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Highly sensitive homogenous chemiluminescence immunoassay using gold nanoparticles as label

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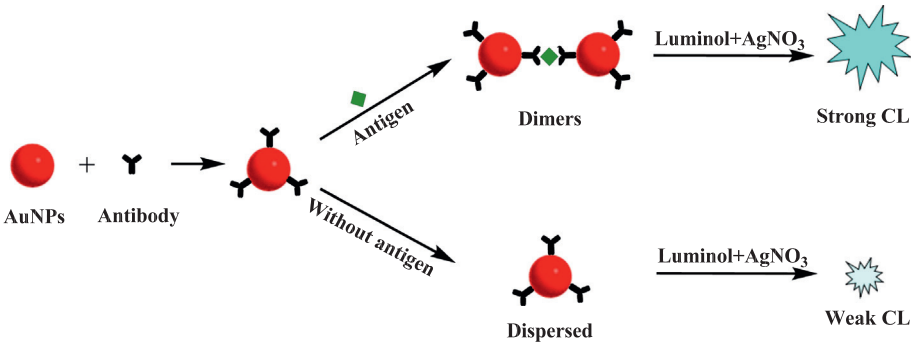
HIGHLIGHTS

- A universal platform for homogeneous immunoassay was proposed using gold nanoparticles (AuNPs) as label.
- This simple protocol consisted of just a one-step incubation followed by injection and reading.
- The assay exhibited excellent sensitivity with a detection limit as low as 3 pg/mL.

GRAPHICAL ABSTRACT

A novel and sensitive strategy to convert the antibody–antigen recognition event into chemiluminescence signal by employing AuNPs as signaling probes is proposed.



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ABSTRACT

Homogeneous immunoassay is becoming more and more attractive for modern medical diagnosis because it is superior to heterogeneous immunoassay in sample and reagent consumption, analysis time, portability and disposability. Herein, a universal platform for homogeneous immunoassay, using human immunoglobulin G (IgG) as a model analyte, has been developed. This assay relies upon the catalytic activity of gold nanoparticles (AuNPs) on luminol–$AgNO_3$ chemiluminescence (CL) reaction. The immunoreaction of antigen and antibody can induce the aggregation of antibody-functionalized AuNPs, and after aggregation the catalytic activity of AuNPs on luminol–$AgNO_3$ CL reaction is greatly enhanced. Without any separation steps, a CL signal is generated upon addition of a trigger solution, and the CL intensity is directly correlated to the quantity of IgG. The detection limit of IgG was estimated to be as low as 3 pg/mL, and the sensitivity was better than that of the reported AuNPs-based CL immunoassay for IgG.

Introduction

Chemiluminescence (CL) immunoassay combines the high sensitivity of CL detection with the high specificity of immunoassay, and has been widely utilized to detect various biomolecules in clinical, pharmaceutical and environmental and biochemical fields [1,2]. The established CL immunoassay methods were mostly based on using luminor (such as luminol, isoluminol and acridinium ester) or enzyme (such as horseradish peroxidase and alkaline phosphatase) as label. Luminol is the best known and one of the most efficient CL reagents. In general, luminol is coupled to antibody or antigen via reactions involving the amino group. However, the resulting conjugates have much lower CL efficiency than that of

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luminol itself [1,3]. Meantime, labeled directly with the CL tags, the biomolecules activity could decrease more or less. Enzyme label is easily denaturalized, short lifetime and low stability [4], and the labeling procedure are complex and require low temperature [3]. Therefore, searching for the stable, easy-to-prepare, low-cost and highly biocompatible labels is of critical importance for the CL immunoassay.

In recent years, nanomaterials have been widely employed as biological labels for immunoassays and other bioanalyses to overcome the safety problems, poor sensitivity, and poor stability associated with the radioisotopic, fluorescent, and enzyme labels [5]. Among these nanomaterial labels, gold nanoparticles (AuNPs) have drawn much attention because of several advantages, such as easy preparation of different nanostructures with special properties, small size and correspondingly large surface-to-volume ratio, chemically tailorable physical properties and good biocompatibility [6,7]. AuNPs have already been used as label in CL immunoassay [4,8–10]. In 2005, Lu group [8] and Li group [9] have separately developed AuNPs-based CL immunoassay using Au^{3+}-catalyzed luminol CL reaction. Recently, Qi et al. [4] proposed a competitive flow injection CL immunoassay for IgG using AuNPs as label. After immunoreaction, the AuNPs, CL label, were dissolved through chemical oxidation to Au^{3+}, and then the CL system was employed to measure Au^{3+} [4,8,9]. Because about 2.3×10^5 gold atoms are theoretically contained in one 20-nm spherical gold particle, the sensitivity of the CL immunoassay could be improved. However, the dissolution of AuNPs was conducted under extremely strict conditions (high concentration HNO_3–HCl or poisonous HCl–Br_2), and it needed a long time to ensure that the dissolution was completed. To avoid the dissolution of AuNPs, Li et al. [11] directly utilized the catalytic activity of irregular AuNPs on luminol–H_2O_2 CL reaction to establish a non-stripping CL immunoassay. Although this protocol avoided the strict stripping procedure, the synthesis of irregular nanoparticles was hard to control, requiring stirring for long time with temperature control (40 °C for 24 h), purging of oxygen and relatively low monodispersity, which may influence the repeatability among different batches, limiting the practical application of this method. Cui group found the catalytic activity of the normal spherical AuNPs on luminol system [12,13], and used the normal spherical AuNPs to label the second antibody for developing a non-stripping CL immunoassay [14].

