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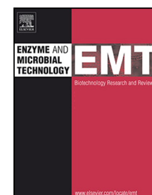

Rapid detection of viral antibodies based on multifunctional *Staphylococcus aureus* nanobioprobes

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ABSTRACT

Biosynthesis of nanoparticles inside *S. aureus* cells has enhanced the sensitivity of immunoassays based on the *S. aureus* nanoparticles. However, the current methods are limited to antigen detection by conjugating IgG antibodies on *S. aureus* nanoparticles. In this study, a simple way to conjugate antigens to the *S. aureus* nanobioparticles was developed by utilizing a cell wall binding domain (CBD) from a bacteriophage lysin PlyV12. Based on this novel design, simple agglutination tests of the IgG antibodies of Ebola virus (EBOV) nucleoprotein (NP) and Middle East Respiratory Virus (MERS) NP in rabbit sera were successfully developed by conjugating the *S. aureus* nanobioparticles with two fusion proteins EBOV NP- CBD and MERS NP-CBD, respectively. The conjugation was done easily by just mixing the fusion proteins with the *S. aureus* nanoparticles. The detection time was within 20 min without any special equipment or expertise. As far as we know, this is the first time to realize the detection of viral antibodies based on *S. aureus* nanoparticles.

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1. Introduction

In recent years, as an emerging field in bio-nanotechnology, the synthesis of nanomaterials by living organisms, such as yeast, fungi and bacteria, have demonstrated great potential for different applications [1,2]. Among bacteria, protein A producing *S. aureus* has been an attractive vector for immunoassays of pathogens, especially for coagglutination tests of bacteria and viruses [3], because it is easy to conjugate IgG antibodies through protein A molecules on the surface of *S. aureus* cells. On the same time, biosynthesis of nanoparticles inside *S. aureus* cells could be exploited to enhance detection sensitivity. For example, biosynthesis of quantum dots inside *S. aureus* cells has been used for sensitive detection of H9N2 virus [2]. In our lab, we established a simple way to transform *S. aureus* into red and fluorescent nanobioprobes by reacting with a monotetrazolium redox dye, 5-cyano-2, 3-ditolyl tetrazolium chloride (CTC) at room temperature [4]. Comparing with the uncolored *S. aureus* nanobioprobes, 100 fold higher sensitivity was achieved in rapid coagglutination test of *E. coli* O157:H7 with the novel red nanobioprobes.

Despite tremendous progress made in this area, the current methods are still limited to antigen detection by conjugating IgG antibodies on *S. aureus* nanoparticles. In this study, we aim to present a simple way to conjugate antigens to the *S. aureus* nanobioparticles by utilizing a cell wall binding domain (CBD) from a bacteriophage lysin PlyV12, which could bind to the surface of the *S. aureus* nanobioparticles [5,6]. Based on this novel design, simple agglutination tests of the IgG antibodies of Ebola virus (EBOV) nucleoprotein (NP) and Middle East respiratory syndrome coronavirus (MERS-CoV) NP were successfully developed by conjugating the *S. aureus* nanobioparticles with two fusion proteins EBOV NP-CBD and MERS NP-CBD.

EBOV and MERS-CoV are highly contagious pathogens which can cause Ebola virus disease and the acute respiratory infectious disease-Middle East respiratory syndrome, respectively [7,8]. Because until now no specific antiviral drugs or vaccines have been approved for clinical use against EBOV and MERS-CoV, rapid and specific diagnosis is essential for controlling the infections. In general, virus-specific antibodies IgM or IgG with rising titers constitute a strong presumptive detection. Therefore, detection of IgM or IgG antibodies in blood are used commonly in epidemic investigation of viral infections [9–14]. Some diagnostic methods, such as Enzyme-linked immunosorbent assay (ELISA) [9–11,15], indirect immunofluorescence assay (IFA) [16–18],

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neutralization test [12,19,20] and protein microarray [21], have been developed for EBOV and MERS-CoV antibody detection. However, the requirement of dedicated instruments limits the application of these assays in field, which is important for fast responses during epidemics. In comparison, agglutination test is more rapid, inexpensive, simple to interpret, and most importantly requires no specialized instrument since naked eyes could read the results [3]. Therefore, agglutination test might be a good choice for EBOV or MERS-CoV antibody detection in field. In our agglutination test, the conjugation is quite simple and could be done easily by just mixing the fusion proteins with the *S. aureus* nanoparticles. As far as we know, this is the first time to realize the detection of viral antibodies based on *S. aureus* nanoparticles.

2. Materials and methods

A monoclonal antibody (clone 4B4) specific against EBOV subtype Zaire NP were developed by our lab previously. Polyclonal rabbit antisera against EBOV subtype Zaire NP and MERS Virus NP were kindly provided by Professor Zhengli Shi, from Wuhan Institute of Virology, Chinese Academy of Sciences.

2.1. Determination of the antibody titer of monoclonal antibody and polyclonal rabbit antiserum against EBOV subtype Zaire NP by ELISA

ELISAs were performed as described by Nakayama et al. using the recombinant NP antigens [9]. Briefly, ELISA plates were coated with a predetermined optimal quantity of recombinant EBOV subtype Zaire NP antigens (50 ng/well). Then purified monoclonal antibody (clone 4B4) and polyclonal rabbit antiserum diluted in series 2 fold from 1:10 to 1:81920 with PBS containing 1% BSA were added into the wells and incubated with the coated NP antigen for 30 min at 37 °C, respectively. After washing, the bound monoclonal antibody (clone 4B4) and the polyclonal rabbit antiserum were detected by using horseradish peroxidase (HRP) conjugated rabbit anti-mouse IgG and goat anti-rabbit IgG at a dilution of 1:5000, respectively. Finally, the optical density (OD) was measured at 450 nm using a microplate reader (Synergy H1, BioTek, USA). Each dilution was tested in triplicates.

