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Detection of ricin by using gold nanoclusters functionalized with chicken egg white proteins as sensing probes

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ABSTRACT

Ricin produced from the castor oil plant, *Ricinus communis*, is a well-known toxin. The toxin comprises A and B chains. Ricin A chain can cause toxicity by inhibiting protein synthesis, and ricin B can bind to the galactose ligand on the cell membrane of host cells. Inhalation or ingestion of ricin may even lead to death. Therefore, rapid and convenient sensing methods for detecting ricin in suspicious samples must be developed. In this study, we generated protein encapsulated gold nanoclusters (AuNCs@ew) with bright photoluminescence by using chicken egg white proteins as starting materials to react with aqueous tetrachloroaurate. The generated nanoclusters, which were mainly composed of chicken ovalbumin-encapsulated AuNCs, can recognize ricin B because of the presence of Galβ(1→4)GlcNAc ligands on chicken ovalbumin. The generated conjugates of AuNCs@ew and ricin B were heavy and readily settled down under centrifugation (13,000 rpm, 60 min). Thus, bright spots resulting from the conjugates at the bottom of the sample vials were easily visualized by the naked eye under ultraviolet light illumination. The limit of detection (LOD) was ~4.6 μM. The LOD was reduced to ~400 nM when fluorescence spectroscopy was used as the detection tool, while the LOD can be further improved to ~7.8 nM when using matrix-assisted laser desorption/ionization mass spectrometry as the detection method. We also demonstrated the feasibility of using the proposed approach to selectively detect ricin B chain in complex samples.

1. Introduction

Molecular recognitions between carbohydrates and lectins (carbohydrate binding proteins) are significant in cellular interactions (Sharon, 1993; Sharan and Lis, 1995; Bertozzi and Kiessling, 2001), immune system responses (Lis and Sharon, 1998), host–pathogen interactions (Sequeira, 1978), and tumor metastasis (Nakahara and Raz, 2008). Thus, identification of lectins by using specific carbohydrates has been extensively studied (Liener, 1986; Mandal and Brewer, 1992; Weis and Drickamer, 1996; Harona and Shaanan, 1997; Vijayan and Chandra, 1999; Lee and Lee, 2000; Barre et al., 2001; Hirabayashi, 2008; He et al., 2011). Hydroxyl groups on the carbohydrates can bind to lectin through hydrogen bonding (Sharona and Shaanan, 1997; Barre et al., 2001). In nature, glycan ligands, such as mannose, glucose, and galactose, are covalently attached to the polypeptide chains of glycoproteins. Thus, glycans on glycoproteins can be used as probes to bind to their corresponding carbohydrate moieties on specific lectins.

Some of the plant toxins found in nature are lectins. For example, ricin is a lectin produced by castor bean (*Ricinus communis*) plant and widely known for its highly toxic nature (Olsnes and Kozlov, 2001). This compound is considered as a potential biological weapon and listed as a category B bioterrorism agent by the Centers for Disease Control and Prevention in the USA (Rotz et al., 2002). Several incidents in which ricin was used have been confirmed as bioterrorist attacks (Mayor and Mayor, 2003; Audi et al., 2005; Papaloucas et al., 2008; Zilinskas, 1997). For example, a terrorist attack on the senator office in Washington, D.C. occurred through post mails containing ricin in 2003 (Mayor and Mayor, 2003). The most recent case involved sending an envelope laced with ricin to the White House (https://archives.fbi.gov/archives/jackson/press-releases/2013/update-fbis-ongoing-investigation-into-letters-containing-ricin). Thus, sensing methods such as using nanoparticles (Diaz-Diestra et al., 2017) as the sensing probes must be developed for rapid identification of ricin in suspicious samples.

