Electrochimica Acta 222 (2016) 580–586

Contents lists available at ScienceDirect

Electrochimica Acta

journal homepage: www.elsevier.com/locate/electacta

Research Paper

Rapid sensing of antioxidant capacity based on electrochemiluminescence induced by electrochemically generated reactive oxygen species

Naoki Nagatani[a], Yuki Inoue[b], Akiko Araki[b], Hiromi Ushijima[c], Gen Hattori[d], Yasuhiro Sakurai[d], Yutaka Ogidou[d], Masato Saito[b], Eiichi Tamiya[b,*,1]

[a] Department of Applied Chemistry, Graduate School of Engineering, Okayama University of Science, 1-1, Ridai-cho, Kita-ku, Okayama 700-0005, Japan
[b] Department of Applied Physics, Graduate School of Engineering, Osaka University, 2-1, Yamadaoka, Suita, Osaka 565-0871, Japan
[c] Biodevice Technology, Ltd., 2-13, Asahidai, Nomi, Ishikawa 923-1211, Japan
[d] Designer Foods Co., Ltd., 22–8 Chikusa, Chikusa-ku, Nagoya, Aichi 464-0858, Japan

ARTICLE INFO

Article history:
Received 27 September 2016
Received in revised form 1 November 2016
Accepted 4 November 2016
Available online 5 November 2016

Keywords:
electrochemiluminescence
antioxidant capacity
screen-printed electrode
ORAC
ESR
ROS
luminol

ABSTRACT

A simple and rapid electrochemiluminescence (ECL) method for the measurement of antioxidant capacity using a portable ECL device with a screen-printed electrode is described. The ECL method is based on the luminous reaction of reactive oxygen species (ROS), luminol radicals and antioxidants. ROS are generated by the electrochemical reduction of dissolved oxygen and competitively react with the luminol and antioxidants. The ECL intensity depends on the antioxidant capacity because the radicals are neutralized by the antioxidants, suppressing the luminous reaction. An antioxidant standard curve was generated using different concentrations of known amounts of Trolox by ECL measurement and the detection limit for antioxidant capacity was found to be 0.06 mM of Trolox. An antioxidant capacity of beverages (22 types) was evaluated by comparing with the standard curve of Trolox. The time necessary for the ECL measurement of antioxidant capacity is only two minutes after combining the sample solution with luminol on a screen-printed electrode.

1. Introduction

A number of epidemiological research studies have revealed a positive correlation between eating fruits or vegetables and the reduction of heart disorders, cancer and other degenerative pathologies [1–3]. Fruits and vegetables have a variety of antioxidants and a strong antioxidant capacity, and a large amount of scientific evidence shows that antioxidants reduce the incidence of cancer and other diseases [4–6]. Various antioxidants with different functions play a role in preserving human health and preventing and treating diseases in vivo [7].

Fluorometric or spectrometric methods such as oxygen radical absorbance capacity (ORAC) [8], the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay [9], the ferric ion reducing antioxidant power (FRAP) assay [10] and electron spin resonance (ESR) [11] are widely used in the measurement of antioxidant capacity [12]. These methods have been modified and improved [13–18] and a large number of new approaches to measure antioxidant capacity using nanoparticles, electrochemistry and cultured cells have been reported [19–32]. However, these modifications have resulted in standard measurement methods for antioxidant capacity that are not unified.

Chemiluminescence (CL) using luminol has also been used for the measurement of antioxidant capacity [33]. The principle of the measurement is based on the reaction of radicals with luminol to produce excited states for light emission. The antioxidants that react with radicals inhibit light production. ECL measurements generally provide advantages such as reaction control, higher sensitivity, lower noise and a rapid response. Furthermore, it is a simple method compared to standard CL methods. Luminol ECL dramatically increased by coupling electrochemical reduction and oxidation using linear sweep voltammetry [34]. ECL methods are

* Corresponding author.
E-mail addresses: nagatani@dac.ous.ac.jp (N. Nagatani), inoueyuki@ap.eng.osaka-u.ac.jp (Y. Inoue), araki-a@ap.eng.osaka-u.ac.jp (A. Araki), ushijima@biodevicetech.com (H. Ushijima), g-hattori@designerfoods.net (G. Hattori), y-sakurai@designerfoods.net (Y. Sakurai), y-ogidou@designerfoods.net (Y. Ogidou), saitomasato@ap.eng.osaka-u.ac.jp (M. Saito), tamiya@ap.eng.osaka-u.ac.jp (E. Tamiya).
[1] http://dolphin.ap.eng.osaka-u.ac.jp/nanobio/.

http://dx.doi.org/10.1016/j.electacta.2016.11.012
0013-4686/

directly linked with reactive oxygen species (ROS), which produce intermediate active species leading to luminescent reactions. There have been only a few ECL papers reported previously. The first report evaluated the total antioxidant capacity of grape skin with oligomeric proanthocyanidin as an index and used micro-emulsion enhanced ECL of luminol-H_2O_2, which required the addition of hydrogen peroxide for luminol-induced ECL [35]. Another report demonstrated the radical-scavenging properties of phenolic compounds against superoxide anion radicals ($O_2^{-}\cdot$) using the ECL of lucigenin [36].

