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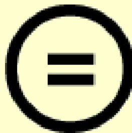

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A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Rapid visible detection of Ara h1, major peanut allergen, using functionalized gold nanoparticle aggregation

성형 금나노입자 응집을 이용한 **Ara h1** 검출법 연구

August, 2016

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Department of Agricultural Biotechnology

Seoul National University

농학석사학위논문

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지도교수 최영진

이 논문을 석사학위 논문으로 제출함

2016년 8월

서울대학교 대학원 농생명공학부

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김웅희의 석사 학위논문을 인준함

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ABSTRACT

There has been a lot of research aimed to develop an easy, rapid and sensitive detection method for useful/harmful biomaterials and microorganism in food system. However, current techniques have their own problems such as complicate sample preparation of time-consuming steps, low sensitivity, and necessity of trained experts or sophisticated instruments. In the preceding studies, a rapid and simple detection method using gold nanoparticle (AuNPs) was developed. This method is carried out in two steps. In the first, the target is mixed with switchable linker (SLs) with various concentrations. After the first step, streptavidin coated AuNPs (stAuNPs) are added to the above solutions. Because of high affinity of biotin to streptavidin, biotins on the SLs connect with the streptavidin on the stAuNPs and form huge aggregates. Without the target, region of forming aggregates is naturally appeared by the relationship between the concentrations of the SLs and stAuNPs. However, when the targets are added, the SLs covered with targets have less ability to connect with stAuNPs than naked SLs. This resulted in a change of the quantitative relationship mentioned above, which led to shift of the aggregation region. Therefore, in this quantitative relationship, the number of streptavidin on stAuNPs is one of the key factors. In this study, streptavidin on stAuNPs was modified to improve this detection system.

Firstly, the effect of the number of streptavidin on stAuNPs on the range exhibiting a visible color change (REVC) and reaction time was investigated. The less number of streptavidin on stAuNPs allowed REVC forming region to shift to lower concentrations of SLs and it took more time for making enough aggregation. However, by agitating during they are forming REVC, time for forming REVC was reduced. Finally, after optimized streptavidin modifying level on stAuNPs. Obtained particles are used to detect Ara h1, major peanut allergen. This detection system using modified stAuNPs could detect very low concentration of Ara h1 (10 nM), without sophisticated equipment. It was also tested with peanut extract and could be distinguished by naked eye in solution to 100-fold diluted extract. Thus, the result of this study could be used as valuable references for improving and applying this detection system to real food system.

Keywords: detection, modified gold nanoparticle, aggregation, visible detection, Ara h1

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Ⅰ. INTRODUCTION

Food allergy is a significant worldwide issue in not only food industries, but also public health problem. Estimates for the prevalence of peanut allergies are around 0.5−2% of the total population, and this trend appears to be increasing (Bindslev-Jensen, Briggs, & Osterballe, 2002). Especially, peanut is one of the common allergenic foods. Peanut allergy affects approximately 1% of the population worldwide and many of them contacting with even very small as 100 μg of the allergens are sufficient to lead allergic reactions (Peng et al., 2013). Because no treatment has been developed for peanut allergy (Wen, Borejsza-Wysocki, DeCory, & Durst, 2007), avoidance of peanuts is the only way to prevent accident and labeling of food containing peanut ingredients has proven to be an effective and simple method for avoiding allergic reactions (Vierk, Koehler, Fein, & Street, 2007). The Food and Drug Administration (FDA) implemented a new regulation which named Food Allergen Labeling and Consumer Protection Act (FALCPA) in Jan 2006, requiring food allergen labeling of all foods sold in the USA (FDA, 2004). Similar regulations have been established in other developed regions (Li et al., 2009). For this reason, proper methods for

detection of peanut allergens are necessary for ensuring the compliance of food labeling and for improving consumer protection.

Ara h1 is an ideal biomarker for peanut allergen detection for a number of reasons (Pomés et al., 2003). Firstly, Ara h1 is the major component of peanut, comprising 12–16% of the total peanut protein (Koppelman et al., 2001). Secondly, it has a stable structure so that it does not degrade during food processing procedures, such as heating or roasting (Beyer et al., 2001). Thirdly, Ara h1 is the most important major allergen from peanuts.

Because of its advantages on peanut allergen detection, a lot of study for Ara h1 detection has been conducted. The current reference method for detecting food allergens is an enzyme-linked immunesorbent assay (ELISA) (Matthias, 2001; van Hengel, 2007), and several immuno-assay techniques also have been developed for detection of Ara h1 including RP-HPLC (Singh, Cantoria, Malave, Saputra, & Maleki, 2016), lateral-flow assay (Wen, Borejsza-Wysocki, DeCory, & Durst, 2005), surface enhanced Raman spectroscopy (Gezer, Liu, & Kokini, 2016; Xiaoyan et al., 2014) and immune-sensors (Alves et al., 2015; Yin, Melissa, & Ian, 2008). However, these techniques have their own problems such as complicate sample preparation of time-consuming steps, and necessity of trained experts or

expensive sophisticated instruments.

Nowaday, colorimetric detection methods which can verify result with naked eye have been got interest due to its simplicity (P. & L., 2008; Wang, Pu, & Liu, 2010; Z., F., & L., 2010) and these methods did not require sophisticated detection equipment or techniques (Aili, Selegard, Baltzer, Enander, & Liedberg, 2009; Kaittanis, Santra, & Perez, 2010; Sener, Uzun, & Denizli, 2014). For these reasons, our group reported study about development of novel colorimetric detection system (Lim et al., 2012). This system is signal-based amplification strategy introducing switchable linkers (SLs) to mediate aggregation of AuNPs. The SLs are an element allowing multiple bindings, which can be selectively enabled or disabled. When SLs recognize the target material, they lose their multiple binding forming capabilities, and result in forming the range exhibiting a visible color change (REVC) at higher concentration of SLs with AuNPs than when they could not recognize target. This system could be a powerful tool to detect target materials, however, many SLs are required for forming aggregate. Because SLs are fairly expensive, it is preferable to use less amount of SLs for detecting peanut allergens.

Here, I describe a new method of modifying the number of streptavidin, functional protein which has an affinity to biotin, on AuNPs for

decrease of SLs in this system. Optimization was carried out for shifting REVC forming region to lower concentration of SLs and reducing reaction time. After optimization, this system was applied to detect Ara h1 in buffer and real sample.

Ⅱ. MATERIALS AND METHODS

2.1. Materials

Tetrachloroauric acid, bovine serum albumin (BSA) and biotinylated bovine serum albumin (B·BSA) were purchased by Sigma Aldrich (St. Louis, MO, USA.). Tri-Sodium citrate was purchased by Yakuri Pure Chemicals Co., Ltd (Kyoto, Japan). Tetraborate pH standard solution was purchased by Wako Pure Chemicals Industries, Ltd (Osaka, Japan). Phosphate Buffered Saline (PBS) was purchased by Thermo Fisher Scientific (Waltham, MA, USA). Thiolated streptavidin was purchased by Protein mods (Madison, WI, USA) purified natural Ara h1 and its biotinylated monoclonal antibody, 2F7 Anti Ara h1 were purchased from Indoor Biotechnologies (Cardiff, UK). Syringe filter was purchased from Merck (Frankfurt, Germany). Roasted peanut was purchased from Lotte, Ltd (Seoul, Korea).

2.2. Instrumentation

Extinction measurements were performed on a UV-visible spectrophotometer (Shimadzu, Kyoto, Japan). The pH of all of the buffer solution was examined with a Professional meter PP-15 (Satorious, Göttingen,

Germany).

