Biosensors and Bioelectronics 43 (2013) 366–371

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

A gold nanoparticles-based colorimetric assay for alkaline phosphatase detection with tunable dynamic range

Chun Mei Li [a], Shu Jun Zhen [a], Jian Wang [b], Yuan Fang Li [a], Cheng Zhi Huang [a,b,*]

[a] Education Ministry Key Laboratory on Luminescence and Real-Time Analysis, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, PR China

[b] College of Pharmaceutical Sciences, Southwest University, Chongqing 400715, PR China

ARTICLE INFO

Article history:
Received 5 October 2012
Received in revised form
3 December 2012
Accepted 8 December 2012
Available online 14 December 2012

Keywords:
Gold nanoparticles (AuNPs)
Alkaline phosphatase (ALP)
Colorimetric
Metal ions
Tunable dynamic range

ABSTRACT

In this report, a simple and label-free colorimetric assay was developed for detecting alkaline phosphatase (ALP). Based on the conjugated gold nanoparticle/adenosine triphosphate (AuNP/ATP) sensing system, this assay is highly sensitive and selective. In this system, ATP induces the aggregation of cetyltrimethylammonium bromide (CTAB)-capped AuNPs and ALP stimulates the disaggregation of AuNPs by converting ATP into adenosine through an enzymatic dephosphorylation reaction. Hence, the presence of ALP can be visually observed (gray-to-red color change) and monitored by the shift of the surface plasmon resonance (SPR) absorption band of AuNPs. Furthermore, the dynamic range of the method can be varied by addition of different metal ions (e.g. 100–600 unit/L to 5.0–100 unit/L and 0.2–20 unit/L in the presence of Ca^{2+} and Pb^{2+}, respectively). The feasibility of this sensitive and specific assay with a tunable dynamic range was demonstrated to be consistent even in human serum samples.

1. Introduction

In the development of sensors for practical applications, tunable dynamic range is of great significance, as the content of the analyte may vary considerably under different circumstances. A number of sensors with tunable dynamic range have been developed for various kinds of analytes, including metal ions (e.g., Hg^{2+} (Chen et al., 2011; Xu et al., 2009), Pb^{2+} (Wang et al., 2008b; Wen et al., 2011), Ca^{2+} (Kim et al., 2011)), small molecules (e.g., adenosine (Xiang et al., 2009)), and amino acids (Pu et al., 2010)). To the best of our knowledge, few sensors have been reported for the detection of proteins with tunable dynamic range, especially for the proteins whose concentrations vary among different human organs.

Alkaline phosphatase (ALP) is one of the most commonly assayed enzymes in clinical practice to diagnose different types of diseases and it is commonly used as a biomarker for the development of enzyme immunoassays, gene assays, molecular biology and related affinity sensing methods for monitoring nucleic acids and other analytes (Bronstein et al., 1996; Fanjul-Bolado et al., 2007; Nistor and Emnéus, 1998). It is distributed at various levels in human organs, more in the liver and bones, and less in placenta, intestines and kidneys (Gyurcsányi et al., 2002; Hartwell et al., 2007). Under-expressed levels of serum ALP have been reported as a rare form of rickets (known as hypophosphatasia), while overexpressed levels of serum ALP are usually associated with several diseases, including bone disease, liver dysfunction, prostatic cancer and bile duct blockage (Gyurcsányi et al., 2002; Jia et al., 2010; Ooi et al., 2007). In addition, the normal levels of ALP may differ by gender and age (Eastman and Blxler, 1977). For instance, the normal range of serum ALP in adults is about 46–190 unit/L, while children and pregnant women tend to have higher levels (more than 500 unit/L) (Hausamen et al., 1967). Therefore, developing a strategy to detect ALP with high sensitivity, selectivity and with tunable dynamic range is very important in diagnostic and clinical assays.

