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Single Interaction Force of Biomolecules Measured with Picoforce AFM

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The interaction force between biomolecules (DNA-DNA, antigen-antibody, ligand-receptor, protein-protein) defines not only biomolecular function, but also their mechanical properties and hence bio-sensor. Atomic force microscopy (AFM) is nowadays frequently applied to determine interaction forces between biological molecules and biomolecular force measurements, obtained for example using AFM can provide valuable molecular-level information on the interactions between biomolecules. A proper modification of an AFM tip and/or a substrate with biomolecules permits the direct measurement of intermolecular interactions, such as DNA-DNA, protein-protein, and ligand-receptor, *etc.* and a microcantilever-based sensor appeared as a promising approach for ultra sensitive detection of biomolecular interactions.

Keywords: Atomic Force Microscopy; Biomolecules; Force Measurements; Inter-and Intra-Molecular Interactions; Microcantilever-Based Sensor

Ⅰ. Introduction

Understanding the biomolecular recognition force that drives specific molecular interaction is an important task in molecular and structural biology, because it is the most direct means of obtaining information about biomolecular structure and function as well as mechanical properties [1]. The interaction between biological molecules has been traditionally investigated by using tools that report the ensemble average. Therefore, new analytical techniques should be employed to gain the information on the molecular recognition event at single molecule level.

Recently, various techniques including biomembrane force probe (BFP), optical tweezers, and atomic force microscopy (or AFM) have emerged which are capable of direct measuring the single biomolecular interaction. Among these, the AFM is a well-established technique capable of high resolution imaging of individual biomolecules and measuring biomolecular interaction at the single molecule level. In particular, the spectroscopy measuring the force of a picoforce range has been utilized for a variety of single molecule measurements [2~5], and protein unfolding processes [6~8], DNA- DNA [9~11], protein-protein [12, 13], and ligand- receptor interaction [14] are examples.

The measurement of the biomolecular interaction has made information on mechanical properties, subtle structural changes and interaction forces in biological systems available at the single molecular level. Its high vertical resolution and speed, combined with its suitability for applications in liquid environment, provide the AFM with excellent qualities for the measurement of single interaction force.

Ⅱ. Functionalization of Sample for Biomolecular Force Spectroscopy

In order to sense interaction force, the force probing AFM uses a fine tip on a cantilever. The cantilever arm serves as a soft spring to measure forces between the tip and a substrate surface. A typical experiment to investigate biomolecular recognition events comprises the

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functionalization of the probe and substrate with their respective binding partners, and then takes the probe through approach/retraction cycles. Here, it is important that the binding of the molecule to the tip or sample is much stronger than the intermolecular bonding being investigated. Two techniques dominate the chosen approaches, the chemisorption of the thiols on gold and silanes on silicon oxide surfaces [15, 16]. These covalent or quasi covalent bonds ensure that the weakest bonds are the noncovalent ones between the biomolecules.

When biomolecular recognition experiments with AFM, the success of a force experiment depends on force resolution, which is limited by suitable functionalization of the tip and sample to detect single molecular interactions, which is an important toward developing new analytical and biomedical devices (e.g., biosensors). In order to achieve single molecular interactions between complementary biomolecules a number of approaches can be employed. Traditional approaches to single molecule studies are that the binding site availability of biomolecules can be minimized, by applying low concentrations of biomolecules to a surface followed by force-distance spectroscopy. In case of low concentration of biomolecules, however, more often than not, the biomolecules tend to aggregate, form films, or associate on the surface. This results in the vast majority of such traces being representative of multiple molecules. In addition to this lack of control on the surface distribution, the presence of such complexes on the surface exacerbates the tip-surface adhesion that presents confounding factors in the analysis of force curve [17].

However, single molecule can be achieved to some degree by the addition of flexible polymer molecules to the probe and/or sample architecture. The presence of a flexible crosslinker is advantageous not only for the measurement of single molecule interactions, but it also reduces steric hindrance and provides a means of distinguishing between specific and non-specific intermolecular interactions. A crosslinker spaces the biomolecule from the surface of the probe, and it doing so it allows the attached biomolecule to move without restriction in the identification of its binding site. This also allows the molecule to assume the correct orientation and conformation for binding. Furthermore, if a crosslinker of a known fixed length is employed then upon the formation of multiple complexes it is probable that the final rupture event to occur under an external force is due to the rupture of a single molecular interaction [18].

Ⅲ. Biomolecular Interactions by Picoforce AFM

3.1 Intermolecular Interaction.

Intermolecular interactions occur during the formation of double-stranded DNA, in antigen-antibody recognition, hormone-receptor reactions, the signal transduction, the initial step of the infection of viruses and bacteria, adhesion and migration of cells, and in the cohesion of the cytoskeleton [1]. Besides the thermodynamic and kinetic data, the complex interplay of forces is of prominent interest for the understanding of structure-function relationships of biomolecules. Retraction with constant velocity provides the desired information about rupture events. A lot of experimental effort has gone into studying intermolecular interactions.

