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Sensitive competitive flow injection chemiluminescence immunoassay for IgG using gold nanoparticle as label

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ABSTRACT

A sensitive competitive flow injection chemiluminescence (CL-FIA) immunoassay for immunoglobulin G (IgG) was developed using gold nanoparticle as CL label. In the configuration, anti-IgG antibody was immobilized on a glass capillary column surface by 3-(aminopropyl)-triethoxysilane and glutaraldehyde to form immunoaffinity column. Analyte IgG and gold nanoparticle labeled IgG were passed through the immunoaffinity column mounted in a flow system and competed for the surface-confined anti-IgG antibody. CL emission was generated from the reaction between luminol and hydrogen peroxide in the presence of Au (III), generated from chemically oxidative dissolution of gold nanoparticle by an injection of 0.10 mol L^{-1} HCl–0.10 mol L^{-1} NaCl solution containing 0.10 mmol L^{-1} Br_2. The concentration of analyte IgG was inversely related to the amount of bound gold nanoparticle labeled IgG and the CL intensity was linear with the concentration of analyte IgG from 1.0 ng mL^{-1} to 40 ng mL^{-1} with a detection limit of 5.2×10^{-10} g mL^{-1}. The whole assay time including the injections and washing steps was only 30 min for one sample, which was competitive with CL immunoassays based on a gold nanoparticle label and magnetic separation. This work demonstrates that the CL immunoassay incorporation of nanoparticle label and flow injection is promising for clinical assay with sensitivity and high-speed.

1. Introduction

Chemiluminescence (CL) immunoassay has been developed rapidly for the analysis of various biomolecules because of its high sensitivity, safety and lower detection limit at the order of nanograms, and has been widely utilized in clinical, pharmaceutical and environmental and biochemical fields [1]. The established CL immunoassay methods were mostly based on using luminor or enzyme as CL label. Disadvantages of enzyme label are easily denaturalized, short lifetime and low stability. Disadvantages of luminor label have low sensitivity and complicated labeling-process. CL immunoassay employing nanoparticles, especially gold nanoparticle, as label, has received much attention because of its advantages such as higher specific activity, stability and sensitivity [2–5]. Lu [3] reported a CL immunoassay method employing gold nanoparticle in which a sandwich-type complex was formed by the primary antibody immobilized on the surface of magnetic beads, the antigen in the sample, and the second antibody labeled with gold nanoparticle. Then, a large number of Au^{3+} ions from each gold nanoparticle anchored on the surface of magnetic beads were released after oxidative dissolution of gold nanoparticle and then quantitatively determined by a simple and sensitive Au^{3+}-catalyzed luminol CL reaction. In this protocol, each gold nanoparticle contains thousands of atoms (e.g., 2.3×10^5 gold atoms are theoretically contained in a 20-nm spherical gold nanoparticle), and consequently, picomolar detection limit is attained. Li [5] reported a sensitive CL immunoassay method by using rabbit–anti-goat IgG-functionalized gold nanoparticle. However, these CL methods in stationary state required several incubations, washing and dissolved steps, suffered from the tedious process.

To avoid the dissolution of gold nanoparticle under these conditions, various strategies were proposed for the CL method by using gold nanoparticle [6–10]. A non-stripping CL immunoassay based on gold nanoparticle was reported by Wang [7], which was established according to the fact that the irregular gold nanoparticle could greatly enhance the CL intensity of the luminol–H_2O_2 system. Recently, Li [9] found that the triangular AuNPs could significantly enhance the CL intensity for the luminol–H_2O_2 system and a CL method was developed to detect aminothiols. Although this protocol avoided any strict stripping procedure, the synthesis process of special nanoparticle was tedious and hard to control. It is necessary to develop fast-speed, simple and sensitive CL immunoassay for clinical assay.

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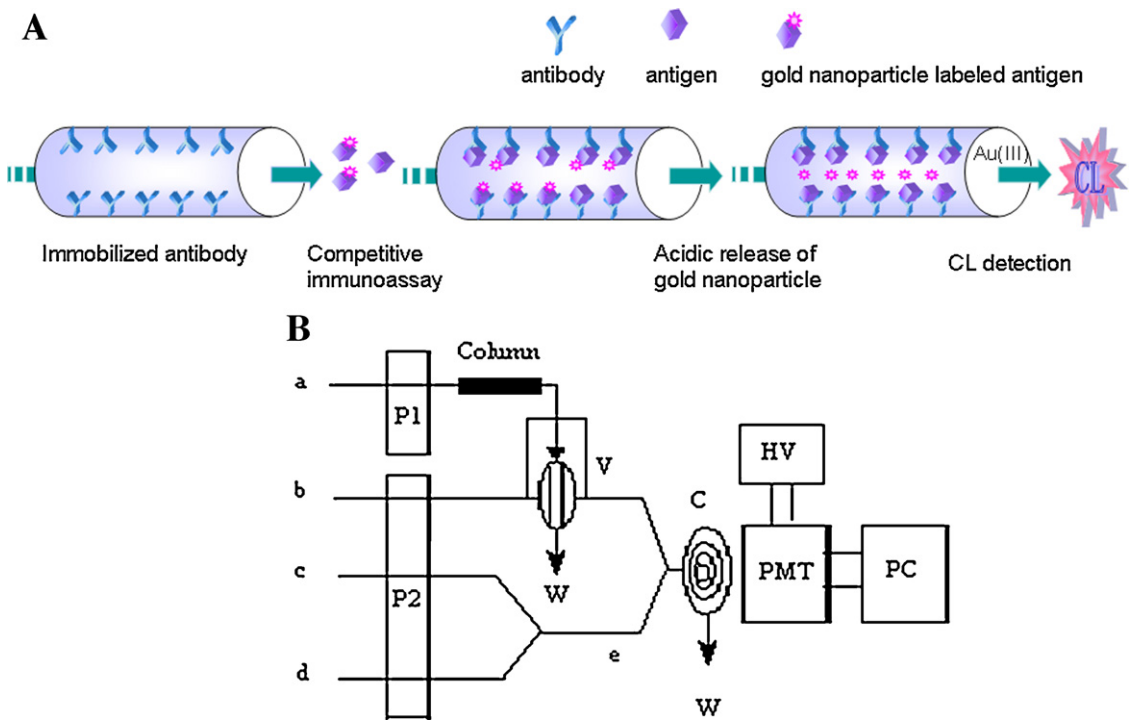

