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Selective extraction of thiol-containing peptides in seawater using Tween 20-capped gold nanoparticles followed by capillary electrophoresis with laser-induced fluorescence

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ABSTRACT

This study combines Tween 20-capped gold nanoparticles (Tween 20-AuNPs) with capillary electrophoresis (CE) for ultrasensitive detection of thiol-containing peptides, including glutathione (GSH), γ-glutamylcysteine (γ-GCS), and phytochelatin analogs. By forming Au—S bonds, Tween 20-AuNPs can selectively extract and enrich these thiols from a complicated matrix. A Tween 20 capping layer not only suppresses nonspecific adsorption, but also enables NPs to disperse in a highly salinity solution. Dithiothreitol removes thiol-containing peptides from the NP surface through ligand exchange. The released peptides are selectively derivatized with o-phthaldialdehyde (OPA) to form tricyclic isoindole derivatives. Extraction efficiency of five thiol-containing peptides with Tween 20-AuNPs was highly reliable in the Tween 20-AuNP concentration, time of extraction and desorption thiols, and sample volume. After injecting a large sample volume, the OPA-derivatized peptides migrate against the electroosmotic flow (EOF) and enter the polyethylene oxide (PEO) zone. The sensitivity of these peptides was improved by stacking them at the boundary between the sample and PEO zones. As a result, limits of detection (LODs) for five peptides were down to 0.1–6 pM. Not only is the proposed method probably the first CE example for detecting dissolved thiols in seawater; it also has the lowest LODs for GSH, γ-GCS, and phytochelatins compared to other reported methods.

1. Introduction

Thiol-containing peptides, including phytochelatins and glutathione (GSH), guard against toxicity of heavy metal ions [1] and maintain the balance between oxidation and antioxidation [2]. Phytochelatins, with the general structure of (γ-Glutamate–Cysteine)$_n$–Glycine (PC$_n$), are mainly present in plants and algae [3], while GSH is ubiquitous in a broad range of organisms, from plants to vertebrates [4]. When heavy metal ions migrate into the cells of plants or algae, phytochelatin synthase consumes GSH and catalyzes phytochelatin production [5] (Fig. 1A). Phytochelatins detoxify heavy metal ions by coordinating between thiol groups of phytochelatins and heavy metal ions [6]. Thus, the concentration of phytochelatins in plants or algaes responds to heavy metal pollution [7]. Because phytochelatins are liberated from algal cells into surrounding seawater by exudation or cell breakage, the level of phytochelatins can reflect the concentration of aqueous free metal ions, influencing the biogeochemical cycling of metal in seawater [8–11]. Additionally, compared with dissolved metals, the measurement of dissolved thiols in seawater may provide more complete information on the potential toxicity of these contaminants on living organisms.

Because of the environmental significance of GSH and phytochelatins, many researchers have attempted to quantify them in plants and algae. Electro-analytical techniques, including different pulse voltammetry [6,12] and stripping chronopotentiometry [13,14], can be used to probe the interaction of metal ions and phytochelatins. Circular dichroism [12,15] and electro-spray ionization mass spectrometry [12,16] were useful in complementing and clarifying results of electro-analytical techniques. Separation procedures are necessary to quantify different length phytochelatins and GSH. Reversed-phase high-performance liquid chromatography (RP-HPLC) is the most frequently used method in phytochelatins and GSH analysis. This method is commonly coupled with amperometric detection [17,18], electrospray

