Electrochemical Detection of Sesamol Dimer and its Application to Measurement of Radicals

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Abstract

In this paper, we propose a novel measurement for NO and •OH by electrochemical detection using sesamol. Standard samples of sesamol monomer and dimer were subjected to differential pulse voltammetry, resulting in their peaks being clearly separated and detected. Based on the oxidative dimerization of sesamol, the current simple, sensitive and selective method was successfully applied to the preliminary measurement for NO and •OH, respectively.

Keywords: sesamol, radical, NO, •OH, electrochemical detection, differential pulse voltammetry
Introduction

Sesame is widely known for its nutritional value, and sesame oil contains oleic acid and linoleic acid as major compounds. Furthermore, there are minor compounds such as sesamin, sesamolin and sesamol of sesame lignan showing strong antioxidant properties.\textsuperscript{1–3} In our previous reports,\textsuperscript{4,5} we found sesamol (3,4-methylenedioxyphenol) as a fluorogenic reagent by screening the reactivity between \textit{p}-phenol derivatives and NO. Sesamol was rapidly and selectively reacted with NO and •OH to yield fluorescent sesamol dimer in aqueous solution, as shown in Fig. 1. Therefore, this reaction was applied to the fluorometric determination of NO released from NO donors such as NOC7 (1-hydroxy-2-oxo-3-(N-methyl-3-aminopropyl)-3-methyl-1-triazene) and NOR1 ((\textpm)-(\textit{E})-4-methyl-2-[(\textit{E})-hydroxyimino]-5-nitro-6-methoxy-3-hexenamide) in the biological samples containing 20\% human plasma and cell extract.

In this study, we propose a novel selective measurement for sesamol dimer by electrochemical detection, which is a simple, highly sensitive and selective technique for oxidative and/or reductive reactions. Sesamol monomer and dimer consist of phenol moiety (Ph-OH) showing electrochemical activity, and their oxidation potentials were measured by differential pulse voltammetry (DPV). Consequently, it was demonstrated that sesamol monomer and dimer gave the different oxidation peaks, respectively, in the voltammograms of DPV. As sesamol monomer and dimer are easily oxidized, DPV was suitable for their sensitive and reproducible determination. Finally, based on the reaction of sesamol with radicals to yield sesamol dimer, we carried out the preliminary measurement for NO and •OH using sesamol by DPV to confirm the feasibility of the proposed method.
Experimental

Reagents and chemicals

Sesamol was purchased from Tokyo Chemical Industry. Iron (II) sulphate heptahydrate and hydrogen peroxide (30%) were obtained from Wako Pure Chemical. NOC7 was from Dojindo Laboratories.

According to our previous report, sesamol dimer was prepared with minor modifications as follows: Sesamol (850 mg, 6.15 mol) and 0.2 mg/mL horse radish peroxidase (HRP, TOYOBO) 4 mL were dissolved in 0.05 mol/L PBS (pH 7.4) 360 mL. To the reaction mixture, 0.05% hydrogen peroxide 36 mL was slowly added, and the resulting mixture was stirred for 2 h at room temperature. The final reaction mixture was extracted with ethyl acetate, and the organic extracts were washed with water, dried over Na$_2$SO$_4$, filtered and concentrated. The crude product was purified by column chromatography on silica gel (30% ethyl acetate in $n$-hexane) to afford sesamol dimer (54.5 mg, 6.5%). $^1$H NMR spectrum was identical to that of our previous report.

Apparatus

DPV was performed using an electrochemical analyzer (ALS model 660B; BAS, Tokyo, Japan), glassy carbon electrode (diameter, 3 mm) as the working electrode, a platinum wire as the counter-electrode, and an Ag/AgCl (3 mol/L KCl) as the reference electrode. DPV was then conducted at a step potential of 4 mV, a pulse amplitude of 50 mV, a pulse width of 60 ms, a pulse period of 200 ms, a sample period of 20 ms, and a voltage range from −0.1 to 0.7 V.
Sample preparation

For the determination of NO (NOC7): Sesamol (final concentration, 1 mmol/L) and each concentration of NOC7 (final concentration, 4.0–200 µmol/L) were dissolved in 0.05 mol/L PBS (pH7.4), and the reaction mixture was held at 37°C for 30 min. The resultant mixture was subjected to DPV measurement in triplicate.

For the determination of •OH (Fenton’s reaction): Sesamol (final concentration, 1 mmol/L) and each concentration of H$_2$O$_2$ and FeSO$_4$ (final concentration, 1.0–20 µmol/L) were dissolved in H$_2$O. To the reaction mixture, PBS powder was added to adjust pH and salt concentration for DPV measurement.

