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Fluoroimmunoassay for Antigen Based on Fluorescence Quenching Signal of Gold Nanoparticles

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A unique, sensitive, and highly specific fluoroimmunoassay system for antigen detection using gold and magnetic nanoparticles has been developed. The assay is based on the fluorescence quenching of fluorescein isothiocyanate caused by gold nanoparticles coated with monoclonal antibody. To demonstrate its analytical capabilities, the magnetic nanoparticles were coated with anti-α-fetoprotein polyclonal antibodies, which specifically bound with α-fetoprotein. Gold nanoparticles coated with anti-α-fetoprotein monoclonal antibodies could sandwich the α-fetoprotein captured by the magnetic nanoparticle probes. The sandwich-type immunocomplex was formed on the surface of magnetic nanoparticles and could be separated by a magnetic field. The supernatant liquid, which contained the unbound gold nanoparticle probes, was used to quench the fluorescence, and the fluorescence intensity of fluorescein isothiocyanate at 516 nm was proportional to the α-fetoprotein concentration. The result showed that the limit of detection of α-fetoprotein was 0.17 nM. This new system can be extended to detect target molecules with matched antibodies and has broad potential applications in immunoassay and disease diagnosis.

Gold nanoparticles (GNPs), as a class of nanomaterials with many unique properties such as colorimetric, conductivity, and nonlinear optical properties, have been explored for potential applications in biomolecular detection.[1−3] GNPs can be used to quantitatively detect nucleic acids and proteins in clinical samples. For example, a DNA-based method for rationally assembling gold nanoparticles into macroscopic materials has been reported by Mirkin and co-workers.[4] It can sensitively detect oligonucleotides using colorimetric characterization of gold nanoparticles and hybridization of nucleotides.[5,6] For protein detection, an aggregation-based immunoassay for anti-protein A using gold nanoparticles has been developed.[7] The hyper-Rayleigh scattering signals of aggregated gold nanoparticles labeled with immunoglobulin G could be used to quantify antibody/antigen in aqueous solutions.[8] However, the two methods could only detect proteins at the microgram level, which limits their applications in immunoassay, especially in early cancer diagnosis. In recent years, fluorescent detection methods have achieved major improvements in bioanalytical applications because of their extraordinary sensitivity and selectivity.[9] Fluorescence immunoassays employ a fluorescent signal for analytical detection.[10] GNPs have a very strong ability for fluorescent quenching, which can advantageously replace 4-[(4′-(dimethylamino)phenyl)azo]benzoic acid, quench fluorescence as much as 100 times better, and have higher quenching efficiency for dyes emitting near the infrared region.[11]

Magnetic nanoparticles (MNPs), as a special biomolecule immobilizing carrier, have been broadly used in immunoassays, enzyme, DNA, protein immobilization, DNA purification, and magnetically controlled transport of anticancer drugs.[12,13] Several new ultrasensitive measurements of proteins using magnetic beads have been developed.[14] Fully using the advantageous properties of magnetic nanoparticles and gold nanoparticles, several new ultrasensitive detection methods for proteins and nucleic acids can be developed.[15,16]

A well-known, α-fetoprotein (AFP) is the serum biomarker of hepatocellular carcinoma (HCC), which has become a public health concern in recent years largely due to the worldwide epidemic of hepatitis B and hepatitis C.[17] Serum levels of AFP often increase under conditions such as periods of rapid liver

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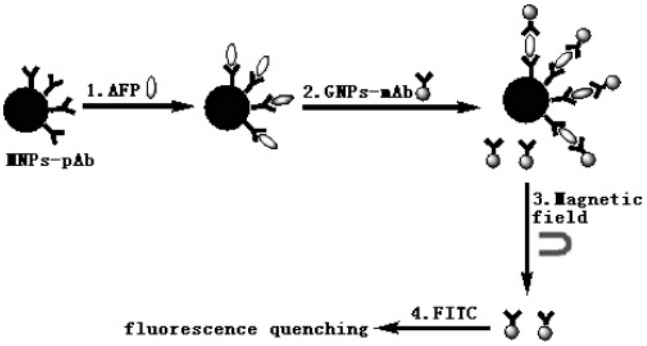

Figure 1. Schematics of the GFQIA.

