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Determination of IgG by electron spin resonance spectroscopy using Fe_3O_4 nanoparticles as probe

Sizhu Tian[a], Jia Jiang[a], Shuang Zang[a], Kun Wang[a], Yong Yu[b], Xuwen Li[a], Hanqi Zhang[a], Aimin Yu[a], Ziwei Zhang[a,*]

[a] College of Chemistry, Jilin University, Qianjin Street 2699, Changchun 130012, PR China
[b] College of Instrumentation and Electrical Engineering, Jilin University, West Minzhu Street 938, Changchun 130061, PR China

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ABSTRACT

A novel electron spin resonance (ESR) spectroscopy method for determination of rabbit IgG was presented. Anti-rabbit IgG antibody was bound to Fe_3O_4 nanoparticles (FeNPs). The FeNPs were used as probe to give ESR signal. After the optimization of the assay, a molar ratio 500:1 of antibody to FeNPs was selected. The sepharose beads were used as solid phase and used to separate the analyte from the sample solution. Two typical immunoassay formats, sandwich and competitive formats, were applied to the determination of IgG. The analytical sensitivities obtained by sandwich and competitive formats were 0.13 μg mL^{-1} and 0.56 μg mL^{-1}, respectively. The two formats were applied to the determination of the rabbit IgG in rabbit serum. The diluted sample was directly analyzed and other additional sample treatment was not required. The analytical results of the spiked samples indicated that the recoveries of the analytes were acceptable. Furthermore, the sample preparation time used in this method is shorter than most existing immunoassay methods.

1. Introduction

Electron spin resonance (ESR), also referred to as electron paramagnetic resonance (EPR), an effective analysis tool that can be directly applied to the detection of substances which contain one or more unpaired electrons [1]. The substances with ESR signal contain free radicals, such as DPPH· and other paramagnetic species, such as transition metal ions (e.g., Fe^{3+}, Mn^{2+} and Cu^{2+}) [2, 3]. So far, ESR has been applied widely in several research fields including physics, chemistry, biology, nutraceutical, and food science [3, 4].

The application of quantitative ESR to detect transition metal ions was largely pioneered in the middle of last century [5]. In the following decades, a number of works were reported in which different methods were developed to analyze transition metal ions by ESR spectroscopy [6–9].

Immunoassay is a high-sensitivity detection technology in modern clinical laboratories to measure many different biological molecules [10, 11]. Till now, a series of immunoassay methods including radioimmunoassay (RIA) [12], enzyme-linked immunosorbent assay (ELISA) [13], fluorescence immunoassay (FIA) [14], colloidal gold immunochromatographic assay (CGIA) [15], and chemiluminescence immunoassay (CLIA) [16] have been widely applied. ESR has been reported as a tool for immunoassay since 1970s [17, 18].

In recent years, nanosized materials have been widely applied to various disciplines [19]. Among the nanosized materials, Fe_3O_4 nanoparticles (FeNPs) as magnetic nanomaterial, were commonly used. FeNPs have been demonstrated to be the most promising materials because of: (1) their magnetic properties to isolate and purify the analytes by external magnetic field from complex sample solutions [20]; (2) their essential surface functionality and high specific surface to functionalize with biorecognition molecules such as antibody, bioprotein, and carbohydrates as well as to immobilize various biomolecules [21–23]; (3) low cytotoxicity [19]; and (4) good water solubility. In this work, FeNPs was used as ESR probe in immunoassay.

In this work, antigen (Ag) rabbit immunoglobulin G (IgG) was determined by ESR spectroscopy using FeNPs as probe. Both sandwich and competitive immunoassay formats were evaluated [15, 24]. In these formats, the antibody (Ab) was immobilized on FeNPs and FeNPs were used to give signal. In previous work, we used 10 nm and 30 nm iron oxide nanoparticles and in this work we used even larger 180 nm iron oxide nanoparticles as the probe [25]. It helps to reduce the background signals. The sepharose beads were used as solid phase and can be isolated by centrifugation. In sandwich format, sepharose bead-Ab was interacted specifically first with Ag and then with the conjugated FeNP-Ab. Similarly, sepharose bead-Ag was interacted with FeNP-Ab in competitive formats. The rabbit IgG in rabbit serum was

* Corresponding author.
E-mail address: zzw@jlu.edu.cn (Z. Zhang).

