Journal of Microbiological Methods 142 (2017) 41–45

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

A label-free biosensor based on localized surface plasmon resonance for diagnosis of tuberculosis

Wenxia Sun[a], Shishan Yuan[b,*], Haowen Huang[c], Ning Liu[b], Yunhong Tan[d]

[a] The Clinical Laboratory of the First Affiliated Hospital of Hunan Normal University, Changsha, China
[b] Medical College, Hunan Normal University, Changsha, China
[c] School of Chemistry and Chemical Engineering, Hunan University of Science and Technology, Xiangtan, China
[d] The Clinical Laboratory of Hunan Province Chest Hospital, Changsha, China

ARTICLE INFO

Keywords:
LSPR
Tuberculosis
CFP10-ESAT6
Gold nanorods
Biosensor

ABSTRACT

A biosensor based on localized surface plasmon resonance (LSPR) was developed to detect the antibody of *Mycobacterium tuberculosis* using the fusion protein CFP10-ESAT6 as an antigen. To explore the diagnostic potential of the biosensor for tuberculosis (TB), the fusion protein CFP10-ESAT6 was immobilized on gold nanorods (Au NRs) by chemical modification, and the functionalized Au NRs were subsequently incubated with serums collected from TB patients, non-tuberculous pulmonary disease patients or healthy individuals. The change in the LSPR properties of Au NRs from the specific interaction between the antigen and antibody was monitored, and detection of the target antibody was completed based on the proposed biosensor. Serum analysis showed that the sensitivity of the biosensor was 79% and the specificity was 92%. Therefore, the LSPR biosensor is a valuable tool for serodiagnosis of TB.

1. Introduction

Tuberculosis (TB) is one of the world's deadliest infectious diseases mainly caused by infection with *Mycobacterium tuberculosis*. It remains a major public health problem worldwide, as one-third of the world's population is estimated to be infected with *Mycobacterium tuberculosis* and more than 9 million new cases occur annually (Getahun et al., 2015). The major reasons for the high prevalence rate of TB include poor access to effective diagnostic methods and treatments. Furthermore, it is still challenging to detect TB patients with drug-resistant strains of *Mycobacterium tuberculosis* and patients who are co-infected with human immunodeficiency virus (HIV). Therefore, the development of rapid, reliable and effective diagnostic methods for TB is of vital importance for disease control.

Localized surface plasmon resonance (LSPR) technology is a new bio-sensing technology with high sensitivity (Unser et al., 2015) that can be used to monitor the interaction between bio-molecules, such as antigens and antibodies, in real-time and in situ without using any labels (Kim et al., 2007; Raghu et al., 2015). LSPR is a phenomenon caused by the resonant excitation of noble (e.g., Ag and Au) metal nanoparticles' free electrons (Jia et al., 2014; Mayer et al., 2010). Gold nanorods (Au NRs) possess an anisotropic configuration and unique optical properties that exhibit transverse and longitudinal plasmon bands. The longitudinal plasmon band is extremely sensitive to changes in the dielectric properties of the surroundings, producing strong, characteristic absorption bands in the visible to infrared spectrum (Xiang et al., 2010). A significant change in the plasmon spectra in response to a change in the refractive index in the vicinity of the Au NRs arises from LSPR properties and can be effectively utilized to detect specific target binding events. Therefore, we can use the wavelength shift of the LSPR spectrum to detect molecular adsorption to Au NRs.

The fusion protein CFP10-ESAT6 has been shown to be a great candidate antigen for the immunodiagnosis of TB due to its high specificity in enzyme linked immunosorbent assay (ELISA) (Zhang et al., 2010). However, some disadvantages, including tedious washing procedures and antibody labeling, may limit wide use of the method in the detection of TB. In the present study, we established a simple and rapid method for determination of the anti-CFP10-ESAT6 antibody for TB diagnosis based on a LSPR biosensor, and our results demonstrated that the specific CFP10-ESAT6-based LSPR biosensor could potentially be used for TB diagnosis.

