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One-step homogeneous non-stripping chemiluminescence metal immunoassay based on catalytic activity of gold nanoparticles

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ABSTRACT

The catalytic activity of gold nanoparticles (AuNPs) on a luminol–H_2O_2 chemiluminescence (CL) system is found to be greatly enhanced after its crosslinking aggregation induced by immunoreaction. Based on this observation, a one-step homogeneous non-stripping CL metalloimmunoassay was designed. In the presence of corresponding antigen (Ag), the immunoreaction caused the aggregation of antibody (Ab)-modified AuNPs, and these crosslinking aggregated AuNPs could catalyze luminol–H_2O_2 CL reaction to produce a much stronger CL signal than dispersed Ab-modified AuNPs. The assay, including immunoreaction and detection, can be accomplished in homogeneous solution. In the assay, no tedious and strict stripping of metal nanoparticles, difficult synthesis of labels, multiple steps of immunoreactions and washings, and complicated magnetic separation process were required. The detection limit of human immunoglobulin G (IgG, 3σ) was estimated to be as low as 3.2×10^{-11} g ml^{-1}. The sensitivity was increased by two orders of magnitude over that of other AuNP-based CL immunoassay. The current CL metalloimmunoassay offers the advantages of being simple, cheap, rapid, and sensitive.

Sensitive, rapid, and selective immunoassays are applied widely in various biomolecule detections, which are critical for clinical applications and biochemical studies [1–3]. After the radioimmunoassay was introduced [4], other immunoassays employing enzyme, chelate complex of metal ions, chemiluminophore, and fluorescent dye labels were successively developed [5–9]. However, these methods have some drawbacks such as being a health hazard, waste disposal problems, bad stability, and poor sensitivity [5,10].

Gold nanoparticles (AuNPs)[1] are used widely as labels because of several advantages such as easy preparation, correspondingly large surface-to-volume ratio, special physicochemical properties, and good biocompatibility [11–13]. On the other hand, chemiluminescence (CL) analysis has become an important detection method because of the high sensitivity, wide linear response range, low consumption of inexpensive reagents, and use of a simple instrument [14]. Therefore, the CL immunoassay (CLIA) using AuNPs as the biological tags attracted great interest [15–22]. In 2005, Lu's group [18] and Li's group [15] separately developed two CL metalloimmunoassays exploiting AuNPs as labels in which, after immunoreactions, AuNPs bound to the antibody (Ab) or antigen (Ag) were dissolved and stripped to produce Au(III) or $AuCl_4^-$ so as to catalyze luminol CL reaction; thus, the indirect measurement of Ab or Ag was realized. However, the dissolution of AuNPs required extremely strict conditions (highly concentrated HNO_3–HCl or poisonous HBr–Br_2), which resulted in high CL background so as to reduce sensitivity. In 2006, the other CL metalloimmunoassay was developed using silver precipitation on colloidal AuNP tags for the determination of human immunoglobulin G (IgG) [19]. Although this work avoided the dissolution of gold and improved the sensitivity, a stripping process was still inescapable. Among the above CL metalloimmunoassays, the dissolution of gold or silver was required to be conducted under extremely strict conditions for more than 12 h to ensure complete dissolution. Hence, the stripping CL metalloimmunoassays were tedious and time-consuming. To address the issue, the development of non-stripping CLIA attracted tremendous interest [20,23]. Li and coworkers [20] first established a non-stripping CLIA based on the phenomenon that the irregular AuNPs could greatly enhance the CL intensity of a luminol–H_2O_2 system. Although this assay avoided the strict stripping procedure, it still had an important disadvantage in that the synthesis of irregular nanoparticles was hard to control, which was required to react under 40 °C for

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[1] *Abbreviations used:* AuNP, gold nanoparticle; CL, chemiluminescence; CLIA, CL immunoassay; Ab, antibody; Ag, antigen; IgG, immunoglobulin G; UV–Vis, ultraviolet–visible; TEM, transmission electron microscopy; BSA, bovine serum albumin; HRP, horseradish peroxidase; OPD, *O*-phenylenediamine; PBS–BSA, phosphate buffer containing BSA; ELISA, enzyme-linked immunosorbent assay; PBS-T, phosphate buffer containing Tween 20; SPR, surface plasmon resonance.

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24 h during purging of oxygen, limiting the practical application of this method. Recently, a non-stripping microplate-compatible CLIA was developed for the determination of human IgG based on a luminol–$AgNO_3$–AuNP CL system [23]. Normal spherical AuNPs can be used in this method, but it still needs extraordinary apparatus such as a microplate luminometer and several cycles of consecutive binding and washing steps. So, it was sophisticated and had a long dwell time. In addition, some researchers [21,24,25], using a magnetic separation/mixing process and the amplification feature of AuNP labels, also developed the non-stripping CL immunoassays. These protocols involved a sandwich format in which an extraordinary CL enhancer and several labeling and washing steps were indispensable, and the preparation of antibody-immobilized magnetic beads and a magnetic separation process made these methods complicated. In addition, the analytic time was very long, with even dozens of hours being needed. Moreover, the use of a CL enhancer and magnetic beads greatly increased the analytic cost. Very recently, a capillary electrophoresis-based CLIA using AuNPs as a label also was developed [22].

