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DNA-based sensor against nitrite oxide radical: Evaluation of total antioxidant capacity in beverages

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ABSTRACT

Reactive nitrogen and oxygen species are produced in cells and play an important role in the oxidative metabolism; when in abnormal concentrations, they are able to induce oxidative damage on biomolecules, namely in DNA. In this paper it is described, an electrochemical DNA-based sensor against NO• radical developed for total antioxidant capacity (TAC) evaluation. The sensor consisted on dA_{20} (adenine-rich oligonucleotide) physically adsorbed into carbon paste electrode (CPE). When this dA_{20}-CPE was damaged, by immersion in a freshly generated NO• radical, a protective effect onto dA_{20} was observed in the presence of antioxidants (ascorbic, gallic, caffeic, *p*-coumaric acids). Electrochemical studies were performed through square wave voltammetry. The construction of the sensor is simple, fast and the results indicated that the DNA-based sensor is suitable, accurate, and can be used to the assessment of TAC in commercial samples of juices.

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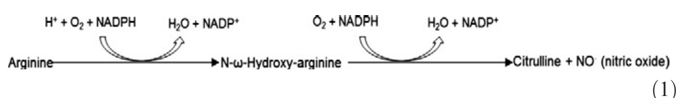
1. Introduction

Oxidant compounds (radicals and non-radicals), which may be generated as a consequence of normal aerobic metabolism, are able to induce damage to cells by reacting with biomolecules (proteins, lipids, among others), thus causing serious DNA injuries [1].

The nitric oxide or nitrogen monoxide radical (NO•) is a short-life hydrophobic endogenously synthesized free radical molecule, whose generation is mediated by multiple oxidative stress mechanisms responsible for changes in the cells and tissues [2]. In living organisms, NO• is produced by the oxidation of one of the terminal guanido nitrogen atoms of L-arginine. This reaction is catalyzed by the enzyme nitric oxide synthase (NOS), often stimulated by intracellular calcium, which catalyzes the conversion of L-arginine to nitric oxide and L-citrulline, in a two-step NADPH-dependent monoxygenation process. In the first step, NOS converts L-arginine into Nω-hydroxy-L-arginine (NHA), and in the second step, NOS converts NHA to L-citrulline and nitric oxide (Eq. (1)) [3].



(1)

NO• radical is of utmost importance, since it was identified as transcellular messenger in many physiological and pathophysiological processes [4]. However, this radical also contributes to pathologies as Parkinson and Alzheimer diseases [5]. In fact, according to Kohen and Nyska [6], NO• and its derivatives are powerful oxidizing agents that might cause depletion of sulfhydryl groups in biomolecules, and can also cause DNA damage, protein oxidation, and nitration of aromatic amino acid residues in proteins.

Since nature has developed complex antioxidant systems to counteract and prevent the deleterious effect of oxidants, thus minimizing the oxidative stress in living beings [1], the usual approach to determine the effect of a specific radical into a system consists in the measurement of its effects in the system in the presence or absence of antioxidant compounds.

Several electrochemical DNA-based biosensors have been developed in order to assess the total antioxidant capacity (TAC) in biological and food samples [7–12]. The principle behind these biosensors is close to the antioxidant activity in biological systems [1]. Briefly, these approaches are based on immobilization of DNA-based materials onto active electrode surfaces, by adsorption [13] or electrodeposition [14], which are then exposed to oxidant (radicals or non-radicals) and antioxidant compounds, similarly to what occurs within the cell [15,16]. Actually, the electrochemical oxidation current of DNA nucleobases (guanine or adenine) dramatically decreases in the presence of oxidant compounds when comparing with their native electroactivity. Nevertheless, when an antioxidant is added to the radical-containing solution, the current is partially recovered, which is attributed to the scavenging activity of these compounds [1].

An indirect eletrocatalytic method using DNA-modified sensors was also reported, in this strategy the oxidation of adenine

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homopolynucleotides by radicals led to the formation of a common oxidized product that catalyzed the oxidation of NADH. Therefore, the oxidative lesions generated after immersion of the DNA modified carbon paste electrode (DNA-CPE) in free radicals can be indirectly quantified after the electrochemical oxidation of the adenines that remained unoxidized on the electrode surface [17].

The ability of antioxidants (namely ascorbic acid, polyphenols) to scavenge or inactivate free radicals and protect DNA from oxidative damage has also been performed [18,19]. Polyphenols are widely distributed in plants (vegetables and medicinal herbs) and fruits and they usually have the possibility to scavenge reactive oxygen or nitrogen radicals [20].

The effect of some free radicals on the induction of oxidative damage in DNA-based sensors has already been studied, namely the hydroxyl radical generated by UV irradiation [21,22] or the Fenton reaction [23, 24], and the sulfate and superoxide radicals generated by chemical and enzymatic reactions, respectively [24,25]. However, considering that the antioxidant scavenging efficiency against free radicals is strongly dependent on the source of the free radical, the development of different analytical methodologies based on several sources of free radicals is required for the complete knowledge of the antioxidant profile. For instance, it would be interesting to study the effect of reactive nitrogen species (NO•) or hypochlorous acid on DNA-based biosensors [1].

As far as we know, there are no electrochemical studies concerning the effect of NO• radicals into DNA layers. In this work, NO• used was obtained by chemical synthesis, because it seems to have no differences compared to the NO• obtained by organic or biological synthesis [2].

