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Determination of the affinity constant of streptavidin-coupled magnetic particles and a biotinylated antibody for high performance of magnetic solid carrier in immunoassays

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ABSTRACT

Streptavidin-coupled magnetic particles (SAMPs) usually serve as a separation tool in immunoassays to specifically recognize and capture biotinylated protein. Determining the affinity constant (K_A) of SAMPs and biotinylated proteins and further evaluating the performance of SAMPs as a solid-phase carrier are of great importance. Towards this goal, we present a simple method to detect the K_A of SAMPs and a biotinylated antibody (Ab). A combined equilibrium equation derived from fluorescence labeling experiments was employed to evaluate the binding capacity between SAMPs and a biotinylated antibody. The main experimental conditions were optimized, and the K_A of SAMPs and a biotinylated antibody was determined to be $3.64 \pm 0.31 \times 10^7\ M^{-1}$ with a coefficient of variation (CV) of 8.38%. The initial effective biotinylated antibody binding sites of as-synthesized SAMPs were found to be 132 pmol/mg SAMPs. In addition, the K_A of Ab towards SAMPs with four different streptavidin coating densities (16–83 μg/mg MPs) was studied, and the results showed no significant difference for the K_A among these four SAMPs. This suggests that the binding activity of SAMPs with Ab would not be affected by the streptavidin coupling density at least below 83 μg/mg MPs. However, when the four SAMPs were used as the capture reagent in sandwich immunoassays, the SAMPs with streptavidin coating density of 83 μg/mg MPs had a lower affinity than the other three SAMPs with less streptavidin coating density.

1. Introduction

The streptavidin–biotin system has been used for many years in a variety of applications including immunoassays [1–5], protein separations [6–8], nucleic acid hybridization [9–12] and drug delivery [13–17]. In the immunoassay field, the streptavidin–biotin system is widely employed to improve the sensitivity and specificity of an immunoassay due to its ultra-high affinity (the streptavidin–biotin affinity constant in solution is approximately $10^{15}\ M^{-1}$ [18,19]) and signal amplification effect (four biotins could be combined per streptavidin). Most clinical immunoassay technologies such as enzyme-linked immunosorbent assay (ELISA) and chemiluminescent immunoassay (CLIA) rely on the recognition and capture of specific analytes by a solid-phase carrier such as plastic plates, latexes and magnetic particles (MPs) [20,21]. In contrast to homogeneous analysis technologies, the reaction thermodynamics and kinetics of solid phase-based immunoassay are dramatically affected by the solid-phase carrier [22–24]. Compared with plastic plates, latexes and MPs usually exhibit faster reaction kinetics due to their uniform dispersion in solution and their larger specific surface area. The micron and submicron MPs are usually coupled by streptavidin in clinical immunoassays, while the antibody or antigen is labeled with biotin. The binding capacity between the streptavidin-coupled magnetic particles (SAMPs) and a biotinylated antibody (Ab) is one of the most important factors that affect the sensitivity and reaction efficiency of immunoassays. It is critical to determine the affinity constant (K_A) of SAMPs and Ab to understand the performance of SAMPs in immunoassays. Such measurements also guide the preparation of high performance SAMPs to enhance detection sensitivity, linear range, shorten detection time, etc.

Currently, capillary electrophoresis [25] and isothermal titration calorimeters (ITC) [26] are widely used to study the K_A of molecule–molecule or molecule–nanoparticle interactions in solution while other methods such as surface plasmon resonance technology (SPR) [27], surface-enhanced Raman scattering (SERS) [28] and the quartz crystal microbalance (QCM) [29] are used to study the affinity between molecules in solution and molecules fixed onto a solid chip or membrane. However, the above methods are suboptimal for measuring the K_A of micron or submicron particle surface molecules and their ligands in suspension.

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Table 1
Comparison of the methods for the determination of K_A.

	NM	CE	ITC	SPR	SERS	QCM
Advantage	Simple operation No need of large specific instruments Suitable for interaction between micron sphere surface molecule and its ligands	Little sample consumption	Sensitive, simple operation	Real time, label free, sensitive		
Limits	Need a little more sample	Limited detection sensitivity Only for molecule–molecule interaction in solution	Need specific instrument Time-consuming Only for molecule–molecule or molecule–nanoparticle interaction in solution	Need specific instrument One of the molecules needed to be immobilized on a solid chip or membrane		

Note: NM, the method we established in this paper; CE, capillary electrophoresis; ITC, isothermal titration calorimetry; SPR, surface plasmon resonance; SERS, surface-enhanced Raman scattering; and QCM, quartz crystal microbalance.

