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A Novel Electrochemical Immunosensor Based on Mesoporous Graphitic Carbon Nitride for Detection of Subgroup J of Avian Leukosis Viruses

Dandi Zhou, Min Wang, Jing Dong*, Shiyun Ai*

College of Chemistry and Material Science, Shandong Agricultural University, Tai'an, Shandong 271018, China

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ABSTRACT

The rapid and sensitive detecting technique for subgroup J of avian leukosis viruses (ALVs-J) needs to be developed as soon as possible in order to not only reduce economic losses but also ensure the food safety. In this paper, a novel electrochemical immunosensor was built based on mesoporous graphitic carbon nitride (mpg-C_3N_4). Mpg-C_3N_4 was used as the sensor platform to bond with the primary antibodies (Ab_1). The compound of thionine and mpg-C_3N_4 (Th-mpg-C_3N_4) was synthesized for the first time to serve as the electroactive probe as well as the carrier of secondary antibodies (Ab_2). Compared to bulk g-C_3N_4, mpg-C_3N_4 possesses larger specific surface area, smaller electrochemical resistance and abundant active sites. So the proposed electrochemical immunosensor exhibited amplified detective signals which realized the sensitive detection of ALVs-J. Under the opitimized conditions, the immunosensor exhibited outstanding analytical performance for the detection of ALVs-J whose titer ranged from $10^{2.08}$ to $10^{4.0}$ $TCID_{50}$/mL ($TCID_{50}$: 50% tissue culture infective dose) with a low detection limit of 120 $TCID_{50}$/mL (S/N = 3). The sensitivity of the immnosensor was 6.15 μA/($TCID_{50}$/mL). This high sensitive immunosensor also displayed good selectivity, reproducibility and stability. Last but not least, this new strategy may be of great promise for clinic application in the future.

1. Introduction

Avian leukosis viruses (ALVs) are the most common [1] avian retroviruses occurring in chickens which cause neoplastic diseases and some other production problems. They are divided into six subgroups (A to E and J) [2]. Among them, subgroup J of avian leukosis virus (ALVs-J) [3] has the strongest infectiousness which is extremely harmful to our food industry [4]. As far as we are concerned, there are no efficient vaccine or drugs for the eradication of ALVs-J until now. Selecting the healthy chicken from the population is the most effective measure [5]. Therefore, the effective methods for the detection of ALVs-J need to be developed as soon as possible. So far, a few detecting ways has been reported, for example enzyme-linked immunosorbent assay (ELISA) [6], polymerase chain reaction (PCR) [7] and surface plasmon resonance (SPR) [8]. However, the methods mentioned above usually take several days to complete the whole progress. Unlike the traditional ways, electrochemical immunosensors with sandwich-type structure are getting more and more popular for their advantages such as rapidness, high sensitivity, low cost and etc.

The analytical performance of electrochemical immunosensors is partly dependent on the modification material. Recent years, graphitic carbon nitride (g-C_3N_4) composed of only C, N, and some impurity H [9] has attracted much attention since it is similar to graphene [10]. Because g-C_3N_4 is a medium semiconductor and an effective photocatalyst, the application of g-C_3N_4 for biosensors mostly focuses on photoelectrochemistry (PEC) and electrochemiluminescence (ECL) up till now. Li et al. developed a PEC immunoassay using caboxylated g-C_3N_4 as the platform [11]. Chen et al. designed an ECL immunosensor for the detection of carcinoembryonic antigen (CEA) based on the gold nanoparticle-g-C_3N_4 nanohybrids [12]. However, the g-C_3N_4 they used were bulk materials [13] synthesized by self-condensation of organic precursors. Due to the intrinsic properties of bulk g-C_3N_4, the detective signal was weak and unstable.

Unlike bulk g-C_3N_4, mesoporous g-C_3N_4 (mpg-C_3N_4) possesses not only the properties of bulk g-C_3N_4 but lots of other features such as large surface areas, tunable pore diameters and large pore volumes [14,15]. What's more, compared to bulk g-C_3N_4, mpg-C_3N_4

* Corresponding author. Tel.: +86 538 8249248; fax: +86 538 8242251.
E-mail addresses: dongjing@sdau.edu.cn (J. Dong), ashy@sdau.edu.cn (S. Ai).

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has faster electron transfer rate, better biocompatibility and more sensing sites [16,17]. Besides, the strong electron donor nature of N promotes the π-π electron transition which improves the stability [18,19]. These particular characteristics authentically make it more appropriate to be the platform and conjugate with other nanomaterials or biomolecules.

Thionine (Th) is an electron transfer mediator which has been widely used [20,21] in the development of electrochemical immunosensors. Composites of graphene and Th have been synthesized successfully by former researchers [22,23]. Since mpg-C_3N_4 shares common with graphene, we integrated mpg-C_3N_4 and Th to be the signal probe to enhance the detecting sensitivity. As far as we know, there is few published data related to the Th-mpg-C_3N_4 nanocompound. Herein, we designed a novel electrochemical immunosensor with sandwich structure to detect ALVs-J. Mpg-C_3N_4 was used as the platform which extremely increased the specific surface area of the electrode. As a result, considerable primary antibodies (Ab_1) were bonded onto the electrode. Furthermore, Th-mpg-C_3N_4 was synthesized for the first time to act as the carrier of secondary antibodies (Ab_2) as well as the electroactive probe of the electrochemical immunosensor. Because mpg-C_3N_4 has smaller resistance and bigger specific surface area than g-C_3N_4, the immunosensor fabricated using mpg-C_3N_4 exhibited improved electron transfer rate and amplified detection signals. Consequently, the sensitivity of the immunoassay is significantly enhanced. Besides, the great detection performance towards ALVs-J makes this original immunosensor have great promise in clinical analysis.

