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Capillary Electrophoresis Detection of Hydrogen Peroxide by Using Titanium Ion and 4-(2-thiazolylazo)resorcinol

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A novel method for the detection of hydrogen peroxide in aqueous solution was developed via reaction between H_2O_2, trivalent titanium ion (Ti^{3+}) and 4-(2-thiazolylazo) resorcinol (TAR), resulting in a ternary complex with a maximum UV absorbance at 530 nm. The CE detection of H_2O_2 was fast, sensitive and cost-effective without pretreatment procedures. H_2O_2 was detected within 15 min at 1 to 100 μM range with the lowest detection limit at 1.0 μM. Under the optimized CE conditions, the concentration of H_2O_2 in coffee or tea extract was quantitatively determined. Our results show that CE detection of the ternary complex of H_2O_2-Ti^{3+}-TAR has potential applications for the detection of H_2O_2 in aqueous sources.

Key words: Hydrogen peroxide, capillary electrophoresis (CE), titanium ion (Ti^{3+}), 4-(2-thiazolylazo)resorcinol (TAR).

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Introduction

Hydrogen peroxide is an integral part of chemical and biological systems as temporary reservoirs for $HO_x\cdot$ and $RO_x\cdot$ radicals [1]. In biological systems, H_2O_2 is produced in various reactions catalyzed by numerous enzymes [2,3], functioning as a messenger in cellular signal transduction [3]. H_2O_2 mediates various cellular injuries and may cause mutagenesis or carcinogenesis [4]. In dental field, H_2O_2 is used for tooth whitening purpose by degrading colored pigments on the enamel [5].

Detecting hydrogen peroxide in aqueous environment has been difficult due to short half-life time and instability. There has been several methods developed for the detection of H_2O_2. Ti-PAPS (titanium 2-((5-bromopyridyl)azo)-5-(N-propyl-N-sulfopropylamino) phenol) reagents were used for the spectrophotometric detection of H_2O_2 [6]. The Fox assay, developed in 1990's, uses ferrous ion oxidation in the presence of the ferric ion indicator, xylenol orange, under acidic condition [7]. Tanner and co-workers detected H_2O_2 through a reaction with pyridine-2,6-dicarboxylic acid and vanadate (V) in acidic solution to form chelate complex, oxo-peroxo-pyridine-2,6-dicarboxylato vanadate (V) [8]. Recently, the ternary complex of Ti-H_2O_2 and 4-(2-pyridinylazo)resorcinol (PAR) was used for H_2O_2 detection [9]. Other methods using florescent probes, chemiluminescence or enzyme peroxidase are also known [10,11].

4-(2-Thiazolylazo)resorcinol (TAR) used in this study is a

derivative of PAR which has high affinity to titanium ion. TAR also functions as an indicator of spectrometric detection of complexes. Here, we present novel CE detection method of hydrogen peroxide in aqueous solution by using the interaction of H_2O_2, TAR and trivalent titanium ion. CE detection of Ti(III)–TAR–H_2O_2 ternary complex was fast, sensitive, cost-effective without requiring pre-treatment steps. We show that this method could be successfully applied for the accurate measurement of H_2O_2 in beverages such as tea and coffee.

Material and Methods

Hydrogen peroxide (35 %) was purchased from Junsei Chemical Industries (Tokyo, Japan). Sep-Pak catridges and filters (0.45 μm) were from Waters (Milford, MA, USA). Commercial tea or coffee product was purchased from a local shop. Catalase from bovine liver was obtained from Sigma Chemical (St. Louis, MO, USA). CE background electrolyte and standard solutions were prepared in Milli-Q water produced by a purification system (Millipore, Molsheim, France). The background electrode (BGE) was 75 mM boric acid and 35 mM NaOH, pH 9.1.

Preparation of Ti(III)−TAR−H_2O_2 ternary complex

Trivalent titanium ion (Ti^{3+}) solution was prepared by dissolving a titanium disc into concentrated HCl (10 M) on hot plate and then diluted to 10 mM solution. The mixed solution of Ti(III) ion and 4-(2-thiazolylazo) resorcinol (Ti(III)–TAR) was prepared by mixing 1:1 volume ratio of stock titanium (10 mM) and 10 mM TAR solutions (Alfa Aesar, Seoul, Korea). The appropriate concentration of aqueous H_2O_2 was added into the Ti(III)-TAR complex solution to form ternary complex of Ti(III)–TAR–H_2O_2. The complex of Ti(III)–TAR–H_2O_2 was detected at 530 nm by UV-Vis spectrophotometer (Hitachi, Tokyo, Japan).

