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Abstract

Preparation of Antibody-Conjugated Gold Nanotriangles for Immunochromatographic Test †

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Abstract: Gold nanotriangles (GNTs) for producing a test strip of human chorionic gonadotropin (hCG) tests were reported in this paper. The GNTs were simply synthesized by non-thermal liquid-phase reduction with sodium citrate and tannic acid composition as a reducing reagent. The antibody-conjugated GNTs were prepared using the synthesized GNTs and anti-hCG beta antibodies. The experimental results confirmed that the use of GNTs can decrease the volume of antibodies required for the use of a labeling reagent compared with spherical GNPs for the first time.

Keywords: antibody; antigen; hCG; nanoparticles; synthesis

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1. Introduction

The spherical gold nanoparticles (GNPs) with a mean diameter of tens of nanometers and the localized plasmon resonance (LSPR) peak can be applied to a labeling reagent for antibodies in antigen tests such as influenza tests and human chorionic gonadotropin (hCG) tests by immunochromatography [1]. However, in the case of antigen tests using spherical GNPs, a testing solution with a low antigen concentration cannot be detected, because the amount of GNP-labeled antibodies connecting to the antigen is low, and a large amount of the GNP-labeled antibodies cannot be fixed on the test line to indicate a positive. Therefore, the GNPs with a strong LSPR peak and low volume ratio of antibodies to Au in the antibody-conjugated GNPs were required to improve the sensitivity of the antigen test. To meet this issue, we proposed the use of gold nanotriangles (GNTs) that exhibited a strong LSPR peak compared with GNPs [2]. In this paper, we produced the antibody-conjugated gold nanotriangle (ACGNTs) using GNTs synthesized by liquid-phase reduction with sodium citrate acid and tannic acid composition as a reducing reagent. Moreover, we revealed the molar ratio of antibody to Au required for use as a labeling reagent of hCG tests.

2. Materials and Methods

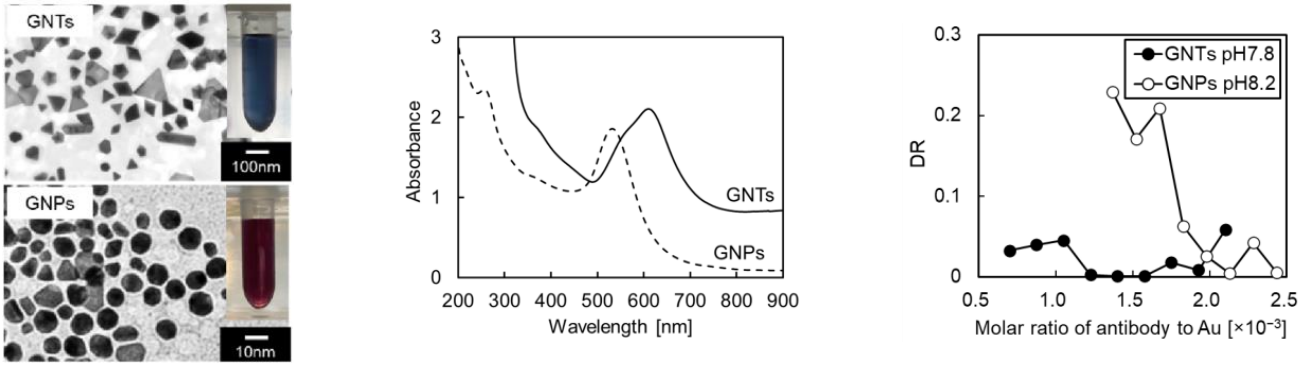
The GNTs were synthesized with liquid-phase reduction developed by authors [2]. A mixture of the aqueous solution of sodium citrate (128 µL, 100 mM) and tannic acid (7.7 mL, 0.153 mM) was prepared as solution A. A mixture of the aqueous solution of hydrogen tetrachloroaurate (III) tetrahydrate (0.21 mL, 29.7 mM) and CTAB (4.8 mL, 9.15 mM) was prepared as solution B. Subsequently, solution A was added to solution B and stirred for 10 min at room temperature (23 °C). The solution was allowed to stand at room temperature for three days.

Monoclonal antibody (Medix Biochemica, Espoo, Finland, Anti-hCG beta 5006) with a molecular weight of 23.5 kDa, the synthesized GNTs solution (Au concentration of 0.486 mM), and the buffer solutions (12 types of aqueous solution with pH = 5.7 to 9.8) were used for preparing ACGNTs. A 100 µL amount of GNTs solution, antibody solution

(50 μg/mL, 2.128 nM), and 4 μL buffer solution were mixed in a 98-well microplate. Subsequently, 10% NaCl solution of 100 μL was added to the wells. The mixture solution was measured with the absorbance ratio of the wavelength of 750 nm to that of 600 nm using a microplate reader (Corona Electric, Hitachi, Japan, SH-1300Lab). In this study, the volume of antibodies varied from 16 to 64 μL, and the wells without antibodies and NaCl were prepared as references. The smallest difference value in absorbance ratio DR between each well and the reference well in the column, where no absorbance changes were indicated, was determined as a suitable composition. The result was compared with that using a GNP solution (Au concentration of 0.559 mM and a diameter of 15 nm) [3]. In the case of using GNPs, the absorbance ratio was measured using the wavelength of 690 nm and that of 522 nm.

3. Results and Discussion

The synthesized GNTs showed an edge length of 126 nm (Figure 1a) and a blue-colored solution with an LSPR peak wavelength of 610 nm (Figure 1b). The value of DR depends on the volume of the antibody, and the suitable volume of antibody indicated to minimum DR and pH of buffer solution were 32 μL (DR = 0.001) and 7.8. On the other hand, in the case of using GNPs, the suitable antibody volume and pH of buffer solution were 56 μL (DR = 0.004) and 8.2.



(**a**) (**b**) (**c**)

Figure 1. (**a**) TEM images of GNTs and GNPs. (**b**) Absorption spectra of the synthesized GNTs and GNPs solution. (**c**) Difference in absorbance ratio DR of antibody-conjugated GNTs and GNPs as a function of the molar ratio of antibody to Au.

The molar ratios of antibody to Au at the minimum DR in the case of using GNTs and GNPs were calculated as 0.0021 and 0.0014, respectively. The molar ratio at the use of GNPs was 1.5 times compared to that of GNTs (Figure 1c). These results confirmed that the molar ratio of antibody to Au required for use as a labeling reagent was decreased by using GNTs, and the application of GNTs to the labeling reagents realized a low-cost hCG test strip.

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