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Short communication

Magnetic bead and gold nanoparticle probes based immunoassay for β-casein detection in bovine milk samples

Y.S. Li [a,1], X.Y. Meng [a,1], Y. Zhou [a,*], Y.Y. Zhang [a], X.M. Meng [b], L. Yang [a], P. Hu [a], S.Y. Lu [a], H.L. Ren [a], Z.S. Liu [a], X.R. Wang [a]

[a] Key Laboratory of Zoonosis, Ministry of Education, Institute of Zoonosis, College of Veterinary Medicine, Jilin University, Changchun 130062, PR China
[b] Grain and Oil Food Processing Key Laboratory of Jilin Province, Jilin Business and Technology College, Changchun 130062, PR China

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ABSTRACT

In this work, a double-probe based immunoassay was developed for rapid and sensitive determination of β-casein in bovine milk samples. In the method, magnetic beads (MBs), employed as supports for the immobilization of anti-β-casein polyclonal antibody (PAb), were used as the capture probe. Colloidal gold nanoparticles (AuNPs), employed as a bridge for loading anti-β-casein monoclonal antibody (McAb) and horseradish peroxidase (HRP), were used as the amplification probe. The presence of β-casein causes the sandwich structures of MBs-PAb–β-casein-McAb–AuNPs through the interaction between β-casein and the anti-β-casein antibodies. The HRP, used as an enzymatic-amplified tracer, can catalytically oxidize the substrate 3,3′,5,5′-tetramethylbenzidine (TMB), generating optical signals that are proportional to the quantity of β-casein. The linear range of the immunoassay was from 6.5 to 1520 ng mL^{-1}. The limit of detection (LOD) was 4.8 ng mL^{-1} which was 700 times lower than that of MBs-antibody-HRP based immunoassay and 6–7 times lower than that from the microplate-antibody-HRP based assay. The recoveries of β-casein from bovine milk samples were from 95.0% to 104.3% that had a good correlation coefficient (R^2=0.9956) with those obtained by an official standard Kjeldahl method. For higher sensitivity, simple sample pretreatment and shorter time requirement of the antigen–antibody reaction, the developed immunoassay demonstrated the viability for detection of β-casein in bovine milk samples.

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1. Introduction

Protein content is one of particularly important milk quality parameters for characterizing its nutritional value (Kucheryavskiy et al., 2014). Casein (CN) is the main protein component of bovine milk which account for approximately 80% of the total milk protein content (Choi et al., 2011). There are four kinds of CNs in bovine milk and the concentration of each CN is consistent, namely αs1-, αs2-, β-, and κ-CNs in the ratios of 37%, 10%, 37% and 10%, respectively (Johansson et al., 2009). Therefore, each CN could be used as an index to evaluate the quality of bovine milk.

Various analytical techniques have been proposed for determination of individual CN content, such as electrophoresis for κ-CN (Kaminaridesa and Koukiassa, 2002), liquid chromatography for β-CN (1–25) (Gaucheron et al., 1995), surface plasmon resonance (SPR) measurement for β-CN (Muller–Renaud et al., 2004) and αs1-CN (Muller-Renaud et al., 2005), reverse-phase high-performance liquid chromatography, hydrophobic interaction chromatography and ion-exchange chromatography for αs1-, αs2-, β-, and κ-CNs (Bonfatti et al., 2008; Bramanti et al., 2003; Holland et al., 2010). Although these techniques are reliable for determination of CNs, they require expensive instruments and specific technical skills for inconvenient sample pre-treatment processes.

Immunoassays based on antibody are sensitive and high throughput procedure for quantitative detection and also successfully applied to measurement of milk protein content, such as enzyme-linked immunosorbent assay (ELISA) for αs1-CN (Black and Reynolds, 1998) and β-CN (Song et al., 2011; Zhou et al., 2013), immunomagnetic beads-based immunoassay for β-CN (Song et al., 2014), and nephelometric immunoassay for αs-CN and κ-CN (Collard-Bovy et al., 1991).

Due to having high surface to volume ratio, nanoparticle-based sensing provides a comparably large surface area available for reaction within a small sample volume. On the other hand, the accelerated analyte species transport to the nanoparticle surface significantly reduce the time that is required to perform a measurement (Katelhon and Compton, 2014). Magnetic beads (MBs) can be easily separated from the reaction mixtures with a magnet and re-dispersed immediately following removal of the magnet

* Corresponding author.
E-mail address: zhouyu69@sina.com (Y. Zhou).
[1] Both authors contributed equally to the preparation of this manuscript.

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(Wei et al., 2012) which allow for a nearly 'in solution' reaction (Kim et al., 2009), leading to shorter reaction times (Song et al., 2014). Various protocols based on MBs have been developed in a variety of research fields, such as environment monitoring (Schreier et al., 2012), clinical diagnosis (Eguilaz et al., 2010; Wei et al., 2012) and food safety (Xu et al., 2012). For having high surface areas, high chemical stability and unique size-dependent optical properties, colloidal gold nanoparticles (AuNPs) can be conjugated with more number of biomolecules for synthesis of probe to improve detection sensitivity (Jia et al., 2009), such as DNAs (Orza et al., 2010), antibodies (Omidfar et al., 2011) and enzymes (Zhou et al., 2012).

