Measurement of Binding Force between Microtubule-Associated Protein and Microtubule *

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Microtubule-associated proteins (MAPs) are important proteins in cells. They can regulate the organization, dynamics and function of microtubules. We measure the binding force between microtubule and a new plant MAP, i.e. AtMAP65-1, by dual-optical tweezers. The force is obtained to be $14.6 \pm 3.5\,pN$ *from the data statistics and analysis. This force measurement is helpful to understand the function and mechanism of MAPs from the mechanical point of view and lays the groundwork for future measurements of the mechanical properties of other biological macro-molecules.*

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Cytoskeleton systems exhibit the main structure to generate mechanical force in eukaryotic cells. Microtubule, which is composed of protein subunit, i.e. tubulin, is an important kind of cytoskeleton in eukaryotic cells. Microtubule-associated proteins (MAPs) coexist with tubulin during tubulin purification. MAPs including many kinds of proteins that can bind to microtubules and/or tubulin regulate the organization, dynamics and function of microtubules, even change the mechanical properties of microtubules.[1] Different MAPs bind to microtubules in different manners, and have different functions. AtMAP65-1 is a new MAP found from Arabidopsis. It can induce the formation of microtubule bundles by forming cross-bridges between microtubules evenly along the whole length of microtubules.[2]

Up to date, the ways to investigate MAPs are almost limited in biochemical, cell biological and molecular biological methods. These traditional methods can only study the physiological functions, cell location and expression regulation of the proteins. However, the mechanical property is of great importance for MAPs. Binding strength between the microtubule and MAP may influence the mechanical characteristics of microtubules. For instance, the flexural rigidity of microtubule will increase while MAP-2 or Tau exists.[1] There are few articles about the binding force between MAPs and microtubule so far, so the knowledge about how the binding strength influences the function of MAPs is almost blank. In the past years, optical tweezers were used to investigate the mechanical properties of cytoskeleton and its motor proteins,[3−6] but the research about the interaction forces between proteins was largely focused on the interaction between antigen and antibody.[7−9] In this study, we quantitatively measure the binding force between the AtMAP65-1 and microtubule by dual-optical tweezers for the first time.

Tubulin is extracted by temperature cycle from porcine brain and purified by polymerizing under 0.5 mol/L PIPES and 10% DMSO to throw off MAPs.[10] Then the purified tubulin is labelled by rhodamine and biotin, and microtubule is polymerized in vitro as described in Ref. [11]

AtMAP65-1 is prepared according to Mao *et al.*[12] We add it to the microtubule solution with a proportion of tubulin: AtMAP65-1= 7:1 in units of mol. AtMAP65-1 can bundle microtubules by forming cross-bridge between microtubules as shown in Fig. 1.

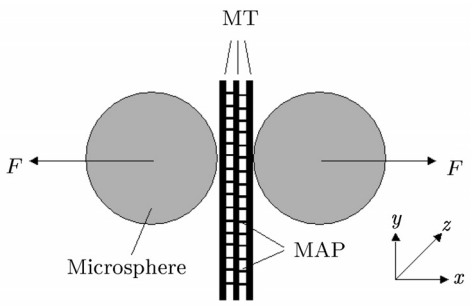

Fig. 1. Microtubule bundle manipulated by dual-optical tweezers.

In our experiment, the system of dual-beam optical tweezers consists of inverted microscope (Leica DMIRB, Germany, with 50 mW mercury lamp) and

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the Nd-YVO_4 laser (1064 nm, Coherent, USA) with the output power of 4 W. Two parallel laser beams enter the back aperture of the microscope objective (HCXAPO 100, 1.40 NA, oil immersion) and are focused at the specimen, forming dual-beam optical traps. The detailed parameter of the equipment has been described before.[13] One trap could move in x and y directions by rotating a mirror driven by a dc servomotor (Newport, LTA-HL Actuator) while the other is fixed. A quadrant photodiode detector (QD) is used to measure the displacement of the microsphere in the fixed trap and a high-resolution cooled CCD camera (CoolSNAP-fx, USA) is for acquiring the whole image of the trapped microspheres and the microtubule bundle. The method of force calibration and displacement measurement has been described in Refs. [14,15] In our experiment, the stiffness of both optical traps is 0.25 pN/nm when the power of each beam at the objective back aperture is 320 mW. The resolution of QD is 1 nm.