Results and Discussion

Differential pulse voltammetry for sesamol monomer and dimer

Since differential pulse voltammetry (DPV) provides narrow and sensitive redox peaks, it was useful for the simultaneous determination of catechol and hydroquinone of structurally similar isomers. In order to characterize the oxidative reaction of sesamol, standard samples of sesamol monomer and dimer were subjected to DPV ranging from −0.1 to 0.7 V. As shown in Fig. 2, sesamol monomer and dimer gave different oxidation peaks around 0.3 and 0.1 V, respectively, in PBS (pH 7.4). Although sesamol dimer is an oxidative product obtained from sesamol monomer, its oxidation potential was lower than that of sesamol monomer. Until now the electrochemical determination of sesamol (monomer) was already achieved, in this study, we found that their oxidation peaks were clearly detected and separated under the same conditions. Next, we examined the effects of pH on DPV for sesamol dimer bearing two -OH group. As a result, the peak was positively shifted around 0.35 V in acidic solution (pH 3) and was negatively
shifted around 0.0 V in alkaline solution (pH 10), but their peak heights were decreased (See Figs. 3 and S1 in the Supporting Information). The calibration curve for sesamol dimer was obtained over the concentration range of 1.7 to 67 µmol/L, as shown in Fig. 3. Thus, the optimum pH condition was set at pH 7.4 in the following experiment.

**Calibration curves for NO and •OH using sesamol by DPV**

As mentioned above, we successfully demonstrated that the peaks of sesamol monomer and dimer were separately detected by DPV. According to our previous reports,\(^4,5\) therefore, it was applied to the determination of NO and •OH. Figs. 4a and 5a show NO and •OH concentration-dependent DPV responses resulted from sesamol dimer, while there are saturation peaks around 300 mV from the excessive amounts of sesamol monomer (data not shown). It was suggested that sesamol was oxidized with NO and •OH released from NOC7 and Fenton’s reaction, respectively, to yield sesamol dimer. Based on the peak height of sesamol dimer, the calibration curve for NO using sesamol monomer and NOC7 was obtained over the concentration range of 4.0 to 200 µmol/L, exhibiting good linearity of correlation coefficients (\(r\)) of 0.97, as shown in Fig. 4b. The electrocatalytic responses were evaluated from the difference in oxidation peak current between the absence and presence of NOC7 (Δ\(I_p\)). In the same manner, the calibration curve for •OH was obtained by Fenton’s reaction. As a result, the concentration range was 1.0 to 15 µmol/L, exhibiting good linearity of correlation coefficients (\(r\)) of 0.99, as shown in Fig. 5b.

In conclusion, we developed the novel selectively electrochemical detection of sesamol dimer for the determination of NO and •OH by DPV. The current approach demonstrates a simple, sensitive and selective method to detect sesamol monomer and
dimer, which appears to be useful for precise ratiometric measurement of radicals. Further work regarding the addition of some functional molecules, surface modification of electrode and effects of biological samples such as human serum and cell extracts on electrochemical detection is ongoing.
References

1. M. Uchida, S. Nakajin, S. Toyoshima, and M. Shinoda, *Biol. Pharm. Bull.*, **1996**, *19*, 623.

2. K. P. Suja, A. Jayalekshmy, and C. Arumughan, *J. Agric. Food Chem.*, **2004**, *52*, 912.

3. R. Joshi, M. S. Kumar, K. Satyamoorthy, M. K. Unnikrisnan, and T. Mukherjee, *J. Agric. Food Chem.*, **2005**, *53*, 2696.

4. Y. Makino, S. Uchiyama, K. Ohno, and H. Arakawa, *Anal. Chem.*, **2010**, *82*, 1213.

5. S. Abe, S. Nakabayashi, J. Murayama, Y. Sano, *Luminescence*, **2010**, *25*, 456.

6. H. Arakawa, S. Nakabayashi, K. Ohno, and M. Maeda, *J. Pharm. Anal.*, **2012**, *2*, 156.

7. C. Walling, *Acc. Chem. Res.* **1975**, *8*, 125.

8. L. Chen, Y. Tang, K. Wang, C. Liu, and S. Luo, *Electrochem. Commun.*, **2011**, *13*, 133.

9. B. Aslışen, Ç. C. Koçak, and S. Koçak, *Anal. Lett.*, **2020**, *53*, 343.
Figure Captions

Fig. 1  Chemical reaction of sesamol with NO and •OH.

Fig. 2  Voltammograms of DPV for sesamol monomer and dimer.

Fig. 3  Calibration curves for sesamol dimer by DPV measurements.

Fig. 4  Typical voltammograms of DPV for reaction mixture of sesamol (1 mmol/L) and NOC7, (i) 0, (ii) 10, (iii) 20, (iv) 40, (v) 80, (vi) 100, (vii) 150, and (viii) 200 µmol/L (a) and calibration curve for NOC7 (b).

Fig. 5  Typical voltammograms of DPV for reaction mixture of sesamol (1 mmol/L) and H₂O₂, FeSO₄ (i) 0, (ii) 1, (iii) 2, (iv) 5, (v) 7.5, (vi) 10, (vii) 15, and (viii) 20 µmol/L (a) and calibration curve for H₂O₂, FeSO₄ (b).

Fig. S1  Voltammograms of DPV for sesamol dimer (100 µmol/L) at pH 3, 7.4 and 10.
Fig. 1
Fig. 2
Fig. 3
Fig. 4
Fig. 5