2. Experimental

2.1. Reagents and apparatus

Cyanamide ($CN\text{-}NH_2$) and ammonium bifluoride (NH_4HF_2) were from Aladdin (Shanghai, China). 30% dispersion of SiO_2 with an average diameter of 12 nm was obtained from Jiangsu Guolian Technology Co., Ltd. Thionine (Th) was purchased from Chengdu Ai Keda Chemical Technology Co., Ltd. Bovine serum albumin (BSA) was from Sigma (USA). A serum specimen with ALVs-J and ALVs-J antibodies (Ab) were provided and purified by the skilled colleagues from the College of Animal Science and Technology, Shandong Agricultural University (Taian, China). Other chemicals were analytical reagent grade and used without further treatment.

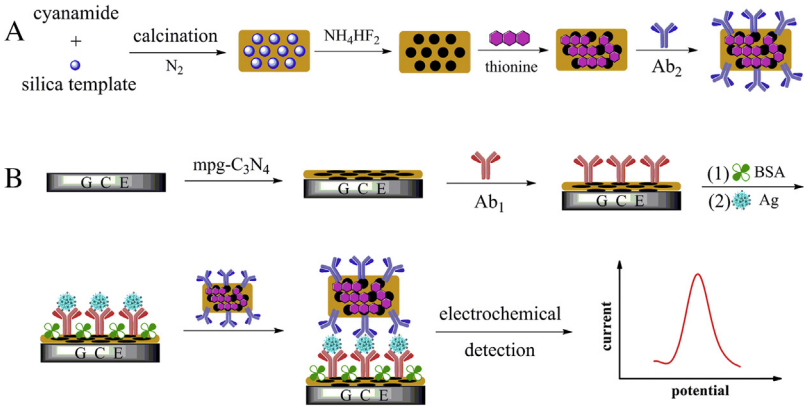
Electrochemical experiments were performed on a CHI660C electrochemical workstation (Shanghai Chenhua Co., China) with a conventional three-electrode system. A bare or modified glassy carbon electrode (GCE) with a diameter of 3 mm was used as the working electrode. A saturated calomel electrode (SCE) and a platinum wire were employed as the reference electrode and auxiliary electrode, respectively. The transmission electron microscope (TEM) images were obtained at a 100CX II transmission electron microscope (JEM, Japan). X-ray diffraction (XRD) pattern was recorded by a D8 Advance X-ray diffractometer using Cu Kα radiation (λ = 0.15416 Å, Bruck, Germany), ranging from 5 to 80 with a speed of 4/min. Fourier transform infrared (FT-IR) spectra were recorded on a Nicolet 380 FT-IR spectrometer (Thermo electron Co., USA). N_2 absorption-desorption isotherm was taken with an NOVOE 4000/TriStar II 3020. All the measurements were carried out at room temperature (25 ± 0.5 °C).

2.2. Preparation of mpg-C_3N_4 and bulk g-C_3N_4

The mpg-C_3N_4 was synthesized by the hard template method as previous reports [24]. An amount of 6.0 g cyanamide was added dropwise into 10 g of a 30% dispersion of 12-nm SiO_2 particles, which were used as the hard template. The mixture was kept at 60 °C over night to remove water. Then, the acquired white powder was heated to 550 °C (2.3 °C min^{-1}) and kept at the specific temperature for 4 h. The yellow product was treated with 4 M NH_4HF_2 for 36 h under stirring to remove the silica template. The solution was centrifuged at 4000 rpm for 15 min to eliminate the supernatant liquid and the precipitate was washed by double distilled water for three times. In the end, the obtained mpg-C_3N_4 was dried at 60 °C overnight. TEM was used to study the morphologies and structures of the prepared sample. The crystallography phase and composition of the mpg-C_3N_4 was assayed by XRD. The bulk g-C_3N_4 was prepared under the same conditions by employing melamine as precursor without adding hard template.

2.3. Preparation of the Th-mpg-C_3N_4-Ab_2 bioconjugates

Since mpg-C_3N_4 is similar to graphene, the Th-mpg-C_3N_4 composite was prepared following the synthetic procedure of the Th-graphene [25]. As shown in Scheme 1A, the obtained mpg-C_3N_4 was re-suspended in double-distilled water after ultrasonication for 30 min to form the mpg-C_3N_4 suspension (1 mg/mL). Th was added to double-distilled water to get the Th solution (1 mg/mL). 10 mL of the Th solution was added dropwise into 10 mL of the mpg-C_3N_4 suspension and stirred for 48 h at room temperature. The mixture was centrifuged at 12000 rpm and washed thoroughly to collect the sediment. In this process, Th was covalently bonded onto mpg-C_3N_4 through π-π stacking. The synthesized Th-mpg-C_3N_4 was dispersed in 20 mL 0.075 wt% glutaraldehyde (GA) solution, stirred for 4 h, and washed with double-distilled water for three times. Next, a certain amount of Ab_2 was added and the



Scheme 1. Schematic representation of the preparation of Th-mpg-C_3N_4-Ab_2 (A) and fabrication of the immunosensor (B).