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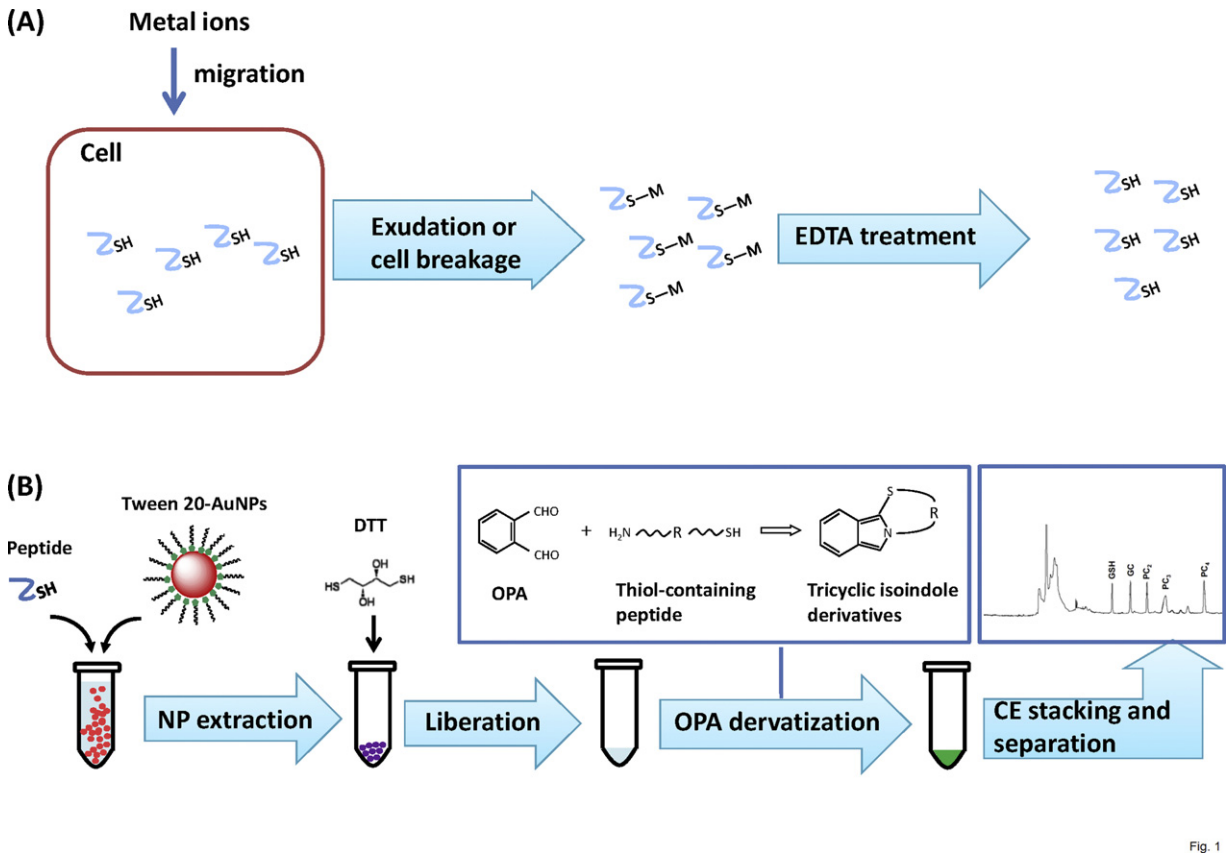

Fig. 1. (A) Metal ion-induced production of thiol-containing peptides from algal cells. (B) Ultrasensitive detection of thiol-containing peptides by the combination of Tween 20-AuNP extraction, OPA derivatization and CE stacking.

ionization mass spectrometry [19], inductively coupled plasma mass spectrometry [20,21], and fluorescence [22–24]. However, these methods are insufficiently sensitive to detect dissolved thiols in seawater [10]. For example, concentrations of GSH and PC_2 in the Elizabeth River range from 10–2390 to 3–98 pM, respectively [25]. To determine dissolve thiols in seawater, Tang et al. used solid-phase extraction to enrich monobromobimane-derivatized thiols prior to RP-HPLC with fluorescence detection; the limits of detection (LODs) for GSH and PC_2 were reduced to 30 and 60 pM, respectively [26,27]. Because solid-phase extraction is unable to selectively extract derivatized thiols, several interfering peaks appeared in the chromatogram. Additionally, this method suffered from long separation time and slow derivatization reaction. Capillary electrophoresis (CE) is an alternative method of determining phytochelatins and GSH, providing the advantages of small required sample volume, quick analysis, low capillary cost, and low solvent consumption. Still, CE combined with laser induced fluorescence (LIF) [28,29] or electrospray ionization mass spectrometry [30] cannot detect dissolved thiols in seawater.

Recent research verifies that Tween 20-modified gold nanoparticles (Tween 20-AuNPs) have strong binding to aminothiols by forming Au—S bonds [31–33]. Because Tween 20-AuNPs possess a high surface-area-to-volume ratio, they can extract and enrich aminothiols from a complex matrix such as urine or plasma. CE with UV and LIF detection then quantified the extracted aminothiols. This study assumes that Tween 20-AuNPs can be employed for selective extraction and enrichment of thiol-containing phytochelatins.

This study presents an ultrasensitive method to analyze thiol-containing peptides, including GSH, γ-glutamylcysteine (γ-GCS), PC_2, PC_3, and PC_4. Detecting thiol-containing peptides requires four steps (Fig. 1B). (A) Tween 20-AuNPs extract and enrich thiol-containing peptides from an aqueous solution. (B) After centrifuging and washing, a displacement reaction between dithiothreitol (DTT) and thiol-containing peptides liberates thiol-containing peptides adsorbed on the NP surface. (C) Because of their own sulfhydryl (SH) and amine groups, the released peptides react with o-phthaldialdehyde (OPA) to form tricyclic isoindole derivatives in the absence of nucleophile (inset in Fig. 1B). (D) OPA-derivatized peptides were stacked and separated by CE-LIF with a polyethylene oxide (PEO) additive. The extraction, derivatization, and separation conditions in terms of separation efficiency and sensitivity were carefully optimized. This method was successful in determining GSH, γ-GCS, and PC_2 in seawater, which may be considered an additional tool for the assessment of water quality.

2. Experimental

2.1. Chemicals and preparation

$Na_2B_4O_7$, poly(ethylene oxide) (PEO) (M_w 8,000,000 g/mol), OPA, GSH, γ-GCS, DTT, H_3PO_4, NaH_2PO_4, HCl, NaOH, disodium dehydrate ethylenediamine tetraacetate (EDTA), trisodium citrate, hydrogen tetrachloroaurate(III) dehydrate, and Tween 20 were purchased from Sigma–Aldrich (St. Louis, MO, USA). PC_2, PC_3, and PC_4 were purchased from AnaSpec (San Jose, CA, USA). Fused-silica capillaries (75 μm I.D., 365 μm O.D.) were purchased from Polymicro (Phoenix, AZ, USA). Buffer solutions were 10 mM $Na_2B_4O_7$ (pH 9.2). Milli-Q ultrapure water (Milli-Pore, Hamburg, Germany) was used in all of the experiments.

