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Analytical Methods

A competitive immunoassay for biotin detection using magnetic beads and gold nanoparticle probes

Wen-Zhi Lin[a,c], Yi-Hsiang Chen[b], Chih-Kai Liang[b], Cheng-Che Liu[d,*], Shao-Yi Hou[b,*]

[a] Graduate Institute of Engineering Technology Doctoral, National Taipei University of Technology, Taipei, Taiwan
[b] Graduate Institute of Biochemical and Biomedical Engineering, Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, Taipei, Taiwan
[c] Institute of Preventive Medicine, National Defense Medical Center, Taipei, Taiwan
[d] Graduate Institute of Physiology, National Defense Medical Center, Taipei, Taiwan

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ABSTRACT

Haptens are small molecules with low molecular weight that include biotin and many toxins in food. In this study, we used biotin as a model molecule for hapten detection. In this competitive immunoassay anti-biotin antibody-modified magnetic beads (Ab-MBs) and biotinylated thiol-DNA gold nanoparticles (biotin-GNPs) were used. The assay contains three reactions, the mixing of the sample and Ab-MBs, the capture of biotin-GNPs by Ab-MBs and the magnetic attraction. When biotin molecules were absent, the solution was transparent because biotin-GNPs bound to Ab-MBs which were caught by an external magnetic field. When biotin was present, the supernatant was red because the Ab-MBs bound to the analytes and the gold nanoparticles were still in solution. It was possible to complete all the operating steps in 15 min. The limit of detection (LOD) was 2 pmol. This rapid competitive-immunoassay has potential for application in detection of other haptens.

1. Introduction

Haptens are small molecules with low molecular weight (less than 5 kD) such as biotin, dioxin (Lin, Liu, Wai, & Lin, 2008), digoxin (Emrani et al., 2015), morphine (Hao, Zhou, Chang, Zhu, & Wei, 2011), amphetamine (Montesano et al., 2015), ketamine (Chen, Yang, & Tu, 2013), saxitoxin (Handy et al., 2013), and other toxins common in food and abused drugs. In general, an antigen such as a protein contains several epitopes which are recognized by specific antibodies. However, haptens are small in size with only one epitope and rarely induce immunogenicity. They generally only elicit an immune response when coupled to a carrier molecule such as bovine serum albumin, ovalbumin and keyhole limpet hemocyanin.

Recently, many researchers have reported analytical techniques to detect haptens, including through enzyme-linked immunosorbent assay (ELISA) (Kim, Shelver, & Li, 2004), gas chromatography–mass spectrometry (GC–MS) (Ten Noever de Brauw & Koeman, 1973) and high-performance liquid chromatography (HPLC) (Khayoon et al., 2010). Although these methods are sensitive and specific, complex procedures, time-consumption, high equipment cost and personnel training restrict their use.

Immunochromatographic assays based on the antigen-antibody reaction through a solid substrate have been successfully applied to detect pathogens, abused drugs, hormones, and contaminants in the environment, such as pesticides because they are rapid, operationally simple and low cost (Al-Yousif, Anderson, Chard-Bergstrom, & Kapil, 2002; Peng et al., 2007; Qian & Bau, 2003; Rong-Hwa, Shiao-Shek, Der-Jiang, & Yao-Wen, 2010; Tsay, Williams, Mitchell, & Chapman, 2002; Wong, 2002). The two major types of the immunochromatographic assay are the sandwich assay and the competitive assay. The sandwich assay whose analyte, the target molecule, is bound between two different antibodies is used for detection of high molecular weight molecules, such as proteins with multiple epitopes. After being mixed with antibody-modified nanogold and the target, the reagents migrate toward the detection zone where another anti-target antibody is immobilized on the solid substrate and forms a red band (Hou, Chen, Cheng, & Huang, 2007). Although the sandwich assay has some advantages, small molecules like haptens with a single epitope cannot bind two different antibodies. In the competitive assay, which can be used to detect haptens, the analyte analog rather than an antibody is

* Corresponding authors at: Graduate Institute of Physiology, National Defense Medical Center, No. 161, Sec. 6, Minquan E. Rd., Neihu Dist., Taipei 114, Taiwan (C.-C. Liu). Graduate Institute of Biochemical and Biomedical Engineering, Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, No. 1, Sec. 3, Chung-Hsiao E. Rd., Taipei 106, Taiwan (S.-Y. Hou).
E-mail addresses: jasonliu@mail.ndmctsgh.edu.tw (C.-C. Liu), f10955@ntut.edu.tw (S.-Y. Hou).

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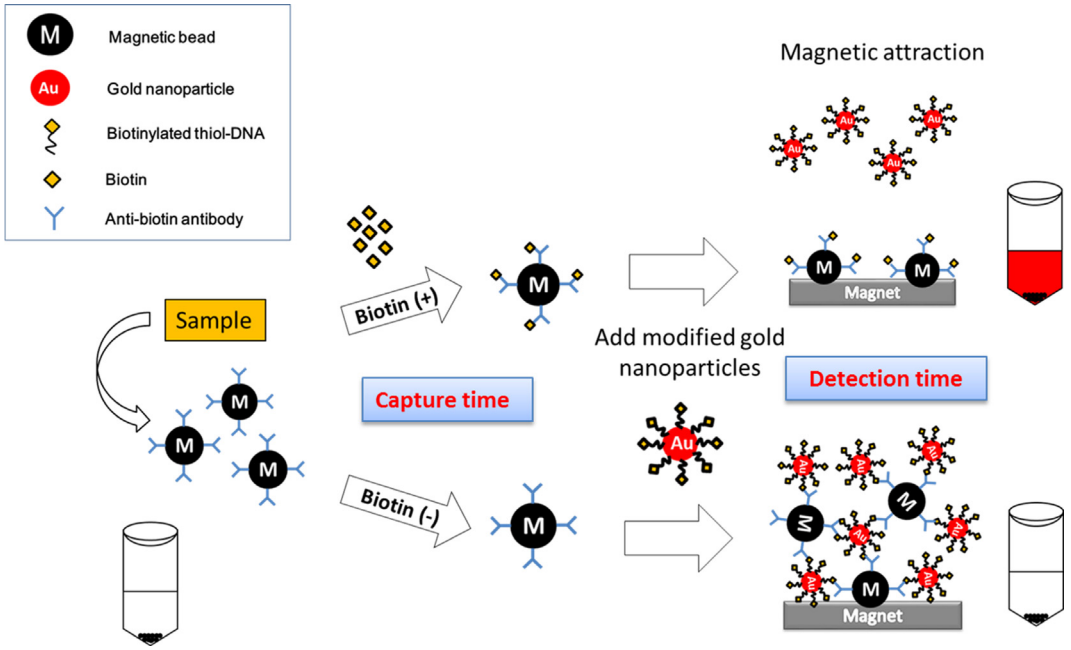