2.3. Preparation of gold nanoparticles (AuNPs)

AuNPs with a diameter of 30 nm were synthesized according to the method established by Bastus et al (Bastus, Comenge, & Puntes, 2011). Briefly, 150 mL of a sodium citrate solution (2.2 mM) heated to boiling. After boiling had begun, 1 mL of a $HAuCl_4$ solution (25 mM) was added into the boiling solution. After color of solution was changed to soft pink, the temperature of solution was cooled down to 90°C in water bath and cooled solution was used as seed solution at size growth process. For AuNPs size growth, 1 mL of a $HAuCl_4$ solution (25 mM) was added to seed solution twice at 30 min intervals. After reaction was finished, 55 mL of sample was extracted. And then, remaining sample was diluted by adding 55 mL of a sodium citrate solution (2.2 mM). After the temperature of diluted solution reached to 90°C again, size growth process was repeated by using diluted solution as seed solution, and the process was repeated three times. After that, AuNPs with a diameter of 30 nm were finally obtained and stored at 4°C before further use. Particle Size of AuNPs was determined by using UV-Visible (UV-Vis) spectrophotometer (UV-1700, Shimadzu, Japan).

2.4. Preparation of streptavidin-coated gold nanoparticles (stAuNPs)

600 µL of AuNPs (Abs. 4.0 @ 526 nm, diameter 30 nm) were incubated with 100 ul of BSA (0, 50 and 100 µg/mL) in borate buffer (pH 7.4) for 20 min. incubated mixture was reacted with thiolated streptavidin (50 µg/mL) in borate buffer (pH 7.4) for 10 min. Unbound streptavidin was removed by repeated centrifugation (5,000xg. 20 min), and pellet of streptavidin coated AuNPs (stAuNPs) was resuspended in PBS buffer (1X, pH 7.4) containing 0.1%(w/v) BSA. Finally, concentration of resuspended stAuNPs was adjusted to Abs. 6.0 @ 536±0.5 with spectrophotometer (Fig.1).

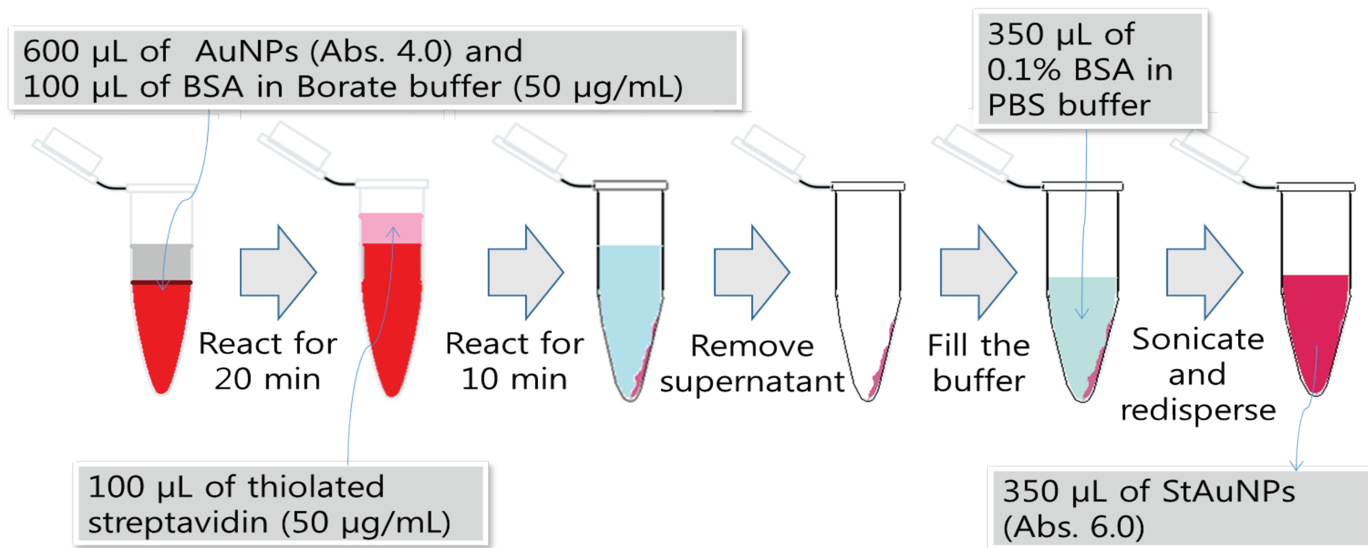


Fig. 1. Schematic of preparation of streptavidin coated AuNPs. AuNPs, gold nanoparticles; BSA, bovine serum albumin.

2.5. Detection of streptavidin in PBS buffer solution

The detection system is composed of two steps. In the first step, 100 µL of streptavidin solution (0, 20, and 200 nM) was mixed with 100 µL of biotinylated BSA in PBS buffer (0.1, 1, 2, 3, 4, 5, 10 and 15 µg/mL), respectively, which the mixture was mildly agitated for 20 min. In second step, 200 µL of stAuNPs (Abs. 6.0 @ 536.0±0.5 nm) was added. In holding test, mixture of second step was held for 14 h. And in agitation test, mixture was agitated for 30 min with vortex mixer at 200 rpm. Shifting of the range exhibiting a visible color change (REVC) was detected by the naked eye and/or by UV-vis spectroscopy at 400-800 nm.

2.6. Extraction of Ara h1 from peanut and preparation of standard Ara h1 solution

Samples were prepared according to previously described procedures (Pomés et al., 2003) with minor modifications. Briefly, three gram of peanut was groud by grinder. And then, grinded sample was dissolved into 30 mL of PBS buffer (pH 7.4) containing 1 M NaCl with stirring for 15 min at 60°C. After extraction, extracted solution was filtered through a 0.22 µm syringe filter to remove solid debris. Filtered solution was used for following experiments. Standard Ara h1 solution was prepared by dilution of

standardized Ara h1 in PBS buffer (pH 7.4) containing 1 M NaCl.

2.7. Detection of Ara h1 in standard solution and peanut extract

The detection system of Ara h1 was the same as the detection system of streptavidin. It composed of two steps. In first step, 100 μL of standard Ara h1 solution (0, 0.2, 0.4 and 0.8 ng/100 μL) was mixed with 100 μL of biotinylated anti-Ara h1 antibody (35, 45, 55 and 65 ng/100 μL), respectively, which the mixture was mildly agitated for 20 min. In second step, 200 μL of stAuNPs (Abs. 6.0 @ 536.0±0.5 nm) was added and then additionally agitated for 30 min. shifting of REVC was detected by the naked eye (Fig. 2). To detect Ara h1 from peanut extract, diluted extract to various concentration (10^0, 10^1, 10^2 and 10^3-fold diluted) were mixed with antibody in the first step. And then, the rest steps were performed in the same way.

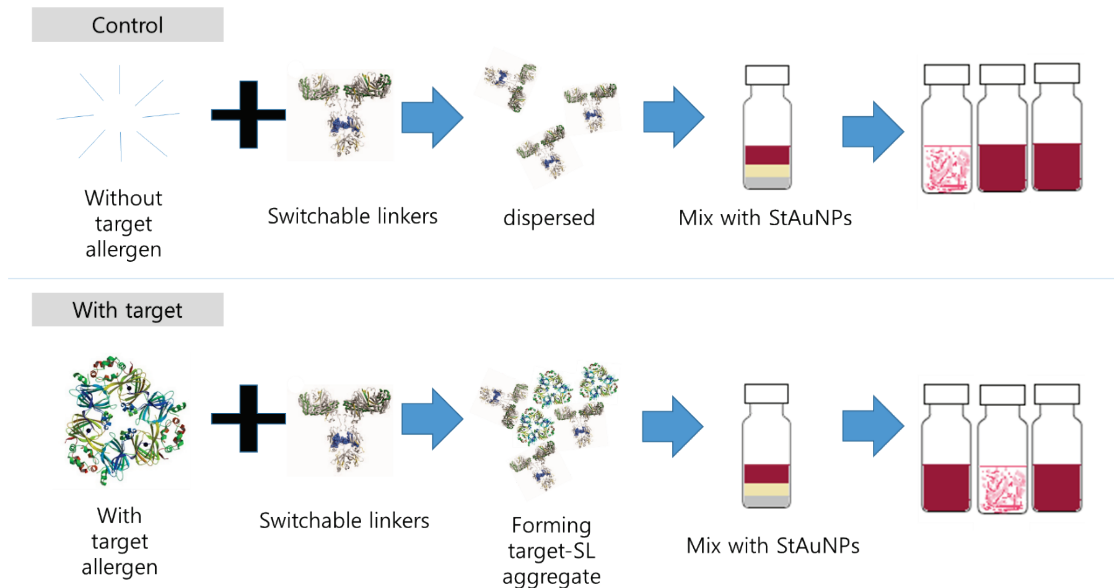


Fig. 2. Schematic of the detection system. Reaction of target materials and SLs in the first step disturbs natural relationship and induces shift of REVC forming region. SLs, Switchable linkers; stAuNPs, streptavidin coated gold nanoparticles; REVC, range of linker concentration exhibiting visual color change.

