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Electrochemical detection of interaction between capsaicin and nucleic acids in comparison to agarose gel electrophoresis

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ABSTRACT

In this study, the biomolecular interaction occurred between nucleic acids and Capsaicin (CPS), the active compound in chilli peppers, which has been reported to have anti-carcinogenic properties, was investigated for the first time herein using disposable electrochemical biosensor. It is aimed to perform the surface-confined interaction between CPS and different types of nucleic acids and under this aim, the experimental conditions were optimized; such as, the concentration of CPS and DNA, DNA immobilization time and interaction time etc. The detection limit of DNA was estimated based on guanine oxidation signal in the linear concentration range of DNA from 1 to 5 μg/mL, and it was found to be 0.62 μg/mL. The effect of time-dependent manner from 1 min to 30 min on the interaction of CPS with nucleic acids was explored upon to the changes at guanine signal coming from double stranded DNA and cDNA as well as PCR samples. The interaction of CPS with double stranded DNA was also determined by agarose gel electrophoresis.

Introduction

There has been an increasing interest in electrochemical devices for DNA biosensing since they allow a lot of advantages like simple, fast, and sensitive analysis by using inexpensive equipment. Electrochemical DNA biosensensor technologies represent another important subject which is the development of fast and accurate methods of DNA damage detection, especially caused by anticancer drugs or hazardous compounds. And also the ways in which drugs interact with DNA, with the goal of understanding the toxic as well as chemotherapeutic effects of many molecules. The interaction of matters with DNA is important topic for studies in drug discovery and pharmaceutical development processes. The mostly known intercalation and groove binding are the two most common modes by which small molecules bind directly and selectively to double-stranded DNA (dsDNA) [1,2]. The binding interactions, which are the topic of several electrochemical studies, result in structural change of both DNA and matter or drug molecules to accommodate complex formation [3–6].

The observation of the pre and post electrochemical signals of DNA or matter interaction provides good evidence for the interaction mechanism to be elucidated. Intercalation, which is an enthalpically driven process, results from deinsertion of a planar aromatic ring system between dsDNA base pairs with accompanying unwinding and lengthening of the DNA helix [7–11].

CPS which is a homovanillic acid derivative (8-methyl-N-vanillyl-6-nonenamide) that has been used in food additives and various pharmaceutical products. Thus, there is a need for the development of conventional methods for monitoring CPS and its interaction with (bio) molecules [12–27].

Under various names such as chilli pepper, red pepper, paprika etc. has a great deal of attention as a chemo-preventive agent against cancer [12]. In this regard, there are many studies that show the anticancer activity of CPS. The effects of CPS are studied in different cancer cell types such as leukemia cells [13], multiple myeloma cells [14], cutaneous cell carcinoma [15], tongue cancer

Abbreviations used: CPS, Capsaicin; cDNA, complemetary DNA; dsDNA, Double stranded DNA; PCR, Polymerase chain reaction; PBS, Phosphate buffer saline; ABS, Acetate buffer solution; DPV, Differential pulse voltammetry; PGE, Pencil graphite electrode; FLR-GCE, Fullerene modified glassy carbon electrode; DL, Detection limit.

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cells [16], gastric cancer cells [17], pancreatic cancer cells [18], small cell lung cancer [19], breast cancer cells [20] and prostate cancer cells [21].

Genotoxicity of CPS is another interesting research topic. Toth et al., in 1984, showed the genotoxicity of CPS in a study. CPS was found to be mutagenic in Salmonella typhimurium strain TA 98 in the presence of Aroclor induced rat liver S9 fraction [22], whereas another study on which S9 from phenobarbital-induced rats were used for metabolic activation was negative [28]. And also Richeux et al., in 1999, showed results of the comet assay and DNA fragmentation assay that CPS was able to induce DNA damage in human neuroblastoma cells. According to their results, DNA damage is increased with the concentration of capsaicin, thus indicating that CPS is able to induce DNA single strand breaks [29].

Qais et al. [30] investigated the spectroscopic detection of interaction between CPS and calf thymus DNA using UV-vis absorbance spectra and fluorescence spectra that indicates the formation of complex between CPS and DNA. It was concluded that the interaction mechanism of CPS with calf thymus DNA would be reported as non-intercalative groove binding.

To understand the interactions between capsaicin and DNA, one of the preferred technique in recent years is electrochemical biosensors. Principle of operation of these type biosensors are basically, devices that integrates an nucleic acid as the biological recognition element and an electrode as the electrochemical signal transducer [31]. More generally, chemical sensors are devices which transform (bio)chemical stimulus from an analyte in relation into analytically useful information. As we mentioned before this type measurements detect the analytes more rapidly, sensitively and selectively [31–33]. The recent studies present increasing advantages of such sensors day by day. There are several publications about the easy use and low cost of electrochemical biosensors [34–37]. For future development in chip technologies, electrochemical biosensors are frequently preferred because of the easy manipulation and application possibilities [38,39].

