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MCM-41 mesoporous material modified carbon paste electrode for the determination of cardiac troponin I by anodic stripping voltammetry

Huishi Guo [a,b], Nongyue He [a,b,c,*], Shuxun Ge [a], Di Yang [b], Jinan Zhang [b,**]

[a] *State Key Laboratory of Bioelectronics (Chien-Shiung Wu Laboratory), Southeast University, Nanjing 210096, China*
[b] *Institute of Cardiovascular Disease, Nanjing Medical University, Nanjing 210029, China*
[c] *Laboratory of NanoBio Medicines and Devices, Zhuzhou Institute of Technology, Zhuzhou 412008, China*

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Abstract

An anodic stripping voltammetric method for the determination of cardiac troponin I (cTnI) at a MCM-41 mesoporous material modified carbon paste electrode (MCM-MCPE) was investigated. The test was based on the dual monoclonal antibody "sandwich" principle using colloidal gold as a labeled substrate. Four main steps were carried out to obtain the analytical signal, i.e. electrode preparation, immunoreaction, silver enhancement, and anodic stripping voltammetric detection. The anodic stripping peak current increased linearly with the concentration of cTnI over the range of 0.8–5.0 ng/ml. A detection limit of 0.5 ng/ml was obtained. The established method was applied to detect cTnI in acute myocardial infarction (AMI) samples using routine enzyme-linked immunoadsorbent assay (ELISA) for comparison analysis, and good results were obtained.

Keywords: Cardiac troponin I; Mesoporous materials; MCM-41; Carbon paste electrode; Anodic stripping voltammetry

1. Introduction

It is a long history to immobilize proteins (enzymes) on solid inorganic materials [1,2]. One of the most widely used methods is to encapsulate them inside sol–gel-derived materials [3]. However, due to the small pore size, most studies showed lower specific activity than that of the free one in solution [4]. MCM-41 mesoporous material is a new kind of molecular sieves, the synthesis of which was first reported by researchers of the Mobil Oil Corporation [5,6]. The unusual properties such as large pore size, uniform pore structure, high surface areas, and high loading capacity of MCM-41 have attracted increasing attention in the immobilization of larger biomolecules [7].

Recently, electrochemical methods based on the specific reaction of antibody and antigen with electrochemical transduction have received considerable attention in clinical diagnosis [8–11]. Most of these methods have been commonly achieved in connection to electroactive indicators or enzyme tags [12–14]. Nanoparticle tracers are the possible substitute for electrical detection. Metal nanoparticles, especially gold nanoparticles (nano-gold), possess many unique physical and chemical properties, and therefore, have been intensively studied in analytical chemistry [15–18]. For example, due to that nano-gold particles can strongly interact with biomaterials, they have been used in non-enzyme immunoassays [19–23].

Cardiac troponin I (cTnI) is a subunit of cardiac troponin complex, which is comprised of troponin I, troponin T and troponin C. The molecular weight of cTnI is 22.5 kDa. Following the myocardial damage, the troponin complex is broken up and the individual protein components are released into the bloodstream [24]. Cardiac troponin I has high tissue specificity because of its structural difference from the corresponding skeletal isoforms in its amino acid composition. This distinction allows the two forms of troponin I to be dis-

* Corresponding author. Tel.: +86 25 83792245; fax: +86 25 83619983.
** Corresponding author.
E-mail addresses: nyhe1958@163.com (N. He), jinanzh@sina.com (J. Zhang).

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tinguished immunologically and thereby ensures an accurate test assay [25–27]. Therefore, cTnI in the serum of patients has been considered as the "gold standard" for diagnosis of myocardial injury [28–30]. And many methods have been developed for detecting cTnI [31–37].

The aim of the present study is to establish a novel electrochemical immunoassay of cardiac troponin I. This assay combines the concepts of the dual monoclonal antibody "sandwich" principle, the nanoparticle-promoted precipitation of silver, the modified carbon paste electrode, and the anodic stripping voltammetry.

2. Experimental

2.1. Reagents and apparatus

cTnI from human myocardium muscle and two monoclonal anti-human cTnI antibodies used in the assay were obtained from Research Institute of Cardiovascular Disease of First Affiliated Hospital of Nanjing Medical University. Colloidal gold was purchased from Sino-American Biotechnology Company (China). Other reagents were commercially available and were all of analytical grade. Deionized water was used in all experiments.