A microsphere is usually adopted as a handle of optical tweezers to manipulate biological materials. In our experiment, 1 μm NeutrAvidin-coated fluorescent polystyrene microspheres (Molecular Probe) are used to attach to biotin-tagged microtubule bundle due to specific interaction between neutravidin–biotin. The binding force between biotin and avidin is about 160 pN, and the interaction between biotin and neutravidin is even larger than that between biotin and avidin, which exceeds the maximum trapping force available. Thus the connections between microtubules and microspheres will not break up during the whole experiment. Rhodamine and biotin labelled microtubule bundle solution is diluted and then mixed with 100 mmol/L NaCl, 8 mmol/L ascorbic acid and some microspheres. The above solution is added to a sample cell and sealed afterward. As shown in Fig. 1, we make each of the laser beams hold one microsphere and then let them bind to the opposite sides of a microtubule bundle which is nearly parallel to the y axis. The process of adhesion takes about 15 s. Then we move the movable trap away from the fixed one along the x axis with a velocity of 74 nm/s until the connections between the two microspheres are thoroughly broken. QD and CCD record the whole course.

Figure 2 shows the time evolution of the trapping force exerted on the microsphere during the whole pulling process, which is calculated from the displacement signal detected by the QD.[14] From the plot we can see that there is no force at the beginning. From point a to point b, with the motion of the movable trap, the force becomes larger and nearly linear. At point b, it reaches the peak, 14.7 pN. Then the connections between MAP and microtubule break up in a moment, and the force turns back to zero rapidly. This suggests that the binding force between AtMAP65-1 and microtubule in this experiment is 14.7 pN.

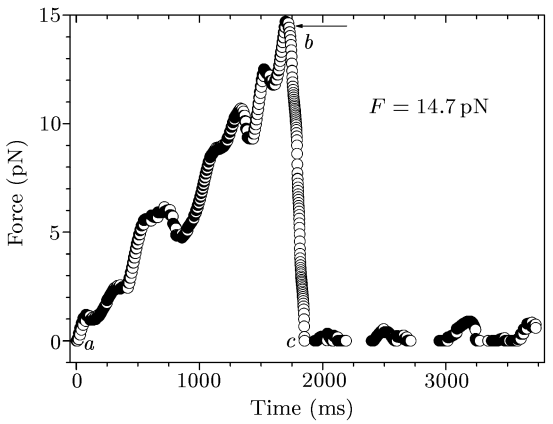

Fig. 2. Time evolution of trapping force detected by the QD.

From Fig. 2 we can see that the force goes back to 0 in the end. This means that the connections between the two beads are thoroughly broken. However, the length of the microtubule bundle used in our experiment is tens of micrometres, so there are hundreds even thousands of MAP molecules in it. The maximum trapping force available is about 60 pN for each trap, so it is difficult to rupture the microtubule bundle completely. Thus the breakup of microtubule must be involved in our measurement. When one or several MAP bonds break, microtubules linking to the beads begin to suffer the force. Thus they should break before the force goes back to 0 and after the MAP bonds break. Although there are dozens or even hundreds of microtubules in a bundle, microtubules connecting to the beads are only one to several because the beads just contact the very outer side of the bundle. On the other hand, the force for breaking single fluorescent microtubule is 3.5 pN,[11] and the shear modulus is 2 orders of magnitude lower than Young's modulus.[16] In our experiment, when the force acting on the microtubules after the MAP bonds break, microtubules will distort to some extent. Thus there must be some shear force included. Therefore, the breaking force of single microtubule should be far smaller than 3.5 pN, and that of several microtubules should be much smaller than 14.6 pN as well. This force will not influence the results of the MAP bond.

