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Dynamic response of HPV16/anti-HPV16 pairs with unbinding events studied by atomic force microscopy



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ABSTRACT

This paper proposes an effective approach to distinguish whether samples include Human Papilloma virus type-16 (HPV16) by Atomic force microscopy (AFM). AFM is an important instrument in nanobiotechnology field. At first we identified the HPV16 by Polymerase chain reaction (PCR) analysis and Western blotting from specimen of the HPV patient (E12) and the normal (C2), and then we used an AFM to observe the surface ultrastructure by tapping mode and to measure the unbinding force between HPV16 coupled to an AFM tip and anti-HPV16 L1 coated on the substrate surface by contact mode. The experimental results by tapping mode show that the size of a single HPV virion was similar to its SEM image from the previous literatures; moreover, based on the purposed methods and the analysis, two obvious findings that we can determine whether or not the subject is a HPV patient can be derived from the results; one is based on the distribution of unbinding forces, and the other is based on the distribution of the stiffness. Furthermore, the proposed method could be a useful technique for further investigating the potential role among subtypes of HPVs in the oncogenesis of human cervical cancer.

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1. Introduction

Recent years have seen increased attention being given to Human papilloma virus (HPV) in biomedicine field literature. Because a number of studies have shown that HPV infection is an important risk factor for cervical cancer [1–3], breast cancer [4–6], and even lung cancer [7]. Especially the relation between suffering from cervical cancer and HPV16/18 has massive empirical support in clinical observation respect [1,8]. Besides, HPV is one kind deoxyribonucleic acid (DNA) virus. In general, HPV itself vanishes after infecting the cervix about 7–8 months, but a small part still survives and proceeds to make the cervix be infected with this type pathogen. HPV is so a type of very tiny pathogen (nanometer level) that finding a suitable observational instrument is a very important task. Traditionally, methods for detecting HPV in cervical samples include Southern blot and dot blot hybridization [9], Hybrid Capture II (HCII) [10], etc. In addition to the above-described methods, the way to detect HPV by atomic force microscope (AFM) is perhaps more efficient, simple and convenient.

AFM, invented by Binning, Calvin Quate, and Gerber in 1986 as

a tool with high-resolution, now has many applications in the fields of molecular biology, semiconductor physics, etc [11–13]. Because AFM has so many applications, in the past 30 years the science and technology for the creation and use of AFM has progressed tremendously. In the field of molecular biology, AFM has proved to be applicable to structural studies of single biomolecules [14–16] and to the measurement of molecular forces at the single-molecule level by contact mode. AFM has the absolute predominance of nano-level spatial resolution for detecting molecular interaction at high lateral resolution combined with immense force sensitivity in an intricately physiologic environment as compared with other biophysical approaches to direct measurements of inter-molecular forces, such as the magnetic torsion, the bio-membrane force probe (BFP), etc. Therefore, AFM has been utilized well to gauge and estimate inter-molecular forces between various ligand and receptor pairs [17–19] and intra-molecular unfolding forces of individual proteins [20].

AFM is perhaps the most versatile member among microscopes known as scanning probe microscopes (SPMs). For example, to achieve specific needs, we can change the characteristics of AFM tips, such as chemical modification [21], and different chemical modification of AFM tips may have different properties. Thus we can acquire the information about specific force between modified tips and local area of samples by contact mode. For measurements of intermolecular binding strengths between antibody and antigen

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binding, antibodies are attached to AFM tips and antigens are attached to substrate surfaces, and vice versa. The modified AFM tip is brought into contact with the substrate surface so as to form antibody/antigen complexes. When the modified AFM tip is retracted from the substrate surface, the force required for dissociation (unbinding force), defined as the rupture force at the moment of sample separation, can subsequently be measured and recorded by pulling on the complexes until the interaction bond breaks. Some studies provide methods for estimation of affinity, rate constants, and bond width of the binding pocket [22–24], and even provide a tool for mapping the energy landscape [25]. In addition, these equipments provide insights into the molecular dynamics of the receptor/ligand recognition process by varying the loading rate of force application [22–25]. In this study, we propose a method for the biosensor application of AFM to studies of the molecular dynamics of the HPV16/anti-HPV16 recognition process based on clinical cantilever mechanical theories.

2. Material and methods

2.1. Polymerase chain reaction (PCR)

PCR is one kind technique in the molecular biology field, and it lets a specific DNA sequence to be copied. Such technique was performed as previously described [26]. This study used PCR technique to copy and produce a large number specific HPV16 E6 primer segments from the HPV16 patient, and then by the same method to produce a large amount of HPV16 E6 gene segments. Next we confirmed whether the specimen includes HPV16 by comparing to above-mentioned two kinds segments electrophoresis patterns.