A large variety of assays have been developed for ALP activity determination, including chromatographic (Hasegawa et al., 2006), chemiluminescent (Blum et al., 2001; Ximenes et al., 1999), fluorometric (Jia et al., 2010; Liu and Schanze, 2008; Nutiu et al., 2004), electrochemical (Limoges and Degrand, 1996; Ruan and Li, 2001), and Surface Enhanced Resonance Raman Scattering (SERRS) (Ruan et al., 2006) assays. Even though some of these assays have shown high sensitivity, most of them suffer from laborious procedures for the synthetic substrates. In contrast, a rapid and cost-effective colorimetric method is relatively easy to perform. For example, ALP activity can be easily detected based on the conversion of *p*-nitrophenylphosphate (PNPP) to *p*-nitrophenol (PNP), which is highly colored with an absorption maximum at 405 nm (Iqbal, 2011; Thompson et al., 1991). However, low selectivity and interferences arising from compounds with absorption wavelengths similar to the reagent still limit the use of this method in clinical applications.

* Corresponding author at: College of Pharmaceutical Sciences, Southwest University, Chongqing 400715, PR China. Tel.: +86 23 68254659; fax: +86 23 68367257.
E-mail address: chengzhi@swu.edu.cn (C.Z. Huang).

0956-5663/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2012.12.015

Recently, metal nanoparticles such as gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) have been used as signal reporters in colorimetric assays for ALP. These methods are normally based on distinguishable colors and colloidal stability in the absence or presence of ALP (Wei et al., 2008; Zhao et al., 2007). In particular, AuNP-based label-free colorimetric assays have received much consideration because of their intrinsic characteristics, such as biocompatibility, high extinction coefficients, and strongly distance-dependent optical properties (Daniel and Astruc, 2004; Ghosh and Pal, 2007). However, the detection limits of these assays (1000 unit/L) are much higher than the normal values in clinical practice. Most importantly, few of these assays have a tunable dynamic range to meet the requirement of ALP detection under different circumstances. As a result, diagnostic assays with high sensitivity, selectivity, and a tunable dynamic range are still desired to match the ALP content in a given sample, thereby avoiding the complicated process of enrichment or dilution.

In this study, a label-free colorimetric assay was developed for monitoring the activity of ALP by using cetyltrimethylammonium bromide (CTAB)-capped AuNPs. Surface coverage by CTAB allows AuNPs to be highly stable in aqueous solution. Adenosine triphosphate (ATP) was chosen as the substrate for the ALP dephosphorylation reaction, because ATP can induce the aggregation of CTAB-capped AuNPs, while the product after dephosphorylation reaction (adenosine) leaves the AuNPs dispersed. By monitoring the shift of the SPR absorption band or by visual observation of the color changes of AuNPs from gray to red, a label-free colorimetric assay for the detection of ALP was developed, with comparable or even better sensitivity and selectivity compared to conventional colorimetric and fluorescent methods (Jia et al., 2010; Song et al., 2010; Wei et al., 2008; Zhao et al., 2007). Furthermore, in this work, just by rationally introducing different metal ions into the sensing system, the detection level and dynamic range can be tuned over several orders of magnitude without having to develop new sensors for clinical diagnosis applications.

2. Experimental

2.1. Chemicals and materials

ATP was obtained from Sigma (St. Louis, MO). Adenosine was supplied by Shanghai Lanji Technology Co., Ltd. (Shanghai, China). Sodium borohydride ($NaBH_4$) was obtained from Huanwei Fine Chemical Co., Ltd. (Tianjin, China). CTAB and hydrogen tetrachloroaurate (III) tetrahydrate ($HAuCl_4 \cdot 4H_2O$) were purchased from Sinopharm Group Chemical reagent Co., Ltd. (Shanghai, China). All of the above reagents were prepared by dissolving commercial products in double distilled water (18.2 MΩ). Calf intestine alkaline phosphatase (ALP) was purchased from Sigma, with the enzyme buffer of 10 mM Tris–HCl, pH 8.2, 50 mM KCl, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 50% (w/v) glycerol. The 1 × reaction buffer for ALP was 50 mM Tris–HCl (pH 9.0) containing 1 mM $MgCl_2$. Thrombin was purchased from Hematologic Technologies Inc. (USA). Lysozyme and human serum albumin (HSA) were obtained from Sigma. Pepsin was purchased from Shanghai Biochemicals (Shanghai, China). Cellular prion protein (PrP^C) was expressed and purified in our laboratory. All protein solutions were prepared by serial dilution with the same buffer as ALP.