2.8. Selectivity of the colorimetric detection

The selectivity of the colorimetric sensor was verified using other extract tree nuts (pistachio and walnut) and their mixture under the above-mentioned conditions. The 10-fold diluted extracts of tree nut were used in this experiments, respectively. In addition, selectivity experiments were performed in the presence of a mixture of all extracts (each 10-fold diluted extracts occupied 10 % (v/v) in mixture).

Ⅲ. RESULTS AND DISCUSSION

3.1. Principle for REVC control by adjusting of the number of streptavidin on stAuNPs

Principle of the two step-based detection system was proposed by Lim et al. (2012). In this system, region of forming REVC was influenced by the quantitative relationship among particle, SL and target. Because particle and SL have multi-functional groups on their surfaces which are conjugated with each other, they form cluster rapidly and settle down. Thus, controlling of multi-functional groups on the particle affects region forming of REVC and settling velocity of the forming REVC. The effect of multi-functional groups

on the particle surface was investigates with stAuNPs and bBSA as particle and SL, respectively. For controlling the number of streptavidin, functional protein which has high affinity to biotin, on particle surface, the number of pre-coated BSA on particle surface was adjusted. Unlike the case of pre-coated with BSA, in case of streptavidin conjugated directly on naked AuNPs, unintended aggregation was occurred. Thus, it could be possible to obtain dispersed stAuNPs, when AuNPs were reacted with excessive quantity of streptavidin only. Unlike BSA, which is monomer and used in the non-specific reagent, streptavidin is a tetrameric protein. That being so, it can make a linkage with other particles after conjugated with a particle. By pre-coating the various concentrations of BSA solution, it could be obtained stAuNPs with various number of streptavidin. As the number of streptavidin on AuNPs was reduced, the region of forming REVC was shifted to lower bBSA concentration (Fig. 3).

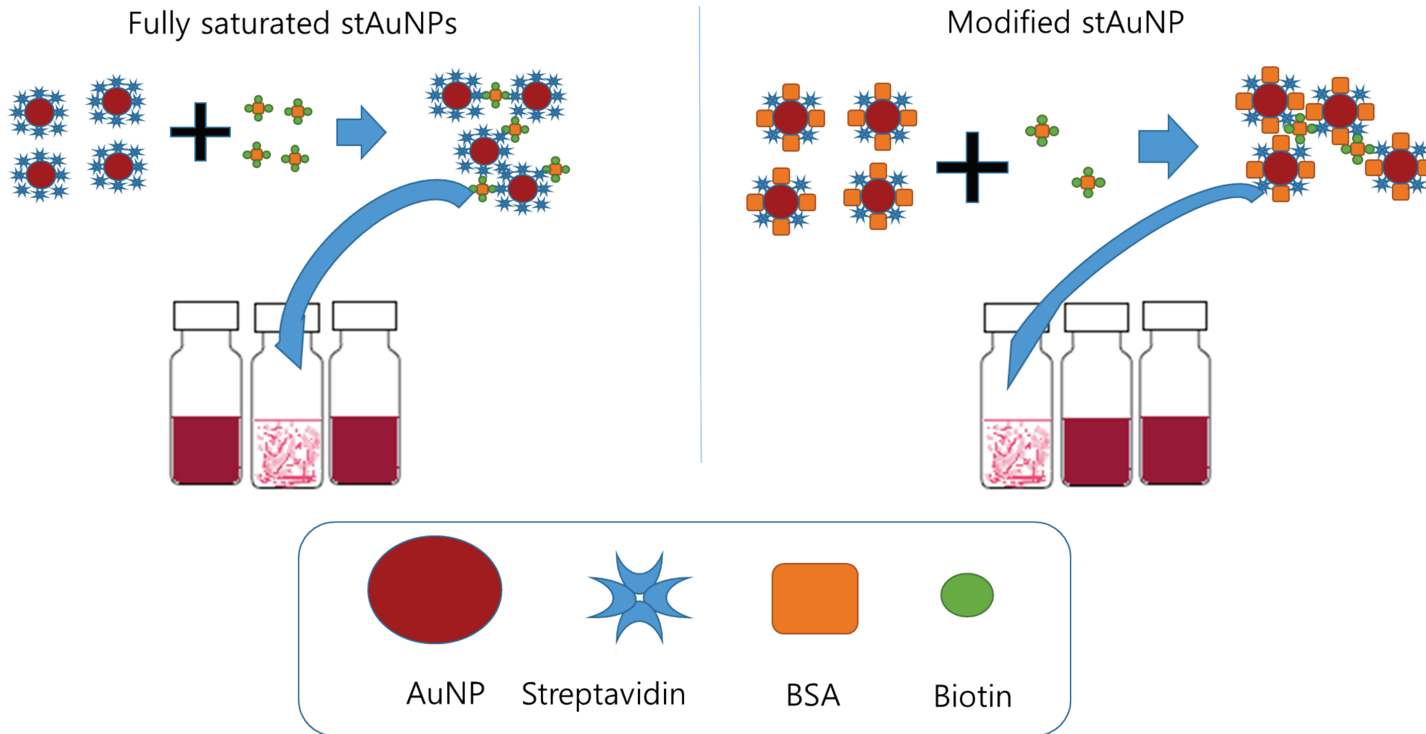

Fully saturated stAuNPs
Modified stAuNP
AuNP
Streptavidin
BSA
Biotin

Fig. 3. Schematic representation of difference of needed SLs quantity for forming aggregates between fully saturated stAuNPs between modified stAuNPs. AuNPs, gold nanoparticles; BSA, bovine serum albumin; stAuNPs, streptavidin-coated AuNPs.

3.2. Effect of the number of streptavidin of AuNPs surface on the detection system

3.2.1. Determination of streptavidin on stAuNPs in the detection system

Naked AuNPs with a diameter of 13 nm showed λ_{max} at 520 nm. The greater concentrations of BSA solutions (from 10 μg/mL to 100 μg/mL) were reacted with bare AuNPs solution, λ_{max} of BSA coated AuNPs increased gradually. However, when concentration of BSA was greater than 100 μg/mL, λ_{max} did not increase anymore (Fig. 4). At this concentration, the surface of AuNPs was fully saturated with BSA. For this reason, I further experiments were carried out in the range of lower than 100 μg/mL of BSA.