2.2. Western blot analysis

Western blot analysis is widely used method to confirm specific proteins in a sample, and the detailed analysis procedure was performed as previously described [27]. This study used western blot analysis and the results of electrophoresis to confirm whether HPV16 existed in the specimen.

2.3. AFM contact mode and tapping mode

AFM contact mode is often used to measure the force interaction between the AFM tips and samples [22–25]. But AFM contact mode is easy to destroy the surface appearances of samples. To overcome the problem, we can use AFM tapping mode as a solution to obtain original AFM images [28].

2.4. AFM images of HPV16 acquired by tapping mode

The images of HPV16 were obtained by using an Atomic force microscopy (SPA 300HV, Seiko Instruments Inc., Japan). Commercial silicon cantilevers (SuperSharpSilicon™ SSS-SEIH, NANOSENSORS, Switzerland) were used in tapping mode in air, and then topographic images were acquired by using a resonant frequency of 140 kHz for the probe oscillation.

2.5. Functionalization of AFM tips and the substrate surface

The mica substrate surface coated with HPV16 from the specimen that had checked by PCR and western blot analysis and AFM tips (PointProbePlus, Nanosensors, Switzerland) were coupled with anti-HPV16 L1, and moreover, the functionalization procedure is described as follows. AFM tips and the substrates were immersed in 65% solution of nitric acid for 10 min and then were

taken out. These AFM tips and the substrates were then immersed in 5% ethanol solution for 10 min at room temperature, followed by several rinses with solution of 5% ethanol/95% distilled water, and then these AFM tips and the substrates were immersed in 5% 3-APTES solution in phosphate-buffered saline (PBS, pH 7.2) for 45 min and then extensively rinsed with PBS solution. Next, these AFM tips and the substrates were immersed in a 2.5% Glutaraldehyde solution for 75 min. In the last step, the anti-HPV16 L1 solution (200 µg/ml in PBS, pH 7.2) bound covalently to the AFM tips for overnight incubation at 4 °C. On the other hand, before AFM force measurements, the mica substrates were covalently bond with HPV16 sample (E12) solution in PBS (pH 7.2) for 2 h.

2.6. AFM force measurement acquired by contact mode

A scanning probe microscope (SPM) system measuring head (SMENA liquid head, NTMDT, Moscow, Russia) was used to measure the interaction force between HPV16 and anti-HPV16 L1 in liquid environment. All AFM force measurements were executed at the same loading rate 333.3 nm/s in PBS buffer (pH 7.2). Besides, the interaction force between HPV16 and anti-HPV16 probe was measured by approaching the AFM tip towards the HPV16 sample perpendicularly.

3. Results and discussion

3.1. AFM images of HPV16

The detailed surface topographies of HPV16 samples on the substrate were observed by use of AFM tapping mode (Fig. 1); moreover, Fig. 1A–F shows the AFM images of the HPV particles on the mica substrate. Fig. 1A shows the AFM image (scanning area 2000 nm × 2000 nm) of the HPV samples, and the HPV particles appear to be mainly spherical and uniformly distributed on the mica substrate. By zooming into the square area which displayed in Fig. 1A and B respectively, this higher resolution images (Fig. 1B and C) were obtained. This higher resolution images (Fig. 1 B and C) clearly display many separate spherical viral particles which exist on the surface. Besides, Fig. 1A', B', and C' are three-dimensional images of Fig. 1A, B, and C, respectively. To research the dimension of a single in Fig. 1C, three viral particles which were marked by squares in Fig. 1C were zoomed in Fig. 1D–F (topographic images). More than 50 single HPV16 particles were then examined and analyzed by AFM imaging software, and their average diameter from the AFM images was 51.6 ± 1.8 nm (mean ± SD, n=51), in agreement with the size estimated from electron microscopy apparently [29].

3.2. AFM force measurements acquired by contact mode and analysis

3.2.1. The specificity of HPV16/anti-HPV16

To understand the dynamic response relation between HPV16 and anti-HPV16 L1, we used AFM contact mode to measure the unbinding force required to separate complementary HPV16/anti-HPV16 L1 pairs. Besides, all AFM force measurements were implemented at the same loading rate of 333.3 nm per second in PBS buffer (pH 7.2). The interaction force between HPV16 and anti-HPV16 L1 probe was measured by approaching the tip towards the sample perpendicularly. Before executing the force vs. distance measurements of the antigen-antibody interaction, we must design and carry out experiments of control group and experimental group of unbinding events. The detailed all kinds of unbinding events results are described as follows.

Fig. 2A–C show typical examples of the force-distance curves

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