A combination of fluorometric and flow cytometric methods was used by Buranda et al. [22] to study the binding behavior of fluorescein biotin to streptavidin-coated polystyrene beads with a diameter of 6.2 μm. Huang et al. [24] studied the K_A and reaction kinetics of biotinylated DNA with streptavidin-coated polystyrene latex using a facile method based on a combined equilibrium equation derived from fluorescence labeling experiments between biotinylated DNA and streptavidin-coated polystyrene latex. These two methods have been utilized to study the binding capacity between streptavidin-coated particles and biological molecules such as free biotin or biotinylated DNA. As for immunoassays, the SAMPs usually recognize and capture biotinylated proteins rather than free biotin or biotinylated DNA, and the binding capacity between the SAMPs and the biotinylated protein directly affects the sensitivity and reaction efficiency of immunoassay. However, few studies have been performed to determine the K_A of SAMPs and biotinylated proteins, which is a specific thermodynamic parameter for evaluating the actual performance of SAMPs in immunoassays. In the present study, we established a rapid and simple method to do this based on the same principle that was described in the previous study [24]. We then applied this tool to study four kinds of SAMPs with different streptavidin coating densities. Finally, the performance of the four batches of SAMPs in chemiluminescence immunoassay was studied. A comparison between the established method and others is shown in Table 1.

2. Materials and methods

2.1. Reagents

Recombinant streptavidin and hepatitis B surface antigen (HBsAg) were purchased from YeaMin Biotech (Shanghai, China). Biotin was obtained from Sangon Biotech (Shanghai, China) and biotinylated horseradish peroxidase (biotin-HRP) from BIOS (Beijing, China). Biotinylated fluorescent antibody (antibody labeled with both biotin and FITC) was custom-made by PL Laboratory (Canada), and the biotin, FITC and goat anti-mouse IgG were purchased from SIGMA (USA). Biotin-labeled anti-HBsAg monoclonal antibody-1 (biotin-MAb1) and horseradish peroxidase labeled anti-HBsAg monoclonal antibody-2 (HRP-MAb2) were all from Hua'an Biotech (Hangzhou, China). Bovine serum albumin (BSA) was obtained from Genview (USA). Tween-20 was provided by BBI (Canada). N-Hydroxysuccinimide (NHS), BCA protein assay kit and chemiluminescent substrates were purchased

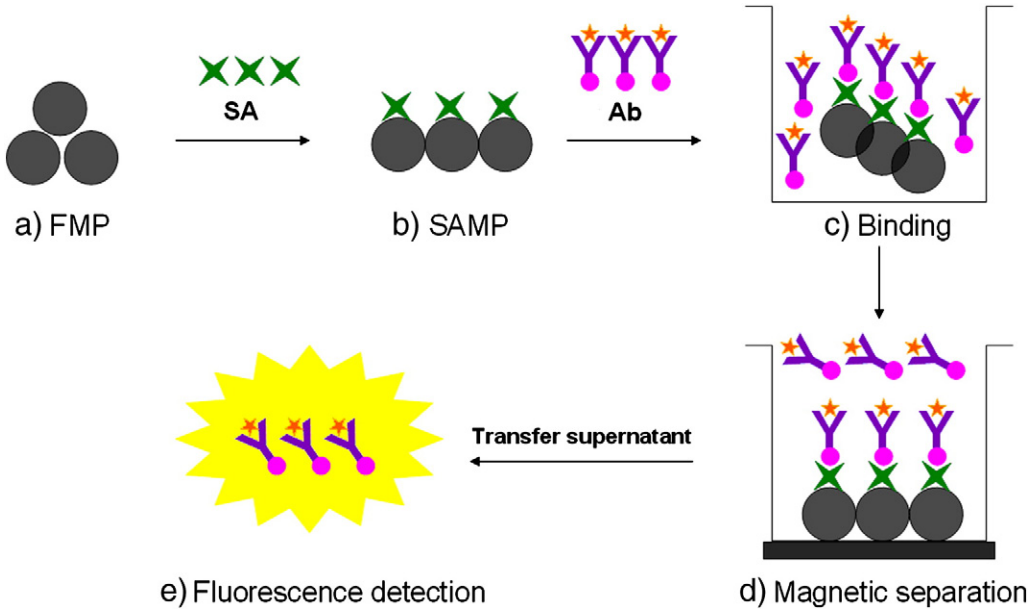

Fig. 1. Schematic illustration of the experimental procedure. FMP: magnetic particle modified by carboxyl group, SA: streptavidin, SAMP: streptavidin-coupled magnetic particle, Ab: antibody labeled with both biotin and FITC. (a) First, the surface carboxyl-modified MPs were prepared via a modified seed hybrid emulsion polymerization method developed by our group. (b) Second, the MPs were conjugated with streptavidin through a chemical conjugation method to obtain SAMPs. (c) Third, the SAMPs were incubated with Ab at room temperature. (d) Fourth, after incubation for 30 min, the mixture was separated on a magnetic plate. (e) Finally, the fluorescence intensity of Ab in the supernatant was measured. The amount of Ab bound to SAMPs was calculated by a subtraction method.