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Improvement of Protein Chip Sensitivity by Increasing Salt Concentration

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Abstract

The protein chip is a powerful emerging technology for biomedical applications. In contrast to the DNA chip, however, great challenges face the development of optimal surface materials for protein chips that are able to maintain the activity of embedded proteins with increased sensitivity, while being produced at sufficiently low cost to replace the materials used in current technology. We previously developed a sol-gel protein chip technology with significantly improved physical properties and sensitivity. Here, in addition to modifying the protein chip materials, we found that the addition of more salt with proteins in the preparation of 3-dimensional protein chips can increase the sensitivity of the protein chip. The present study results will support the technological advancement for protein chip developers and biochip research.

Keywords: Protein chip, Salt concentration, Sensitivity

Text

Protein chip technology provides a novel tool for studying various complex protein interactions, such as protein-protein, protein-nucleic acid, and protein-small molecule interactions, with increased sensitivity and throughput, even when using small sample volumes[1-3]. The current major challenge for the development of protein chips is the design of optimal chip materials that can maintain the nature of the embedded proteins. Unlike other biomolecules such as DNA, proteins tend to unfold when immobilized, which often causes loss of activity and binding sites that are dependent on the 3-dimensional structure[4-6].

Sol-gel materials have been widely investigated for the entrapment of proteins and have proven to maintain protein activity over months or even longer[7-9]. Especially, the immobilization of proteins within sol-gel-derived materials is expected to provide a potential advantage over other protein chip materials because additional protein modification, such as affinity captured agents or tagged recombinant proteins, is not required. This advantage will support the use of a wide variety of proteins in their native state[8-13]. Furthermore, the use of various silicates or additives allows for the potential optimization of materials for optimum biocompatibility and activity of the immobilized proteins[14].

Previously, unique screening and selection methods were used to obtain the best formulation for a novel, three-dimensional, sol-gel chip with optimal physical properties, including strong spot adhesion and good protein activities. The selected formulations were then used to detect protein-antibody and protein-protein interactions on the sol-gel-derived protein chips[14].

Here, we used the same formulation[14] for immobilizing the P24 proteins. As sketched in figure 1, we tested the salt effect on sensitivity by adding 3 different salt solutions when preparing the sol-gel protein chip. We expected the cations to play a role in amplifying the fluorescence signals by ionic energy transfer. We used three different cations containing salts, Na^+, K^+ and NH_4^+.

As shown in Figure 2A, we assayed protein chips by immobilizing P24 proteins with different NaCl concentrations. As a control, we washed the spots before assay to remove the salts from the spots (Pre-Wash). The unwashed P24 spots (with cations) showed higher signals than the Pre-Wash spots (without cations). This effect was linearly increased up to a Na^+ level of 200 ppm, but further increase in salt concentration beyond 200 ppm had little effect. We further tested the salt effects with other solutions, as shown in Figure 2. The addition of K^+ had a similar effect to that of Na^+, as shown in Figure 2B. The level of signal enhancement was similar for both Na^+ and K^+. Lastly, the addition of NH_4^+ ions to the protein chips, as shown in Figure 2C, gave a significant signal enhancement compared to the Pre-Wash spots (no NH_4^+). Especially, the NH_4^+ addition showed the highest enhancement among the three salt solutions in Figure 2.

To apply this effect on protein-antibody interactions

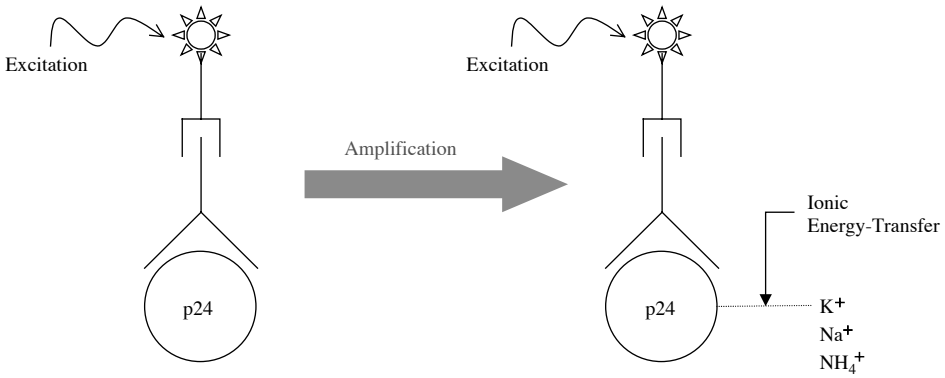

Figure 1. Schematic diagram of sensitivity enhancement through salt addition. In case of P24 proteins, after incubation with primary anti -p24 antibody and Cy3-labeled secondary antibody, signal amplification was observed by salt addition.