2. Materials and methods

2.1. Regents

Cetyltrimethylammonium bromide (CTAB), hydrogen tetrachloroaurate

* Corresponding author at: Department of Medical Laboratory, Medical College of Hunan Normal University, 371 Tong Zi po Avenue, Yue Lu District, Changsha 410013, China.
E-mail address: 30939198@qq.com (S. Yuan).

http://dx.doi.org/10.1016/j.mimet.2017.09.007
Received 29 May 2017; Received in revised form 28 August 2017; Accepted 10 September 2017
Available online 11 September 2017

(III) trihydrate ($HAuCl_4{\cdot}3H_2O$), sodium borohydride ($NaBH_4$), silver nitrate ($AgNO_3$) and L-ascorbic acid (AA) were purchased from Sinopharm Chemical Reagent Co., Ltd. 11-mercaptoundecanoic acid (MUA) was purchased from Sigma. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) were purchased from Aladdin. Chemiluminescence detection kit was purchased from Auragene Bioscience Inc., China. Double distilled water was used in all experiments.

2.2. Antigen

The fusion protein CFP10-ESAT6 was prepared as the antigen in this study. The genes encoding CFP10 and ESAT6 proteins were amplified by PCR using the CFP10 primers (forward primer P1: 5′-CCGGA TCCATGGCAGAGATGAAGAC-3′; reverse primer P2:5′-TTCCACTGC TGCTCTGTGAAGCCCATTTGCGAGG-3′) and the ESAT6 primers (forward primer P3: 5′-CCTCGCAAATGGGCTTCACAGAGCAGCAGTGGAA-3′; reverse primer P4:5′-ATCGTCGACCTATGCGAACATCCCAG-3′) from the genomic DNA of *Mycobacterium tuberculosis* reference strain H37Rv, and the CFP10-ESAT6 fusion gene was subsequently amplified by Gene SOEing. After sequence analysis, the fusion gene was further subcloned into expression vector pQE-30 in the B*am*H I/*Sal* I site. The fusion protein CFP10-ESAT6 was expressed in *E. coli* JM109 and purified with affinity chromatography, and can be stored with 20 M Tris-HCl buffer (pH 8.0) at − 20 °C for six months. The antigenicity of the fusion protein was analyzed by Western blotting.

2.3. Serums

Serum samples from 52 clinically diagnosed TB patients were provided by the clinical laboratory of Hunan Province Chest Hospital following informed consent and approval by the Ethics Committee of Hunan Province Chest Hospital. Serums from 29 non-tuberculous pulmonary disease patients and 39 healthy individuals were provided by the clinical laboratory of Hunan Province People's Hospital following informed consent and approval by the Ethics Committee of Hunan Province People's Hospital. All subjects in the study were aged between 18 and 70 years. Serums were stored at − 20 °C before use.

2.4. Synthesis of Au NRs

The seed-mediated growth methods were described previously (Garcia-Ramirez et al., 2016; Orendorff et al., 2006). In the present study, some experimental parameters were slightly modified as follows: 10 ml of 0.10 M CTAB solution was mixed with 0.24 ml of 0.02 M $HAuCl_4$. 5 ml of double distilled water was added, followed by the addition of 1.2 ml of ice-cold 0.01 M $NaBH_4$. All the regents were added with stirring, resulting in the formation of a light-brown solution. The seed solution was vigorously stirred for 2 min and then kept at 25 °C for 2 h before use.

To create the growth solution, 0.8 ml of 0.01 M $AgNO_3$ and 1.5 ml of 0.02 M $AuCl_3$ were added to 30 ml of 0.10 M CTAB solution in a separate conical flask at 25 °C, followed by the addition of 350 μl of 0.10 M ascorbic acid with stirring for 2 min to make the orange solution fade. Subsequently, 70 μl of the seed solution was added to the growth solution. The resulting mixture was left undisturbed and aged for approximately 2 h at 25 °C. The as-synthesized Au NRs were stored in a refrigerator at 4°C until they were used for analysis within 3 months.

