Simultaneous Electrochemical Determination of Isoquercitrin and Epigallocatechingallate at a Carbon Nanotube Electrode

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ABSTRACT

Simultaneous electrochemical determination of isoquercitrin (ISQ) and epigallocatechingallate (EGCG) at a carbon nanotube electrode is presented. The carbon nanotube electrode for ISQ and EGCG sensing has attractive properties in terms of well-defined current peaks, high sensitivity, and high reproducibility. Two well-distinguished anodic peaks +0.23 V and +0.38 V due to EGCG and ISQ are observed by cyclic voltammetry and those peaks are independent from each other. The linear ranges of ISQ and EGCG are 0.76–94 μ M and 4.0–94 μ M, respectively. The ISO and EGCG values of real sample determined by this method show good agreement with the actual ones.

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1. Introduction

Isoquercitrin (ISQ) and epigallocatechingallate (EGCG) are ones of polyphenols and their chemical structure are shown in Fig. 1. ISQ is a glycosylated flavonoid which is a subclass of polyphenol and naturally exist in vegetables and fruits.¹ EGCG is major catechin and also one of flavonoid. It exists in green and black tea.² The intake of those polyphenols has healthy benefits, for example, reduction of obesity, delay of degenerative diseases, and defense from virus infection.^{3–5} Recently, the beverage containing abundant ISQ and catechins are approved for the labeling as food for specified health uses (FOSHU) by Consumer Affairs Agency, Japan. Therefore, simultaneous detection of ISQ and EGCG is beneficial in terms of the quality control of food function and nutrition.

Although high-performance liquid chromatography (HPLC)^{6–8} is the present technique used for simultaneous detection of ISO and EGCG, HPLC requires bulky equipment, sample pre-treatment, harmful solvent, skill, time, and cost. Thus, electrochemical analysis has many benefits overcoming the HPLC's drawbacks. Carbon nanotube (CNT)-based electrodes have been promising because it has excellent properties for electrochemical behavior. Since we have already reported sensing of total polyphenol in green tea⁹ and black tea¹⁰ with CNT electrode, we would like to extend the possibility of the CNT electrode. Although there are some reports of EGCG sensing with CNT-based electrodes,^{9–11} there are few reports of ISQ sensing. Although there have been many reports on the electrochemical determination of solo ISQ (as a quercetin glucoside)^{12,13} and EGCG,^{11,14} the simultaneous analysis for ISQ and EGCG in food has not been done. In this report, we demonstrate the simultaneous electrochemical quantification of ISQ and EGCG with the CNT electrode for the first time.

2. Experimental

2.1 Materials

ISQ was purchased from was purchased from Sigma-Aldrich (St. Louis, USA). EGCG was purchased from Nagara Science Co. (Gifu, Japan). Citric acid buffer solution was purchased from Wako

Figure 1. Molecular structures and electrochemical reaction schemes of ISQ and EGCG.

(Osaka, Japan). Multi-walled CNT (10–30 nm in diameter, length 200 μ m) was produced by Taiyo Nippon Sanso Corporation (Tokyo, Japan).

2.2 Electrode fabrication

The electrode was formed on a screen-printed carbon paste on a plastic substrate. The area of the opening for the working electrode was 9 mm². The details are in literature.⁹ In brief, the CNT was dispersed in 0.3% carboxymethylcellulose (CMC) aqueous solution. The CNT concentration was 0.1%. 5 μ L aliquots of the CNT and

CMC solution was dropped onto the screen printed carbon paste electrode surface and dried. Finally, 5 μL aliquots of 1.0% (w/v) CMC solution were dropped onto the CNT and CMC layer and dried.

2.3 Measurements

Electrochemical measurements were performed with an electrochemical analyzer (ALS Instruments, 701A West Lafayette, IN, USA) using a three-electrode configuration. Reference (Ag/AgCl saturated KCl, RE-1C) and counter (platinum wire) electrodes were purchased from BAS Inc. (Tokyo, Japan). Electrochemical measurements were conducted in a 5 mL vessel at 20°C using a citric acid buffer (50 mM, pH 5.3) as the supporting electrolyte, to which stock polyphenol solutions of 0.2 mM were successively added to prepare samples with designated concentrations. Food sample was purchased at a retail store in Japan.