within a reasonable range of salt concentrations, we used five different salt concentrations (0, 10, 20, 50, 100 and 200 ppm of NaCl) up to 200 ppm, which was considered to be a saturated salt concentration showing signal enhancement in Figure 2. From the previous study, the detection limit of our sol-gel protein chip was tested using known amounts of HIV antibodies against p24 antigens immobilized on the slide, along with BSA proteins. The HIV antibody was detected down to the femtogram level in 1 mL of serum, which is 1,000-fold higher than the sensitivity of any currently available methods[14]. In Figure 3, the five spots with BSA proteins (200 pg/mL) were assayed without pre-washing, along with the no-protein spots, and then incubated at the femtogram level with Cy3-labeled BSA antibodies (fg/mL). As shown in Figure 3A, the highest signal enhancement was observed at 200 ppm NaCl concentration. Therefore, salt addition to the material formulation was found to increase the interaction signal level (BSA signal) while decreasing the background fluorescent signal (no-protein signal), thereby enhancing the sensitivity for protein-antibody interactions.

A protein chip developed for the sensitive detection of protein-protein interactions underwent surface or material modification to increase the sensitivity while maintaining protein activities[15-18]. The study results indicated that the addition of more salt solution to the proteins during preparation of the 3-dimensional protein chips, in addition to the surface and material modification, can increase the protein chip sensitivity. Therefore, this result promises to assist in the technological advancement in the biochip research field.

Methods

Protein Preparations

The HIV P24 protein, anti-P24 antibodies and Cy3-labeled secondary antibodies (goat, rabbit and mouse) were purchased from Abchem (UK), and the BSA protein and anti-BSA antibodies from Sigma-Aldrich (USA). Protein labeling was performed using the Fluorescent Labeling Kit according to the standard protocol (Molecular Probes, USA).

Protein Chip Preparation

The sol-gel protein chip method was used as described previously[14]. In brief, using our optimized sol-gel materials [25.5% TMOS (Tetramethyl orthosilicate, Gelest), 12.5% TEOS (Tetraethyl orthosilicate, Sigma), TrEOS (ethyltriethoxysilane, Gelest), 5.0% PEG8000 (Sigma), 10 mM HCl, 10 mM sodium phosphate (pH 7.5)][14], we immobilized BSA and P24 proteins onto PMMA (polymethylmethaacrylate, SPL) plates using an Arrayer according to the manufacturer's protocol (GeneMachine, Genomic Solutions, USA).

Salt Dependency Assays

To measure the salt dependency of the sensitivity[19-23], p24 proteins (200 ng/mL) were immobilized as described above. When we made p24 protein chips, each spot was made from different concentrations of additional salts (NaCl, KCl, NH_4Cl) from 0-1,000 ppm. Then, immunoassays were performed with antibody against p24 proteins (1 pg/mL). As described in Figure 1, the p24 spots with different salt concentrations were then incubated for an hour with the p24 antibody and washed following the standard procedure as described previously. The secondary antibody labeled with Cy3 (Sigma) was then incubated for another 30 mins. After thorough washing and drying, the resulting spots on the slides were scanned and analyzed using GenePix Pro 4.0 software (Axon Instruments) for quantification of the signals versus the background ('Sensitivity'). For comparison, we washed the spots (Pre-Wash in figure 2) before antibody addition.