2.2. Derivatization

Thiol-containing peptides were derivatized with a solution containing 10 mM OPA and 1 mM $Na_2B_4O_7$ at pH 3.0–11.0 for 2 h. The fluorescence spectra of OPA derivatives were obtained using a Hitachi F-7000 fluorometer (Hitachi, Tokyo, Japan).

2.3. Synthesis of Tween 20-AuNPs

Citrate-capped AuNPs are prepared by reducing metal salt precursor (hydrogen tetrachloroaurate, $HAuCl_4$) in a liquid phase. Briefly, 10 mL of 38.8 mM trisodium citrate was added rapidly to 100 mL of 1 mM $HAuCl_4$ that was heated under reflux. Heating under reflux was continued for an additional 15 min, during which time the color changed to deep red. The images of transmission electron microscopy (TEM) (H7100, Hitachi High-Technologies Corp., Tokyo, Japan) confirmed that the size of citrate-AuNPs is 13 ± 1 nm. The extinction spectra of citrate-AuNPs were recorded using a UV–vis spectrophotometer (V-530, Jasco, Hachioji, Japan). The surface plasmon resonance (SPR) peak of 13 nm AuNPs was 520 nm, indicating that their extinction coefficient is $2.78 \times 10^8\ M^{-1}\ cm^{-1}$. The particle concentration of the as-prepared AuNPs was estimated to be 4.8 nM using Beer's law. Tween 20-AuNPs were obtained when 240 μL of 10% (v/v) Tween 20 was added to a solution of 4.8 nM citrate-capped AuNPs (60 mL). The resulting mixture was stored at 4 °C until further use. Batch-to-batch reproducibility of synthesis of Tween 20-AuNPs was evaluated by measuring their SPR wavelength and extinction value, which correspond to the particle size and concentration, respectively. The SPR wavelength and extinction value of Tween 20-AuNPs almost unchanged when the synthesis of Tween 20-AuNPs was repeated five times (Fig. S1, supplementary).

2.4. NP extraction

Tween 20-AuNPs and thiol-containing peptides were prepared in 10 mM phosphate at pH 3.0 prior to extraction. Note that Tween 20-AuNPs are dispersed in a wide range of pH and a high-ionic-strength solution [31–33]. We mixed thiol-containing peptides (0.2–10 mL, 10–250,000 pM) with Tween 20-AuNPs (200 μL, 0.48–24 nM) and equilibrated the resulting solution for 0.3–16 h. After that, the mixture was collected by centrifugation at 17,000 rpm ($32{,}310 \times g$) for 15 min. The supernatant was carefully removed up to a residual volume of 10 μL. To liberate thiol-containing peptides from the Au surface, the NPs were resuspended in a fresh prepared solution of DTT (1 μL, 800 mM). After 0.3–4 h, the released peptides were separated from NPs by centrifugation at 17,000 rpm ($32{,}310 \times g$) for 10 min. The obtained supernatant (4 μL) was derivatized with a solution (1 μL) containing 10 mM OPA in 1 mM $Na_2B_4O_7$ (pH 9.2) for 2 h. On-line stacking and separation of OPA-derivatized peptides were performed using 0.6% (w/v) PEO in the presence of electroosmotic flow (EOF).

2.5. CE separation and stacking

The CE-LIF setup used in this study was described in the supporting information. To obtain high EOF, new fused-silica capillaries (50 cm) were filled with 500 mM NaOH solution overnight [34]. The detection window was located at 40 cm from the capillary end. Prior to CE separation, the capillary was filled with 10 mM $Na_2B_4O_7$ (pH 9.2). A mixture of OPA-derivatized peptides was injected into the capillary by raising the capillary inlet 20-cm height for a period of time up to 60 s. The injection volume was calculated from the capillary radius and the injection length, which was determined by monitoring the baseline shift at detection window during sample injection. The ends of the capillary were immersed in the cathodic and anodic vials containing 0–0.6% (w/v) PEO solutions and 10 mM $Na_2B_4O_7$ (pH 9.2). The preparation of PEO was described in the supporting information. When the separation was performed at 250 V/cm (the electric current is 40 μA), neutral PEO molecules migrated from buffer vial to the capillary via EOF. The bulk EOF mobility was determined by the time at which the baseline shifts as a result of detection of PEO [34]. Note that EOF was greater than effective electrophoretic mobility of OPA-derivatized peptide (negatively charged molecules). At the end of each run, the capillary was rinsed with 500 mM NaOH by syringe pumping (KD Scientific, New Hope, PA) at a flow rate of 3 mL/h for 10 min and subsequently filled with 10 mM $Na_2B_4O_7$ (pH 8.0–11.0). Because PEO is unstable under alkaline conditions (hydrolysis occurs above 7.4), high concentration of NaOH can remove PEO molecules from the capillary wall and generate high EOF [37–39].