2.5. Chemical modification of Au NRs

Au NRs were interacted with 0.005 M MUA overnight at 25 °C, centrifuged at 14,000 rpm and dispersed with 0.01 M CATB. Next, 0.075 M EDC and 0.015 M NHS were added to the solution for 40 min at 25 °C to activate the Au NRs by linking amidogen to the Au NRs. Then, Au NRs were centrifuged at 8000 rpm and dispersed with double distilled water.

2.6. Conjugating antigen to Au NRs

To conjugate the fusion protein CFP10-ESAT6 to the particles, the activated Au NR solution was mixed with CFP10-ESAT6 (0.456 mg/ml) at a volume ratio of 50:1 and allowed to react for 1 h at 25 °C. The carboxyl group of the protein was connected with the amidogen of the Au NRs to complete the conjugation between the antigen and Au NRs. After attachment of CFP10-ESAT6 onto the Au NRs, the modified Au NRs were collected by centrifugation at 14,000 rpm for 10 min and then resuspended in a 10 mM PBS buffer solution (pH 7.4) containing 0.005 M CTAB. The solution could be stably stored for 3 to 4 weeks at 4 °C.

2.7. Seroanalysis of the biosensor

Seroanalysis was performed to explore the diagnostic potential of the CFP10-ESAT6-based LSPR biosensor for TB. Serum samples from TB patients, non-tuberculous pulmonary disease patients and healthy individuals were diluted at a ratio of $1{:}10^4$ in double distilled water. Serums were incubated with CFP10-ESAT6-conjugated Au NRs at 25 °C for 2 min.

2.8. UV–Visible absorbance

The absorption spectra were collected using a Lambala 35 UV–Visible spectrophotometer over the range from 400 to 1100 nm. The samples were measured against water as a reference. All samples were loaded into a quartz cell for measurements.

3. Results

To establish a biosensor by detecting the antibody of *Mycobacterium tuberculosis* with the fusion protein CFP10-ESAT6 as an antigen, the first step was to express and purify the CFP10-ESAT6 fusion protein. We cloned the CFP10-ESAT6 fusion gene into the pQE30 vector, named the plasmid pQE30-CFP10-ESAT6, and validated the recombinant plasmid by PCR and restriction enzyme digestion with *Bam*HI and *Sal*I. Consistent with the predicted band size, the PCR and restriction enzyme digested products were approximately 600 bp (Fig. 1A). The sequence of the CFP10-ESAT6 fusion gene in pQE30-CFP10-ESAT6 was also confirmed by direct sequencing (data not shown). Moreover, pQE30-CFP10-ESAT6 was transformed into *E. coli* JM109, and CFP10-ESAT6 fusion protein was heterologously expressed and purified using a Ni.NTA column. Subsequently, SDS-PAGE was used to detect the His-CFP10-ESAT6 fusion protein. The Coomassie brilliant blue staining results showed that His-CFP10-ESAT6 fusion protein was specifically purified (Fig. 1B).

To further determine whether the fusion protein His-CFP10-ESAT6 is a detectable antigen for establishment of the biosensor, Western blotting analysis was further preformed. The results showed that the expressed fusion protein His-CFP10-ESAT6 can be specifically detected using anti-His antibodies and that the protein's molecular weight is approximately 25 KD (Fig. 1C). Moreover, Western blotting analysis showed that the His-CFP10-ESAT6 fusion protein can be detected using the serums of TB patients (Fig. 1D), but not the serums of normal subjects (Fig. 1E), indicating that His-CEP10-ESAT6 acted as the detectable antigen, reacting with the corresponding antibodies in the serums of TB patients.

When the antigen was prepared, the key steps for the preparation and serological reaction of the CFP10-ESAT6 LSPR biosensor included the synthesis and chemical modification of Au NRs, conjugation of the fusion protein CFP10-ESAT6 to the Au NRs and seroanalysis of the biosensor (Fig. 2). Serum analysis was performed to explore the diagnostic potential of the CFP10-ESAT6 LSPR biosensor for TB. The as-prepared Au NRs were first characterized by transmission electron microscopy (TEM) image as shown in Fig. 3A, and the corresponding