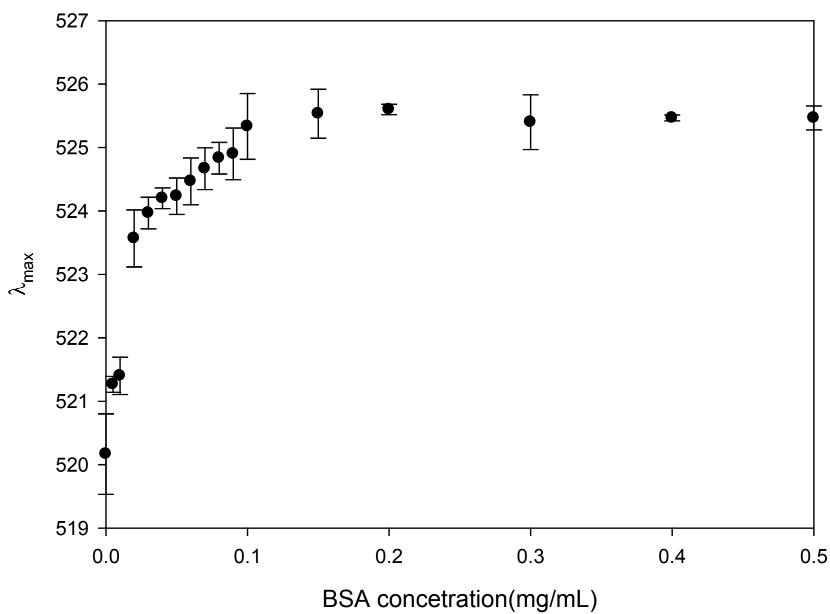


Fig. 4. Change of λ_{max} according to the amount of added BSA. BSA, Bovine serum albumin.

3.2.2. Change of REVC forming region in the detection system

The number of streptavidin is much critical for this detection system. This system is based on the streptavidin-biotin affinity for making aggregation. In the same concentration, the more number of streptavidin gives the more chances for binding with bBSA, so it needs less bBSA for making the same scale of aggregation for the detection, if the stAuNP has less streptavidin on it. AuNPs which is fully saturated by BSA were not attached on microtube (Fig. 5). However, the greater number of streptavidin was coated on AuNPs by reducing concentration of BSA used in pre-coating, stAuNPs were adhered on microtube because of streptavidin on their surface.

When less streptavidin coated AuNPs reacted with bBSA, REVC was formed at lower region than fully saturated stAuNPs was reacted with bBSA (Fig. 6). However, even by increasing pre-coating degree of BSA on AuNPs above certain level, REVC region did not shift to lower than 2 μg/mL of bBSA and time of forming REVC was increased more than 2 times. As a result of the experiments with several conditions (data not shown), using stAuNPs pre-coated by 50 μg/mL of BSA was determined as optimal condition. In this condition, bBSA concentration of formed REVC region was minimalized, but time of forming REVC was not increased than the time it took when fully saturated stAuNPs was used.

In streptavidin detection, REVC was formed different region from previous study. When detected for 0.1 μg/ 100 μL of streptavidin by fully saturated stAuNPs, REVC formed at shifted region of bBSA (1.0-1.5 μg/100 μL) than performed without streptavidin (Fig. 7). However, when modified stAuNPs was used in the same detection, REVC was formed at lower and narrower concentrations of bBSA (0.4-0.6 μg/100 μL) than reported at previous study (1.0-1.5 μg/100 μL). Similarly, in case of detecting 1.0 μg/ 100 μL of streptavidin, REVC forming region was shifted more than detecting 0.1 μg/ 100 μL of streptavidin and this REVC forming region (0.6-1.0 μg/100 μL) also lower and narrower concentrations of bBSA than reported at previous study (1.5-2.0 μg/100 μL). As a result, REVC forming region wwa appeared at about 3 times lower and narrower concentrations than reported at previous study.

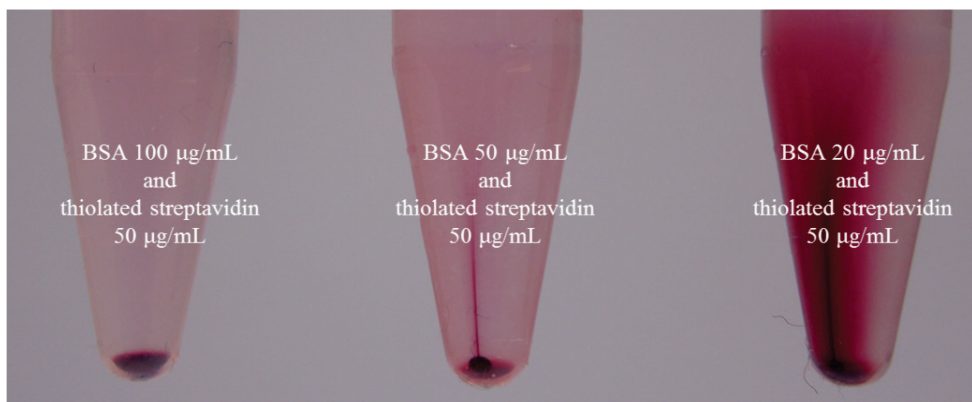
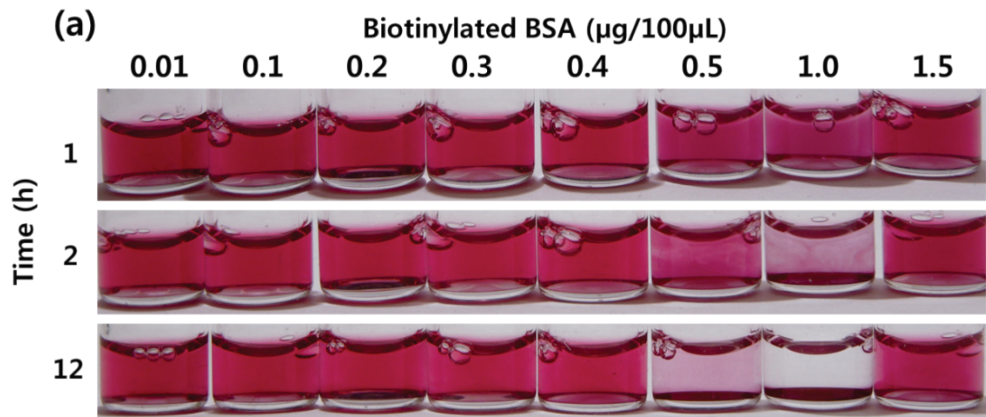
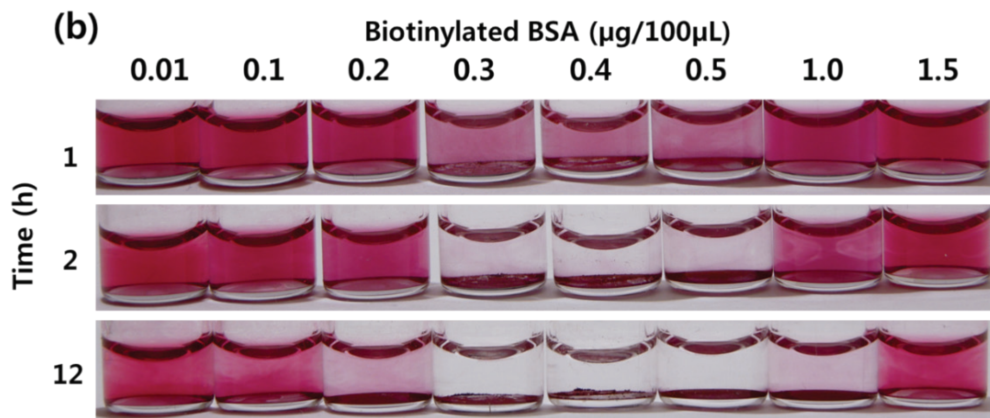


Fig. 5. Change of adhesive property according to the number of streptavidin on streptavidin-coated gold nanoparticle. BSA, bovine serum albumin.