2.2. Experimental instrumentation

UV–vis spectra were recorded with a Hitachi U-3010 spectrophotometer (Tokyo, Japan). Scanning electron microscope (SEM) images of AuNPs were obtained using a Hitachi S-4800 scanning electron microscope (Tokyo, Japan) at 30.0 kV. A Zetasizer Nano-ZS System (Malvern Inc.) was used for DLS measurements with the detector angle of 173° and Zeta potential measurements. The data analysis was carried out by calculating the mean of three successive measurements. A vortex mixer QL-901 (Haimen, China) was employed to blend the solution.

2.3. Preparation of AuNPs

Positively-charged AuNPs were prepared following literature protocol by a seed-mediated method with a two-step procedure, in which CTAB was employed as a stabilizer capped on the AuNP surfaces (Sau and Murphy, 2004; Wang et al., 2008a). The diameters of the prepared nanoparticles were 26 ± 3 nm, and they could be synthesized in high quality with good reproducibility. In this contribution, the concentration of as-prepared AuNPs solution is calculated to be 0.7 nM based on the extinction coefficients of $1.3 \times 10^9\ M^{-1}\ cm^{-1}$ for 23 nm diameter gold spheres (Orendorff and Murphy, 2006).

2.4. Colorimetric detection of ALP

The AuNP-based colorimetric assay was performed as follows (referred to as procedure I): First, the mixture of 5 μL ATP (5 mM) and 10 μL different concentrations of ALP (0–1000 unit/L) were placed in a 1.5 mL plastic tube. The solution was further diluted to 50 μL with 1 × reaction buffer, and then incubated in a 37 °C water bath for 45 min. Second, 200 μL of AuNPs (0.7 nM) was added into the solution mentioned above, and then incubated at room temperature for 10 min. Finally, the mixture was diluted to 500 μL with double distilled water and the absorption spectra were recorded using a Hitachi U-3010 spectrophotometer.

To examine the influence of metal ions on the enzymatic dephosphorylation reaction, 10 μL Ca^{2+}, Pb^{2+} or other metal ions (1 mM) was added to the mixture during the first step. All other conditions were kept identical to procedure I (referred to as procedure II).

For the detection of ALP in human serum, 19 freshly obtained human blood samples (from the Ninth People's Hospital of Chongqing, Chongqing, PR China) were treated immediately to obtain the corresponding serum samples. Before each test, the serum samples were ultrafiltered to remove the redundant salt with a Microcon centrifugal filter device (Ultracel YM-10 membrance, Millipore). Then the samples were stored in a refrigerator at 4 °C for further use. The feasibility of this AuNP-based colorimetric assay for testing human serum samples was examined in the presence of Pb^{2+} following procedure II.

3. Results and discussion

3.1. Design strategy

Alkaline phosphatase plays a crucial role in removing the phosphate group efficiently from different types of substrates, including proteins, nucleotides and other bio-molecules (Choi et al., 2007). In this study, ATP was chosen as the substrate for the enzymatic dephosphorylation reaction to avoid the complicated and time-consuming laborious procedures for synthesis of other substrates. As shown in Scheme 1A, ALP converts ATP into adenosine and phosphate (Pi) through an enzymatic dephosphorylation reaction (Fernley, 1971; Wei et al., 2008; Zhao et al., 2007). However, it is difficult to monitor this dephosphorylation reaction directly by conventional spectroscopic methods, because of the identical