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determined by two formats and the obtained results was compared with those reported previously.

2. Material and methods

2.1. Reagents and materials

FeNPs with carboxylic acid group (Product PM3-020 for 180 nm, 10 mg mL^{-1}) were purchased from Shanghai Allrun Nano Science & Technology Co., Ltd. Rabbit IgG (Product No. AGRIG-0100, 15.18 mg mL^{-1}) and goat anti-rabbit IgG polyclonal antibody (Ab) (Product No. ABGAR-0500, 7.88 mg mL^{-1}) were provided by Arista Biologicals. N-hydroxysuccinimidyl-Sepharose 4 Fast Flow beads (sepharose beads) (mean particle size 90 μm) was purchased from Sigma-Aldrich, Co. Normal rabbit serum was provided by Shanghai Yeasen Biological Technology Co., Ltd. Bovine serum albumin (BSA) was purchased from Beijing Xiasi Biotechnology Co., Ltd. 2-Aminoethanol was purchased from TCI Chemicals. N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and 2-(N-morpholino)ethanesulfonic acid (MES monohydrate) were provided by Aladdin Industrial Corporation. Triton-X-100 was purchased from Seebio Biotech, Inc. Magnet for isolating iron oxide nanoparticles was obtained from Shanghai Da Xue Electromagnetic Devices Co., Ltd. All other reagents were of analytical grade.

2.2. Preparation of FeNP-Ab solution

In a centrifuge tube, 100 μL of 10 mg mL^{-1} FeNPs were washed by 500 μL of MEST solution (5 mmol L^{-1} MES solution containing 0.05% Triton X-100, pH 5.4). After the separation of FeNPs with magnet, 600 μL of MEST solution containing 0.2 mg mL^{-1} EDC and 0.1 mg mL^{-1} NHS were added as coupling reagents, and the mixture was shook on a translational shaker at room temperature for 10 min to activate the –COOH groups of FeNPs. 500 μL of HEPES solution (50 mmol L^{-1}, pH 8.3) were added to wash the activated FeNPs. Subsequently, the activated FeNPs were isolated with magnet and then mixed with 498.5 μL of HEPES solution. 1.5 μL of goat anti-rabbit IgG antibody (7.88 mg mL^{-1}) were added into the mixture and the resulting mixture was shaken at room temperature for 2 h. The obtained FeNP-Ab conjugate was collected by magnetic separation within 1 min, followed by washing with phosphate-buffered saline (PBS) containing 0.05% Triton X-100 to wash off unbound antibody. Then the FeNP-Ab conjugate was treated with 500 μL of PBS containing 0.05% Triton X-100 and 0.1% (w/v) BSA for 40 min to block nonspecific binding sites. Finally, the FeNP-Ab conjugate was isolated and suspended in 1 mL of PBS containing 0.05% Triton X-100 and 0.1% (w/v) BSA. The resulting mixture was referred as FeNP-Ab solution and stored at 4 °C before use.

2.3. Preparation of sepharose bead-Ab solution

100 μL of NHS-activated sepharose beads particles were added into a centrifuge tube, followed by washing with 500 μL of HCl (1 mmol L^{-1}) for 3 times. Then the sepharose beads were washed with 500 μL of HEPES solution (10 mmol L^{-1}, pH 8.3) for 1 time. 450 μL of HEPES solution and 50 μL of Ab (7.88 mg mL^{-1}) were added into the obtained sepharose beads and the mixture was incubated at room temperature with gentle shaking. After 1.5 h, 1 mL of bloking buffer (0.5 mol L^{-1} 2-aminoethanol, 0.5 mol L^{-1} NaCl, pH 8.3) was added to block the nonbinding sites. 10 min later, the sepharose beads were washed with 1 mL of washing buffer (0.1 mol L^{-1} acetic acid, 0.5 mol L^{-1} NaCl, pH 4.0) to remove the unbound antibody. Subsequently, 1 mL of water was used to wash the sepharose beads 3 times. Finally, PBS containing 0.05% Triton X-100 and 0.1% w/v BSA was injected into the obtained sepharose beads until the final volume was 1 mL. The resulting mixture was referred to as sepharose beads-Ab solution and stored at 4 °C for further use in sandwich format.