(a)
Biotinylated BSA (μg/100μL)
0.01
0.1
0.2
0.3
0.4
0.5
1.0
1.5
Time (h)
1
2
12


(b)
Biotinylated BSA (μg/100μL)
0.01
0.1
0.2
0.3
0.4
0.5
1.0
1.5
Time (h)
1
2
12

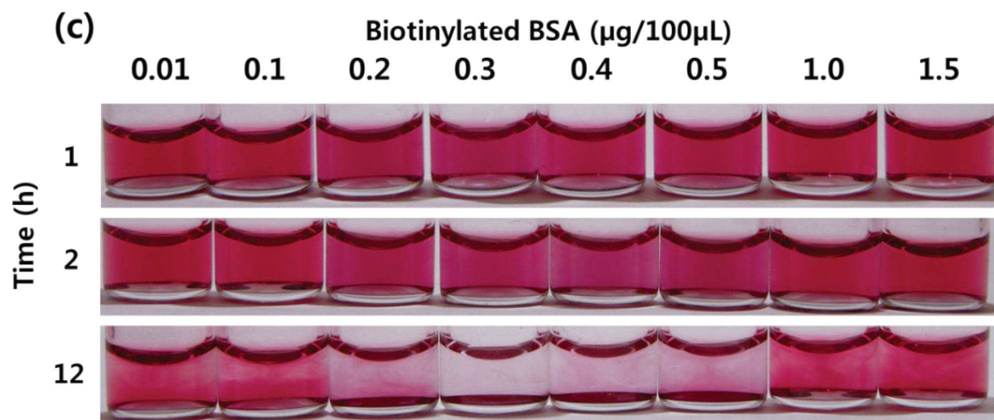

(c)
Biotinylated BSA (μg/100μL)
0.01
0.1
0.2
0.3
0.4
0.5
1.0
1.5
Time (h)
1
2
12

Fig. 6. Change of REVC forming region by modified level on stAuNPs for 12 h. (a) fully saturated stAuNPs, (b) pre-coated with 50 μg/mL of BSA, and (c) pre-coated with 100 μg/mL of BSA. stAuNPs, streptavidin coated gold nanoparticles; REVC, range of linker concentration exhibiting visual color change.

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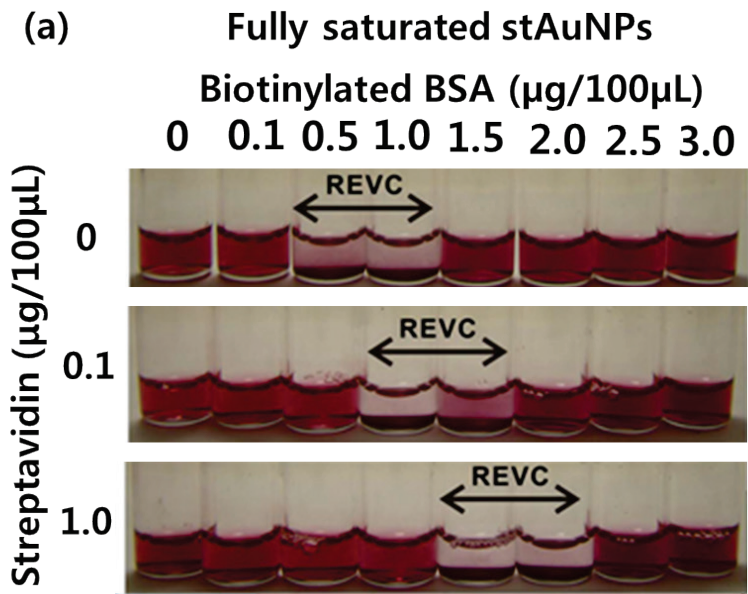

(a)
Fully saturated stAuNPs
Biotinylated BSA (μg/100μL)
0 0.1 0.5 1.0 1.5 2.0 2.5 3.0
Streptavidin (μg/100μL)
0
0.1
1.0
REVC
REVC
REVC

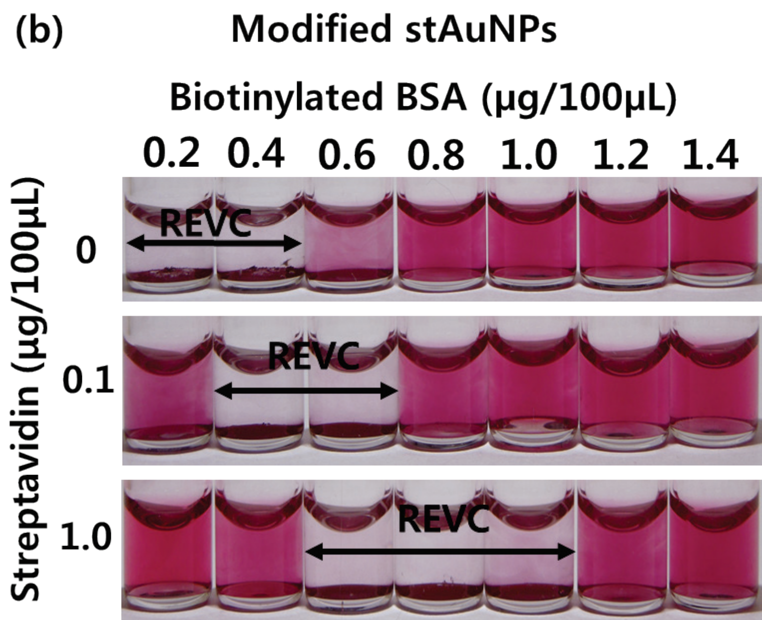

(b)
Modified stAuNPs
Biotinylated BSA (μg/100μL)
0.2 0.4 0.6 0.8 1.0 1.2 1.4
Streptavidin (μg/100μL)
0
0.1
1.0
REVC
REVC
REVC

Fig. 7. Change of REVC forming region in the presence of streptavidin for 2 h. (a) Mixed with PBS buffer in first step and (b) Mixed with 0.1 μg/ 100 μL of streptavidin solution in first step. REVC, range of linker concentration exhibiting visual color change; stAuNPs, streptavidin coated gold nanoparticles.

3.2.3. Effect of agitation on the detection system

To differentiate the REVC clearly by naked eye, the second step was needed for approximately more than 150 min. However, in case of adding mild agitation in the second step, the REVC was formed at the same concentrations of bBSA within 20 min. The shifting of REVC region was not affected by agitation (Fig. 8). As a result, it was possible to reduce the time by 5 times or more than the previous study.