3. Results and Discussion

3.1 Cyclic voltammetry of mixture solution of ISQ and EGCG at the CNT electrode

Cyclic voltammetry (CV) was performed to investigate the electrochemical response of the CNT electrode toward ISQ and EGCG. Advantage of the CNT electrode is presented. Figure 2 shows the CVs of ISQ and EGCG at conventional carbon paste and CNT electrodes. The stronger and more distinct peaks due to ISQ and EGCG (assignments are mentioned as following) at the CNT electrode than at the carbon paste counterpart were observed. The response current ratio to background current at the CNT (ISQ: 4.1, EGCG: 2.9) was much larger than that of the carbon paste electrode (ISQ: 2.3, EGCG: 1.4) in spite that non-Faradic (background) current at the CNT electrode is larger than that of the carbon paste electrode. This demonstrates that the CNT electrode exhibits the best performance for ISQ and EGCG quantification.

Red line in Fig. 3A is CV profile of ISQ. In forward sweep, the peak at +0.38 V is assigned to oxidation of the *ortho*-diphenol on B-ring in flavonoid skeleton to quinone (defined as Peak I, Fig. 1). In backward sweep, the peak at +0.34 V is assigned to reduction of quinone to the *ortho*-diphenol. An observed peak-to-peak separation (ΔE_p) was estimated as ca. 40 mV, which was within a range for a quasi-reversible process. Blue line in Fig. 3B is CV profile of EGCG. In the forward sweep, two oxidative peaks are observed. The one peak at +0.23 V is assigned to the oxidation of triphenol in B-ring of EGCG on the flavonoid skeleton to the semiquinone radical (defined as Peak II, Fig. 1), and the other peak at +0.33 V is assigned to triphenol on the gallate to the semiquinone radical (defined as Peak III, Fig. 1).^{15,16} In the cathodic scan, there is no distinctive peak, which indicates that the oxidation of triphenol is irreversible.

Black line in Fig. 3A is CV profile of various concentration of EGCG (11–180 μM) in the presence of 77 μM ISQ. Peak II due to EGCG increases while Peak I due to ISQ changes only within 5% up to 75 μM EGCG addition. When the concentration of EGCG surpasses 130 μM , the peak position negatively shifts for +0.33 V, whose peak position corresponds to Peak III due to EGCG. Black line in Fig. 3B is CV profile of various concentration of ISQ (5.3–75 μM) in the presence of 46 μM EGCG. Peak I due to ISQ increases while Peak II due to EGCG does not change. When the concentration of EGCG surpasses 130 μM , the peak position negatively shift for +0.33 V, whose peak position corresponds to Peak I due to ISQ. This suggests that the peak around at 0.35 V (0.3–0.4 V) discriminate whether it is dominated by ISQ or EGCG. There was no distinctive difference of the current intensity and shape of the CV at any pH except the fact that the peak position shift lower with increasing pH. Based on the sensitivity, reproducibility, and stability of CV profiles and the fragility of the catechins in alkaline media, the following experiment was conducted at pH 5.3.

Figure 2. CVs of (A) ISQ and (B) EGCG at carbon paste (black line) and CMC-CNT (red line) electrodes. Concentrations are 0 (dot line) and 65 (solid line) μM . The electrolyte used was in 50 mM pH 5.3 citric acid buffer solution. The scan rate was 0.1 V s^{-1} . Peak assignments were shown in Fig. 1.

Figure 3. CVs for the CNT electrode at different concentration of (A) EGCG in the presence of 77 μM ISQ and (B) ISQ in the presence of 46 μM EGCG. Altering analyte concentrations were (A) 11, 22, 42, 75, 130, 180 μM and (B) 5.3, 11, 22, 42 μM . Dot lines are backgrounds. The electrolyte used was in 50 mM pH 5.3 citric acid buffer solution. The scan rate was 0.1 V s^{-1} . Peak assignments were shown in Fig. 1.

Our anxiety is that the contamination of electrode due to analyte adsorption badly affects the sensing performance. We claim that using CV is the solution because a repeat scan can enhance the reproducibility by stability of the electron transfer rate and the cleaning effect of the electrode surface. We have already reported this cleaning effect by consecutive CV where adsorbed analyte on the electrode are removed by more than three CV cycles.

