Talanta 156-157 (2016) 112–118

Contents lists available at ScienceDirect

Talanta

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

A single use electrochemical sensor based on biomimetic nanoceria for the detection of wine antioxidants

Veronica Andrei [a], Erica Sharpe [b], Alina Vasilescu [a,*], Silvana Andreescu [b,**]

[a] International Centre of Biodynamics, 1B Intrarea Portocalelor, 060101 Bucharest, Romania
[b] Clarkson University, Department of Chemistry and Biomolecular Science, Potsdam, NY 13699-5810, United States

ARTICLE INFO

Article history:
Received 26 February 2016
Received in revised form
26 April 2016
Accepted 27 April 2016
Available online 7 May 2016

Keywords:
Enzyme mimetic
Nanoceria
Oxidizible phenolics
Wine antioxidants
Disposable sensors
Amperometry

ABSTRACT

We report the development and characterization of a disposable single use electrochemical sensor based on the oxidase-like activity of nanoceria particles for the detection of phenolic antioxidants. The use of nanoceria in the sensor design enables oxidation of phenolic compounds, particularly those with ortho-dihydroxybenzene functionality, to their corresponding quinones at the surface of a screen printed carbon electrode. Detection is carried out by electrochemical reduction of the resulting quinone at a low applied potential of −0.1 V vs the Ag/AgCl electrode. The sensor was optimized and characterized with respect to particle loading, applied potential, response time, detection limit, linear concentration range and sensitivity. The method enabled rapid detection of common phenolic antioxidants including caffeic acid, gallic acid and quercetin in the μM concentration range, and demonstrated good functionality for the analysis of antioxidant content in several wine samples. The intrinsic oxidase-like activity of nanoceria shows promise as a robust tool for sensitive and cost effective analysis of antioxidants using electrochemical detection.

1. Introduction

Cerium oxide nanoparticles (CeO_2 NPs) or nanoceria particles have received significant attention due to their unique catalytic and free radical scavenging properties [1] which make them attractive for a variety of applications in biology, medicine, environment and catalysis. The useful properties of nanoceria are a consequence of their dual reversible oxidation states of cerium Ce^{3+}/Ce^{4+} onto the NP surface, which allows them to act as catalysts and to mimic the activity of oxidase and peroxidase enzymes such as superoxide dismutase [2] and catalase [3]. Polymer coated nanoceria were found to have an intrinsic oxidase-like activity [4]. As compared to systems based on biocatalytic enzymes, CeO_2 NPs are robust, inexpensive and not susceptible to denaturation and temperature variations. Their redox properties and inherent stability provide attractive opportunities for development of new analytical devices with increased performance for field analysis [5].

Several types of inorganic NPs have been studied for their enzyme mimetic properties including Fe_3O_4 NPs [6–8], Co_3O_4 [9] and CuO [10] among others [11]. Ferromagnetic iron oxide NPs have been explored as materials in colorimetric assays to detect H_2O_2 and glucose [7,12]. Previously, these particles have been used in conjunction with biomolecules (e.g. redox proteins, enzymes, antibodies) to enhance bio-stability [13,14], facilitate electron transfer [15], enhance biocatalytic signals [16] and provide an oxygen buffering capacity for oxidase enzymes [17]. We have studied the catalytic activity of nanoceria towards H_2O_2 and have demonstrated the use of these particles as redox active material for the electrochemical [18] and colorimetric [19] detection of H_2O_2 and glucose. Nanoceria has been used as a colorimetric indicator for antioxidants activity taking advantage of color changes induced by modifications in NP surface properties on a paper platform [20,21]. In a recent study, we have demonstrated superior oxidase-like activity of nanoceria against catechol and dopamine as compared to tyrosinase when used in a colorimetric assay [22], and the use of these particles as catalytic amplifiers [23] and labels in bioaffinity assays [24].

Here, we propose a disposable electrochemical sensor with immobilized nanoceria on screen printed electrodes (SPE) for analyzing phenolic antioxidants. These compounds have received considerable attention due to their ability to scavenge free radicals and protect against oxidative stress thus providing health benefits [25,26]. A variety of antioxidant sensors with electrochemical detection have been reported [27–29]. A preferred sensing design is to utilize tyrosinase [30,31] or laccase enzymes [32–34] to convert the phenolic compound into its quinone derivative that can then

* Corresponding author.
** Corresponding author.
E-mail addresses: avasilescu@biodyn.ro (A. Vasilescu), eandrees@clarkson.edu (S. Andreescu).

http://dx.doi.org/10.1016/j.talanta.2016.04.067
0039-9140/

be easily reduced and detected at a low applied potential, thus minimizing interferences. Here we describe a novel approach that utilizes inorganic biomimetic nanoceria with oxidase-like activity to replace laccase and tyrosinase enzymes in the construction of an electrochemical sensor for the detection of readily oxidizable phenolic compounds. The method is particularly applicable for analysis of phenolics with ortho-dihydroxybenzene functionality. The sensor was fabricated on a SPE platform, which facilitates development of inexpensive detection systems and portable instrumentation for food and environmental analysis [35,36]. We demonstrate that nanoceria can be successfully used as electrode material for the detection of oxidizible phenolics in wine samples in a single step procedure, providing enhanced stability and comparable characteristics to previously reported electrodes which utilize the enzyme counterpart. The biomimetic sensor shows great promise as a robust tool for sensitive, rapid and cost-effective analysis of antioxidants. The sensor can find a wide range of potential applications in food chemistry and biotechnology.