2.6. Analysis of dissolved thiols in seawater

Samples of seawater (pH 7.9) were collected from National Sun Yat-sen University campus. The concentrations of Cu^{2+}, Cd^{2+}, and Hg^{2+} in seawater were determined by inductively coupled plasma mass spectroscopy (ICP-MS) (Perkin-Elmer-SCIEX, Thornhill, ON, Canada). Artificial seawater (salinity, 35%) was prepared by dissolving NaCl (23.477 g), $MgCl_2$ (4.981 g), Na_2SO_4 (3.917 g), $CaCl_2$ (1.102 g), KCl (0.664 g), $NaHCO_3$ (0.192 g), KBr (0.096 g), H_3BO_3 (0.026 g), $SrCl_2$ (0.024 g), and NaF (0.003 g) in 1000 mL of distilled water. Two kinds of samples (9.8 mL) were mixed with a solution (200 μL) containing 500 mM phosphate (pH 3.0) and 500 μM EDTA. We then prepared a series of samples by spiking them with standard solution (200 μL) of GSH (0.1–5 nM), γ-GCS (0.1–5 nM), PC_2 (0.1–5 nM), PC_3 (0.2–8 nM), and PC_4 (0.1–5 nM). These spiked samples were added to a solution of Tween 20-AuNPs (200 μL, 9.6 nM). The mixture was equilibrated at room temperature for 4 h. After centrifugation, the obtained precipitates (10 μL) were resuspended in a fresh prepared solution of DTT (1 μL, 0.8 M) for 1 h. The supernatant (4 μL) was derivatized with a solution (1 μL) containing 10 mM OPA and 1 mM $Na_2B_4O_7$ (pH 9.2) for 2 h. OPA derivatives are hydrodynamically injected by raising the capillary inlet 20 cm in height for 60 s. Five peptides were separated and stacked by CE-LIF in the presence of 0.6% PEO.

3. Result and discussion

3.1. Derivatization of phytochelatins

Previous studies report that OPA react with GSH and γ-GCS to form highly florescent derivative without the addition of nucleophiles [35,36]. The authors suggest that phytochelatins containing the repeating units of γ-GCS may also be derivatized with OPA. To demonstrate this assumption, several phytochelatins were derivatized with 10 mM OPA in a solution of 1 mM $Na_2B_4O_7$ at pH 9.2. The fluorescence bands of OPA-derivatized GSH, γ-GCS, PC_2, PC_3, and PC_4 all centered at 428 nm, respectively, when they were excited at 355 nm (Fig. S2, supplementary). Obviously, OPA can also react with phytochelatins to form a fluorescent derivative in the absence of nucleophiles. In analogy with the reaction of GSH and γ-GCS with OPA, Fig. S3 (supplementary) shows that the predominant products in the reaction with phytochelatins and OPA should be the tricyclic derivative. We next used a fluorometer to examine the effect of pH on the OPA derivatization of phytochelatins. The derivatization of phytochelatins with OPA was only successful under alkaline conditions (Fig. S4, supplementary). A similar result occurred in the case of OPA derivatization of GSH and γ-GCS. In terms of fluorescence intensity of OPA derivatives, optimal pH was 9.2.