Capillary electrophoresis

CE analyses were carried out with a P/ACE 5500 system (Beckman, Kraemer, CA) equipped with photodiode array (PDA) detector and interfaced with the Karat 8.0 software. A fused-silica capillary (60 cm length, 100 μm i.d, 365 μm o.d) was used for all separations (Polymicro Technologies, Phoenix, USA). Before injection, the capillary was reconditioned by rinsing with 0.1 N HCl for 1 min, 0.1 N NaOH for 1 min and BGE solution for 1 min. Electrophoresis was carried out at a constant current mode in a positive polarity (+20 kV) at 25 ^{0}C. Hydrodynamic injections at the pressure of 0.5 psi for 3s were applied for all analytical operations.

Preparation of coffee or tea extract

100 mg of coffee / tea leaves in 1 ml of water were boiled for 5 minutes and centrifuged at 10,000 rpm for 20 min. The supernatant was filtered through Sep-pak C18 cartridge and microfilter (0.45 μm), and then diluted by BGE. Injection samples for CE were prepared by mixing 2 μl of Ti-TAR (100 μM) reagent and 48 μl of the extract. All CE operations were triplicated.

Results

Ternary complex formation of Ti−TAR−H_2O_2

Doubly deprotonated TAR^{2-} formed a complex with trivalent titanium ion, predominantly in 2:1 ratio of TAR to titanium ion (Fig. 1A). As aqueous H_2O_2 was added into the Ti(III)–TAR solution at pH 3, stable ternary complexes of Ti(III)–TAR–H_2O_2 were rapidly formed with the maximum absorbance at the wavelength of 531 nm (eq. 1 & Fig. 1B) [12].

$$Ti\ (III) + TAR + H_2O_2 \rightarrow Ti\text{-}TAR\text{-}H_2O_2 \qquad (eq.\ 1)$$

The aqueous TAR at pH 3.0 displayed the absorbance spectrum with λ_{max} = 400 nm (Fig. 2). In the presence of trivalent Ti ion, new absorbance band was appeared at 450 nm which belongs to Ti(III)-TAR complex. Finally, when 25 μM of aqueous H_2O_2 was titrated into the 50 μM of Ti(III)-TAR solution, significant band shift of maximum absorbance (450 to 530 nm) was observed with the rapid increase of pH to 9.1 within minutes (Ti(III)-TAR-H_2O_2, Δ = 80 nm). Newly formed ternary complex was stable for more than 24 hours at room temperature (data not shown).

CE detection of ternary complex Ti−TAR−H_2O_2

The ternary complex was detected by observing a CE migration peak of Ti(III)-TAR-H_2O_2 on electropherogram (Fig 3A). The peak intensity of ternary complex was proportionally

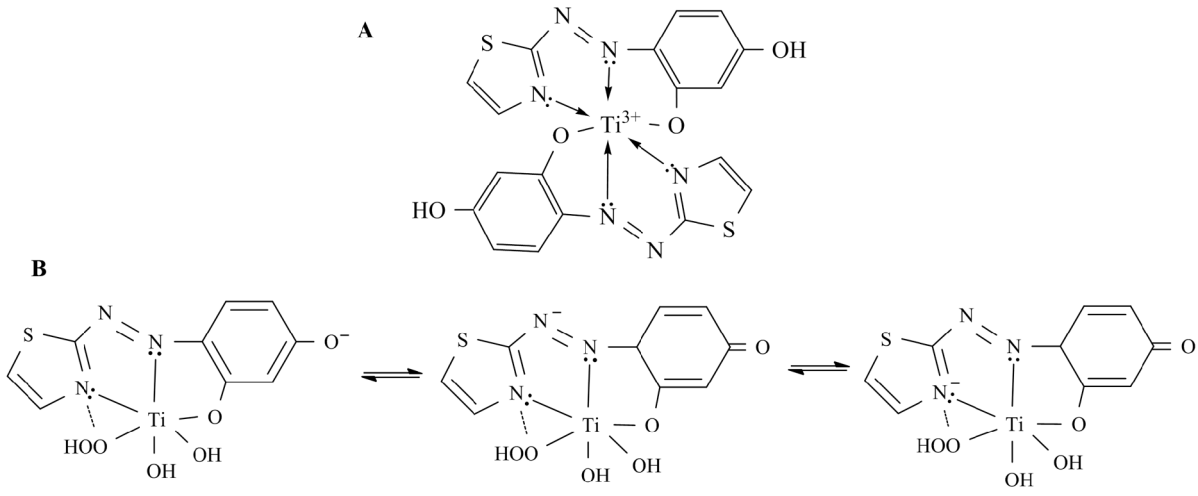

Fig. 1. The interaction scheme of Ti(III)-TAR (A) and Ti(III)−TAR−H_2O_2 complex (B).