The reported AuNPs-based CL immunoassays [4,8–11,14] were performed in heterogeneous format. A typical heterogeneous immunoassay involves many steps, such as antibody immobilization, incubation, multiple separation and washing cycles, signal amplification and measurement. From the initial antibody immobilization to the final reading of the assay results, the entire immunoassay can usually take several hours to complete. The heterogeneous immunoassay is rather time-consuming and labor-intensive. To overcome these problems, there is an increasing need for homogeneous immunoassays without any separation steps, especially in the field of modern diagnosis because of their simplicity, ease of automation, and high throughput [15–17].

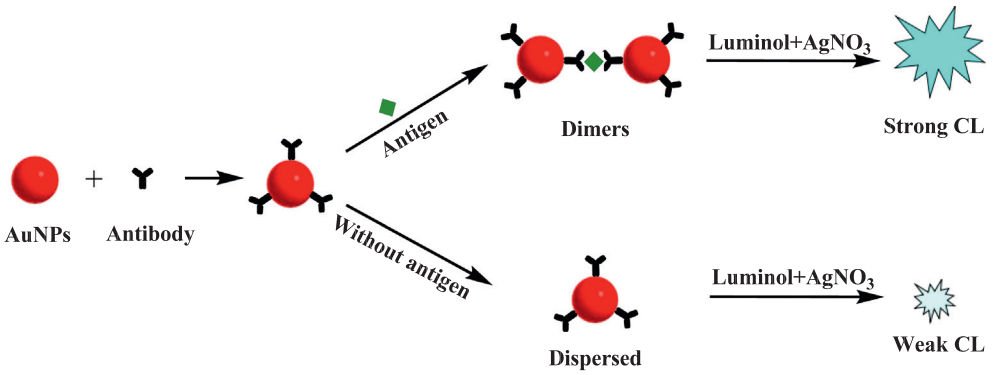
Recently, it is reported that the normal spherical AuNPs or silver nanoparticles can catalyze luminol–$AgNO_3$ reaction to produce a CL [13,18], and this novel CL reaction has the merits of low background, good stability and good reproducibility. In this work, an interesting phenomenon is observed that the catalytic activity of the aggregated AuNPs on luminol–$AgNO_3$ CL reaction is much higher than that of dispersed AuNPs (ca. 13 nm). By taking advantage of this phenomenon, a homogenous CL immunoassay is proposed. A diagram of this method is shown in Scheme 1. Human immunoglobulin G (IgG) was taken as the model analyte to provide the "proof-of-principle" verification of the concept. Aqueous AuNPs were prepared and modified with antibody (goat-anti-human IgG). In the presence of antigen (IgG), the functionalized AuNPs can bind antigen to form dimers (or oligomers) [16,19], and the aggregated AuNPs induce a strong CL signal of luminol system. In the absence of antigen, the dispersed AuNPs induce weak CL emission. The increased CL emission intensity will be positively related to the concentration of the antigen added in the assay solution. Homogeneous assays offer some unique advantages compared to heterogeneous assays, especially in the simplicity of the assay. However, the sensitivity of homogeneous assays is often not as high as heterogeneous amplification assays [20]. In this work, the low background of luminol–$AgNO_3$–AuNPs CL system made the homogenous CL immunoassay exhibit high sensitivity. The detection limit of IgG was estimated to be as low as 3 pg/mL, and the sensitivity was better than that of the reported AuNPs-based heterogeneous CL immunoassay for IgG [4,8–11,14].

Experimental

Reagents and materials

Chloroauric acid ($HAuCl_4$) and silver nitrate was purchased from Sinopharm Chemical Reagent Company (Shanghai, China). Luminol was obtained from Sigma. Sodium citrate was purchased from Xi'an Chemical Reagent Company (Xi'an, China). Human IgG, goat-anti-human IgG, bovine serum albumin (BSA) and HRP-labeled goat-anti-human IgG were purchased from Beijing Dingguo Biotechnology Company (Beijing, China). All the measurements of IgG have been performed in 0.01 M sodium phosphate buffer (pH 7.4). The human serum, provided by Shaanxi Normal University Hospital, was used as the sample to evaluate the reliability of the proposed immunoassay. Polystyrene 96-well microtiter plates were used to perform the immunoreactions.

The luminol stock solution (50 mM) was prepared by dissolving luminol (0.4428 g) in 0.1 M NaOH and then diluting to 50 mL with 0.1 M NaOH. The luminol solution was stored in the dark for one



Scheme 1. Schematic illustration of CL immunoassays based on the catalytic effect of aggregated AuNPs to luminol–$AgNO_3$ CL system.