2. Materials and methods

2.1. Reagents and equipment

Cerium (IV) oxide NPs 20 wt% colloidal dispersion in 2.5% acetic acid with an average particle size of 10–20 nm (catalog number 289744) and sodium phosphate were from Sigma Aldrich. Gallic acid (GA) was purchased from Acros, quercetin (Q) from Alfa Aesar and caffeic acid (CA) from Spectrum Chemical. Potassium phosphate monobasic was supplied by Fisher Scientific. Sodium phosphate (dibasic, anhydrous) was purchased from J. T. Baker (Phillipsburg, NJ, USA). A solution of 20 mM potassium hexacyanoferrate (III) prepared in 0.2 M potassium chloride was used in cyclic voltammetric experiments. Stock solutions of 10 mM phenolic antioxidants were prepared daily before use: CA in ethanol, Q in acetone and GA in water. Working solutions were prepared in phosphate buffer (PBS) at pH 7.4. All studies were performed at a pH of 7.4, where the particles have the highest oxidase-like activity against phenolic compounds [22].

Experiments using cyclic voltammetry (CV) were performed with an Epsilon potentiostat (BASi, West Lafayette, IN, USA). Amperometric experiments were carried out with a portable electrochemical analyzer from Dropsens (Dropsens μ8400 Potentiostat, Dropsens, Spain, equipped with 8 channels). All experiments were carried out using a SPE three electrode system from Dropsens (Dropsens DRP-C110) with the nanoceria modified carbon electrode as the working electrode. Fourier Transform Infrared Spectroscopy (FTIR) spectra were recorded using a Mattson Galaxy 2020 spectrometer. FTIR samples included bare nanoceria particles and nanoceria exposed to CA, dried for minimum 48 h, mixed with KBr powder (~5% sample in KBr) and pressed into a pallet. FTIR spectra were recorded immediately. High resolution transmission electron microscopy (HR-TEM) images were taken using a high-resolution JEOL 2010 transmission electron microscope.

2.2. Procedures

To fabricate the nanoceria functionalized electrode a colloidal NPs suspension of 2% (w/v) nanoceria was prepared by dispersing particles in distilled water. 3 μL of this mixture was casted onto the working electrode surface of the SPE and allowed to dry for two days at room temperature until use. Electrodes were stored at room temperature with no further treatment. CV measurements were carried out in phosphate buffer solution at pH 7.4 in the potential range from −0.4 to 0 V vs Ag/AgCl, at a scan rate of 100 mV s^{-1}. Amperometric experiments to obtain calibration curves were carried out at a constant applied potential of −0.1 V vs Ag/AgCl reference electrode, with the nanoceria sensor placed vertically in a 10 mL electrochemical cell under constant magnetic stirring at 500 rpm. All experiments were performed at room temperature and electrodes were used for single analysis, i.e. used for the analysis of one sample, after which they were discarded. The variation in current intensity after addition of antioxidants/wine samples in the cell was measured upon signal stabilization, when the variation in current was not more than 2 nA/min. The data reported represent average measurements of n=3 independent sensors fabricated using the same procedure.

2.3. Analysis of real samples

Two varieties of red wine (Negru Aromat and Feteasca Neagra) from Valea Calugareasca vineyard in Romania from the 2012 and 2013 harvest were tested for their antioxidant content with this method. These wines correspond to various maceration techniques as follows: classical maceration - V1, maceration with partial running-off of the juice –V2, maceration with use of pectolytic enzyme-V3 and maceration with addition of exogeneous tannin-V4. The wine samples were used without any pre-treatment and were added directly in the electrochemical cell containing PBS buffer at pH 7.4. The antioxidant potential of wine samples was explored in parallel by the nanoceria-modified electrodes and the TEAC assay for total antioxidant capacity. The antioxidant activity of the wine samples measured with the nanoceria sensors was expressed as gallic acid equivalents (GAE), obtained by interpolation of the determined amperometric currents of the sample into the calibration curve for GA. The TEAC assay was carried out as described in literature [35]. In short, a 7×10^{-3} M ABTS solution prepared in 2.5×10^{-3} M potassium persulfate was kept in the dark for 12–16 h at room temperature to generate ABTS radicals. This solution was then diluted 400 times with water. The red wines were diluted 100 times with water. Next, 100 μL of either diluted sample, Trolox or ethanol were mixed with 2.5 mL of ABTS radical solution and 0.4 mL H_2O and the absorbance was measured after 3 min at 731 nm. The results were expressed as Trolox equivalents. The measurements are expressed as mean of 3 replicate determinations.

3. Results and discussion

3.1. Electrochemical study of nanoceria functionalized sensors

The nanoceria-modified electrodes with immobilized particles were first characterized by CV to test their behavior for the oxidation of a common antioxidant, GA (Fig. 1A). To avoid any possible electrochemical oxidation process of GA, the nanoceria-induced oxidation of GA was tested in the potential range between −0.4 and 0 V. The CVs of bare SPE carried out in PBS (pH 7.5) in the absence and in the presence of 0.5 mM GA showed a similar profile with no oxidation/reduction peaks. The CV of nanoceria-modified SPE obtained in absence of GA showed no peaks in PBS indicating lack of electrochemical activity of these particles in the potential range tested (Fig. 1 – blue). When the CV was recorded in the presence of 0.5 mM GA, a reduction wave was observed at −0.11 V. This behavior is consistent with a fast, almost instantaneous GA oxidation by nanoceria resulting in formation of a quinoic compound that is then reduced electrochemically in the negative potential range starting from around −0.05 V vs the Ag/AgCl reference electrode.

The reduction current was proportional with the amount of GA present in solution as illustrated in Fig. 2A. By increasing GA concentration from 0.05 to 0.5 mM, the reduction peak potential