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Specific binding of antigen-antibody in physiological environments: Measurement, force characteristics and analysis

Xin Gu[a], Jun Zhou[a,*], Lu Zhou[a], Shusen Xie[b], Lucia Petti[c,*], Shaomin Wang[d], Fuyan Wang[e]

[a] Institute of Photonics, Faculty of Science, Ningbo University, Ningbo, 315211, China
[b] Key Laboratory of Optoelectronic Science & Technology for Medicine of Ministry of Education, Fujian Normal University, Fuzhou 350007, China
[c] Institute of Applied Sciences and Intelligent Systems -ISASI, CNR, Via Campi Flegrei, 34-Comprensorio Olivetti, 80078 Pozzuoli, Napoli, Italy
[d] Affiliated Hospital, School of Medicine, Ningbo University, Ningbo 315020, China
[e] Diabetes Center, Zhejiang Provincial Key Laboratory of Pathophysiology, School of Medicine, Ningbo University, Ningbo, 315211, China

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ABSTRACT

The specific recognition of the antigen by the antibody is the crucial step in immunoassays. Measurement and analysis of the specific recognition, including the ways in which it is influenced by external factors are of paramount significance for the quality of the immunoassays. Using prostate-specific antigen (PSA)/anti-PSA antibody and α-fetoprotein (AFP) /anti-AFP antibody as examples, we have proposed a novel solution for measuring the binding forces between the antigens and their corresponding antibodies in different physiological environments by combining laminar flow control technology and optical tweezers technology. On the basis of the experimental results, the different binding forces of PSA/anti-PSA antibody and AFP/anti-AFP antibody in the same phosphate-buffered saline (PBS) environments are analysed by comparing the affinity constant of the two antibodies and the number of antigenic determinants of the two antigens. In different electrolyte environments, the changes of the binding force of antigens-antibodies are explained by the polyelectrolyte effect and hydrophobic interaction. Furthermore, in different pH environments, the changes of binding forces of antigens-antibodies are attributed to the role of the denaturation of protein. The study aims to recognise the antigen-antibody immune mechanism, thus ensuring further understanding of the biological functions of tumour markers, and it promises to be very useful for the clinical diagnosis of early-stage cancer.

1. Introduction

In 1845, the Bence-Jones protein was first discovered to be the biomarker of multiple myeloma [1]. Since then, a variety of proteins have been found as tumour markers such as carcinoembryonic antigen (CEA), prostate-specific antigen (PSA), α-fetoprotein (AFP), and carbohydrate antigen 125 (CA125) [2]. Many techniques have been developed to detect the tumour markers in blood, urine or body tissues for early diagnosis, therapy and tracing of cancers [3–6]. For example, the enzyme-linked immunosorbent assay (ELISA) and the fluorescence-based immunoassay (FBIA) have been widely applied in clinical test of cancers [7,8]. The advantages of ELISA and FBIA are cost-effective, with high-throughput, specific selectivity and high sensitivity. However, they are neither quantitatively nor qualitatively used to measure and analyse the binding forces between antigens and antibodies. In fact, the measurement and analysis of the specific binding between antigens and antibodies are important for further understanding the biological functions of tumour markers.

In recent decades, many works have focused on researching the specific binding between antigens and antibodies for a deep comprehension of the immunologic mechanism. For example, with the help of the atomic force microscope (AFM), Allen et al [9] directly measured the specific binding forces between the ferritin linked on the AFM probe and the anti-ferritin antibody coated on the silicon substrate. Dufrene's group [10] used AFM to measure the specific binding force of antigen and anti-lysozyme Fv fragments on a single molecule basis. Hua Jin et al [11] measured the unbinding force between the AFM probe modified with anti-human cluster of differentiation (CD44) antibody and B16 cells. Coppair et al. [12] measured the unbinding force between the β2 microglobulin (B2M)-functionalised tip and anti-B2M monoclonal antibodies adsorbed on mica substrate. Although the AFM has been frequently applied to measure the specific binding force, there are three main limits: (1) the measurement precision of controllable force is tens of pN, which is not enough to evaluate the specific antigen and antibody binding ability of; (2) the probe tip is vulnerable to pollution when exposed to outer environment; and (3) the target analyte can be easily damaged during the scanning process. To avoid the mentioned limit,

* Corresponding authors.
E-mail addresses: zhoujun@nbu.edu.cn (J. Zhou), l.petti@isasi.cnr.it (L. Petti).