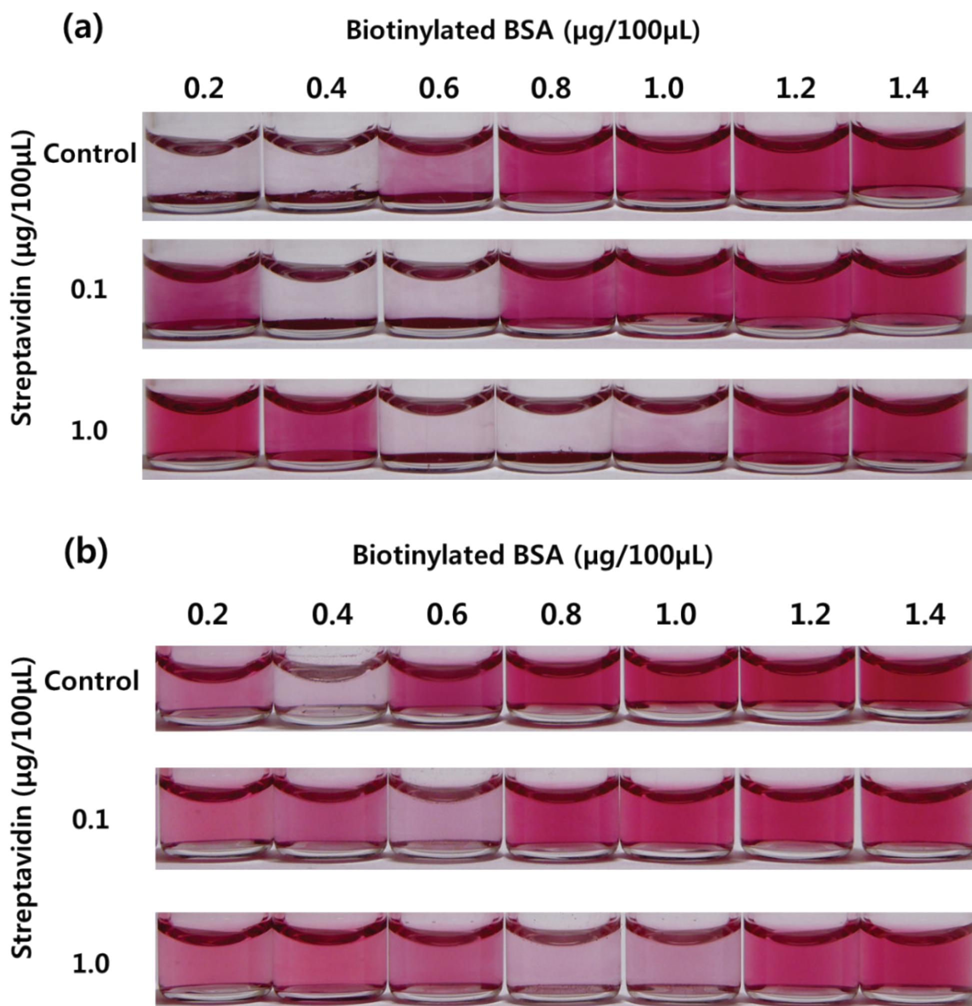
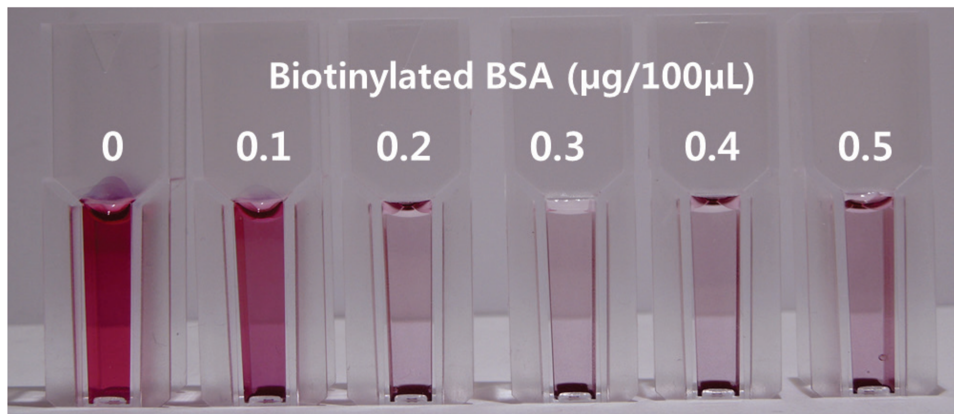


Fig. 8. Comparison of REVC forming region in the presence of streptavidin (a) without agitation for 2 h and (b) with agitation for 20 min. REVC, range of linker concentration exhibiting visual color change.

3.2.4. Analysis of normalized absorbance ratio in the detection system

When stAuNPs and bBSA were mixed, in order to form aggregates, SPR peak was shifted to upper wavelength (from 536 nm to 550 nm). This led to a change in color. However, it was too small change to be determined by naked eyes. In contrast, change of absorbance value was much more critical. The measured absorbance value at formed REVC region showed the same results as seen by naked eye (Fig. 9a, b). Moreover, in order to harmonize visible information and absorbance data, obtained results were normalized. The ratio of absorbance at 536 nm and 550 nm was used for normalizing. The normalized absorbance ratio value (A_{536}/A_{550}) was fitted well in which is distinguished by naked eye (Fig. 10a, b).


(a)
Biotinylated BSA (μg/100μL)
0
0.1
0.2
0.3
0.4
0.5

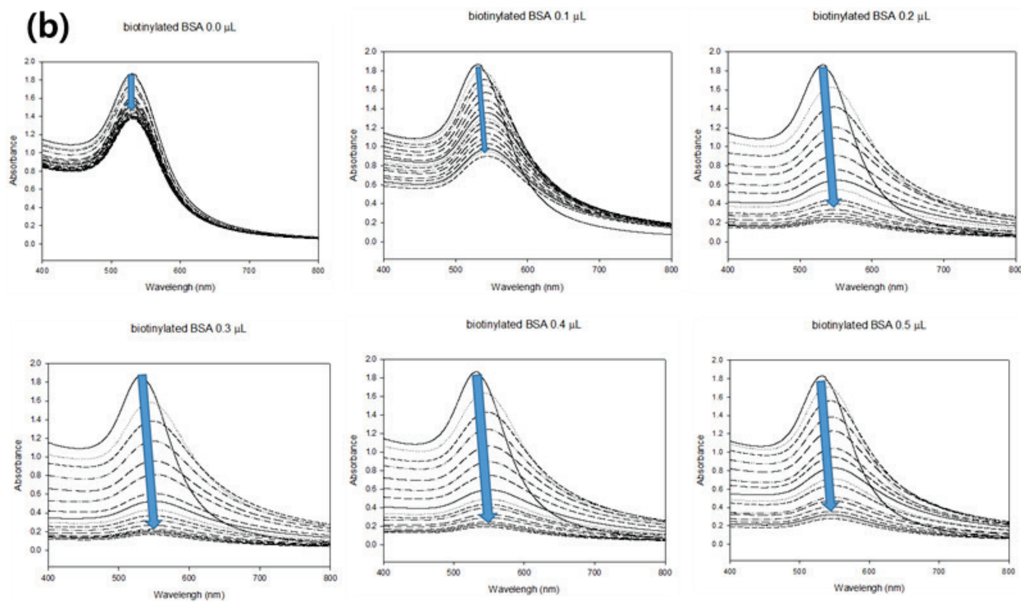
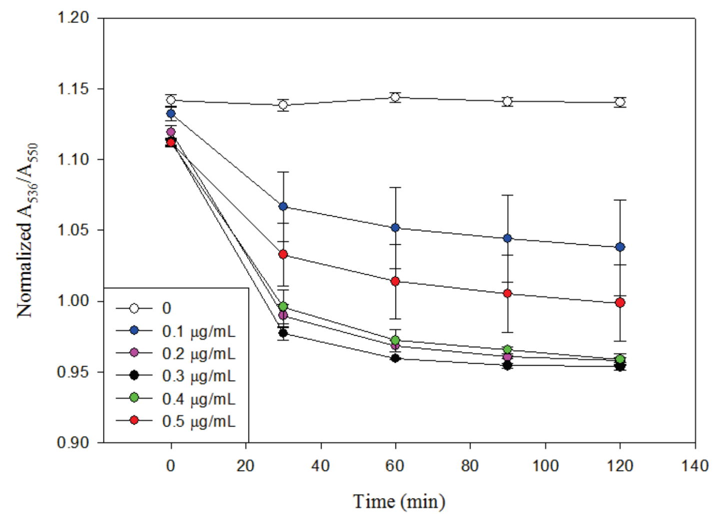

(b)
biotinylated BSA 0.0 μL
biotinylated BSA 0.1 μL
biotinylated BSA 0.2 μL
biotinylated BSA 0.3 μL
biotinylated BSA 0.4 μL
biotinylated BSA 0.5 μL
Absorbance
Wavelengh (nm)

Fig. 9. Comparison of visual image and UV-vis spectra data. (a) image of mixture of stAuNPs with different concentrations of bBSA (0~0.5 μg/100 μL) in REVC forming region at 120 min. at 120 min (b) concentration of bBSA and time-dependent change of UV–vis absorption spectra upon mixture of stAuNPs with different concentrations of biotinylated BSA (0~0.5 μg/100 μL) for 120 min

(c)



(d)