The colloidal gold-labeled detection antibody (cAu–IgG_2) conjugates were centrifuged at a BECKMAN Avanti^{TM} J-25 Centrifuge. Transmission electron micrographs were recorded with a Hitachi 8100 transmission electron microscope (TEM). All electrochemical experiments were carried out on a CHI600A electrochemical workstation (CH Instruments, Inc., USA) with a platinum wire used as the counter electrode and a Ag/AgCl electrode as the reference. The enzyme-linked immunoadsorbent assay (ELISA) comparison analysis was performed on a EL_X808_{IU} Ultra microplate reader (Bio-tek Instruments, Inc. USA).

2.2. Synthesis and characterization of MCM-41 mesoporous material

The MCM-41-type mesoporous material was synthesized in ethylenediamine aqueous solution medium referred to the procedure described by Yang et al. using cetyltrimethylammonium bromide (CTAB) as templates [38]. The physical properties of MCM-41 mesoporous material were characterized using transmission electron microscopy, X-ray powder diffraction, and nitrogen physisorption isotherm. The structural data are summarized in Table 1.

2.3. Preparation of the modified carbon paste electrode

Glass tubes (4 mm inner diameter) were served as the electrode body. Electrical contact was made with a copper wire through the center of the tube. Unmodified carbon paste was prepared by adding 0.5 g of paraffin oil to 2.0 g of high purity graphite powder. Modified carbon pastes were prepared by substituting corresponding amounts of the graphite powder (1.0%, 2.0%, 5.0%, 10.0%, and 20.0%, w/w) with MCM-41 mesoporous material and then adding the paraffin oil and thoroughly hand-mixing in a mortar with a pestle. The pastes were packed into the hole of glass tube and the surface was polished on a sheet of graph paper while a slight manual pressure was applied to the piston.

Table 1
Pore characterization of MCM-41 mesoporous material

Surface area, BET (m^2/g)	961.9
Pore volume (cm^3/g)	0.75
d_{100}-Spacing (Å)	38.4
a_0[a] (Å)	44.3
Pore size[b] (Å)	31.0

[a] $a_0 = 2d_{100}/\sqrt{3}$.
[b] Calculated from N_2 adsorption isotherms.

2.4. Preparation and characterization of the colloidal gold-labeled detection antibody

Both solutions of colloidal gold and detection antibody (IgG_2) were adjusted to pH 8.2 before the label procedure. To 50 ml of colloidal gold solution, 1.13 mg of IgG_2 was added dropwise under constant stirring at room temperature. After the addition of an appropriate concentration of bovine serum albumin (BSA), 15 min incubation under constant stirring was required. The mix was centrifuged in the cold at 300 × *g* for 20 min and the pellet was discarded. The supernatant was then centrifuged in the cold at 10,000 × *g* for 1 h and the pellet of the colloidal gold–IgG_2 complex was resuspended in 5 ml of 0.05 M Tris(hydroxymethyl)aminomethane (Tris)–C1 buffer solution (TB, pH 8.2) containing 0.1% BSA. Finally, the mix was centrifuged in the cold at 300 × *g* for 20 min, and the supernatant was harvested and stored in a refrigerator for further use. The cAu–IgG_2 conjugates could be preserved for at least 6 months under this condition.

TEM technique was used to analyze the size and structure of the unlabeled and labeled colloidal gold (Fig. 1). A brief description of the procedure was as follows. Gold particle samples were prepared by dropping 10 μl of nanoparticle solutions onto a formvar membrane coated on a copper grid.

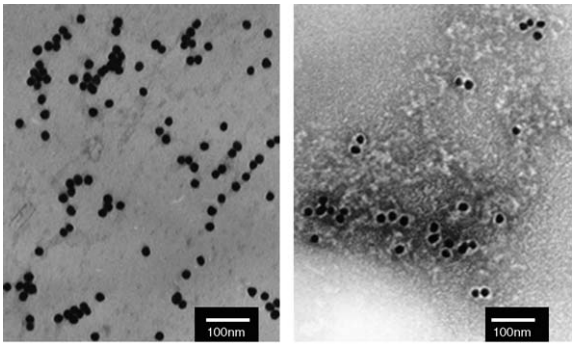

Fig. 1. TEM images of (A) unlabeled and (B) labeled colloidal gold after uranium acetate negative staining.