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we propose the use of optical tweezers to measure the specific binding forces between antigens and antibodies more accurately due to their unique functions. Optical tweezers are not only able to non-invasively capture the target analyte but also to detect much weaker forces (0.1–10 pN) with a small error of 0.01 pN, which is adapted for probing the dynamic characteristics of bio-macromolecular systems [13–15]. The merits of optical tweezers have led to a burst of activity in the biomedical field, for example, trapping and manipulating of virus or bacteria [16], imaging and analysing the cell [17], measuring the displacement of the motor protein [18], studying the mechanical properties of DNA at the single-molecule level [19] and monitoring the stretching force of single chromatin fibres [20].

In this paper, we propose a novel solution to measure the binding forces between the antigens and their corresponding antibodies in different physiological environments by combining laminar flow control technology and optical tweezers technology. First, a self-designed multichannel laminar flow cell was fabricated by heating and mounted on the dual-beam optical tweezers system. Then, with the aid of the amino groups, PSA, anti-PSA antibody, AFP and anti-AFP antibody were stabilised on the surface of the prepared SiO_2 microspheres (MPs) with different sizes, respectively. Subsequently, these modified SiO_2 MPs were separately injected into the self-built multichannel laminar flow cell and distributed in different laminar flows, and then captured into the different optical traps to measure the binding forces between the antigens and the antibodies. The experimental results demonstrate that the binding forces of antigens-antibodies depend on the types of protein, the electrolyte and pH environments. It improves our understanding of the biological function of tumour markers as well as aids in the clinical diagnosis of early-stage cancer.

2. Experiments and methods

2.1. Chemicals

Ethanol (99.5%) and aqueous ammonia (28%) were purchased from Sinopharm Chemical Reagent Co., Ltd. Tetraethoxysilane (TEOS, 98%) and 3-(Aminopropyl)-trimethoxysilane (APTMS) were obtained from J&K Scientific, Ltd. Prostate-specific antigen (PSA), anti-PSA antibody, α-fetoprotein (AFP) and anti-AFP antibody were purchased from Beijing Key-Bio Biotech Co., Ltd. 1-(3-dimethyl aminopropyl) -3-ethylcarbodiimide hydrochloride (EDC), hydrogen chloride (HCl) and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich. Sodium hydroxide (NaOH), sodium chloride (NaCl), calcium chloride ($CaCl_2$) and sodium sulphate (Na_2SO_4) were purchased from Zhejiang Zhongxing Chemical Reagent Co., Ltd. Bovine serum albumin (BSA) was purchased from Nanjing Sunshine Biotechnology Co., Ltd. Phosphate buffer solution (PBS, pH = 7.0, 136 mM NaCl, 2.7 mM KCL, 15.6 mM Na_2HPO_4, 17.6 mM KH_2PO_4) was purchased from Sigma-Aldrich Co. LLC. Milli-Q water (resistivity 18.2 MΩ cm-1) was used to prepare all solutions.

2.2. Synthesis of SiO_2 MPs

By using the Stöber method, the SiO_2 nanoparticles were synthesised by hydrolysis and condensation of TEOS [21]. First, the solution A (9 mL TEOS, 45.5 mL ethanol) and the solution B (9 mL ammonia, 16.25 mL ethanol, 24.75 mL water) were rapidly mixed by magnetic stirring. Then, the mother solution was gently agitated for 2 h at room temperature until displaying a white colour, which demonstrated that the SiO_2 nanoparticles are generated. Subsequently, the reaction solution was centrifuged at 6500 rpm for 6 min, and the extracted sediment was put into the mother solution composed by solutions A and B to synthesise larger SiO_2 nanoparticles. Repeating the above steps several times, SiO_2 MPs with diameters of 2 or 3 μm were obtained. Finally, the 2- and 3- μm SiO_2 MPs were filtered through the microfiltration membrane and dispersed in 5 mL of PBS solution ready to be used.