Ricin consists of two subunits, namely, A and B chains, which are linked by a disulfide bond. The molecular weights of ricin A and B chains are ~32 and ~34 kDa, respectively (Audi et al., 2005). The A subunit can inactivate ribosomes, leading to the occurrence of toxicity. The B chain binds to galactose ligands on the cell surface to promote internalization of ricin into the target cells, leading to inhibition of

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protein synthesis (Endo and Tsurugi, 1986). Inhalation and injection are the most common routes that cause ricin poisoning (Challoner and McCarron, 1990). The lethal dosages for ricin in humans are approximately 5–10 μg kg^{-1} (per kilogram of body weight) through inhalation and ingestion (Bradberry et al., 2003). Thus, a person weighing 70 kg may die by accidentally inhaling 0.35–0.7 mg of ricin. Although no effective treatment is available for ricin intoxication, analytical methods for rapid ricin detection are helpful for taking prompt medical treatment, conducting food/water safety tests, and monitoring the presence of biological warfare. To date, several methods, including immunoassays (Ler et al., 2006; Lubelli et al., 2006; Singh et al., 2016), antibody sandwich assay (Stern et al., 2016), chemiluminescence (Poli et el., 1994), microarray biosensor assay (Dill et al., 2004), fluorescence anisotropy (Xiao et el., 2016), electrochemical impedance spectroscopy (Komarova et al., 2010), fluorescent aptamer/quantum dots (Bogomolova and Aldissi, 2015), and piezoelectric detection (Carter et al., 1995) have been used to detect ricin. However, most of these methods require the use of antibodies as probes for ricin detection. Antibodies are expensive and have limited availability; thus, alternative probe molecules and sensing methods should be developed.

Chicken egg white contains abundant proteins, primarily ovalbumin (~54%) (Mine, 1995). Chicken egg white is used as starting material for generating chicken egg white protein-encapsulated gold nanoclusters (AuNCs@ew) through one-pot reactions (Selvaprakash and Chen, 2014). Furthermore, AuNCs@ew is mainly composed of ovalbumin-encapsulated AuNCs (AuNCs@ova) (Selvaprakash and Chen, 2014). Ovalbumin is a glycoprotein containing various glycan ligands on the protein structure (Yamashita et al., 1978). Ricin B can specifically bind to Galβ(1→4)GlcNAc-terminated ligands (Hennigar et al., 1987; Sharona and Shaanan, 1997). Considering that chicken ovalbumin contains Galβ(1→4)GlcNAc-terminated glycans on its structure (Yamashita et al., 1978; Harvey et al., 2000), we believe that the generated AuNCs@ew can be used as suitable probes for targeting ricin B, which possess binding sites for Galβ(1→4)GlcNAc termini (Scheme S1). This study demonstrated the feasibility of using the generated AuNCs@ew as affinity probes for ricin detection based on probing ricin B.

2. Experimental

The details of the synthesis of AuNCs@ew and the steps of sample preparation for detection of ricin B are provided in Supplementary Information.