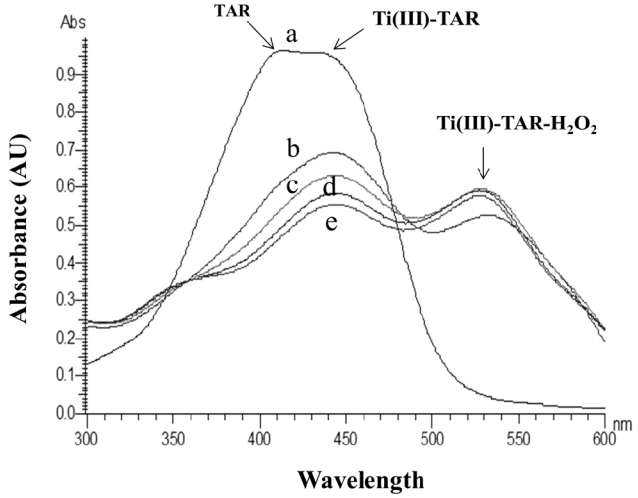

Fig. 2. UV absorbance spectra of Ti(III)-TAR (50 μM) and Ti(III)−TAR−H_2O_2 complex. (a) Ti(III)-TAR (50 μM); (b-e) H_2O_2 (25, 50, 100, 200 μM in pH 3.0) titration into Ti(III)-TAR (50 μM) solution. Band shift of maximum absorbance (450 to 530 nm) was observed.

dependent on the mole ratio of H_2O_2 to Ti-TAR (Fig. 3B). For the sensitive detection, we tested the background electrode (BGE) in different ion strengths (50 to 125 mM) and pHs (8.0 to 10.0) as well as the voltages supplied (15 to 25 kV) at room temperature. Among the tested BGE conditions (data not shown), 75 mM boric acid and 35 mM NaOH was the most suitable buffer condition showing the highest resolution of ternary complex peak at the migration time of 8.0 minutes (Fig. 3).

Detection of H_2O_2 in coffee or tea extract

In order to test the detection condition of H_2O_2 we optimized is appliable to other sources, the aqueous extracts of coffee or green tea were prepared as described in Materials and methods. As shown in Figure 4A, the complex of Ti-TAR-H_2O_2 was detected on CE electropherogram with expected migration time, convincing that Ti-TAR formed the ternary complex with H_2O_2 in coffee extracts. Similarly the same peak of Ti-TAR-H_2O_2 complex was observed in green tea extract when Ti-TAR was added (Fig. 4B). The intensity of the Ti-TAR-H_2O_2 peak was decreased as the unit of catalase was added to the extract prior to CE running, indicating that H_2O_2 in extract was slowly decomposed by the action of catalase. From the analysis of a Ti-TAR-H_2O_2 peak area by using standard fitting curve generated, H_2O_2 concentration in the extracts of instant coffee, roasted coffee and green tea was 75.3, 85.1 and 97.5 μM, respectively.

Discussion

In this study, we investigated CE detection of hydrogen peroxide in aqueous solution by using trivalent titanium ion (Ti^{3+}) and TAR. The detection was based on the ternary interaction of H_2O_2, trivalent titanium ion (Ti^{3+}) and TAR, forming stable ternary complex of Ti(III)−TAR−H_2O_2. TAR provides benefits as a chelating agent and also as an indicator of spectrometric detection of complexes. Among the known methods, the most common method for H_2O_2 detection was based on the redox reaction. This method requires pre-treating steps to eliminate the redox substances such as ascorbic acid or polyphenols in samples since they interfere accurate