Strunz et al. investigated the dynamics of the rupture of complementary DNA strands at different pulling velocities (16~4000 pNs^{-1}) under physiological conditions (Fig. 1) [11]. Both 5'end of double stranded DNA were linked to the sample and probe through a polyethyleneglycol (PEG) spacer in order to suppress nonspecific interactions in the vicinity of the substrate. Depending on the pulling velocities and sequence length, the unbinding forces of single DNA strands varied from 20 to 50 pN. These single duplexes results allowed a quantitative comparison with data from thermodynamic ensemble measurements and a discussion of the analytic applications of unbinding force measurements for DNA.

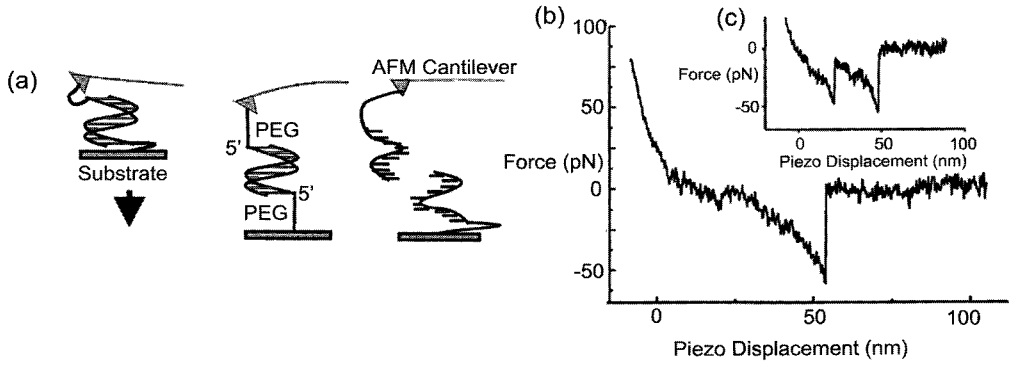

Fig. 1. Measurement of DNA unbinding forces. (a) Force Measurement of complementary DNA strands (b) A typical force versus piezo displacement for the DNA duplex (c) A force displacement curve in which two molecules unbind one after the other

Dufrêne et al. investigated the antigen binding forces of individual Fv frgments of antilysozyme antibodies [19]. To detect single molecular recognition events, genetically engineered histidine-tagged Fv frgments were coupled onto AFM tips modified with mixed self-assembled monolayers (SAMs) of nitrilotriacetic acid and tri(ethyleneglycol) terminated alkanethiols while lysozyme (Lys) was covalently immobilized onto mixed SAMs of carboxyl-and hydroxyl terminated alkanethiols. They observed unbinding forces composed of integer multiples of an elementary force quantum of ~50 pN that they attributed to the rupture of a single antibody-antigen pair at a loading rate of 5000 pN/s between Fv and Lyso-modified surfaces.

Ratto's group showed evidence of multivalent interactions between a single protein molecule and multiple carbohydrates at a pH where the protein can bind four ligands [13]. The evidence is based not only on measurements of the force required to rupture the bonds formed between concanavalinA (ConA) and α-D-mannose but also on an analysis of the polymer-extension force curves to infer the polymer architecture that binds the protein to the cantilever and the ligands to the substrate. They found that although the rupture forces for multiple carbohydrate connections to a single protein are larger than the rupture force for a single connection, they do not scale additively with increasing number. Specifically, the most common rupture forces were approximately 46, 68, and 85 pN at a loading rate of 650 ± 25 pN/s which we argued corresponds to 1, 2, and 3 ligands being pulled simultaneously from a single protein as corroborated by an analysis of the linkage architecture.

3.2 Intramolecular Interactions.

The structure of biomolecules can be investigated by circular dichroism spectroscopy, IR spectroscopy, X-ray analysis, and NMR spectroscopy, while their stability can be studied by calorimetry. In most cases, the energy hypersurface is unknown, which makes a direct measure of intramolecular force necessary. This measurement can be done using force spectroscopy on single molecules. The polymer is immobilized on the substrate and interacts with the tip specifically or nonspecifically. On retraction of the cantilever the molecules are stretched revealing important information about their conformation.

Rief et al. measured sequence-dependent mechanical properties of DNA by stretching individual dsDNA by AFM [20]. Native dsDNA adopts the so-called B conformation and changes to the S conformation (S: stretched) at 65 pN. The transition occurs fast within a fore range of approximately 5 pN, which is indicative of a process of high cooperativity. At this point, the molecule can be stretched to almost twice its contour length. The