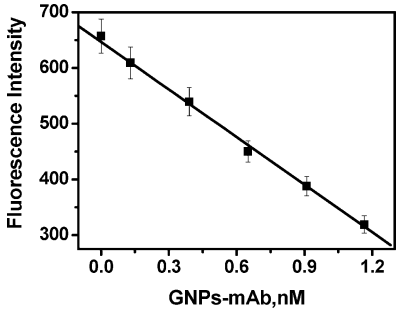

Figure 2. Plot of GNPs-mAb probes quenching FITC.

cancer cell growth, cirrhosis, and chronic active hepatitis, as well as carbon tetrachloride intoxication.[18] Therefore, detecting serum levels of AFP can help to diagnose early HCC. There are several conventional methods to detect AFP, such as radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), and chemiluminescence assay.[19] The detection limit of AFP is ~0.01 and ~0.14 nM for RIA[20] and ELISA,[21] respectively. Although both RIA and ELISA have advantages of high sensitivity, they are time-consuming and the operations require a well-trained technician and special equipment in a laboratory.[20]

We reported herein a novel immunoassay named gold fluorescence quenching immunoassay (GFQIA), which could be developed using the fluorescent quenching property of gold nanoparticles. AFP was chose to prove the novel immunoassay method as a typical example. Compared with the traditional method of the AFP radioimmunoassay, the GFQIA method had widely potential applications in immunoassay and pathogenic detection.

EXPERIMENTAL SECTION

Materials and Reagents. The preparation of GNPs, MNPs, MNPs coated with anti-AFP polyclonal antibodies (MNPs-pAb), and GNPs coated with anti-AFP monoclonal antibody (GNPs-mAb) were described in the Supporting Information. AFP was purchased from Sigma Co. FITC was purchased from Shanghai Chemical Reagent Corp. (Shanghai, China).

Fluorescence Quenching by GNPs-mAb Probes. Different concentrations of GNPs-mAb probes (0.13−1.17 nM) were mixed with the same concentration of fluorescein isothiocyanate (FITC, 6 μg/mL, pH9.0), respectively. The fluorescence intensity of FITC was detected immediately with Perkin-Elmer LS-55 spectrofluorometer. The excitation wavelength of FITC was at 490 nm, and the emission spectra were recorded from 500 to 600 nm. The GNPs-mAb probes were not excited by 490-nm wavelength and did not emit fluorescence. The excitation and emission slit widths were set to 10 nm. Samples were put in 1-cm path length quartz cuvettes.

Detection of AFP by GFQIA. The methodology of AFP detection by GFQIA is shown in Figure 1. MNPs-pAb and GNPs-mAb probes were used in this method. After the immunoreaction, the sandwich-type immunocomplex was formed on the surface of magnetic nanoparticles. The unbound GNPs-mAb, which could cause the fluorescence quenching of FITC, were obtained by magnetic field and the fluorescence quenching signal of FITC was monitored by spectrofluorometer. The procedure is described as follows: 0.05 mL of MNPs-pAbs probes (1 mg/mL) were reacted simultaneously with 0.05 mL of AFP samples or standards (the different concentrations of AFP were 0, 20, 50, 100, 200, and 400 ng/mL, respectively) for 30 min in the polystyrene tubes. After magnetic separation, the probes were redispersed in 0.05 mL of 0.01 M phosphate buffer solution (pH 7.4), 0.1 mL of GNPs-mAb probes (1.17 nM) was added, and the mixture was incubated for 2 h at room temperature. A 0.1-mL sample of unbound GNPs-mAb probes in the supernatant liquid was obtained by magnetic separation after the immunoreactions, to which was added the same volume of FITC (6 μg/mL, pH 9.0), and the fluorescence quenching signal was measured by a Perkin-Elmer LS-55 spectrofluorometer immediately. The quenching signal was proportional to the amount of AFP present in standards and samples.

RESULTS AND DISCUSSION

Measurement of Fluorescence Quenching. GNPs-mAb probes could quench the fluorescence of FITC, and the fluorescence intensity of FITC at 516 nm decreased linearly when the concentration of GNPs-mAb probes increased as shown in Figure 2. The correlation coefficient was −0.998 56. One can speculate on the point of binding of FITC to the Au surface.[22] Gold atoms on the surface of the particle are coordinately unsaturated; that is, unoccupied orbitals are available for nucleophiles to donate electrons. Stronger electron donors such as amines and alkanethiols could displace citrate ions from colloidal gold surfaces. The isothiocyanate group of the FITC molecule is a strong nucleophile, which forms stable complexes with gold(I). It is reasonable to suggest that FITC binds to gold via the sulfur atom as well.