2.4. Preparation of sepharose bead-Ag solution

100 μL of NHS-activated sepharose beads were added into a centrifuge tube, followed by washing with 500 μL of HCl (1 mmol L^{-1}) for 3 times. Then the sepharose beads were washed with 500 μL of HEPES solution (10 mmol L^{-1}, pH 8.3) for 1 time. 473.6 μL of HEPES solution and 26.4 μL of rabbit IgG (15.18 mg mL^{-1}) were added into the obtained sepharose beads. The resulting mixture was incubated at room temperature with gentle shaking. After 1.5 h, 1 mL of bloking buffer (0.5 mol L^{-1} 2-aminoethanol, 0.5 mol L^{-1} NaCl, pH 8.3) was added to block the nonbinding sites. 10 min later, the sepharose beads were washed with 1 mL of washing buffer (0.1 mol L^{-1} acetic acid, 0.5 mol L^{-1} NaCl, pH 4.0) to remove the unbound antigen. Subsequently, 1 mL of water was used to wash the sepharose beads 3 times. Finally, PBS containing 0.05% Triton X-100 and 0.1% w/v BSA was injected into the obtained sepharose beads until the final volume is 1 mL. The resulting mixture was referred to as sepharose beads-Ag solution and stored at 4 °C for further use in competitive format.

2.5. Immunological reaction

2.5.1. Sandwich immunoassay format

The schematic diagram of the sandwich format is shown in Fig. 1(a). 50 μL of sepharose beads-Ab solution and 50 μL of standard solution or sample were added into a centrifuge tube. After mixed completely, the mixture was incubated at 37 °C for 35 min with slight shaking. Then the mixture was centrifuged for 1 min at 600 rpm to separate the sepharose beads from the solution, followed by removing the supernatant. Then 100 μL of FeNP-Ab solution were added and the immunological reaction was performed within 60 min with shaking. The resulting mixture was referred as the reaction solution.

2.5.2. Competition immunoassay format

The schematic diagram of the competitive format is shown in Fig. 1(b). 100 μL of FeNP-Ab solution and 50 μL of standard solution or sample were added into a centrifuge tube. After mixed completely, the mixture was incubated at 37 °C for 15 min with slight shaking. Then 50 μL of sepharose beads-Ag solution were added and the immunological reaction was performed within 35 min with shaking. The resulting mixture was referred as the reaction solution.

2.6. Preparation of analytical sample for ESR measurement

1 mL of PBS was added into the reaction solution to wash for 3 times. The mixture was centrifuged for 1 min at 600 rpm and the supernatant was removed. The sepharose beads were referred to as analytical sample and collected with a glass capillary tube from Kimble Chase (0.8 mm i.d., 1.1 mm o.d., 100 mm length). The bottom of capillary was sealed with the sealing clay. The sum height of the analytical sample in the capillary tube was 2 cm.

2.7. ESR measurement

An X-band Bruker A300 spectrometer was used at room temperature (20 °C) at microwave frequency of 9.85 GHz. The microwave power was set at 2.3 mW. The modulation amplitude was 4 G and the modulation frequency was 100 kHz. The number of scans of 0 μg mL^{-1} in sandwich format and 0.8 μg mL^{-1} in competitive format are 5 and 2 scans, respectively. In other measurements, good signal-to-noise could be achieved with 1 scan. Other instrumental setting was set as followed: center magnetic field, 3340 G; sweep width, 4000 G; conversion time, 80 ms; time constant, 164 ms; receiver gain, 5×10^3. A quartz tube (Wilmad LabGlass) with the glass capillary tube inside was place into the resonator cavity. The top end of the glass capillary tube was inserted in a long plastic tube, which is used to replace the glass capillary tube after completing a test with the quartz tube stayed in the