In this study, MBs were immobilized with anti-β-CN polyclonal antibody (PAb) as the capture probe. In the probe, MBs were as carrier for PAb allowing easily manipulation of the antibody for improving the kinetic of the antibody–antigen immunointeraction. AuNPs were immobilized with anti-β-CN monoclonal antibody (McAb) and horseradish peroxidase (HRP) as the amplification probe to improve detection sensitivity with the assumption that the interference of sample matrix could be neglected by diluting the sample. As shown in Fig. 1, in the presence of β-CN, it was firstly captured by MBs probe. Followed by adding of AuNPs probe, the analyte was directly recognized by the McAb immobilized on AuNPs and detected by the amplified colorful products produced by the catalyzing oxidation of HRP and 3,3′,5,5′-tetramethylbenzidine (TMB). The absorbance value of the colorful products is proportional to the concentration of the target.

2. Materials and methods

2.1. Chemicals and apparatus

β-CN, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), horseradish peroxidase (HRP), 3,3′,5,5′-tetramethylbenzidine (TMB), and N-hydroxysuccinimide (NHS) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Activated HRP (type B) kits and MBs (350 nm in diameter) were obtained from Tai Tianhe (Beijing, China) and Wa Wasaina (Wu Han, China), respectively. Anti-β-CN McAb and PcAb were produced in our previous study (Zhou et al., 2013). All reactions were carried out in 96-well polystyrene microtiter plates (Stripwell plate 2592, Costar, Changchun, China). The absorbance value was read with a MK3 microplate reader (Thermo, Shanghai, China).

2.2. Solutions and buffers

Dilution buffer [phosphate buffered saline (PBS)], 10 mmol L^{-1} sodium phosphate buffer (pH 7.4) containing 140 mmol L^{-1} NaCl; PBST solution, PBS containing 0.05% (v/v) Tween 20; TMB solution, 50 mmol L^{-1} sodium citrate buffer (pH 5.0) containing 0.01% (w/v) TMB and 0.005% (v/v) H_2O_2. Distilled water was used throughout the experiments.

2.3. Preparation of MBs probe

The MBs (400 μL, 10 mg mL^{-1}) were firstly activated by using the mixture solution of EDC (300 μL, 50 mg mL^{-1}) and NHS

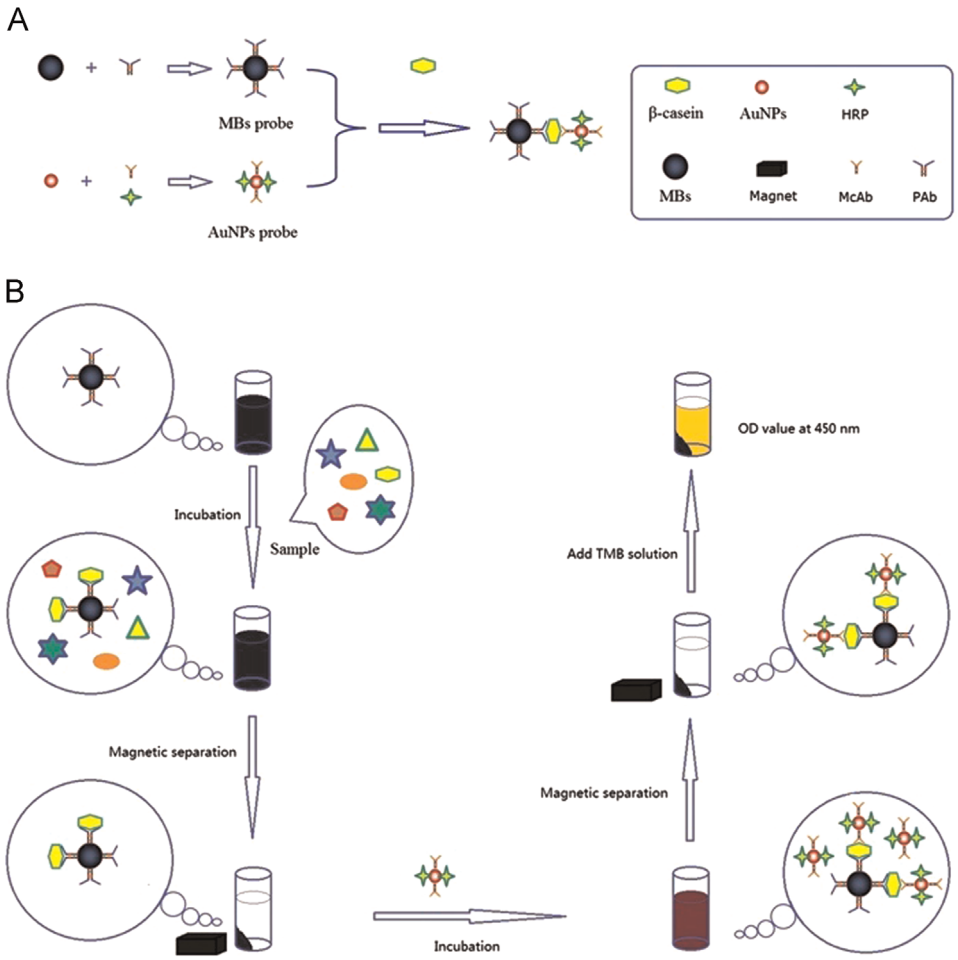

Fig. 1. Synthesis illustration of MBs and AuNPs probes (A). Principle of the double-probe based immunoassay for rapid and sensitive detection of β-CN (B). The details are described in Section 2.5.

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