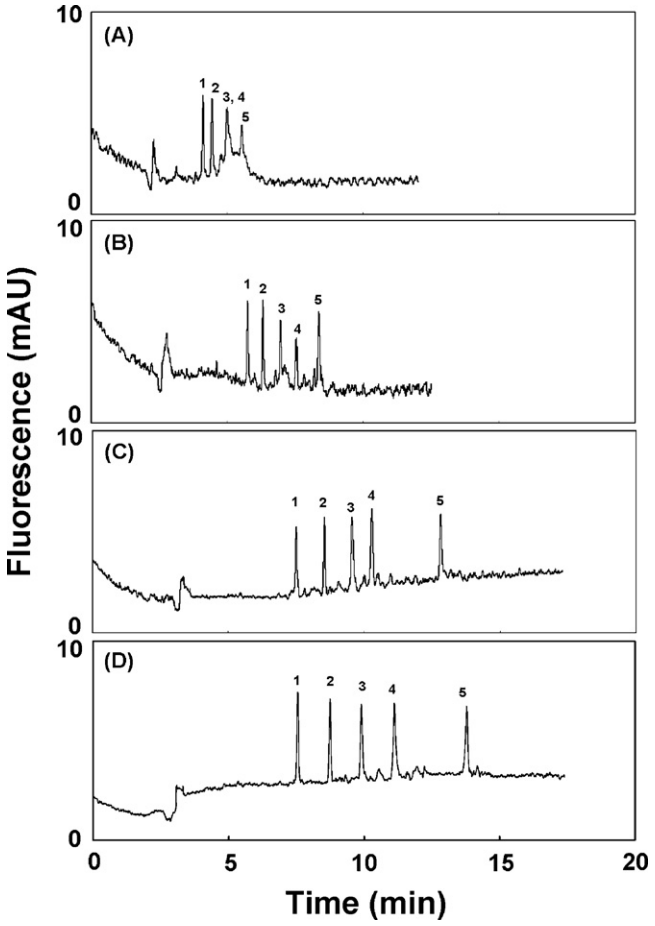

Fig. 2. Effect of PEO concentration on the separation of thiol-containing peptides in the presence of EOF. A capillary is filled with 10 mM $Na_2B_4O_7$ at pH 9.2, while buffer vials contain (A) 0%, (B) 0.2%, (C) 0.4%, and (D) 0.6% (w/v) PEO. Thiol-containing peptides were derivatized with a solution containing 10 mM OPA and 1 mM $Na_2B_4O_7$ (pH 9.2) for 2 h. OPA derivatives are hydrodynamically injected by raising the capillary inlet 20 cm in height for 10 s. Peak identified: 1, GSH (0.2 μM); 2, γ-GCS (0.1 μM); 3, PC_2 (1 μM); 4, PC_3 (2 μM); 5, PC_4 (1 μM).

3.2. Role of PEO

Because OPA reacts with thiol groups of peptide and its amine groups to form a tricyclic isoindole derivative, OPA-derivatized peptide containing carboxylic groups has a negative charge in a solution of 10 mM $Na_2B_4O_7$ (pH 9.2) (Fig. S3, supplementary). This study supposes that OPA-derivatized peptides with negative charges can enter into neutral PEO zone in the presence of EOF and their separation efficiency can be improved by varying PEO concentration. When the capillary and buffer vial both contained 10 mM $Na_2B_4O_7$ (pH 9.2), two peaks corresponding to OPA-derivatized GSH and γ-GCS were resolved (Fig. 2A). However, separation of OPA-derivatized phytochelatins was unsuccessful. In contrast (Fig. 2B–D), resolution of adjacent peaks increased upon raising PEO concentration in the buffer vial. An increase in PEO concentration resulted in EOF decay and longer migration times of OPA-derivatized peptides. EOF mobility changed from 11.6 to 7.9×10^{-4} cm^2 V^{-1} s^{-1} when the PEO concentration varied from 0 to 0.6%. Decreased EOF is a result of PEO adsorption on the capillary through hydrogen bonding between hydroxyl groups of capillary wall and the ether oxygen of PEO molecules [39]. During the separation, it is possible that the earlier and later detected peptides migrated in the presence of higher and lower EOF, respectively. As a result, the difference in migration time between any two adjacent peptides increased with time, thereby improving resolution.

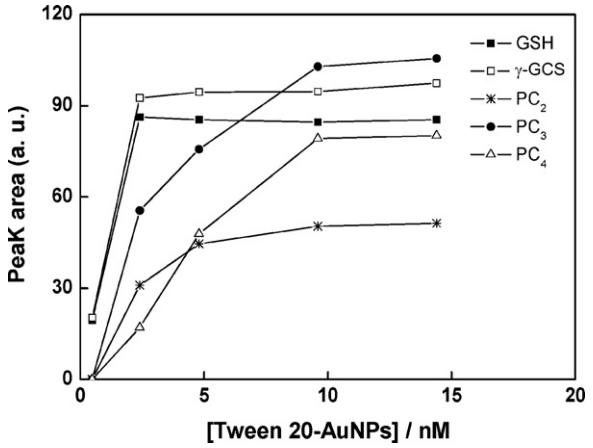

Fig. 3. Effect of the concentration of Tween 20-AuNPs on the extraction of thiol-containing peptides. A solution (200 μL) of 10 mM NaH_2PO_4 (pH 3.0) containing five peptides was incubated with Tween 20-AuNPs (0.48–14.4 nM, 200 μL) for 4 h. After centrifugation, five peptides onto the AuNP surface were mixed with DTT (800 mM, 1 μL) for 1 h. The released peptides were derivatized with a solution containing 10 mM OPA and 1 mM $Na_2B_4O_7$ (pH 9.2) for 2 h. OPA derivatives were separated by CE-LIF in the presence of 0.6% PEO. The other separation conditions are the same as Fig. 1. Peak identified: 1, GSH (25 nM); 2, γ-GCS (25 nM); 3, PC_2 (250 nM); 4, PC_3 (500 nM); 5, PC_4 (250 nM).

Besides, the order of migration of OPA-derivatized phytochelatins depended on the relative magnitude of molecular length, suggesting that the proposed method can determine molecular length of phytochelatins based on migration time. This result may be due to the increase in the number of carboxylic groups of OPA-derivatized phytochelatins with more repeating units of γ-GCS (Fig. S3, supplementary). In terms of separation and speed, 0.6% PEO was optimal. Under this condition, limits of detection (LODs) at a signal-to-noise (S/N) ratio of 3 for GSH, γ-GCS, PC_2, PC_3, and PC_4 were estimated to be 2, 1, 18, 30, and 20 nM, respectively. However, this sensitivity cannot detect dissolved thiols in seawater.

