Biosensors and Bioelectronics 35 (2012) 313–318

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

The application of Au nanoclusters in the fluorescence imaging of human serum proteins after native PAGE: Enhancing detection by low-temperature plasma treatment

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ARTICLE INFO

Article history:
Received 1 February 2012
Accepted 6 March 2012
Available online 16 March 2012

Keywords:
Au nanoclusters
Fluorescence imaging
Human serum protein
Low-temperature plasma
Polyacrylamide gel electrophoresis

ABSTRACT

Proteins in human serum are increasingly being studied for their roles in a wide variety of biochemical interactions. To improve the sensitivity of the detection of human serum proteins after native polyacrylamide gel electrophoresis (PAGE), we have developed a fluorescence imaging detection technique for the detection. BSA (bovine serum albumin)-stabilized Au nanoclusters (NCs) were applied as fluorescent probes for imaging, and low-temperature plasma (LTP) treatment of the Au NCs was introduced to enhance the fluorescence imaging. Here, a series of optimization experiments (e.g. those to optimize for pH) were conducted for protein detection after 1-DE and 2-DE, and several types of discharge gases (He, O_2, and N_2) were selected for the LTP treatment. The possible mechanism of interaction between the proteins and the Au NCs was demonstrated by an isothermal titration calorimetry experiment. Using the present method, a sensitivity of 7–14 times higher than that of traditional staining detection methods was observed in the oxygen LTP-treated Au NCs fluorescence images, and some relatively low abundance proteins (identified by the MS/MS technique) were easily detected. In addition, this fluorescence imaging method was applied to distinguish between the serum samples of patients with liver diseases and those of healthy people. Thus, this fluorescence imaging method is suitable for the highly sensitive detection of various serum proteins, and it shows potential capabilities for clinical diagnosis.

1. Introduction

Human serum proteins are useful diagnostic tools, and the alteration of the expression of some serum proteins is an early sign of an altered physiology (Ahmed et al., 2003; Tombelli et al., 2005). The detection of proteins in serum is important to public health. Proteins in human serum have been studied since before we knew genes existed. The application of current proteomic technologies is limited by the presence of high abundance proteins, such as albumin and immunoglobulins, that constitute approximately 60–97% of the total serum protein content; such proteins hinder the characterization of the hundreds of low abundance proteins (Anderson and Anderson, 2002). Typically, polyacrylamide gel electrophoresis (PAGE), including one-dimensional gel electrophoresis (1-DE) and two-dimensional gel electrophoresis (2-DE), is considered to be a classic measure for the separation of human serum or other complex biological samples (He and Herr, 2009; Taoka et al., 2000). Of the existing protein detection methods which are performed after PAGE, Coomassie Brilliant Blue R250 (CBB-R250) staining is the most frequently used method, whereas silver staining is regarded as the most sensitive technique (Chromy et al., 2004; Huang et al., 2010; Na et al., 2009; Suzuki and Yokoyama, 2005; Xiong et al., 2007). Colorimetry is mainly based on the absorption of visible light by colored matter; therefore, these colorimetric staining detection methods have various deficiencies, such as tedious and complicated staining and destaining steps and high backgrounds, which lead to low sensitivity and resolution. More advanced probes and detection methods must be developed to reap superior benefits from serum protein detection.

Noble metal nanoclusters (NCs; which consist of several to tens of atoms) of sizes comparable to the Fermi-wavelength of the conduction electrons exhibit molecular-like properties such as luminescence and unique charging properties. NCs are considered to be a new variety of fluorophores because of the bright fluorescence the NCs emit following their discrete and size-tunable electron transition from the excited states (Sakamoto et al., 2009; Zheng et al., 2007). Compared with the traditional semiconductor quantum dots (DQs), which are larger in dimension and typically contain toxic metal species, noble metal nanoclusters are

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doi:10.1016/j.bios.2012.03.010

attractive because of their photostability, ultrafine size and non-toxicity. Recently, many types of noble metal nanoclusters have been synthesized (Huang et al., 2009; Xie et al., 2009; Zhang et al., 2009; Zhou et al., 2011), however, some of these syntheses often produced large nanoparticles as by-products. Recently, novel BSA-stabilized Au nanoclusters have been synthesized by a one-pot, "green" method, with bovine serum albumin (BSA) as the protecting agent and the reducer in the reaction (Xie et al., 2009). These BSA-stabilized Au NCs have been used in solutions with a broad of pH values (3–12) and in various buffer solutions, and the BSA-stabilized Au NCs have already been employed in many applications (Li et al., 2009; Liu et al., 2010; Retnakumari et al., 2010; Zhou et al., 2006). Furthermore, the BSA-stabilized Au NCs have a red emission at approximately 640 nm which is far from the emission of some fluorescent proteins; the Au NC-based fluorescence detection method has little interference, low background and high sensitivity. Therefore, the use of BSA-stabilized Au NCs as fluorescent probes for the detection of human serum proteins provides the possibility for developing a new advantageous detection method after PAGE.

Low-temperature plasma (LTP) treatment is regarded as a selective process to modify the chemical and topographical properties of biomaterials and polymers (Alves et al., 2007; Boulares-Pender et al., 2009; Favia et al., 2006; Forte et al., 2007; Kan and Yuen, 2006). As a popular modification technique, the LTP process has an amazing ability to change the surface characteristics of materials with a negligible influence on their bulk properties or on the environment. Of the different types of discharge gases, oxygen, which mainly introduces a large amount of various reactive functionalities (e.g. $-NH_2$, $-OH$) onto the surfaces of treated materials, is widely used. It has been established that the LTP technique can be used to alter surface functionality of the treated materials and to improve further reactions (e.g. immobilization) (Gancarz et al., 2003; Gates et al., 2008; Molina et al., 2003; North et al., 2010; Sophonvachiraporn et al., 2011; Svorcik et al., 2010). Therefore, LTP treatment has the potentials to improve the complexation of BSA–Au NCs and proteins in gels, which may effectively enhance the Au NC-based fluorescence imaging detection.

