quad (1)$$

where V is the volume of spherical particle, S is the inner section of the capillary cell, σ_f is the electric conductivity of solution and σ_p is the apparent conductivity of the particle. The electromagnetophoretic force F_{EMP} is the difference of the Lorentz forces on the particle and those in the medium; therefore, it is actually the electromagnetic buoyancy working on the particle, which direction is reversed to the Lorentz force on the medium. The apparent conductivity of particles was the surface conductivity of particles produced by the electric double layer on the particle surface, and was obtained from the measurement of electromagnetophoretic migration velocity.^{17,18} The apparent conductivity of yeast cells in 1 M KCl solution was determined as 0.075 S cm^{-1} .

Yeast cells in solution injected by a syringe are all transferred to the Con A functionalized capillary wall surface by the electromagnetophoretic force. Then, the detachment behavior of yeast cells from the wall surface by the electromagnetophoretic force was observed. In the force measurement, the yeast cells were gradually pulled from the wall by increasing the applied current. The particle is desorbed when the electromagnetophoretic force becomes larger than the adsorption force. Therefore, according to Eq. (1), the interaction force can be calculated from the desorption current, i_D , which is the current at the time when the yeast cell is just desorbed. In the present experiment, the applied current was increased in the range from 0 to $1200 \mu\text{A}$ during the time of 15 s (with the rate of $80 \mu\text{A s}^{-1}$) under the fixed magnetic field of 10 T. In this condition, the range of the electromagnetophoretic force pulling up a yeast cell from the wall was 0 - 60 pN and its loading rate was 4 pN s^{-1} . Moreover, the dissociation rate of polysaccharide-Con A complex on yeast cell surface was measured by this method. A constant electromagnetic force was applied to the adsorbed yeast cells during the measurement, and the number of detached particles was counted as a function of time.

All measurements were carried out in a thermostated room at $25 \pm 1^\circ\text{C}$.

Results and Discussion

The binding force between yeast cell and Con A

In our experiments, 1 M KCl solution was used as a buffer. For higher ionic strengths, the electrophoretic motion of the yeast cell and the electroosmotic flow of the buffer could be neglected in the present conditions. Therefore, the adsorption force was governed simply by the electromagnetophoretic force.

Fluorescence images of Con A fixed capillary were obtained with a confocal microscope. Figure 1 shows fluorescence intensity of the capillary cell wall measured from these images after introducing the Con A solution in the range from 0.01 to 1 mg ml^{-1} . To estimate the surface concentration of fixed Con A, we measured the fluorescence intensities of Con A solution with various concentrations under similar conditions. A calibration

Scheme 2 Schematic illustration of the adsorption-desorption of a particle by electromagnetophoretic force. When a current was applied, the particle adsorbed on the capillary wall was desorbed. Desorption-adsorption cycle was repeated by switching the direction of the current.

Fig. 1 The fluorescence intensity of Con A functionalized capillary wall after being treated by Con A solution of 0.01, 0.1, 0.5 and 1 mg ml⁻¹. The surface concentration of fixed Con A was estimated from a calibration curve (date not shown). A, B and C in this figure correspond to the capillaries treated by 0.1, 0.5 and 1 mg ml⁻¹ Con A solutions.

curve for the fluorescence intensity vs. the amount of Con A was determined by taking into account of the focus depth of our confocal system (2.3 μm)¹⁹ (data not shown). Con A functionalized capillaries treated by the solutions of 0.1, 0.5 and 1 mg ml⁻¹ were named as capillary A, capillary B and capillary C, respectively. From the calibration curve, the surface concentrations of fixed Con A on capillaries A, B and C were estimated to be about 200, 600 and 2800 molecule μm⁻², respectively. These concentration values were corresponding to the ratio of 1:3:14 for capillaries A, B and C.