This work describes, for the first time, the construction and optimization of a DNA-based sensor designed to measure the oxidative damage induced by chemically generated NO• radicals. The DNA-based biosensor consisted on adsorptive immobilization of a deoxyadenylic acid oligonucleotide (dA_{20}) onto CPE surface. Several antioxidants such as ascorbic (AA), gallic (GA), caffeic (CafA) and coumaric (CouA) acids were used to evaluate the protective effect against NO• radicals into the DNA-based sensor. Electrochemical measurements were carried out by square wave voltammetry (SWV).

After optimization of all analytical features, the developed system was used to quantify TAC of beverages, and the results were compared with those obtained using conventional techniques, namely, total phenolic content (TPC), oxygen radical absorbance capacity (ORAC), ferric reducing antioxidant power (FRAP), and DPPH-radical scavenging assay (DPPH-RSA).

2. Material and methods

2.1. Chemicals and solutions

Deoxyadenylic acid oligonucleotide (dA_{20}, as a desalted product), Folin Ciocalteu reagent, 1,1-Diphenyl-2-picrylhydrazyl radical, 2,2-Diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH), sodium fluorescein, 2,2′-Azobis (2-amidinopropane) dihydrochloride (AAPH), concentrated saline sodium phosphate EDTA (20× SSPE; 0.2 mol l^{-1} sodium phosphate, 2 mol l^{-1} NaCl, 0.02 mol l^{-1} EDTA), paraffin oil, phosphate buffer (PBS) pH 7,4 were acquired from Sigma-Aldrich. GA, CouA, CafA, AA, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), sodium nitrite and sulfuric acid were from Fluka. Graphite powder was from Ultracarbon (Dicoex, Spain) and the remaining reagents were obtained from Merck (Darmstadt, Germany).

Stock solutions of 1 g l^{-1} dA_{20} were stored at 4 °C and diluted with 2× SSPE buffer solution (prepared by dilution of 20× SSPE solution) prior to use. The NO• radical solution was daily prepared according to the procedure of Wang and Hu [4]. Before the NO• generation, the apparatus was degassed with nitrogen (N_2) for 2 h to exclude O_2, (to avoid the NO• destruction by the O_2). Briefly, NO• radical gas was generated by slowly dropping of 6 mol l^{-1} H_2SO_4 into a glass flask containing saturated $NaNO_2$ solution). The gas generated was forced to pass through a 30% of NaOH solution and then, as current only pure NO, bubbles in the solution that we intend to saturate in NO•. NO• saturated solution was obtained by bubbling NO pure gas through deoxygenated ultrapure water during 1 h and kept under NO atmosphere until use. NO• standard solutions were prepared (fresh and kept in a glass flask with a rubber septum in a dark place) by making serial dilutions of the saturated NO solutions [4].

2.2. Instrumentation

SWV was performed with an Autolab PSTAT 10 controlled by GPES software, version 4.8 (EcoChemie, The Netherlands). A conventional three electrode cell was used, which includes a home-made CPE (3 mm in diameter) as a working electrode, a platinum wire counter electrode and a Ag|AgCl|KClsat reference electrode to which all potentials are referred. The CPE was prepared by mixing 1.8 g of paraffin oil as pasting liquid with 5 g of spectroscopic grade graphite powder. The unmodified carbon paste was introduced into the well of a teflon electrode body provided by a stainless steel piston. The surface was smoothed against a plain white paper while a slight manual pressure was applied to the piston. The CP was discarded and a new electrode surface was freshly prepared for each analysis.

2.3. Voltammetric procedure

Experiments were structured in three steps: DNA layer preparation, damage of oligonucleotide (dA_{20}) by immersion of the dA_{20}-CPE on a NO• radical solution in the absence/presence of antioxidants or real samples, and detection and measurement of the peak current of dA_{20} in a PBS at pH 7.4.

DNA immobilization was performed by dry adsorption placing a 4 μl droplet of dA_{20} (100 mg l^{-1}) in 2× SSPE solution on the electrode surface and evaporating it to dryness under a stream of nitrogen.

DNA damage was carried out by immersing a new prepared DNA-based CPE in a freshly prepared NO• solution in the absence or the presence of antioxidant (standard) or beverage samples in 2× SSPE buffer.

After a fixed period of 30 s of reaction time, the DNA-based CPE was washed with water and immediately immersed in PBS pH 7.4. SWV (frequency = 25 Hz; step potential = 5 mV and amplitude = 20 mV) was then conducted between +0.7 and +1.5 V and the oxidation peak current of dA_{20} obtained was used as a detection signal. For the electrochemical studies it was considered that the maximum signal current obtained was for the dA_{20} electrochemical signal without damage neither antioxidant effect. Each analysis was performed at least in triplicate.

2.4. Evaluation of antioxidant capacity in commercial beverages

2.4.1. Samples, reagents and equipment

Four commercial orange-based beverages (one flavored water with fruit juices and three fruit soft drinks) from different brands were collected in a supermarket, filtered and stored in the dark at 4 °C until use. Their composition is depicted in Table 1.

Measurement of TAC of beverage samples by using the sensor was performed by immersion the DNA-based sensor for 30 s on a vial containing a certain volume of filtered beverage, NO• solution (1.8×10^{-6} mol l^{-1}) and SSPE buffer. After that, A SWV scan was carried out between +0.7 and +1.5 V in PBS at pH 7.4. Each beverage sample was measured in triplicate.

Standards and remaining reagents were all from Sigma Aldrich (Madrid, Spain). The chosen methods for antioxidant capacity measurements (spectrophotometric) were all performed in a Synergy HT W/TRF Multi Mode Microplate Reader (BioTek Instruments, USA).

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