3.3. Sensitivity improvement

To improve detection sensitivity, Tween 20-AuNPs were used as selective probes to extract and enrich thiol-containing peptides from an aqueous solution. Initial experiments investigated the effect of Tween 20-AuNP concentration on extraction efficiency. Prior to CE separation, Tween 20-AuNPs (200 μL) was mixed with a solution (200 μL) containing GSH (25 nM), γ-GCS (25 nM), PC_2 (250 nM), PC_3 (500 nM), and PC_4 (250 nM). After incubation (4 h) and centrifugation, these peptides bound to the NP surface were incubated with 80 mM DTT (10 μL, 80 mM) for 1 h. The sample volume was reduced to 10 μL after extraction. Peak areas of five peptides increased with the addition of Tween 20-AuNPs and reached a saturation level at 9.6 nM Tween 20-AuNPs (Fig. 3). The simultaneous loading of five peptides on a single 13 nm AuNPs was calculated to be ~110 molecules. Chang et al. reported that the loading of five aminothiols (GSH, γ-GCS, cysteine, homocysteine, and cysteinylglycine) on the same single particle was ~670 molecules [31]. The loading difference between aminothiols and peptides is due to the simple fact that peptides have relatively large steric hindrance. Moreover, the peak areas of GSH, γ-GCS, PC_2, PC_3, and PC_4 reached a plateau above 2.4, 2.4, 4.8, 9.6, and 9.6 nM of Tween 20-AuNPs, respectively. Although the number of thiol groups of PC_2 is larger than that of GSH and γ-GCS, the loading of GSH and γ-GCS on the NP surface is faster than that of PC_2. Thus, the adsorption rate of thiol-containing peptide onto the surface of the AuNP clearly relies on molecular size, rather than the number of thiol groups. Similar results emerged when comparing PC_2 with PC_3 and PC_4. In

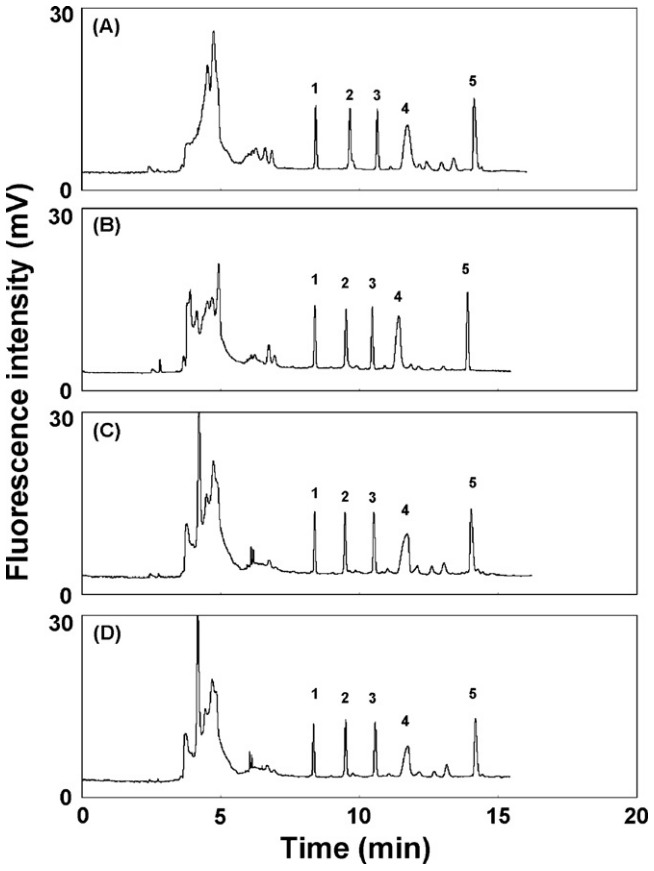

Fig. 4. Extraction of (A) 0.2 mL, (B) 1 mL, (C) 5 mL, and (D) 10 mL of thiol-containing peptides with Tween 20-AuNPs (9.6 nM, 200 μL). The concentrations of GSH, γ-GCS, PC_2, PC_3, and PC_4 are (A) 50, 50, 500, 1000 and 500 nM, (B) 10, 10, 100, 200 and 100 nM, (C) 2, 2, 20, 40 and 20 nM, and (D) 1, 1, 10, 20 and 10 nM, respectively. Peak identified: 1, GSH; 2, γ-GCS; 3, PC_2; 4, PC_3; 5, PC_4. The other conditions are the same as Fig. 2.

addition to the concentration of Tween 20-AuNPs, the time between NPs and thiol-containing peptides as well as between displacing agents and peptide-adsorbed NPs both influence extraction efficiency. Complete extraction time for analytes showed the following trend: $PC_4 \sim PC_3 > PC_2 >$ GSH ~ γ-GCS (Fig. S5, supplementary). This confirms that larger thiol-containing peptides require more time to attach to the NP surface. The time for complete extraction of five peptides was optimized to be 4 h. The peak area of five peptides gradually increased with time and reached a plateau between 60 and 120 min when the excess of DTT (80 mM) was added to peptide-adsorbed NPs (Fig. S6, supplementary). Thus, DTT containing two thiol groups effectively liberated peptides from the NP after 1 h. The time for the extraction of five peptides with Tween 20-AuNPs was about 5 h.