Fig. 1. Schematic description of the competitive immunoassay described in this study. The mechanism of a competitive immunoassay for detection of biotin using anti-biotin antibody-modified magnetic beads (Ab-MBs) and biotinylated thiol-DNA gold nanoparticles (biotin-GNPs).

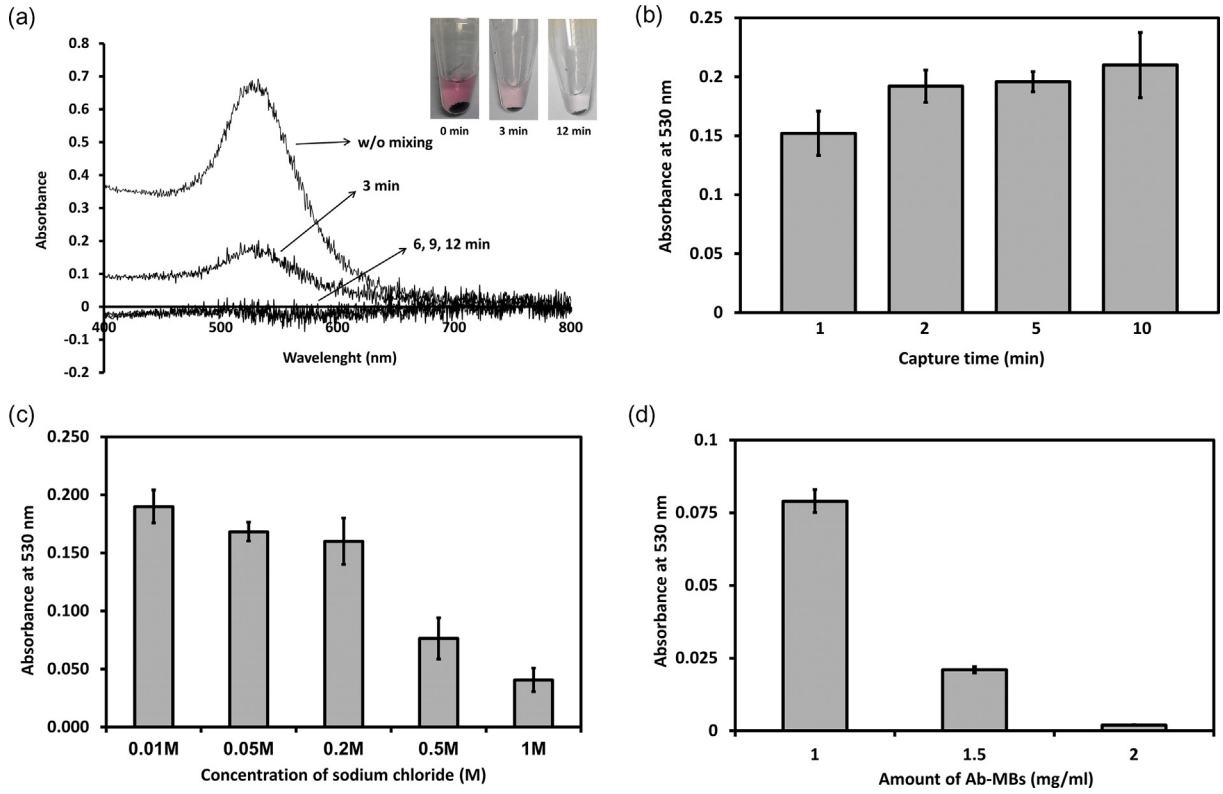

Fig. 2. Optimization of experimental conditions. (a) UV–Vis absorption spectra of supernatant for biotin-GNPs mixing with Ab-MBs at different times (0, 3, 6, 9, 12 min) after magnetic attraction without biotin. (b) UV–Vis absorption spectra of supernatant for biotin captured by Ab-MBs at different times (1, 2, 5, 10 min) then adding biotin-GNPs after magnetic attraction. (c) The relationship between absorbance changes and sodium chloride concentration for biotin-GNPs binding with Ab-MBs after magnetic attraction. (d) The relationship between absorbance changes and amount of Ab-MBs without biotin.

immobilized in the detection zone. The analog competes against the analyte for the binding site of the antibody conjugated with the nanogold. Hence, there is no red band with the presence of the analyte in the detection zone (Kim et al., 2011). The visualization of the results of the competitive assay is thus the exact opposite of the sandwich assay, which may have serious consequences if results are unwittingly confused.

Magnetic beads (MBs) have been broadly applied in bioassays because of their surface functionalization and magnetic separation (Haukanes & Kvam, 1993). Proteins or DNA are conjugated to magnetic