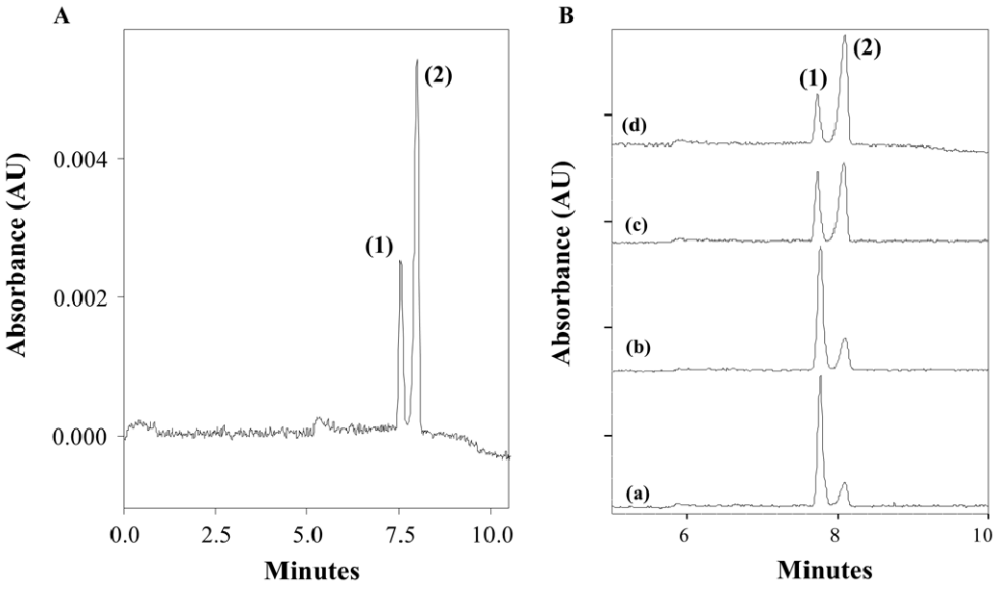

Fig. 3. CE detection of Ti-TAR-H_2O_2 and Ti(III)-TAR complexes. A. (a) Ti-TAR complex (100 μM), (b) Ti(III)-TAR-H_2O_2 complex generated by adding 100 μM of H_2O_2. B. Titration of aqueous H_2O_2 (10, 20, 60, 80 μM) into 100 μM of Ti(III)-TAR solution. The complex was detected at the wavelength of 530 nm. Buffer ground electrode (running buffer) was 75 mM boric acid, 35 mM NaOH, pH 9.1.

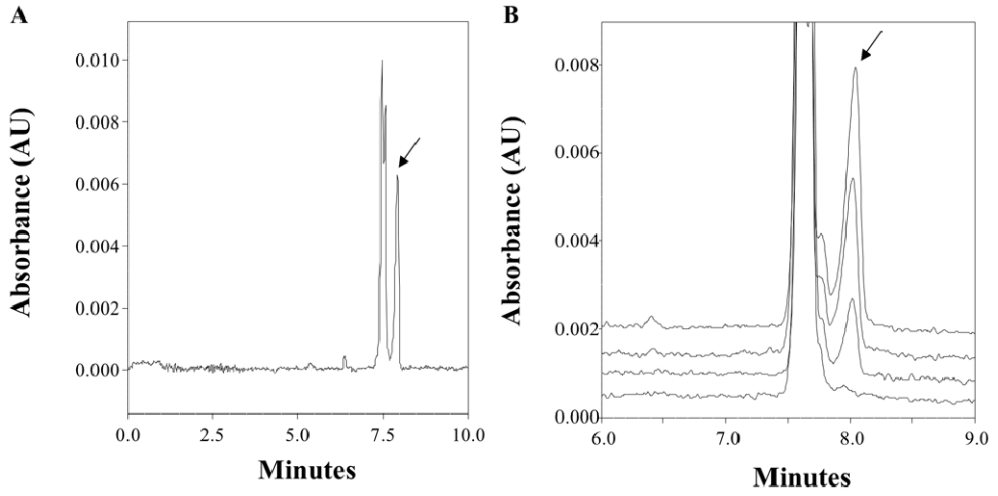

Fig. 4. CE detection of H_2O_2 in coffee / tea extract. A. Ternary complex of Ti-TAR-H_2O_2 in coffee extract. The extract of 48 μl was mixed with 2 μl of Ti-TAR (100 μM) reagent prior to CE injection. B. Ternary complex of Ti-TAR-H_2O_2 in tea extract: catalase (0, 1, 2, 20 unit/ml) was added into the extract solution prior to the complex formation. Arrow indicates Ti-TAR-H_2O_2 complex.

measurement of H_2O_2. The CE detection of H_2O_2 in our work is highly sensitive and specific. The concentration of H_2O_2 in aqueous solution was accurately determined within the concentration range of 1 to 100 μM. Furthermore, the detection of H_2O_2 by ternary complex formation is cost effective without pre-treatment steps. By using this method, the concentration of H_2O_2 in other food sources could be determined. In our trial, H_2O_2 concentrations in instant coffee, roasted coffee and green tea extracts were found to be 75.3, 85.1 and 97.5 μM, respectively, which are consistent with a previous report [13]. Considering that hydrogen peroxide is utilized as sanitizers or disinfectants in food industries [14], various applications of our method might be possible in future for the detection of H_2O_2.

The CE detection and quantitation of H_2O_2 were achieved by using Ti(III)–TAR–H_2O_2 ternary complex formation. Our method may provide potential application for the sensitive detection of H_2O_2 in various aqueous sources.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

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