This study then investigated the increase of sample volume (0.2–10 mL) on the sensitivity improvement. Five peaks corresponding to GSH (50 nM), γ-GCS (50 nM), PC_2 (500 nM), PC_3 (1000 nM), and PC_4 (500 nM) were detected when 200 μL of 9.6 nM Tween 20-AuNPs was employed to extract of 200 μL of sample volume (Fig. 4A). The first peak (around 5 min) could correspond to the hemithioacetal from the reaction between OPA and DTT [40]. When the same sample concentration was diluted in 2, 5, and 10 mL of phosphate solution (pH 3.0), respectively, the peak area of five peptides remained almost constant with varying sample volume (Fig. 4B–D). The molar number of five peptides is constant when sample volume increased from 0.2 to 10 mL. Tween 20-AuNPs are thus efficient for extracting low concentration of peptides from large-volume samples. By combining NP extraction (10-mL sample) with CE-LIF, the LODs for GSH, γ-GCS, PC_2, PC_3, and PC_4 were 0.4, 0.6, 7, 40, and 14 pM, respectively (estimated from Fig. S7A, supplementary). Because PEO is demonstrably useful to stack negatively charged analytes through the viscosity difference between sample zone and PEO solutions [37–39], this study tested whether or not an increase in the sample injection time could further improve detection sensitivity. The peak area of five peaks increased with extension of sample injection times (Fig. S7, supplementary). Importantly, these peaks were well-resolved when the sample was injected for 60 s (~60 nL). Poor separation efficiency was observed when the injection volume was larger than 60 nL. Under optimum extraction conditions and injection volume (~ 60 nL), the LODs for the detection of GSH, γ-GCS, PC_2, PC_3, and PC_4 by CE-LIF were down to 0.1, 0.2, 2, 6, and 1 pM, respectively. These values were lower than the concentration of dissolved thiols in seawater [10]. As compared to RP-HPLC coupled to amperometry, electrospray ionization mass spectrometry, inductively coupled plasma mass spectrometry, or fluorescence and CE combined with fluorescence or electrospray ionization mass spectrometry, the method provided a 300–27,000,000-, 300–42,000,000-, and 30–1,000,000-fold improvement in sensitivity when detecting GSH, γ-GCS, and PC_2, respectively (Table S1, supplementary). Although the analysis time (including extraction, derivatization, and separation) of the proposed method for determining these thiols is approximately 8 h, this method can handle several samples simultaneously.

3.4. Reproducibility and quantification

Under optimum extraction conditions (200 μL of 9.6 nM Tween 20-AuNPs and 10 μL of 80 mM DTT), separation conditions (0.6% PEO, pH 9.2), and injection sample volume (~60 nL), five independent runs were examined for the reproducibility of the separation of five peptides. The relative standard deviations (RSD) of migration times and peak areas for the peptides were smaller than 3% and 5%, respectively (Table 1). Table 1 also shows that the calibration curve obtained by plotting the peak areas of the peptides against the peptide concentration were all linear ($R^2 > 0.9970$). Compared to normal hydrodynamic injection (~10 nL) without NP extraction, the proposed method gives more than a 10,000-fold increase in sensitivity for CE-LIF detection of GSH, γ-GCS, PC_2, PC_3, and PC_4. To test the selectivity, the proposed method was utilized for the analysis of five peptides in artificial seawater. Compared to the proposed method for extracting the peptides in 10 mM NaH_2PO_4 (pH 3.0), the use of the same method for extracting the peptides in artificial seawater provided similar peak areas and migration times (Fig. S8, supplementary). This result indicates that the proposed method is largely free from the matrix effect of artificial seawater.

By applying ICP-MS, the concentrations of Cu^{2+}, Cd^{2+}, and Hg^{2+} in seawater were estimated to be 16.2, 0.21, and 0.07 ppb. Under this condition, the proposed method applies to practical analyses of dissolved thiols in seawater (Fig. 1A). To demonstrate this, Tween 20-AuNPs extracted and enriched dissolved thiols in EDTA-treated seawater. EDTA can inhibit the binding of thiol-containing peptides and metal ions [10]. Without NP extraction and large sample volume injection, direct analysis of EDTA-treated seawater by CE-LIF reveals no peak corresponding to five peptides (Fig. 5A). Under the proposed method, three peaks corresponding GSH, γ-GCS, and PC_2 were detected by CE-LIF (Fig. 5B). To confirm this result, the study identified them by comparing the peak areas obtained from EDTA-treated seawater without (Fig. 5B) and with (Fig. 5C) spiked standard GSH, γ-GCS, and PC_2. RSD of the migration times for these identified thiols were less than 3% (Table 2). Mean recoveries for five peptides at five spiked levels were in the range of 103 ± 3 to 99 ± 4%. Good linearity appeared in the plots of peak areas of five peptides against spiked concentrations. By applying a

Table 1
Quantitative analyses of five peptides by the combination of Tween 20-AuNP extraction, OPA derivatization, and CE stacking.