In this study, after native PAGE, gels were treated with a subsequent BSA-stabilized Au NC-based fluorescence imaging method. These BSA-stabilized Au NCs were synthesized by reducing Au^{3+} in a bovine serum albumin solution; simultaneously, the BSA-stabilized Au NCs were treated with LTP and used in subsequent fluorescence imaging. Oxygen LTP treatment for Au NCs was demonstrated as an effective measure for providing fluorescent probes with higher sensitivity. This procedure combined the BSA-stabilized Au NCs and on-gel proteins via van der Waals force and hydrogen-bonding interaction in an acidic environment (pH 3.3). The fluorescence images were stable, no organic solvent was used during the whole experiment and no additional steps (e.g. destaining, immobilizing) were used after the gels were incubated in the Au NC solution. Hence, a simple and sensitive method for the detection of human serum proteins with noble nanoclusters was established.

2. Materials and methods

2.1. Reagents

All reagents were of analytical reagent grade. Water was deionized and further purified with a Milli-Q water purification system (Millipore, Milford, MA). Acrylamide (Acry), *N*,*N*′-methylenebisacrylamide (Bis), bovine serum albumin (BSA), N, N, *N*′,*N*′-tetramethylethylenediamine (TEMED), aminoacetic acid (glycine), and tris(hydroxymethyl)aminomethane (Tris) were purchased from Dingguo Biotech Company (Beijing, China). glycerine, bromophenol blue, ammoniumpersulfate, auric chloride acid ($AuCl_3{\cdot}HCl{\cdot}4H_2O$), acetic acid (HAc), sodium hydroxide (NaOH), hydrochloric acid (HCl) and sodium acetate (NaAc) were purchased from Beijing Reagents Company (Beijing, China). Human serum samples were obtained from the Affiliated Hospital of the Beijing Normal University (Beijing, China).

2.2. Instruments

The electrophoresis systems were made up of DYCZ-21 and DYCZ-24D vertical electrophoresis tanks, together with the electrophoresis instruments of DYY-6B and DYY-6C, which were purchased from Liuyi Instrument Factory (Beijing, China). The low-temperature plasma (LTP) was produced by dielectric barrier discharge (DBD) reactor. The images were formed by using the fluorescence bioimaging system (UVP EC3 Imaging System, UVP Inc., USA).

2.3. Preparation of BSA-stabilized Au NCs

The BSA-stabilized Au NCs were synthesized according to a preceding report (Xie et al., 2009). In brief, aqueous $HAuCl_4$ solution (5 mL, 10 mM, 37 °C) was added to BSA solution (5 mL, 50 mg/mL, 37 °C) under vigorous stirring. NaOH solution (0.5 mL, 1 M) was introduced after 2 min, and the mixture was incubated at 37 °C for 12 h.

2.4. Gel electrophoresis

The native 1-D PAGE was performed in a vertical discontinuous gel system, including separating (7.5%, m/v) and stacking (4.0%, m/v) gels. Gel stock solution (30%, w/v) contained 29.2 g of acrylamide and 0.8 g of Bis, which were dissolved in 100 mL of H_2O and filtrated. The stacking gel (4%, m/v) solution was prepared by mixing 0.7 mL of gel stock solution, 1.25 mL of Tris–HCl (0.5 M, pH 6.8), 40 μL of $(NH_4)_2S_2O_8$ (10%, w/v), and 4 μL of TEMED and then diluting the mixture to 5.0 mL; 4 mL of gel stock solution was mixed with 4 mL of Tris–HCl (1.5 M, pH 8.8), 8 mL of ultrapure water, 150 μL of $(NH_4)_2S_2O_8$ (10%, w/v), and 15 μL of TEMED to prepare the separating gel solution (7.5%, m/v). The voltage was set at 120 V when the serum sample was in the stacking gel, and tuned to 90 V for about 3 h after it entered into the separating one. For the native 2-D PAGE, proteins in serum were suffered from isoelectric focusing (IEF) in the denaturants employing column gels, run at 200 V for 30 min, and then kept at 400 V for 16 h. The isoelectric focusing gel solution was prepared with 0.45 mL of gel stock solution, 0.135 mL of carrier ampholytes, 11.5 μL of $(NH_4)_2S_2O_8$ (10% m/v), and 2.5 μL of TEMED, then diluted with ultrapure water to 2.25 mL. After IEF, gels were transferred onto the second dimension-slab gels (7.5%, m/v), and the voltage was 100 V. Separating gel solution for the second dimension of 2-DE was the same with that of 1-DE. Sample dilution solution was prepared with 6.67% glycerine and 0.05% bromophenol blue, and the loading volume for each channel was 15 μL.

2.5. Fluorescence imaging via BSA-stabilized Au NCs

The BSA-stabilized Au NCs imaging solution was obtained by adding BSA-stabilized Au NCs into NaAc solution (pH 3.3). After the electrophoresis, the gels were incubated in the imaging solution and stored at 4 °C. The images were acquired by the bioimaging system at 365 nm as the excitation wavelength.