The aim of our study is voltammetric detection of the interaction between CPS and dsDNA, cDNA from total RNA isolated from Huh7 human hepatocellular carcinoma cell line and PCR samples. The surface confined interaction of CPS with different type of nucleic acids was investigated by disposable pencil graphite electrode (PGE) in combination with differential pulse voltammetry (DPV) for the first time in the literature. Firstly, the experimental conditions, such as; CPS and DNA concentration, DNA immobilization time onto the surface of PGE and interaction time were optimized in order to find the optimum analytical performance upon electrochemical detection of the interaction occurred between CPS and DNA. The interaction process was evaluated based on the changes at oxidation signals of CPS and guanine oxidation signal of dsDNA in a time-dependent manner as well as the study performed on cDNA and PCR samples while measuring the changes at guanine signal. The electrophoretic studies were also performed in our study to confirm the interaction of CPS with PCR samples.

Experimental

Apparatus

DPV measurements were carried by using AUTO-LAB PGSTAT 30 electrochemical analysis system supplied with GPES 4.9 software package (Eco Chemie, The Netherlands). All measurements were performed in the Faraday cage (Eco Chemie, The Netherlands). The three electrode system consisted of the PGE, an Ag/AgCl/3M KCl reference electrode (BAS, Model RE-5B, W. La- fayette, USA) and a platinum wire as the auxiliary electrode. As a holder for the graphite lead (Tombow 0.5 HB, Japan) a Rotring Pencil model (Germany) was used. Electrical contact with the lead was obtained by soldering a metallic wire to the metallic part. During each measurement, the pencil lead was held vertically with 10 mm of which was immersed into the solution.

Chemicals

CPS was purchased from Sigma-Aldrich (Gemany). Stock solution of CPS (1000 μg/mL) was prepared in ethanol and kept in dark. Diluted solutions were prepared in 0.05 M phosphate buffer saline containing 20 mM NaCl (PBS, pH 7.40). The calf thymus dsDNA was purchased as lyophilized powder from Sigma. The stock solutions of dsDNA were prepared as 1 mg/mL concentration with Tris–EDTA buffer solution (10 mM Tris–HCl, 1 mM EDTA, TE, pH 8.00) and kept frozen. The diluted solutions of dsDNA were prepared with 0.50 M acetate buffer solution containing 20 mM NaCl (ABS, pH 4.80).

All stock solutions of buffers were prepared with ultrapure water.

Other chemicals and equipments were obtained from Sigma-Aldrich (Germany) and Merck (Germany).

Preparation of cDNA and PCR samples

Total RNA was isolated from Huh7 human hepatocellular carcinoma cell line using High Pure RNA Isolation Kit (Roche Applied Science) according to manufacturer's instructions. RNA concentrations were determined by measuring the absorbance at 260 nm in a spectrophotometer (Nanovette, Beckman Coulter). cDNA was synthesized from total RNA using Transcriptor High Fidelity cDNA Synthesis Kit (Roche Applied Science). Polymerase chain reaction (PCR) was performed using Taq PCR kit (New England and Biolabs) and thermal cycler (Techne).

18S ribosomal 5 (RNA 18S5) spesific primer sequences used in PCR are 5′- CGA CGA CCC ATT CGA ACG TCT-3′ and 5′-G CTA TTG GAG CTG GAA TTA CCG-3′ to generate 312 bp product. GC content of PCR product is 54%. 18S rRNA plasmid cDNA (NR_003286.2) was obtained commercially from Thermo Scientific. The length and the GC content of cDNA used in this study are 1869 bp and 56%, respectively.

Procedure

The experimental procedure on electrochemical detection of biointeraction of CPS with nucleic acids (presented in Scheme 1) was comprised of three steps: (*i*) CPS immobilization and its detection at the surface of PGEs, (*ii*) the immobilization of the nucleic acids and its detection cycle, and (*iii*) electrochemical investigation of the surface confined interaction between CPS and nucleic acids (e.g, dsDNA, cDNA and PCR samples) at the surface of PGEs. All experiments were carried out at room temperature.

Preparation of the PGEs

PGEs were pretreated by applying a potential of +1.40 V for 30 s in ABS (pH 4.80). These pretreated electrodes were used for further experiments.

CPS immobilization on to the surface of PGEs and electrochemical detection of CPS

Each pretreated electrodes were dipped into 100 μL of required amount of CPS solution and kept during required time for immobilization. After rinsing immobilized electrodes with ABS (pH 4.80) for 5 s, measurements were performed.