Fig. 1. (A) Principle of competitive CL-FIA immunoassay. (B) Schematic diagram of the flow injection chemiluminescence system. a: sample; b: carrier solution; c: luminol; d: hydrogen peroxide; P_1, P_2: peristaltic pump; V: injection valve; C: flow cell; W: waste; PMT: photomultiplier tube; HV: high voltage; PC: personal computer.

The combination of flow-injection analysis (FIA) with immunoassay has been shown to be a very efficient technique for increasing speed and repeatability of immunoassays [10,11]. CL immunoassay coupled with flow injection analysis (CL-FIA immunoassay), compared to classical CL immunoassay techniques, shows several advantages regarding speed, precision and costs of automation, such as instrumental simplicity, high speed and reproducibility in signal detection and, of being appropriate for on-line analyses, and has been utilized in many different applications.

The aim of the present work is to develop a high-speed and sensitive flow injection chemiluminescence immunoassay. IgG was employed as a model analyte and gold nanoparticle was used as label. The detection of IgG was based on a competitive CL-FIA immunoassay by following the typical procedure, as shown in Fig. 1A. First, the anti-IgG antibody was immobilized on a glass capillary column surface by 3-(aminopropyl)-triethoxysilane and glutaraldehyde to form immunoaffinity column. Analyte IgG and gold nanoparticle labeled IgG were passed through the immunoaffinity column mounted in a flow system and competed for the surface-confined anti-IgG antibodies. CL emission was generated from the reaction between luminol and hydrogen peroxide in the presence of dissolved Au(III), generated from chemically oxidative dissolution of gold nanoparticle by an injection of 0.10 mol L^{-1} HCl–0.10 mol L^{-1} NaCl solution containing 0.10 mmol L^{-1} Br_2. Along with the increase of the concentration of analyte IgG, the decrease of bound gold nanoparticle labeled IgG conjugate on immunoaffinity column by competitive binding of analyte IgG occurs, and thus the detected CL intensity decreases. The concentration of analyte IgG, inversely related to the amount of bound gold nanoparticle labeled IgG, was indirectly determined by the detected CL intensity. In this work, the CL behavior of luminol–H_2O_2–Au (III) and the dissolution of gold nanoparticle were investigated and the analytical performance of the competitive CL-FIA immunoassay for IgG was examined.

2. Experimental

2.1. Reagents

$HAuCl_4 \cdot 4H_2O$ (99% w/w), 3-(aminopropyl)-triethoxysilane (APTES), glutaraldehyde, immunoglobulin G (IgG, goat), and sheep anti-goat IgG monoclonal antibody were purchased from Sigma–Aldrich Chemical Co. (St. Louis, Missouri, USA). Bovine serum albumin (BSA, Mt = 67,000) and Tween-20 were obtained from Beijing Zhongshan Golden Bridge Biotechnology Co. Ltd. (Beijing, China). All of reagents were of analytical grade and doubly distilled water was used for the preparation of all solutions.

2.2. Apparatus

The CL signal was recorded with a computer employing an IFFL-D flow-injection CL analysis system (Xi'an Remex Electronic Equipment Corporate, Xi'an, China, PMT −400 V). The acquisition and treatment of data were performed with the IFFL-D flow-injection CL analysis system. The CL kinetics was recorded using a computer-controlled Ultra-weak Luminescence Analyzer (Institute of Biophysics, Chinese Academy, China, Beijing, PMT −400 V). PTFE tubing (0.8 mm i.d.) was used to connect all components in the flow system (Xi'an Remex Electronic Equipment Corporate, Xi'an, China).

2.3. Preparation of anti-IgG antibody-immobilized immunoaffinity column

Anti-IgG antibody-immobilized immunoaffinity column was prepared according to the reference with slight modification [12]. First, a capillary glass column (length 5 cm, i.d. 1.6 mm) was cleaned ultrasonically with a mixture of 30% hydrogen peroxide (H_2O_2) and concentrated sulfuric acid (1:4, v/v) for 1 h and then rinsed ultrasonically in doubly distilled water for 10 min, and followed by thoroughly washing with pure ethanol and then water. Second,