2.3. Surface modification of SiO_2 MPs

The amino group was modified on the surfaces of SiO_2 MPs by the dehydration condensation reaction between the Si-OH of SiO_2 MPs and the Si-OH produced by APTMS hydrolysis [22,23]. The modifying process is schematically shown in Fig. S1†. In detail, APTMS was first added into the SiO_2 MP solution (4:1 ethanol/water) in the proportion of 1:100. Then, the mixture was refluxed in an oil bath for 4–6 h under magnetic stirring. Next, the APTMS-modified SiO_2 MPs were centrifugally cleaned 3 times consecutively at 8000 rpm for 10 min to remove the excess APTMS. Subsequently, the centrifuged sediment was scattered into 10 mL of PBS solution by an ultrasonic bath for more than 10 min, resulting in well-dispersed APTMS-modified SiO_2 MPs.

2.4. Linking of antigens/antibodies on SiO_2 MPs

Because of the amino groups on the surface of the APTMS-modified SiO_2 MPs reacting with the carboxylic acid groups of proteins, the antigens (PSA, AFP) or antibodies (anti-PSA antibody, anti-AFP antibody) are linked with the APTMS-modified SiO_2 MPs, as schematically shown in Fig. S2†.

For simplicity, taking AFP and anti-AFP antibody as examples, the linking processes follow these steps. First, 100 μL of AFP was added into 2 mL of PBS solution at room temperature to obtain an AFP solution of 0.001 mg mL^{-1}. Then, 1 mL of APTMS-modified SiO_2 MPs with diameters of 3 μm were added into the above diluted AFP solution under agitation for 2 h and kept overnight at 4 °C for immobilising AFP. The mixture was washed 3 times by centrifugation at 1000 rpm for 15 min to remove the unlinked AFP. Next, the sediment was scattered into 10 mL of PBS solution, and the redundant BSA solution (3%) was added to shield the bare amino-groups on the surfaces of the SiO_2 MPs. After incubation at room temperature for 1.5 h, the mixed solution was washed by centrifugation at 1000 rpm for 15 min to remove the excess BSA. Finally, the SiO_2 MPs linked with AFP were scattered into 10 mL of PBS solution as the experiment samples.

Similarly, the immobilisation process of anti-AFP antibodies is almost the same as that described above. First, 100 μL of anti-AFP antibody was added into 2 mL of PBS solution containing 0.4 mg EDC and 0.2 mg NHS at room temperature [24]. Then, the mixed solution of 0.002 mg mL^{-1} was kept at 37 °C for 30 min to fully activate the anti-AFP antibodies. Here, 37 °C was set to keep the bioactivity of the antibody at the approximate temperature of the human body [25]. Next, 1 mL of the APTMS–modified SiO_2 MPs with diameters of 2 μm was added to the activated anti-AFP antibody solution. The resultant solution was incubated at 37 °C for 2 h under shaking, and then kept overnight at 4 °C. Subsequently, the mixture was centrifuged 3 times at 1000 rpm for 15 min to remove the excess anti-AFP antibody. The sediment was scattered into 10 mL of PBS solution and the redundant BSA solution (3%) was added to shield the bare amino-groups on the surfaces of the SiO_2 MPs. After incubation at room temperature for 1.5 h, the mixed solution was washed by centrifugation at 1000 rpm for 15 min to remove the excess BSA. Finally, the SiO_2 MPs linked with anti-AFP antibody were scattered into 10 mL of PBS solution as another experiment sample.

In the same way, the PSA and the anti-PSA antibody were also immobilised on the surface modified SiO_2 MPs.

2.5. Instruments and experimental method

The scanning electron microscope (SEM) images of the SiO_2 MPs were obtained by field-emission scanning electron microscopy (SU-70, Hitachi) operating at 10 kV accelerating voltage. ζ-potential was measured by the nanoparticle size analyser (Zetasizer Nano S90, Malvern). The force measurements were performed with dual beam optical tweezers (Nano Tracker™ 2, JPK Instruments, Germany) equipped with a continuous wave infrared laser (1064 nm, 3 W). The Nano Tracker™ 2 system has accessible trap stiffness in the range of 0.001–100 pN/nm,