In this study, we propose an ECL method for antioxidant capacity detection with luminol-based ECL linked with electrochemically generated ROS. It is a simple and rapid assay that does not require the addition of ROS generators such as hydrogen peroxide or xanthine-xanthine oxidase. Fig. 1 shows the reaction mechanism of ECL produced from luminol [37,38]. Luminol can be electrochemically oxidized to a luminol radical and diazaquinone. The $O_2^{-\bullet}$, hydroxyl radical and hydrogen peroxide (H_2O_2) can be also generated from dissolved oxygen which electrochemically reduced by the application of negative potentials as estimated by the following redox reactions [34,39–41].

$$O_2 + e^- \rightarrow O_2^{-*} \quad E_0: -0.37\,V \text{ (vs. Ag/AgCl)}$$

$$O_2 + H^+ + e^- \rightarrow HO_2^{*} \quad E_0: -0.32\,V \text{ (vs. Ag/AgCl)}$$

$$HO_2^{*} + HO_2^{*} \rightarrow H_2O_2 + O_2$$

$$O_2^{-*} + HO_2^{*} + H^+ \rightarrow H_2O_2 + O_2$$

The luminescent reaction was induced both by electrochemically generated luminol active species and ROS, which when trapped by antioxidants resulted inhibited the luminescent reaction. Trolox, which is often used to standardize antioxidant capacity in ORAC was used as a reference material in ECL method, and the antioxidant capacity of various beverages was compared using ECL and ORAC standard methods.

2. Experimental

2.1. Materials and reagents

Luminol, Trolox, caffeic acid, chlorogenic acid, ferulic acid, *p*-coumaric acid, 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), fluorescein, hypoxanthine, xanthine oxidase (XOD), superoxide dismutase (SOD) from bovine erythrocytes, riboflavin, L-histidine and other reagents were purchased from WAKO Pure Chemical Industries, Ltd. (Osaka, Japan). 5,5-Dimethyl-1-pyrroline-N-oxide (DMPO, Labotec Co., Ltd. (Tokyo, Japan)) was used as a spin trapping reagent without further purification. Dimethyl sulfoxide (DMSO) was obtained from Sigma-Aldrich. Twenty-two types of beverages were purchased from a retail store in Osaka, Japan. Specifically, 3 types of mixed vegetable and fruit juices, tomato juice, acerola drink, 3 types of fruit juices (apple, orange and grape), 5 types of Japanese tea (green tea), oolong tea, black tea, jasmine tea, 3 types of coffees, a cocoa drink, an isotonic drink and lemonade were used as samples for experiments. The juices and drinks were dispensed into micro tubes and stored at −20 °C until further use.

2.2. Instruments

A portable ECL device was used with BDTeCLP100 (Biodevice Technology, Ltd., Ishikawa, Japan), which comprises a photon detection unit (photomultiplier tube) and a USB-powered hand-held potentiostat that has a trigger circuit to send the trigger signal to the photo detection unit (Fig. S1, A, B). The hand-held potentiostat can perform the following four important types of electroanalytical techniques; cyclic voltammetry (CV), differential pulse voltammetry, square wave voltammetry and chronoamperometry. The portable ECL device can simultaneously record the ECL signals and electrochemistry (Fig. S1, C). A screen-printed electrode (DEP chip; Biodevice Technology, Ltd.) was used [42,43]. The DEP chip consisted of a carbon working electrode, carbon counter electrode and Ag/AgCl reference electrode. The working electrode area was 2.64 mm^2, and the total size including the connection port and the carbon barrier used to prevent the solution from flowing

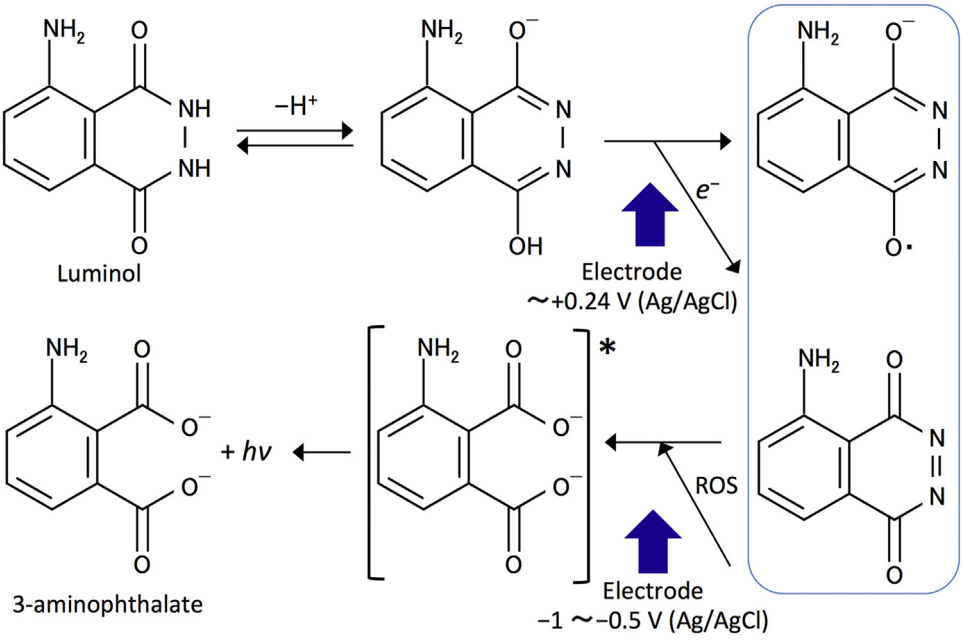

Fig. 1. Scheme of electrochemical luminescence of luminol linked with electro-generated ROS.