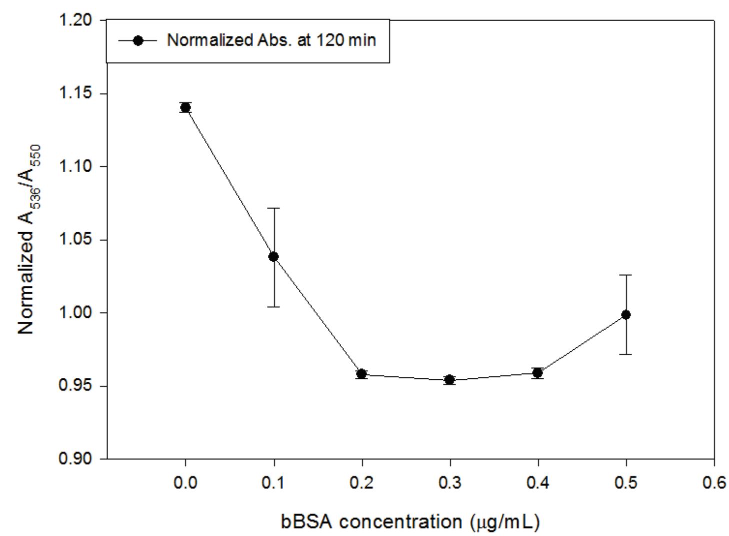


Fig. 10. Change of normalized ratio of absorbance (a) concentration of bBSA and time-dependence of normalized A_{536}/A_{550} ratio of mixture for 120 min, and (d) normalized A_{536}/A_{550} ratio of mixture at 120 min. stAuNPs, streptavidin coated gold nanoparticles; bBSA, biotinylated BSA

3.3. Application for detection of Ara h1

3.3.1 Detection of Ara h1 in standard solution

Detection of Ara h1 was carried out in the same manner without a type of SL. In Ara h1 detection, biotinylated anti-Ara h1 antibody (bAB) was selected as SL. The concentrations of bAB (35, 45 55 and 65 ng/100 µL) used in this study were determined experimentally (data not shown). When there was no Ara h1, REVC was formed from 45 ng/100 µL of bAB because of the natural relationship between bAB and stAuNPs. However, under presence of Ara h1, bAB covered with Ara h1 had less ability to react with stAuNPs than naked bAB. This led to shift of the REVC forming region. When 0.2 µg/100 µL and 0.4 µg/100 µL of Ara h1 were added, the REVC forming region was appeared from 55 and 65 ng/100 µL of bAB, respectively. Moreover, when 0.8 µg/100 µL of Ara h1 was added, the REVC shifted to much higher concentration of bAB, it was not appeared in the range of this study (Fig. 10).

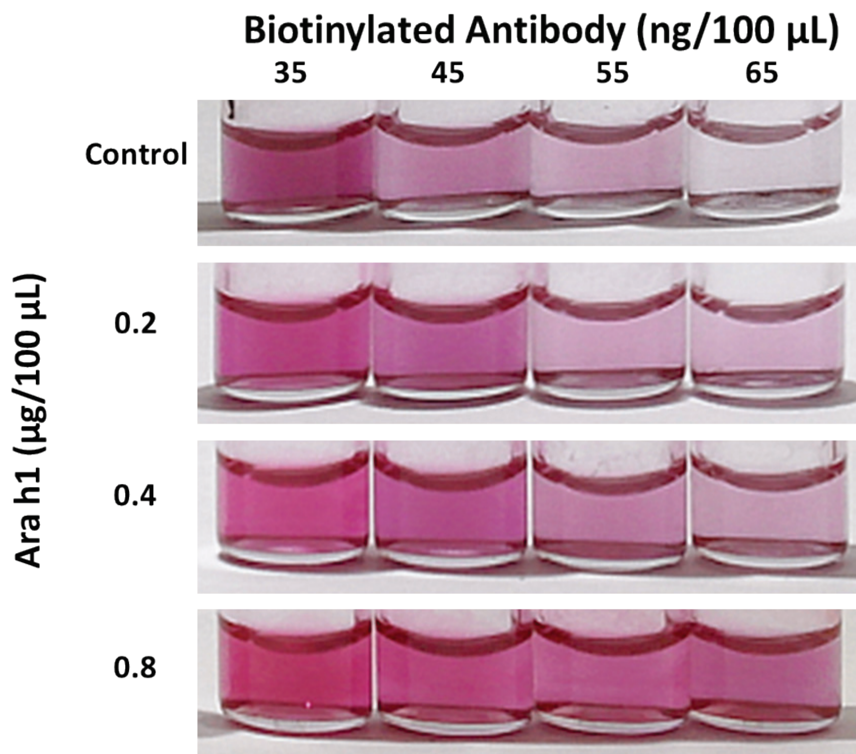


Fig. 11. Colorimetric detection of Ara h1 in standard solution. Visible change of color occurred from 0.2 µg/100 µL of Ara h1 solution.

3.3.2 Detection of Aar h1 in peanut extract

The detection of Ara h1 in peanut sample was performed with same concentration of switchable linker which is used in detection of Ara h1 in buffer. Extracted solution from peanut, which was 10-fold diluted sequentially, was used as target solution. The shift of REVC was observed from undiluted extract to 10^{-2} diluted solution (Fig. 11). According to reference paper, 15 mg/g of Ara h1 was extracted under this condition. The detection limit at buffer condition was 0.2 μg/100 μL of Ara h1. Therefore, if this system worked with diluted peanut extract, as if it operated in the buffer condition, Ara h1 in 10^{-3} diluted extract could detect this system. However, 10^{-2} diluted extract was detection limit in this system. It considered to be hindered bAB and Ara h1 from forming aggregate by fat and other protein in first step.

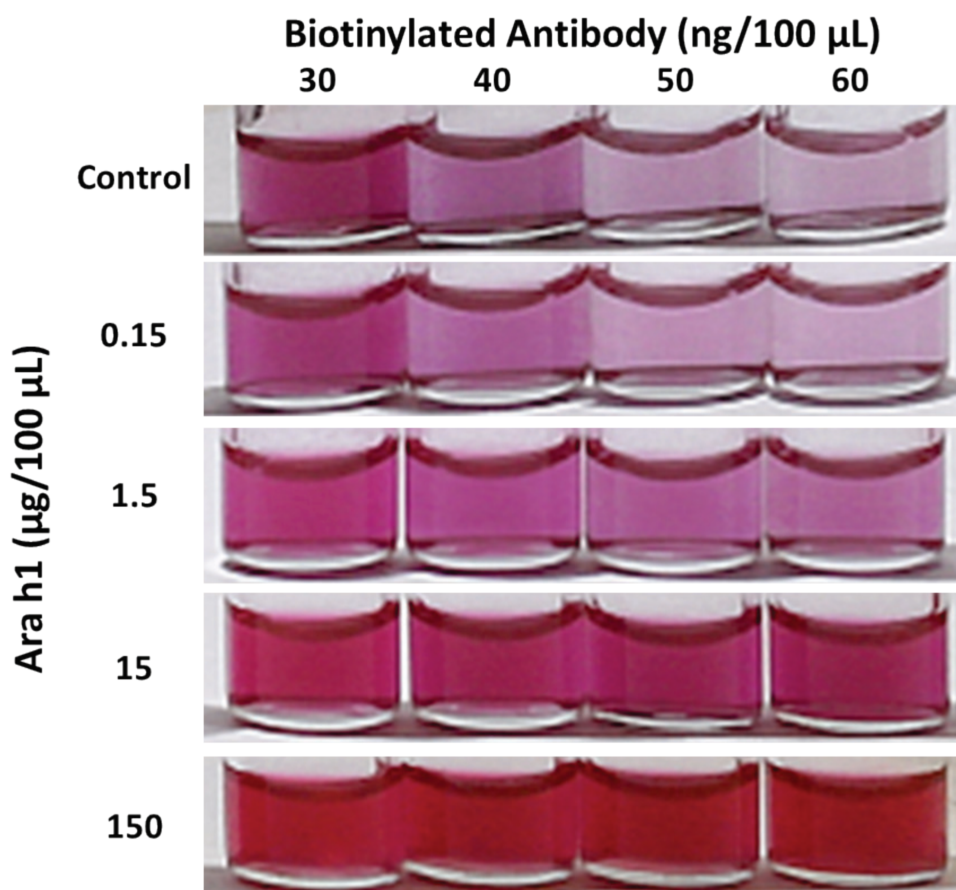


Fig. 12. Colorimetric detection of Ara h1 in peanut extract. Visible change of color occurred from mixture of stAuNPs with 1.5 µg/100 µL of Ara h1 solution (100-fold diluted extract).