Our previous works [26–28] found that the non-crosslinking aggregated AuNPs could display stronger catalytic activity on luminol CL reaction than the dispersed AuNPs. Based on this, label-free homogeneous DNA hybridization detection [26] and a label-free aptamer-based CL biosensor [27] were established. They were based on the fact that different configuration oligonucleotides had different propensities to adsorb on AuNPs in colloidal solution, and DNA hybridization and aptamers' conformational changes could result in non-crosslinking aggregation of AuNPs, producing a strong CL signal. On the other hand, the crosslinking aggregation of AuNPs induced by the immunoreaction between Ab-modified AuNPs and Ag was reported frequently and has been applied for various immunoassays, including the hyper-Rayleigh scattering (HRS) technique, light scattering analysis technique, colorimetric assay, and single AuNP counter (SGNPC) [29–32]. Therefore, the crosslinking aggregation of AuNPs induced by the immunoreaction offers the possibility of applying this phenomenon to direct CLIA. The applicability of crosslinking aggregated AuNPs used in the direct CLIA depends on whether or not the crosslinking aggregated AuNPs conjugated with protein induced by immunoreaction show different catalytic activity on the CL reaction.

In this work, the catalytic ability of crosslinking aggregated AuNPs induced by immunoreaction on the luminol CL system was first studied. It was found that crosslinking aggregated AuNPs surrounded by protein could also enhance the luminol CL signal in comparison with dispersed AuNPs. Based on this finding, the luminol–H_2O_2–crosslinking aggregated AuNP CL system was used to develop a new one-step and non-stripping CLIA protocol for the determination of human IgG. A schematic diagram of this method is shown in Scheme 1. In the absence of Ag, Ab-modified AuNPs were monodispersed and could induce a weak CL signal of the luminol–H_2O_2 system. After the immunoreaction between Ab and Ag, the binding of Ag could cause AuNPs to form dimers, induce their aggregation, and initiate a strong CL signal.

Materials and methods

Apparatus

The CL intensity was measured and recorded on an IFFL-D chemiluminescence analyzer (Xi'an Ruimai Electronic Technology, Xi'an, China). Ultraviolet–visible (UV–Vis) adsorption spectra were recorded on a Hitachi U-3900H UV–Vis spectrophotometer. The transmission electron microscopy (TEM) images of AuNPs were taken by using a JEM-2100 TEM device (Japan Electronics). The pH detections were carried out on a PHS-3E analyzer (Jiangsu, China).

Reagents

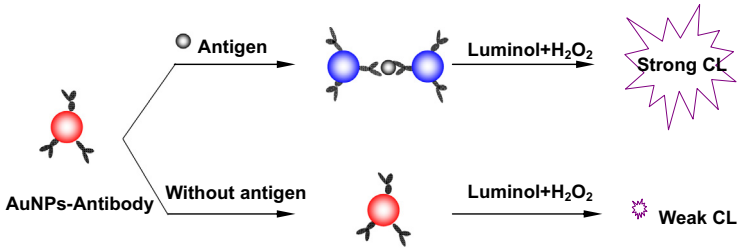
Polystyrene 96-well microtiter plates (FOLCON) were used to perform the immunoreactions. Human IgG, goat anti-human IgG, rabbit IgG, goat IgG, bovine serum albumin (BSA) and horseradish peroxidase (HRP)-labeled goat anti-human IgG were purchased from Beijing Dingguo Biotechnology (Beijing, China).

Chloroauric acid ($HAuCl_4{\cdot}4H_2O$) was purchased from Sinopharm Chemical Reagent (Shanghai, China). Sodium citrate and sodium chloride were purchased from Tianjing Chemical Reagent (Tianjing, China). Other reagents and chemicals were of analytical grade and used without further purification. Doubly distilled and deionized water was used throughout.

Luminol stock solution (2.5×10^{-2} M) was prepared by dissolving 4.43 g of luminol in 20 ml of NaOH solution (0.10 M) and then diluting to 1 L with water. The luminol solution was stored in the dark for 1 week prior to use to ensure that the reagent property had stabilized. Working solutions of luminol were prepared by diluting the stock solution. Working solutions of H_2O_2 were prepared fresh daily from 30% (w/w) H_2O_2. *O*-Phenylenediamine (OPD) served as the substrate of HRP. The OPD solution ($0.4\ mg\ ml^{-1}$) was prepared by dissolving 10.0 mg of OPD in 6.1 ml of 0.1 M citric acid solution and then adding 6.4 ml of 0.2 M Na_2HPO_4 solution and 12.5 ml of deionized distilled water. Next, 40 μl of 30% H_2O_2 was added in the OPD solution immediately prior to use. The human serum (provided by Shanxi Normal University Hospital) was used as the sample to evaluate the reliability of the proposed immunoassay.

Preparation of AuNPs and AuNP-labeled goat anti-human IgG

AuNPs (13 nm) were prepared according to a literature method [33]. Briefly, a sodium citrate solution (0.1 M, 1.94 ml) was rapidly added to a boiled $HAuCl_4$ solution (50 ml H_2O and 0.167 ml 10% $HAuCl_4$) under vigorous stirring. The mixed solution was boiled for 10 min and further stirred for 15 min. The resulting solution was cooled to room temperature (~20 °C) and then stored in the refrigerator (4 °C) before use. The size and shape of the synthesized AuNPs were characterized by TEM. The images showed that their diameter was uniform and their dispersion was very good.



Scheme 1. Schematic representation of the proposed CL immunoassay.