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Electrochemical detection of lipophilic antioxidants with high sensitivity at boron-doped diamond electrode

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ABSTRACT

The electrochemical detection of lipophilic antioxidants, α-tocopherol (vitamin E, VE) and ubiquinone (coenzyme Q_{10}, CoQ_{10}), in methanol and in methanol/hexane mixture solutions, respectively, at boron-doped diamond (BDD) electrodes was investigated by cyclic voltammetry (CV) and flow-injection electrochemical measurements. CV measurements revealed that the overpotential for electrochemical reaction of VE and CoQ_{10} was larger at an oxidized BDD electrode surface than at that of a hydrogenated BDD (H-BDD) electrode. The slope of the CV calibration curve used to determine the VE and CoQ_{10} concentrations was larger at a glassy carbon (GC) electrode than at the H-BDD electrode; however, the signal-to-background ratio was larger at the H-BDD electrode than at the GC electrode, due to the low background feature of the H-BDD electrode. The limit of detection (LOD), defined by a signal-to-noise ratio of three for flow-injection electrochemical detection, was much smaller at the H-BDD electrode (VE: 41 nM and CoQ_{10}: 17 nM) than at the GC electrode (VE: 263 nM and CoQ_{10}: 71 nM). These results confirm that the H-BDD electrode is reliable for the electrochemical detection of lipophilic antioxidants, especially at low concentrations.

1. Introduction

Lipophilic antioxidants (Fig. 1) such as α-tocopherol (vitamin E, VE), ubiquinone (coenzyme Q_{10}, CoQ_{10}), and carotenoids are essential micronutrients used to protect humans against diseases related to oxidative stress, including cardiovascular disease, cancer, cataracts, and age-related problems [1,2]. For example, VE can prevent living cell membranes from decomposing and turning by reacting with reactive oxygen species and lipid peroxide radicals [3]. The reduced form of CoQ_{10}, ubiquinol ($CoQ_{10}H_2$), coexists with CoQ_{10} and is the predominant form in tissues. Ubiquinol is also an important lipophilic antioxidant that is present in inner mitochondrial membranes, which can regenerate VE from its oxidative form (α-tocopheroxyl radical) [4–6]. The $CoQ_{10}/CoQ_{10}H_2$ system also plays an important role in electron and proton transport in the mitochondrial respiratory chain [6]. The reduced level of antioxidants in plasma has been reported to be associated with numerous chronic diseases; therefore, determination of these antioxidants in physiological fluids should be an important subject in analytical chemistry. Besides clinical applications, reliable determination of these antioxidant concentrations in foods, cosmetics, dietary supplements could be beneficial in related industrial fields [7]. VE and CoQ_{10} are electroactive compounds that can be analyzed by electrochemical techniques. The phenolic group of VE can be oxidized at conventional glassy carbon (GC) electrodes [8], and the voltammetric oxidation peak can be used for the determination. Modified electrodes, e.g. polypyrrole-modified platinum electrode [1], carbon nanotube/DNA paste electrode [9] and multi-walled carbon nanotube-modified graphite electrode [10], as well as platinum microelectode [11] were also useful for sensitive electrochemical detection of VE. The $CoQ_{10}/CoQ_{10}H_2$ redox system is based on the redox reaction of the quinone group, showing a well-defined redox peak pair at moderate potential region in cyclic voltammetry (CV). Michalkiewicz demonstrated the determination of CoQ_{10} in acetic acid/acetonitrile solution from the cathodic peak current of differential pulse voltammetry (DPV) curves with a linearity range of 0.12–1.0 mM and a limit of detection (LOD) of 0.014 mM [6]. More practically, high performance liquid chromatography with an electrochemical detector (HPLC-EC) system should be useful for determination of VE and CoQ_{10} with high sensitivity and selectively [7,12–17]. If the analytical accuracy for such compounds,

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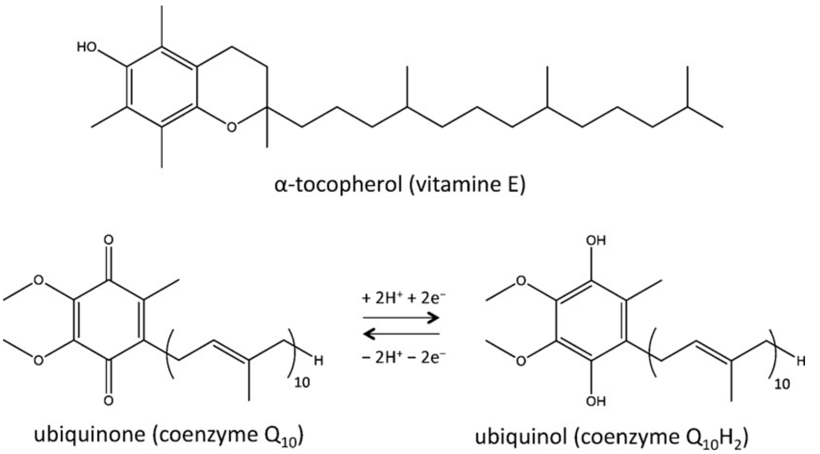

Fig. 1. Chemical structure of α-tocopherol, ubiquinone and ubiquinol. Scheme of ubiquinone/ubiquinol redox couple is also illustrated.

such as the $CoQ_{10}/CoQ_{10}H_2$ ratio measured with a HPLC-EC system, could be improved, then it would also be useful for research into the relationship between oxidative stress and initial diagnoses [14,15,17,18].