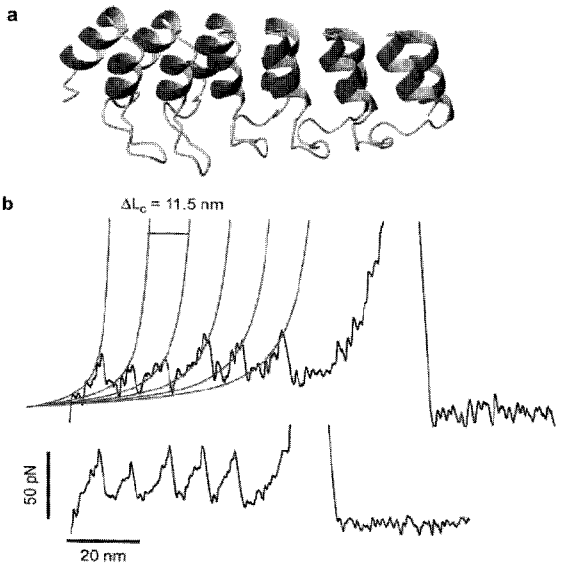

Fig. 2. Mechanical unfolding of the ankyrin repeats in N6C with single-molecule AFM.

melting of the DNA occurs at higher forces of around 150 pN. Both the melting of DNA and B to S transition at low forces are sequence dependent.

Fernandez et al. found that a protein consisting of six identical ankyrin repeat units flanked by N- and C-terminal modulus (N6C) unfolded in a stepwise, unit-by-unit fashion under a mechanical force [21]. Stretching a N6C molecule resulted in a sawtooth pattern fingerprint, with as many as six peaks separated by ~10 nm and an average unfolding force of 50 ± 20 pN (Fig. 2). Their results demonstrated that a stretching force could unfold multiple repeat units individually in a single protein molecule, despite extensive hydrophobic interactions between adjacent units.

3.3 Force Maps.

Individual curves recorded during raster scanning in the x-y direction can be assembled into a force map. For example, Dufrêne et al. reported a method based on AFM with tips bearing biologically active molecules, for measuring the specific binding forces of individual adhesions and for mapping their distribution on the surface of living bacteria (Fig. 3) [22]. They determined

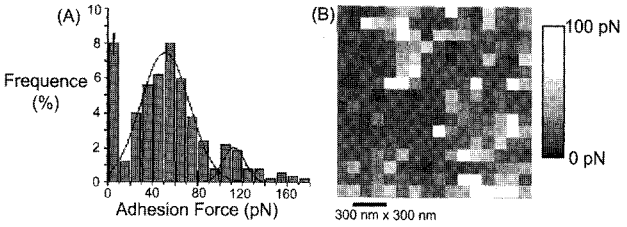

Fig. 3. Histogram (A) and spatially resolved map (B) of adhesion force on living mycobacteria.

the adhesion forces between the heparin-binding haemagglutinin adhesion (HBHA) produced by *Mycobacterium tuberculosis* and heparin, used as a model sulphated glycoconjugate receptor. The adhesion force is 50 ± 23 pN at a loading rate of 10000 pN/s and an interaction time of 500 ms. Both the adhesion frequency and adhesion force increased with contact time, indicating that the HBHA-heparin complex is formed via multiple intermolecular bridges. Dufrêne et al. mapped the distribution of single HBHA molecules on the surface of living mycobacteria and found that the adhesion is not randomly distributed over the mycobacterial surface, but concentrated into nanodomains.

3.4 Microcantilever-Based Sensor.

Recently, a microcantilever-based sensor appeared as a prospecting approach for ultra sensitive detection of biomolecular interactions [23]. The nanomechanical biosensor based on the beam bending method measured the difference in surface stress between the two sides of the microcantilever. Manalis and co-workers [24] reported label-free protein detection using a microfabricated cantilever-based sensor that was functionalized with a DNA aptamer.

Ⅳ. Conclusions

Biomolecular force spectroscopy has rapidly developed, and is now on the verge of becoming a standard technique for the structural and functional investigation

of biomolecules, as recent advancements in AFM technology have allowed to measure inter-and intramolecular forces, force mapping at the level of individual molecules and biosensor. Given extremely high lateral resolution of AFM, wide application of the approach is evident for the fields associated with live biomolecules.

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원자 힘 현미경을 이용한 단일 생분자 힘 측정

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생명현상 발현에 중요한 역할을 하는 생체 분자간 특이적 상호작용을 단분자 수준에서 이해하려는 연구는 매우 중요한 일이다. 나노 바이오 측정기술을 이용하여 여러 복잡한 생명현상을 그 기본 단위인 단일 세포 차원에서 직접 측정하여 응용하려는 시도가 이루어지고 있다. 이런 시도로써, 원자힘 현미경을 이용한 생체분자간의 결합력 측정은 생명현상과 가장 유사한 환경에서 단일 생체 분자간 또는 분자 내 힘을 직접 측정함으로써, 단일 생체분자의 현상을 관찰 할 수 있다는 장점을 가지고 있다. 특히 단분자 힘 분광학을 이용한 단일 생체분자내의 세부 단위체간 상호작용에 대한 연구와 단백질-단백질, 단백질-리간드, DNA-DNA의 분자인지 상호작용에 대한 연구는 많은 생명과학 분야 연구자들의 관심을 끌고 있을 뿐만 아니라 더 나아가 새로운 관련 기술의 개발을 촉진시키고 있다.

주제어 : 원자 힘 현미경, 생분자 힘 측정, 분자간-, 분자내-상호작용 측정, 마이크로 캔틸레버를 이용한 센서

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