3.2 Electrochemical quantification of mixture solution of ISQ and EGCG

Figure 4A shows CV current vs. concentration plot for the selective determination of ISQ in the absence and the presence of EGCG with the CNT electrode. We had varied the concentration of ISQ while keeping the concentration ratio between ISQ and EGCG. The peak at +0.38 V due to oxidation of diphenol in B-ring of ISQ is not affected by up to molar ratio ca. 2 of $[\text{EGCG}]/[\text{ISQ}]$. The linear function of ISQ determination using by I_p vs. concentration plot was $I_p (\mu\text{A}) = 0.45 + 0.11[\text{ISQ}] (\mu\text{M})$ with linear range of 0.76–94 μM ($r = 0.997$).

Figure 4B shows CV current vs. concentration plot for the selective determination of EGCG in the absence and the presence of ISQ with the CNT electrode. We had varied the concentration of EGCG while keeping the concentration ratio between ISQ and EGCG. The peak at +0.23 V due to oxidation of triphenol group in B-ring of EGCG is not affected by the oxidation of ISQ by up to molar ratio ca. 2 of $[\text{ISQ}]/[\text{EGCG}]$. The linear function of EGCG determination using by I_p vs. concentration plot was $I_p (\mu\text{A}) = 0.27 + 0.25[\text{EGCG}] (\mu\text{M})$ with linear range of 4.0–94 μM ($r = 0.997$).

Figure 4. CV current vs. concentration plot for mixture solutions with various contents of individual ISQ and EGCG. Inset: Enlargement of low-concentration region. The black line represents the least-squares fitting line of the solution containing only ISQ or EGCG.

The above results show that the simultaneous determination of ISQ and EGCG is possible when the $[EGCG]/[ISQ]$ ratio are between 0.5 and 2.0. When the $[EGCG]/[ISQ]$ ratio is more than 2, the $[ISQ]$ is overestimated. When the $[EGCG]/[ISQ]$ ratio is less than 0.5, the $[EGCG]$ is overestimated.

3.3 Simultaneous quantification of ISQ and EGCG in a real sample

Figure 5A show a picture of a real sample, Tokucha Lemon produced by SUNTORY corporation. This merchandise is food for special health uses (so called “Tokuho”) authorized by Consumer Affairs Agency Japan and it contains ISQ and catechins. The catechins in real sample are the sum of various kind of catechins. We adopted CV current of real sample due to oxidation of total amount of four catechins as EGCG equivalent because EGCG is often the most major catechin in green tea.^{7,8} Fig. 5B show CV of the real sample and simulated CV of intentionally prepared mixture solution for the component ratio of ISQ and EGCG in real sample based on ingredient indication at the label ($[ISQ] = 22 \text{ mg/dL} = 474 \mu\text{M}$, $[\text{catechins}] = 46 \text{ mg/dL} = 1000 \mu\text{M}$ (EGCG equivalent)) on Tokucha Lemon (Amount of ingredients related with food function must be indicated for FOSHU). When comparing the CVs of real samples and its simulated CV, both the shapes are almost similar. Figure 4C and 4D show the plot of ISQ and EGCG concentration in real sample vs. the CV current, which demonstrates that the current vs. diluted concentration plots have strong relationship with the fitting line based on its simulated CV.

4. Conclusions

We demonstrated simultaneous electrochemical determination of ISQ and EGCG at the CNT electrode. The discrimination of ISO and EGCG is conducted with the difference of peak potential by CV. The peak at +0.23 V due to oxidation of triphenol group in B-ring of EGCG is not affected by the oxidation of ISQ by up to molar ration ca. 2 of $[ISQ]/[EGCG]$. The peak at +0.38 V due to oxidation of *ortho*-diphenol group in B-ring of ISQ is not affected by up to molar

Figure 5. (A) Photograph of the real sample. Food for specified health uses Tokucha Lemon produced by SUNTORY. $[ISQ] = 22 \text{ mg/dL} = 474 \mu\text{M}$, $[\text{catechins}] = 46 \text{ mg/dL} = 1000 \mu\text{M}$ (EGCG equivalent). (B) CVs of real sample and simulated solution contains ISO and EGCG whose concentration is the same as real sample. (C, D) The current vs. diluted concentration based on real sample data are plotted. The fitting line based on CV of solo ISQ or EGCG in Fig. 4. Diluted ratios: 10, 19, 46, 91.

ration ca. 2 of $[EGCG]/[ISQ]$. The linear ranges of ISQ and EGCG are 0.76–94 μM and 4.0–94 μM , respectively. The ISQ and EGCG values of real sample determined by this method show good agreement with the actual ones. The method presented here will be a significant contribution toward the development of electrochemical determination toward the important chemicals in food.

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