3. Results and discussion

3.1. Characterization of the generated AuNCs@ew

AuNCs@ew were generated through one-pot reactions by reacting aqueous tetrachloroaurate and chicken egg white liquid under microwave heating (90 W) for 8–10 successive cycles (5 min/cycle). Figs. S1A and S1B show the absorption and fluorescence spectra of the as-prepared AuNCs@ew, respectively. An absorption band appeared at the wavelength of ~280 nm resulting from the absorption band of proteins immobilized on the surface of the AuNCs@ew (Fig. S1A), whereas the maximum emission band appeared at the wavelength of ~640 nm (λ_{ex}=370 nm) (Fig. S1B). The inset in Fig. S1A shows the photographs of the generated AuNCs@ew taken under room light (left) and UV light (λ_{max}=365 nm) (right). The generated AuNCs@ew emitted red fluorescence under illumination of ultraviolet (UV) light. Fig. S2 shows the high resolution TEM image of the generated AuNCs@ew, with particle size of 2.1 ± 0.3 nm, similar to that obtained in a previous report (Selvaprakash and Chen, 2014). The quantum yield of generated AuNCs@ew was estimated to be ~6.5% when using riboflavin-5′phosphate (QY=~26%) as a reference standard (Fig. S3) similar to that reported previously (Selvaprakash and Chen, 2014). The lifetime of the generated AuNCs@ew was measured to be ~0.76 μs (Fig. S4). X-ray photoelectron spectrum of Au 4f derived from AuNCs@ew confirmed the presence of Au(I) and Au(0) (Fig. S5). To confirm the immobilization of chicken ovalbumin on the AuNCs@ew, we used matrix-assisted laser desorption/ionization (MALDI)-MS to characterize the intact AuNCs@ew. Figs. S6A and S6B show the matrix-assisted laser desorption/ionization (MALDI) mass spectra of egg white proteins and the generated AuNCs@ew, respectively. The peak at *m/z* ~44500 (Juhasz et al., 1993) contributed by chicken ovalbumin dominated the mass spectrum in Fig. S6A. Such result is understandable because ovalbumin is abundant in chicken egg white protein (~54%) (Mine, 1995). However, the ion peak shifted to ~46,500 Da in the MALDI mass spectrum of AuNCs@ew (Fig. S6B). The results indicate that ~10 atoms comprised the core of the generated AuNCs@ew. The intact AuNCs@ew were characterized by surface-assisted laser desorption/ionization (SALDI)-MS, which can be used in verifying the binding site between the gold core and the protective shell (Lai et al., 2015). Fig. S7 shows the SALDI-MS spectrum of AuNCs@ew obtained in negative ion mode. Two series of ion peaks appeared with ~197 mass unit difference. The ions peaks (marked with red dots) appearing at *m/z* 788.3, 984.7, 1182.1, 1379.2, 1576.4, 1773.3, and 1970.1 corresponded to Au_4^-, Au_5^-, Au_6^-, Au_7^-, Au_8^-, Au_9^-, and Au_{10}^-, respectively, with a ~197 mass unit difference and were attributed to the Au cluster ions. The ion peaks (marked with blue dots) appeared at *m/z* 820.3, 1017.1, 1214.5, 1411.3, 1608.4, 1805.1, and 2002.2, corresponding to Au_4S^-, Au_5S^-, Au_6S^-, Au_7S^-, Au_8S^-, Au_9S^-, and $Au_{10}S^-$, respectively (Wu et al., 2009). These two ion groups have ~32 mass unit difference between the adjacent peaks, indicating that Au-S bonding was involved in the formation of the generated AuNCs@ew. That is, 6 cysteines in chicken ovalbumin (Thompson and Fisher, 1978) were involved in the binding of the proteins and the Au core during the synthesis reaction. These results indicate that egg white protein-immobilized AuNCs@ew with red fluorescence were successfully synthesized through the one-pot reactions.

3.2. Examination of binding affinity of AuNCs@ew toward ricin B

We then examined whether the generated AuNCs@ew specifically interacted with ricin B. Optimization experimental conditions including pH (Fig. S8), incubation time (Fig. S9), and the optimized concentration of AuNCs@ew (Fig. S10) were investigated. The results indicated that optimized pH was pH ~7.5, the optimized incubation time was ~2 h, and the suitable concentration of AuNCs@ew for ricin B sensing was 0.25 mg/mL. Thus, the generated AuNCs@ew were vortex-mixed with ricin B at different concentrations (0–29 μM) prepared using 4-(2-hydroxyethyl)−1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.5) for 2 h followed by centrifugation at 13000 rpm at 4 °C for 1 h. Fig. 1A shows the photographs of the samples that were taken under UV light (λ_{max}=365 nm) conditions. When the concentration of ricin B in the sample was > 2.5 μM, precipitates with red emission resulting from the heavy conjugates of the AuNCs@ew and ricin B were apparent. Precipitates were not observed in the sample containing AuNCs@ew only (blank). Furthermore, the fluorescence intensity of the maximum emission wavelength at ~640 nm of the supernatant decreased with increasing concentration of ricin B in the sample solution (Fig. 1B), indicating that AuNCs@ew bonded with ricin B. Fig. 1C shows the correlation plot constructed by plotting the difference of the fluorescence intensity ($(F_0\text{-}F)/F_0$) at the wavelength of ~640 nm obtained before and after incubation of AuNCs@ew and ricin B followed by centrifugation at 13000 rpm for 1 h versus the concentration of ricin B. F_0 stands for the original fluorescence intensity at the wavelength of ~640 nm of the AuNCs@ew, whereas F stands for the fluorescence intensity of the supernatant at the same wavelength. The linear regression coefficient of the concentration range of 0.7–4.6 μM was R^2=0.982. The limit of detection (LOD) was estimated to be ~400 nM based on the calculation of 3 SD/S, whereas SD was the