Yeast cells adsorbed on Con A functionalized capillary wall were desorbed by the electromagnetophoretic buoyancy and the interaction force was measured from the observed desorption current. Figure 2 shows the histograms for the interaction force of yeast cells to Con A functionalized surface. The average value of interaction force of yeast cells to capillary A was obtained as 37 ± 11 pN from Fig. 2(a). On the other hand, the average value of yeast cells to capillary B obtained from Fig. 2(b) was 34 ± 9 pN. Because the mannan polysaccharide on yeast cell surface is thought to bind to the fixed Con A, the desorption force of yeast cells refers to the interaction force between the yeast cells surface and Con A. Apart from the mannose-Con A bond, all other bonds between the capillary inner surface and the yeast cell surface are covalent bonds (Si-O, Si-C, C-C and C-N), whose bonding energies are higher than 300 kJ mol⁻¹.²⁰ On the other hand, the bonding between

Fig. 2 The histograms of desorption events of yeast cells adsorbed on Con A functionalized surface treated by Con A solution of 0.1 mg ml⁻¹, capillary A (a) and 0.5 mg ml⁻¹, capillary B (b) for every increment of the force of 1 pN in a 1.0 M KCl solution.

trimannose, which consists of three mannoses, and Con A was reported to be 31.8 kJ mol⁻¹; this value is much lower than that of covalent bonds.²¹ Therefore, the mannose-Con A bond was thought to be torn apart with the desorption of the adsorbed yeast cells by the pulling force of electromagnetophoretic buoyancy. If a yeast cell has multivalent bonding with Con A, the desorption force will be increased. However, regardless of the different surface concentrations of Con A in capillaries A and B, the average values of the observed interaction force were similar in magnitude, as shown in Fig. 2. Under these concentrations of Con A, we considered that a single binding interaction between yeast cell surface and Con A molecule was being measured.

In the highest surface concentration of Con A, *i.e.* capillary C, very small numbers of yeast cells could be desorbed by the electromagnetophoretic pulling force up to 60 pN. In this case, the adsorption force was larger than 60 pN, because each yeast cell must bind with several Con A molecules. This result supported the conclusion that the adsorption force of yeast cells was governed by mannose-Con A interaction, not by the

Fig. 3 The histogram of desorption events of yeast cells adsorbed on Con A modified surface treated by Con A solution of 1.0 mg ml^{-1} for every increment of the force of 1 pN in a 1.0 M KCl solution containing 0 mg ml^{-1} (a), and 10 mg ml^{-1} D-mannose (b).

physical adsorption interaction. On the other hand, the presence of D-mannose in the solution of yeast cells could remarkably reduce the desorption force because of the decrease of the number of active Con A molecules which could bind to yeast cells. Figure 3 shows the histogram of the observed desorption force of yeast cells in capillary C in the presence of 10 mg ml^{-1} D-mannose. The average value of the interaction force was $40 \pm 10 \text{ pN}$. This value is comparable to the values for capillaries A and B, as shown in Fig. 2. Ratto *et al.* reported using the AFM force measurements that the rupture force of single molecular interaction between mannose and a dimeric-form Con A was $47 \pm 9 \text{ pN}$.²² This value was in good agreement with the values of the adsorption force measured in our experiments. These results confirmed that the adsorption force observed in the present experiments corresponded to the interaction force between a single Con A molecule and a single mannan polysaccharide on the yeast cell surface.

Bonding rupture kinetics

If the desorption force of yeast cells is governed by the rupture probability of the bonding of mannan polysaccharide-Con A complex, Bell's model can be applied to analyze the rupture kinetics of the interaction between mannose and Con A.²³ According to Bell's model, the dissociation rate constant of the yeast cell-Con A binding, k_{off} , exhibits an exponential dependence on the applied pulling force, F :

$$k_{\text{off}}(F) = k_{\text{off}}(0)\exp(Fx/k_{\text{B}}TN_{\text{b}}) \quad (2)$$

where k_{B} is Boltzmann's constant, T is the absolute temperature, x is the critical increment of one binding distance between the mannan polysaccharide and Con A complex for the change from the equilibrium state to the transition state, and N_{b} is the number of bonds between a yeast cell and Con A modified surface. To obtain the dissociation rate of mannan polysaccharide-Con A complexes on the yeast cell surface, the detached number of yeast cells from Con A modified surface was counted under a constant applied electromagnetophoretic force. Figure 4 shows the time dependence of the rate of binding cells. The dissociation rate constant while applying the