	GSH	γ-GCS	PC_2	PC_3	PC_4
RSD of migration time ($n=5$)	1%	1%	2%	2%	1%
RSD of peak area ($n=5$)[a]	3%	4%	3%	4%	4%
Dynamic range (nM)	0.001–20	0.002–20	0.05–30	0.1–30	0.04–16
R^2	0.9991	0.9988	0.9983	0.9974	0.9990
LOD (pM)	0.1	0.2	2	6	1
Sensitivity improvement[a]	20,000	5000	9000	5000	20,000

[a] Comparison of LODs obtained from normal injection without NP extraction.

Table 2
Quantitative analyses of dissolved thiols in seawater by the combination of Tween 20-AuNP extraction, OPA derivatization, and CE stacking.

	GSH	γ-GCS	PC_2	PC_3	PC_4
RSD of migration time ($n=5$)	1%	1%	2%	2%	2%
Spiked concentration (nM)	0.1, 0.5, 1, 3, 5	0.1, 0.5, 1, 3, 5	0.1, 0.5, 1, 2, 5	0.2, 1, 2, 4, 8	0.1, 0.5, 1, 2, 5
R^2	0.9987	0.9995	0.9991	0.9982	0.9989
Mean recovery (%)	103 ± 3	100 ± 4	99 ± 4	102 ± 4	103 ± 3
Mean concentration (nM)	3.02 ± 0.06	0.46 ± 0.03	0.17 ± 0.02	ND	ND

ND: not detected.

standard addition method (regression lines listed in Table 2), we determined the concentrations of GSH, γ-GCS, and PC_2 in the seawater. The order of the concentration of dissolved thiol in seawater is in agreement with reported findings [10].

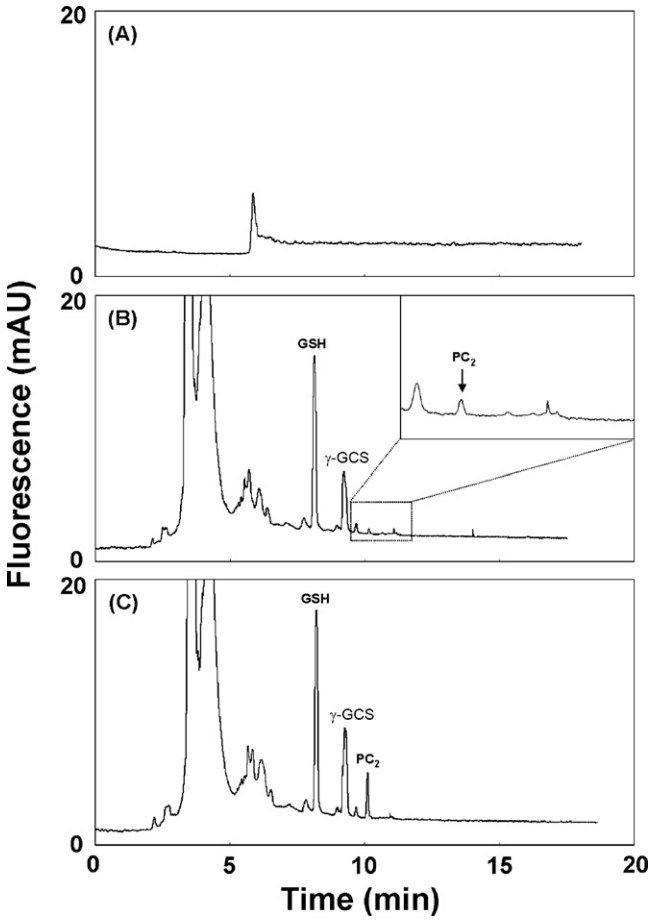

Fig. 5. (A) Analysis of dissolved thiols in seawater by CE-LIF without NP-based extraction and CE stacking. Extraction of (B) dissolve thiols and (C) dissolved thiols spiked with GSH (200 pM), γ-GCS (200 pM), and PC_2 (2000 pM) in seawater by Tween 20-AuNPs, followed by OPA derivatization and CE stacking. The other conditions are the same as Fig. 1.

4. Conclusion

Ultrasensitive and selective detection of GSH, γ-GCS, and PC analogs has been accomplished using Tween 20-AuNPs as an extracting agent, OPA as a derivatizing agent, and CE-LIF as the separation and concentration method. Extraction efficiency of thiol-containing peptides is improved by increasing Tween 20-AuNP concentration, optimizing extraction and desorption time, and using high DTT concentration. Detection sensitivity can be further enhanced based on stacking of extracted thiols in CE. Consequently, LODs of five thiol-containing peptides can be as small as 0.1–6 pM. The proposed method provided a number of distinctive advantages. First, to the best of the authors' knowledge, this is the first use of CE to detect dissolved thiols in seawater. Second, the proposed method provided large linear range and the lowest LOD value for GSH, γ-GCS, and PC_2 compared to other reported methods. Third, Tween 20-AuNPs can selectively extract thiol-containing peptides and effectively inhibit nonspecific adsorption. The proposed method not only allows investigation of interaction between phytochelatins and metal ions in natural water, but also has great potential to detect trace amounts of thiols, such as metalothioneins.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.11.057.

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