2.2. Preparation of recombinant antigens

The fusion protein EBOV NP-CBD was constructed as follows. The EBOV NP gene was amplified from pET32a-EBOV NP with the primers EBOV NP-CBD-F: 5′-GCGT*GCTAGC*GATTCTCGTCCTCAG-3′ and EBOV NP-CBD-R:5′-AATT*GAATTC*TGAACCGCCTCCACCCTGATGATGTTGCAGG-3′ (restriction endonuclease sites were italized) and then digested by Nhe I and EcoR I. Subsequently, the digested fragment was inserted into plasmid pET28a-$(G_4S)_2$-EBOV CBD digested by Nhe I and EcoR I, yielding the plasmid pET28a-EBOV NP-$(G_4S)_2$-CBD. The expression vector pET28a-MERS NP-$(G4S)_2$-CBD for fusion protein MERS NP-CBD was constructed in the same way. Then the cloned plasmids were transformed into *E.coli* BL21 competent cells. Both histidine (His) tagged EBOV NP-CBD and MERS NP-CBD were expressed in *E.coli* and purified by nickel-chelating affinity chromatography. Purity of the eluted EBOV NP-CBD and MERS NP-CBD were confirmed by SDS-PAGE and the concentrations of EBOV NP-CBD and MERS NP-CBD after dialysis were determined with a bicinchoninic acid (BCA) kit (Bi Yun Tian Biotechnology Inc, China).

2.3. Preparation of S. aureus *Nanobioparticles*

The *S. aureus* nanobioparticles were prepared from *S. aureus* strain CCTCC AB91118. Briefly, the *S. aureus* cells were grown in 5 mL of Luria-Bertani broth from the master seed and incubated at 37 °C for 12 h. Then 2 mL of the culture was transferred into 100 mL of Luria-Bertani broth and incubated at 37 °C until OD600 reached 0.6-0.8. The cells were harvested by centrifugation at 8000 rpm for 5 min and washed twice with phosphate buffer saline (PBS, pH 7.4). Following the addition of 4 mM CTC into the culture, the cells were kept at 37 °C for 1 h. Subsequently, the red colored cells were harvested by centrifugation at 6000 rpm for 10 min and washed thrice with PBS. Finally, the cells were heat treated at 80 °C for 20 min in a water bath for complete inactivation and the nanobioparticles obtained were stored at 4 °C until used.

The preparation of *S. aureus* nanobioparticles used for agglutination test of MERS NP IgG antibodies was similar to the above method, except that *S. aureus* cells were stained with another tetrazolium redox dye, triphenyl tetrazolium chloride (TTC), instead of CTC.

2.4. Testing IgG antibodies of EBOV NP (V-bottom 96-well plate)

The *S. aureus* nanobioparticles were sensitized with the fusion protein EBOV NP-CBD for detecting the IgG antibodies of EBOV NP. Briefly, the suspension (1 mL) of the *S. aureus* nanoparticles prepared above was mixed with 20 μg EBOV NP-CBD and then the mixture was incubated at 37 °C for half an hour in a shaker (180 rpm). The nanobioprobes obtained were then washed with PBS (pH 7.4) by centrifugation and resuspended in PBS to a concentration of 10% (w/v). Sodium azide was added to a final concentration of 0.02% and the nanobioprobe suspension was stored at 4 °C until used.

Before the test, the polyclonal rabbit antiserum was inactivated at 56 °C for 30 min. For the agglutination test, 25 μL of the diluted polyclonal rabbit antiserum or the diluted mAb was mixed with an equal volume of the nanobioprobes sensitized with the EBOV NP-CBD in a V-bottom 96-well plate. The mixture was incubated at 37 °C and mixed thoroughly every two minutes. A blank control reaction was simultaneously made by mixing 25 μL of the nanobioprobe suspension with 25 μL of PBS. A sample was considered positive only when it appeared obvious agglutination and the blank control did not show agglutination by naked eyes within 10 min.

2.5. The reaction among the IgG moleucles, protein A on the surface of one staphylococcal particle and antigen molecules fused with CBD on another particle

To demonstrate that the IgG antibodies of MERS NP are sandwiched between protein A on the surface of one staphylococcal particle via their Fc fragments and the MERS NP-CBD fused with another particle after agglutination, two types of *S. aureus* nanobioparticles, one sensitized with MERS NP-CBD and the other non-sensitized, were used. First, 50 μL of the non-sensitized *S. aureus* nanoparticles were mixed with 10 μL of the MERS NP polyclonal rabbit antiserum and incubated at 37 °C for an hour. Then the mixture was centrifuged at 6000 rpm for 5 min to remove the un-bound antibodies and harvest the particles pre-reacted with the IgG antibodies. The pre-reacted particles were washed thrice with PBS, and resuspended in 50 μL of PBS. Finally, 25 μL of the MERS NP-CBD sensitized *S. aureus* nanobioparticles were mixed with 25 μL of the pre-reacted *S. aureus* nanoparticles on a microscope slide. The mixture was incubated at room temperature and mixed thoroughly every two minutes. A negative control was simultaneously made by

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