In the present study, the electrochemical detection of VE and CoQ_{10} lipophilic antioxidants at boron-doped diamond (BDD) electrodes was investigated. BDD electrodes exhibit a wide potential window and low background current, and therefore have potential application to highly sensitive electroanalysis [19–27]. Although there are many reports on electroanalysis at BDD electrodes in aqueous media, there have been few reports for non-aqueous media, which should be also important to be developed. Radovan [28] and Martins [29] reported on sensitive detection of parabens in hydro-alcoholic and water–acetonitrile solutions, respectively. Medeiros reported on voltammetric detection of phenolic antioxidants in aqueous-ethanolic solutions [30]. Zhang investigated sensitive electrochemical detection of 3,6-dihydroxyphenanthrene in methanol solution, based on the lower background current at BDD electrode than at GC electrode [31]. We performed CV of VE and CoQ_{10} at hydrogenated and oxidized BDD electrodes (H-BDD and O-BDD) to investigate the electrochemical reaction properties. Furthermore, the BDD electrodes were applied to a flow-injection electrochemical measurement to investigate the determination of the lipophilic antioxidants at HPLC-EC systems.

2. Experimental

BDD electrodes were obtained by deposition of a polycrystalline BDD thin film on a silicon wafer substrate using the microwave plasma-assisted chemical vapor deposition (MPCVD) method. The detailed conditions have been described in our previous report [32]. Hydrogen plasma treatment using MPCVD apparatus with a microwave power of 500 W and a total pressure of 25 Torr at 800 °C for 5 min allowed hydrogenation of the BDD electrode surface. Surface oxidation of the BDD electrode was performed with a UV/ozone photo surface processor (UVE-110-1H, Sen Lights Corporation) equipped with a low-pressure mercury lamp (185 and 254 nm). The lamp to sample distance was 80 mm. The O/C atomic ratio of the modified BDD surface was estimated using X-ray photoelectron spectroscopy (XPS, Axis Nova, Kratos).

α-Tocopherol (or vitamin E, VE) and ubiquinone (or coenzyme Q_{10}, CoQ_{10}) were purchased from Wako Pure Chemical Industries and were used as received. For CV, 50 mM of sodium perchlorate in methanol was used as an electrolyte solution for VE. On the other hand, we used a mixture of 2 mL sulfuric acid (98%)/230 mL methanol/70 mL hexane for CoQ_{10} because hexane can dissolve CoQ_{10} well. CV was performed using a three-electrode electrochemical cell with platinum wire and Ag/Ag^+ (BAS) as counter and reference electrodes, respectively, and a potentiostat (HZ-5000, Hokuto Denko). The working electrode area was regulated by a Viton O-ring to be 0.07 cm^2 (3 mm in diameter).

Flow-injection electrochemical measurements were conducted using a liquid pump (PU-712, GL Science) and an electrochemical detector (ED-703 pulse, GL Science) installed with GC or BDD electrodes. A Ag/AgCl electode was employed for the reference electrode. The working electrode area was set to 0.15 cm^2 using a silicone rubber gasket. The mobile phases for VE and CoQ_{10} were 50 mM sodium perchlorate in methanol and 50 mM sodium perchlorate in methanol/hexane (76.7/23.3, v/v), respectively. We chose sodium perchlorate as a neutral electrolyte to minimize any damage to the instruments. A 10 μL aliquot of the electrolyte containing VE or CoQ_{10} was injected for the measurement. For the flow-injection electrochemical measurement of CoQ_{10}, a quinone-derivative reduction column (No. 21211, Shiseido), where CoQ_{10} is reduced to $CoQ_{10}H_2$, was connected just before the electrochemical detector, and the oxidation current of $CoQ_{10}H_2$ was detected. The flow rate of the mobile phase was 1 mL min^{-1}.

3. Results and discussion

3.1. Surface modification of BDD

Surface oxidation of the BDD electrode by UV/ozone treatment was investigated. Fig. 2a and b shows XPS spectra of the H-BDD surface and that treated with UV/ozone for 60 min, respectively. The intensity of the O 1s peak (530 eV) in the H-BDD spectrum was rather small, whereas that for the treated surface was significantly increased. The O/C atomic ratios estimated by XPS quantitative analysis were 0.014 and 0.12 for the H-BDD and UV/ozone-treated BDD electrodes, respectively. The O/C ratio is plotted as a function of UV/ozone treatment time in Fig. 2c. The O/C ratio increased significantly within several minutes and became almost saturated at 30 min or longer, which is similar to that previously reported [33]. The results indicate that the surface oxygen content can be controlled according to the UV/ozone treatment time. The O/C ratio became almost saturated by treatment for 30 min or longer; therefore, BDD samples treated by UV/ozone for 30 min were used as oxidized BDD electrodes (O-BDD) unless otherwise specified. The contact angles of water droplets on the H- and O-BDD surfaces were