Figure 3 presents the histogram of the binding forces from 101 measurements. There are 4 obvious peaks in the graph. The lines in the figure are Gaussian fittings of the histogram. The dotted line is the fitting of every peak, while the solid one is the summation of them. According to the fitting line, the statistical averages and standard deviations are 14.6 ± 3.5 pN, 24.6 ± 4.1 pN, 36.1 ± 4.7 pN and 50.0 ± 4.7 pN, respectively. The intervals between every two peaks are almost the same. Because it is possible that the MAPs involving in the measurement are more than one, the force of two or more bonds breaking simultaneously will be integer multiples of that of a single

bond. The first peak means that the binding force of single AtMAP65-1 and microtubule is most probably 14.6 pN. As mentioned above, the maximum force of each optical trap is about 60 pN. If there are 5 or more bonds involved, the force is not sufficient to rupture them. That is why there are only 4 peaks in the graph.

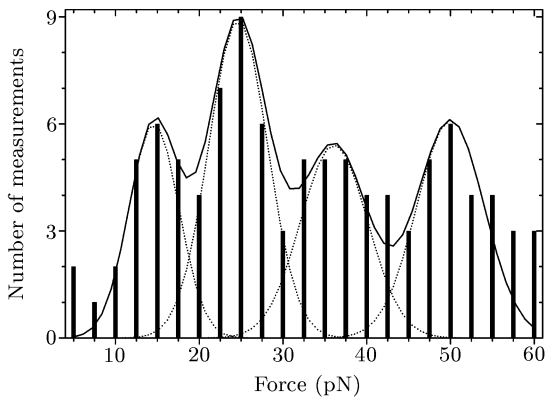

Fig. 3. Histogram of 101 forces. The dotted lines are the fitting to 4 peaks respectively, while the solid one is the summation of them.

According to the velocity of the moving optical trap and the displacements of both microspheres from the trap centres, we can calculate the interaction distance between AtMAP65-1 and microtubule. By analysing the data in the first peak of Fig. 3, the interaction distance is 3 nm. Thus the energy to rupture the bond between AtMAP65-1 and microtubule can be calculated as follows:

$$14.7\,\mathrm{pN} \cdot 3\,\mathrm{nm}/2 = 22.1\,\mathrm{pN} \cdot \mathrm{nm} = 13.3\,\mathrm{kJ/mol}.$$

This bond energy value falls in the range of electrostatic bond that is 10–30 kJ/mol, which indicates that the bond between AtMAP65-1 and microtubule may be electrostatic bond. Previous biochemical experiment has shown that microtubule bundles induced by AtMAP65-1 would scatter in the presence of high concentration of NaCl (above 300 mmol/L).[12] This is also the property of electrostatic bond. The two results are consistent with each other. From the dynamic point of view, the presumption is confirmed such that the bond between AtMAP65-1 and microtubule is electrostatic bond.

The bond energy calculated according to our result falling in the range of electrostatic bond proves that our results are reasonable. The tensile strength of a straight actin filament is about 600 pN,[17] and that to break a microtubule is at least several hundreds of pN.[16] The binding force between antigen and antibody is about 100 pN or more, which is generally considered to be the strongest among protein interactions. For example, the binding force between biotin and avidin is 160 pN, and that between biotin and streptavidin is 257 pN.[18] Compared with the above-mentioned forces, 14.6 pN is not very strong. However, the length of a microtubule can be several microns to tens of micron. AtMAP65-1s distribute along the whole microtubule evenly with an interval of 25 nm without additional NaCl.[12] Thus there are hundreds of AtMAP65-1s on a microtubule. From our result, AtMAP65-1, mainly bundling cortical microtubules at the interphase of the cell cycle is sufficient to stabilize the microtubule array and inhibits the reorganization. However, microtubule is a dynamic structure in cell cycle. There must be some mechanisms to regulate the binding force between MAPs and microtubule. It is impossible that this force is invariable. In fact, there are many mechanisms to regulate this force *in vivo* which leads to microtubule reorganization.

In summary, we have obtained the accurate binding force between AtMAP65-1 and microtubule, which is 14.6 pN. On this basis, we measure the change of this binding force under different physiological conditions. It will afford new view angle to decipher how MAPs influence microtubule function and dynamics, and also is an attempt to exploit a new way to study the interaction of proteins.

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