3.3.3 Selectivity of the detection system

In order to determine the selectivity of the proposed system toward peanut and order tree nuts (pistachio and walnut), 10-fold diluted extracts were respectively added into this system. As shown in fig. 13, REVC in the system with extract of peanut and mixture (peanut, pistachio and walnut) were quite different with other extract. The result indicated that the this system could be used for selective detection of Ara h1.

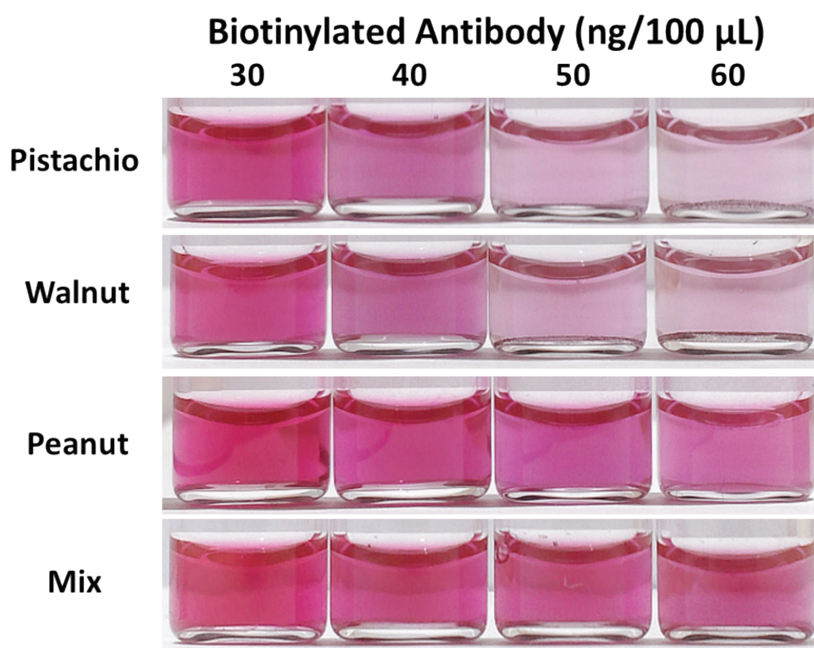


Fig. 13. Selectivity of detection system. Response of the system against other tree nut extract (10-fold diluted) or a mixture of extracts. Walnut and pistachio did not show cross-reactivity.

Ⅳ. CONCLUSION

In this study, the number of functionalized protein on particles was modified for improvement of this detection system. Gold nanoparticle and streptavidin were selected as model particle and protein in this system. Reduction of the streptavidin on stAuNPs changed REVC forming region to less and narrow concentrations of SL. Though modified stAuNPs needed more time for forming REVC, it was minimized through optimization of functionalization level. Moreover, it was confirmed that agitation could decrease reaction time without change of REVC forming region. As a result, by modifiying the number of streptavidin on stAuNPs, I reduced the amount of SLs about 3 times less than previous study. Furthermore, by introducing agitation to reaction, I reduced reaction time about 5 times less than when it was not conducted, without reduction in capability of the detection system.

Detection of Ara h1 was carried out by applying this improved system. I could detect 0.2 μg/ 100 μL of Ara h1 in 100 μL standard solution by naked eye. And 0.4 μg/ 100 μL and 0.8 μg/ 100 μL of Ara h1 were detected through shifting of REVC to higher concentration of bAB. As a results, by distinguishing change of REVC with naked eye, we could detect Ara h1 quantitatively without sophisticated instrument. Ara h1 from peanut extract was detected through this system as well. However, detection limit was 10

times increased than in buffer condition because of disturbance from fat and other protein in real sample. Because this system successfully detects biomaterials, it can be adapted to detect other harmful/useful biomaterials in food.

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VI. 국문초록

식품 내에 존재하는 유용하거나 유해한 물질, 또는 미생물들을 빠르고 쉽고 민감하게 검출하고자 하는 노력들은 오랫동안 계속되어왔다. 그러나 현재까지 연구된 방법들은 복잡하고 시간이 많이 걸리는 시료 처리과정이나 낮은 민감도, 또는 숙련된 인력이나 고가의 기기가 필요해 실제 현장에서 원활하게 사용되지 못하고 있다.

이러한 기존 검출법들의 여러 단점을 극복하는 것을 목표로 하여 금나노입자를 이용한 간편하고 신속한 검출법을 고안하였다. 이 방법은 두 단계로 구성된다. 첫 단계에서는 검출하고자 하는 목표물질과 이를 인지할 수 있는 여러 농도의 switchable linkers(SLs)를 섞어준다. 그 후 streptavidin으로 코팅된 금나노입자(stAuNPs)를 위의 용액에 더해준다. SLs이 가지고 있는 biotin은 금나노입자 표면에 있는 streptavidin과 높은 친화력을 가지므로, SLs와 stAuNPs는 서로 다중으로 연결되고 이를 통해 거대한 응집체를 형성한다. 목표물질이 없는 경우, 시각적으로 확인 가능한 대량의 침전이 형성되는 영역 (REVC)은 SLs와 stAuNPs 간의 내재적인 양적관계에 의해 결정된다. 그러나 목표물질이 들어오면 SLs는 목표물질과 먼저 응집체를 형성하여 stAuNPs와 응집을 형성할 능력을 잃어버린다. 이는

위에서 언급한 내재적인 양적관계의 변화를 일으키고, REVC가 형성되는 영역을 이동시킨다. 그러므로 이 양적관계에 있어서 stAuNPs의 표면에 있는 streptavidin의 수는 관계에 영향을 미치는 중요한 요소 중 하나이다. 이 연구에서, 우리는 검출 시스템의 개선을 위하여 stAuNPs 표면의 streptavidin의 수를 조정하였다. 먼저, stAuNPs 표면의 streptavidin의 수가 REVC가 나타나는 영역과 형성시간을 어떻게 변화시키는지를 조사하였다. 더 적은 수의 streptavidin인 stAuNPs 표면에 있을 때, 더 적은 농도의 SLs에서 REVC가 형성되는 대신 형성 시간은 더 길어졌다. 그러나 REVC가 형성될 때, 혼합액을 흔들어줌으로써 REVC 형성에 필요한 시간을 줄일 수 있었다.

위의 연구에서 얻은 REVC 형성영역과 형성시간이 최적화된 입자를 이용하여, 주요 땅콩 알러젠인 Ara h1을 검출해 보았다. 표면이 조절된 stAuNPs를 적용한 결과, 10 nM 수준의 매우 낮은 양의 Ara h1을 별다른 분석기구의 도움 없이 눈으로 검출할 수 있었다. 또한 땅콩추출액을 통한 실험에서도 추출 원액을 1/100로 희석한 희석액에서까지 Ara h1의 존재유무를 시각적으로 판별할 수 있었다.

결과적으로, 이 연구에서 얻어진 결과는 이 검출 시스템을 강화하고 여러 대상에 적용하는데 있어서 충분한 역할을 할 것으로 기대된다.

주요어 : 검출법; 성형금나노입자; 시각적 검출법; 땅콩 알러젠; Ara h1