Fig. 4 Decrease of the percentage of yeast cells adsorbing on the Con A modified silica surface with time at different pulling forces. The curves show the fitted lines of primary region by Eq. (3).

force F was also given by the relationship between binding number and time as:²⁴

$$N(t) = N(0)\exp(-tk_{\text{off}}(F)) \quad (3)$$

where $N(0)$ and $N(t)$ are the binding numbers of yeast cells at the times 0 s and $t \text{ s}$ under the applied force of $F \text{ (pN)}$, respectively. As shown in Fig. 4, the percentage of remaining yeast cells, $100N(t)/N(0)$, was decreased as the pulling force was increased. Moreover, it was noted in Fig. 4 that the decreasing rate of $100N(t)/N(0)$ against time, t , was faster in the region from $t = 0$ to about 30 s than that after about 30 s . Because the dimeric Con A has two binding sites for the mannan polysaccharide, the binding interaction between dimeric Con A molecules and yeast cells is actually the mixture of the single binding and the double bindings. The single binding interaction is weaker than the double one. Therefore, the decreasing rate of $100N(t)/N(0)$ in the initial stage should reflect mainly the dissociation of the single binding interaction between a site of Con A on a glass surface and a mannose group at a yeast cell surface. The secondary stage of the decreasing after about 30 s suggests the breaking of the interaction between the dimer of Con A and two mannose groups. By fitting Eq. (3) to the decreasing curves in the initial stage, we obtained the dissociation rate constant for the monomeric interaction between Con A and polysaccharide under each applied force.

Figure 5 shows the dependence of the observed dissociation rate constant on the applied force. Equation (2) can be converted into a logarithm form:

$$\ln k_{\text{off}}(F) = \ln k_{\text{off}}(0) + Fx/k_{\text{B}}TN_{\text{b}} \quad (4)$$

As shown in Fig. 5, $\ln k_{\text{off}}(F)$ was proportional to F . This tendency was in good agreement with Bell's ligand-receptor model. Therefore, we confirmed that a single molecular interaction between mannan polysaccharide on a yeast cell and Con A could be detected by the electromagnetophoretic force measurement technique. We calculated the parameters of interaction for mannan polysaccharide-Con A complex on the cell surface from the result shown in Fig. 5 using Eq. (4). The observed k_{off} value was $(4.6 \pm 2.0) \times 10^{-3} \text{ s}^{-1}$ and the x value was $0.33 \pm 0.11 \text{ nm}$ for the breaking of a single mannan polysaccharide-Con A complex on the surface of yeast cells.

Fig. 5 Linear relationship between the logarithmic values of the observed dissociation rate constant and the applied electromagnetic forces.

The observed dissociation rate constant in the present study was well within the range of 1.4×10^{-3} – 6.1×10^{-3} s⁻¹ reported as the dissociation rate constant for high-mannose type oligosaccharide (number of mannose: 5–9)-Con A complex.²⁵ Structural studies of trimannose-Con A complex have been reported as shown in Scheme 3.¹¹ Mannose interacts with Con A by way of hydrogen bonding and/or van der Waals force with the equilibrium distance of 0.26–0.35 nm. The excess elongated distance between mannose and Con A at the transition state was determined as 0.33 nm at 25°C in the present study. This stretch of the binding distance of 0.59–0.68 nm at the transition state seems to be enough to break the interaction forces between mannose and residue of Con A.

Conclusions

We have developed a non-contact pN force measurement method applicable for the desorption of a single micro-particle in a solution from a solid surface in a closed micro-cell by the use of electromagnetophoretic force. This method was applied to measure the binding force between the surfaces of yeast cell and Con A bound to the capillary wall. A single molecular interaction between a mannan polysaccharide on a yeast cell surface and the active position of Con A could be detected. Moreover, the dissociation rate and unbinding excess distance of the mannose-Con A bond could be obtained. These results indicate that the present method can observe directly the interaction of ligand-receptor complex on various kinds of cell surfaces. This method is quite promising as a noninvasive and highly sensitive force measurement method applicable to the study of a single chemical bonding on any biological cells in closed solution systems.