Detection of AFP by GFQIA. The principle of GFQIA was described in Figure 1. The concentration of unbound GNPs-mAb probes decreased as the concentration of AFP increased after the sandwich immunoassay by magnetic separation. Therefore, the ability to quench the fluorescence of FITC decreased because of the linearity between GNPs-mAb probes and FITC. As shown in Figure 3, the fluorescence intensity was enhanced when AFP concentration increased from bottom to top because of the decreasing unbound GNPs-mAb probes, and the values of maximal intensity at 516 nm were proportional to the amount of the AFP.

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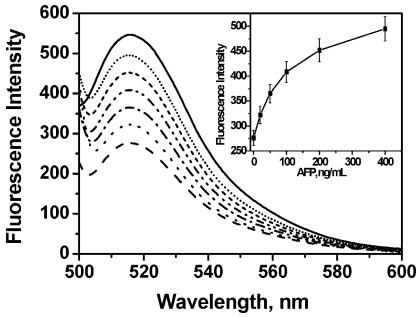

Figure 3. Effects of GNPs-mAb probes on the fluorescence spectra of FITC. AFP concentration increased from bottom to top: 0, 20, 50, 100, 200, 400 ng/mL, and the solid line was the control of FITC without GNPs-mAb probes. The inset showed plots of the fluorescence intensity at 516 nm when AFP concentration increased.

The limit of detection was 0.17 nM (12 ng/mL), which was calculated by the interpolation of the mean plus two standard deviations of 10 replicates of the zero standards (0 ng/mL). The measurement range of the assay was from 15 to 400 ng/mL. The normal concentration of AFP is <20 ng/mL by RIA,[20] so the GFQIA can be used to detect the AFP concentration in the HCC serum samples.

Correlation with Radioimmunoassay. The novel GFQIA method reported herein was compared with RIA. Nine patient serum samples (purchased from Zhongshan Hospital, Shanghai, China) were especially selected to take immunoassay using both methods, and the result was shown in Table 1. The serum samples had been tested with immunoassays by the hospital and were found to be nonreactive for hepatitis B surface antigen and antibodies against HIV1, HIV2, and HVC. The results demonstrated excellent linear correlation, and the correlation coefficient was 0.993.

Conclusion. A new fluoroimmunoassay based on gold and magnetic nanoparticles was developed. The gold nanoparticle probes were highly sensitive to fluorescence quenching, so the fluorescence quenching technique can detect the target at much lower concentrations compared with a gold immunochromatography assay or gold immunofiltration assay. Besides, gold nanoparticle probes possessed the advantage of better stability over radioisotopic labels, and the whole procedure was very simple and did not affect the biochemical activity of the labeled compound.[23] Magnetic nanoparticle probes were separated fast and easily by magnetic field. The two kinds of antibodies, monoclonal and polyclonal, that coupled with different nanoparticles could ensure highly specific reaction to capture the detected target, which did not need to be extracted from serum. Therefore, the new method could be used widely in immunoassays. Although herein we only report that the novel fluoroimmunoassay could detect the AFP very effectively, the assay is general for the detection of the target molecules with known matched antibodies, which could be used widely for detection of tumor markers and pathogens.

Table 1. Results of Nine Patients' Sera Samples Detected by Two Methods

sample	RIA (mean ± 1 SD, ng/mL)	GFQIA (mean ± 1 SD, ng/mL)
1	13.7 ± 2.02	17.6 ± 3.08
2	17.2 ± 1.36	26.4 ± 2.15
3	36.7 ± 1.83	42.6 ± 2.28
4	58.2 ± 1.27	53.7 ± 1.31
5	70.6 ± 2.04	75.3 ± 2.37
6	130.6 ± 5.43	139.9 ± 5.76
7	180.1 ± 4.53	160.2 ± 4.58
8	268.4 ± 9.42	253.8 ± 9.69
9	310.6 ± 10.47	330.2 ± 11.86

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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