Sensitivity Measurement

BSA proteins were also immobilized in the same

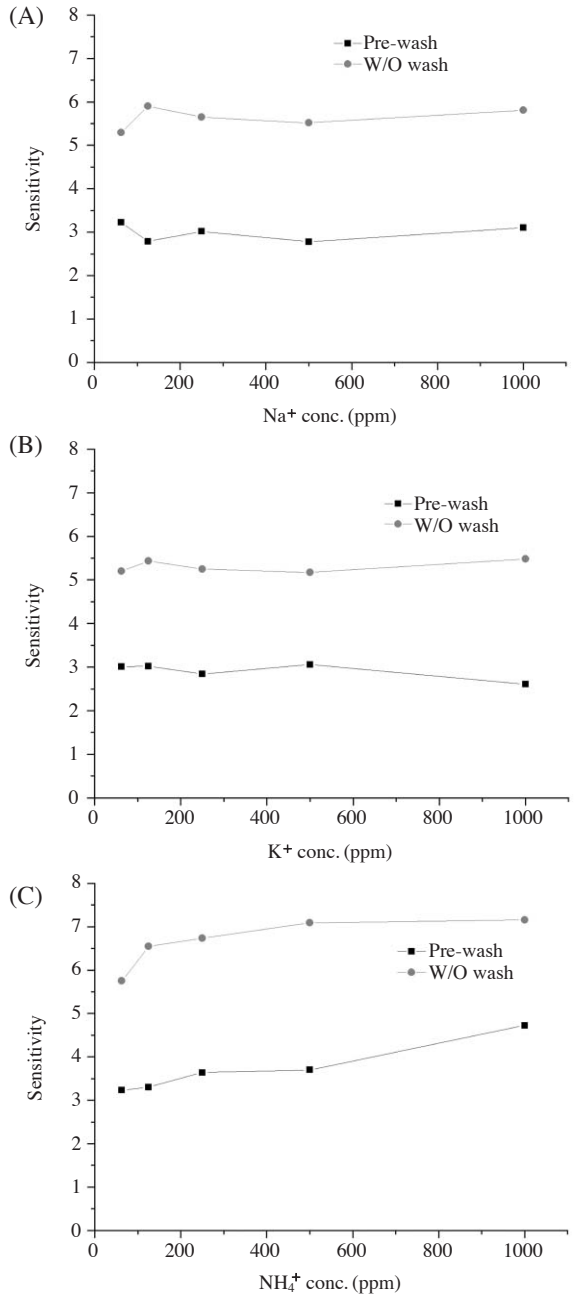

Figure 2. Signal enhancement of protein chip by salt addition. (A) NaCl salt solution containing Na^+ was added to the sol-gel protein chip and the P24 protein signal was compared to that of the no-salt spot (Pre-Wash). Especially, signal amplification was saturated at NaCl concentration above 200 ppm. (B) KCl salt solution containing K^+ was added to the sol-gel protein chip and the P24 protein signal was compared to that of the no-salt spot (Pre-Wash). Especially, signal amplification was saturated at KCl concentration above 200 ppm. (C) NH_4Cl salt solution containing NH_4^+ was added to the sol-gel protein chip and the P24 protein signal was compared to that of the no-salt spot (Pre-Wash). Especially, the signals were amplified by this NH_4Cl salt solution above 200 ppm without saturation.

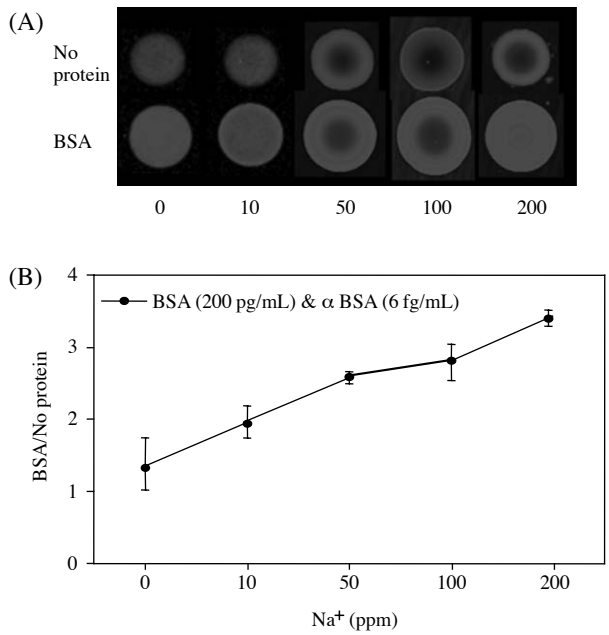

Figure 3. Sensitivity measurement between BSA and BSA antibody. BSA proteins were immobilized with five different NaCl salt concentrations below 200 ppm. As a control, no-protein spots with five different NaCl salt concentrations were immobilized. These different NaCl concentration spots, with or without BSA proteins, were incubated with Cy3-labeled BSA antibodies. The signal of the resulting microspots was measured and analysed. In (A), a representative image of BSA and BSA antibody interaction is shown with increasing salt concentration. The BSA signals, but not the control spot (no protein) signals, were enhanced with increasing salt concentration to 200 ppm. In (B), this BSA signal to no-protein value is plotted versus the salt concentration. This experiment was conducted at least three times to obtain the standard deviation.

chip plate as above in order to observe the effect of salt concentration on sensitivity, in comparison with no-protein spots. For measuring the non-specific interactions with the material, we spotted the material only (no protein) along with BSA proteins (200 ng/mL), and incubated with antibodies against Cy3-labeled BSA (1 fg/mL) for 30 mins. The incubated slides were heavily washed with washing solution (1 × PBS, 0.1% Tween (Sigma)) and then dried. The resulting spots on the slides were scanned and analyzed using GenePix Pro 4.0 software (Axon Instruments) for quantification of the BSA signals versus the no-proteins.

Abbreviations

BSA (Bovine Serum Albumin), fg (femtogram, 10^{-15} g), ag (attogram, 10^{-18} g)

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