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Scheme 3 A schematic representation of the hydrogen bonds between trimannose and Con A binding site reported by Naismith and Field.¹¹ Bold dot line, dot line and dashed line show the hydrogen bonds between trimannose and protein whose lengths were 0.26–0.27, 0.28–0.29 nm and longer than 0.30 nm, respectively. The arrow expresses the direction that mannan polysaccharide is pulled when the force is applied.

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References

1. T. Strunz, K. Oroszlan, I. Schumakovitch, H. J. Güntheridit, and M. Hegner, *Biophys. J.*, **2000**, 79, 1206.
2. A. Chen and V. T. Moy, *Biophys. J.*, **2000**, 78, 2814.
3. A. Pirres, D. Touchard, A. M. Benoliel, and P. Bongrand, *Biophys. J.*, **2000**, 82, 3214.
4. E. Fallman, S. Schedin, J. Jass, M. Anderson, B. E. Uhlin, and O. Axner, *Biosens. Bioelectron.*, **2004**, 19, 1429.
5. A. Touhami, B. Hoffmann, A. Vasella, F. A. Denis, and Y. F. Dufrêne, *Microbiology*, **2003**, 149, 2873.
6. Y. Iiguni and H. Watarai, *Anal. Sci.*, **2003**, 19, 33.
7. Y. Iiguni and H. Watarai, *Bull. Chem. Soc. Jpn.*, **2006**, 79, 47.
8. D. N. Moothoo and J. H. Naismith, *Glycobiology*, **1998**, 8, 173.
9. K. H. Mortell, M. Gimgras, and L. L. Kiessling, *J. Am. Chem. Soc.*, **1994**, 116, 12053.
10. K. D. Hardman and C. F. Ainsworth, *Biochemistry*, **1972**, 11, 4910.
11. J. H. Naismith and R. A. Field, *J. Biol. Chem.*, **1996**, 271, 972.
12. Z. Derewenda, J. Yariv, J. R. Helliwell, A. J. Kalb, E. J. Dodson, M. Z. Papiz, T. Wan, and J. Campbell, *EMBO J.*, **1989**, 8, 2189.
13. C. E. Ballou and W. C. Raschke, *Science*, **1974**, 184, 127.
14. L. N. Amankwa and W. G. Kuhr, *Anal. Chem.*, **1992**, 64, 1610.
15. image J, <http://rsb.info.nih.gov/ij/>.
16. A. Kolin, *Science*, **1953**, 117, 134.
17. M. Namba, H. Watarai, and T. Takeuchi, *Anal. Sci.*, **2000**, 16, 5.
18. Y. Iiguni, M. Suwa, and H. Watarai, *J. Chromatogr., A*,

- 2004**, 1032, 165.
19. G. Jiang, S. Attiya, G. Ocvirk, W. E. Lee, and D. J. Harrison, *Biosens. Bioelectron.*, **2000**, 140, 861.
20. M. Panhorst, P. Kamp, G. Reiss, and H. Brückl, *Biosens. Bioelectron.*, **2005**, 20, 1685.
21. T. K. Dum, R. Roy, S. K. Das, S. Oscarson, and C. F. Brewer, *J. Biol. Chem.*, **2000**, 275, 14223.
22. T. V. Rotto, K. C. Langry, R. E. Rubb, R. L. Balhorn, M. J. Allen, and M. W. McElfresh, *Biophys. J.*, **2004**, 86, 2430.
23. G. I. Bell, *Science*, **1978**, 200, 618.
24. C. Danilowicz, D. Greenfield, and M. Prentiss, *Anal. Chem.*, **2005**, 77, 3023.
25. R. G. Gallego, S. R. Haseley, V. F. L. van Miegem, J. F. G. Vliegthart, and J. P. Kamerling, *Glycobiology*, **2004**, 14, 373.
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