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Electrochemical biosensor for the interaction of DNA with the alkylating agent 4,4′-dihydroxy chalcone based on guanine and adenine signals

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Abstract

The interaction of an alkylating agent, 4,4′-dihydroxy chalcone (DHC) with calf thymus double stranded DNA (dsDNA) and calf thymus single stranded DNA (ssDNA) was studied electrochemically based on the oxidation signals of guanine and adenine by using differential pulse voltammetry (DPV) at carbon paste electrode (CPE). As a result of the alkylation of DHC between the base pairs in dsDNA, the voltammetric signal of guanine and adenine greatly decreased. After the interaction of DHC with ssDNA, a higher decrease in the oxidation signals of guanine and adenine was observed under the same conditions. The partition coefficients of DHC at dsDNA and ssDNA modified CPEs were calculated. The interactions of DHC with synthetic polynucleotides, such as polyguanylic acid and polyadenylic acid were also observed. In addition, the detection limit and the reproducibility were determined by using DPV. The interaction of DHC with dsDNA in solution-phase was also investigated and the results were compared with the ones obtained by surface immobilized dsDNA. The application of electrochemical DNA biosensor for monitoring the DNA–alkylating agent interactions was explored.

Keywords: Electrochemical DNA biosensor; Guanine; Adenine; 4,4′-Dihydroxy chalcone; DNA

1. Introduction

DNA biosensor technologies are currently under intense investigation owing to their great promise for rapid and low-cost detection of specific DNA sequences in human, viral and bacterial nucleic acids [1–6]. The interaction of small molecules with DNA occurs through primarily in three modes: the electrostatic interactions with the negatively charged phosphate backbone, the binding interactions with the two grooves of DNA double helix and the intercalation between the stacked base pairs of double stranded DNA (dsDNA) [1].

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The major cytotoxic and mutagenic effects of the alkylating agents are believed to result from their interactions with DNA. The major mechanism of cytotoxicity is believed to occur as a consequence of a damage to DNA. These agents usually have other sites of action and often react with other cellular targets, such as membranes. Alkylating agents form positively charged carbonium ions, which react with nucleophyllic groups, such as SH, PO_4 and NH_3 on nucleic acids, proteins and smaller molecules. The N^7 of guanine is especially susceptible to alkylation of DNA, cellular damage may occur from single-strand breakage and cross-linking of DNA, thus interfering with cell division [7].

The interactions of some anticancer agents with DNA have been investigated by a variety of techniques [8–10] and there is a growing interest in the electrochemical methods for the determination of anticancer agents [11–14]. Electrochemical DNA hybridization biosensors are mostly employed for determining early and precise diagnosis of infectious agents in various environments [6] and these devices can be used for monitoring sequence-specific hybridization events directly [15–18] or by DNA intercalators (metal coordination complexes, organic dyes, etc.) [19–22]. These studies showed that electrochemical techniques offer a very attractive route for converting the hybridization event into an electrochemical signal.

Marrazza et al. [23] showed the use of daunomycin as an electrochemical hybridization indicator for detecting APO E poylmorphisms from DNA fragments amplified by PCR. Wang et al. [13] reported that the determination of interaction of daunomycin with DNA in the solution and at the surface of carbon paste electrode (CPE) could be observed by using a DNA biosensor. Erdem and Ozsoz [15] reported that epirubicin could be used as a promising hybridization indicator. Epirubicin was used for the detection of mismatched bases in 17-bases oligonucleotides. Hashimoto et al. [24] observed that the anodic peak potentials of daunomycin and doxorubicin shifted to more positive values in the case of the dsDNA modified basal plane pyrolytic graphite electrode.

Kamei et al. [25] studied chalcone or 1,3-diphenyl-2-propen-1-one, which is considered to be a precursor of all flavonoids, for its antitumor growth activity in vitro and in vivo. Their results suggested that the chalcone induced abnormal DNA synthesis and mitosis in the cultured cells. El-Subbagh et al. [26] synthesized a new series of α, β-unsaturated ketones and their corresponding fused pyridines. These compounds were screened for the in vitro antiviral and antitumor activities. In their report, the pyrano[3,2-c]pyridines heterocyclic system proved to be the most active antitumors among the investigated heterocycles. The growth inhibitory activity of several flavonoids against B16 mouse melanoma 4A5 cells was studied by Iwashita et al. [27]. In their report, isoliquiritigenin and butein, which belong to the chalcone group, markedly suppressed the growth of B16 melanoma cells and induced cell death. The condensation of nuclei and the fragmentation of nuclear DNA, which are typical phenomena of apoptosis, were observed by Hoechst 33258 staining and by agarose gel electrophoresis of DNA in cells treated with isoliquiritigenin or butein.

The antimicrobial, anticancer, diuretic, anticonvulsant and anesthesia activities of a variety of acyclic and cyclic α, β-saturated ketones and related Mannich bases such as 4,4′-dihydroxy chalcone (DHC) are well described [28–30]. The activities of these compounds were attributed, in part, to alkylating ability of olefinic groups conjugated with a carbonyl function to guanine bases in DNA [28–30]. The ability of these alkylating agents to attack guanine bases in DNA results in the cross-linking of the DNA. Thus, the voltammetric signals of the agent changes when it interacts with DNA. The changes monitored in the guanine and adenine signals indicate the behavior of electroactive and also non-electroactive agents towards DNA [31,32]. The voltammetry of mitoxantrone at DNA modified CPE was performed by Erdem and Ozsoz [33] and the electrochemical data supported the report of Mao et al. [34] about the piezoelectric impedance analysis of mitoxantrone at bare and DNA modified surfaces.

In this article the electrochemical interaction of DHC with dsDNA and single stranded DNA (ssDNA) at CPE surface based on the differences in the guanine and adenine oxidation signals is

presented. The electrochemical determination of the interaction of DHC with dsDNA and ssDNA by using differential pulse voltammetry (DPV) and cyclic voltammetry in connection with CPE based on the differences in the DHC signal had been reported [35]. The features of the method are discussed.

2. Experimental

Caution: DHC is a hazardous chemical and should be handled carefully in accordance with NIH guidelines.

2.1. Apparatus

The DPV measurements were done by using an AUTOLAB PGSTAT 30 electrochemical analysis system and GPES 4.8 software package (Eco Chemie, The Netherlands). The three electrode system, consisted of the CPE as the working electrode, the reference electrode (Ag/AgCl) and a platinum wire as the auxiliary electrode. The body of CPE was a glass tube (3 mm i.d.) tightly packed with the carbon paste. The electrical contact was provided by a copper wire inserted into the carbon paste. Carbon paste was prepared in the usual way by hand-mixing graphite powder (Fisher) and mineral oil (Acheson 38) in a 70:30 mass ratio. The surface was polished on a weighing paper to a smoothed finish before use. The convective transport was provided by a magnetic stirrer.

2.2. Chemicals

Double stranded calf thymus DNA (dsDNA, activated and lyophilized) and single stranded calf thymus DNA (ssDNA, activated and lyophilized) were purchased from Sigma. Polyguanylic acid (5′) (poly[G]) and polyadenylic acid (5′) (poly[A]) were also obtained from Sigma.

All dsDNA and ssDNA stock solutions (100 mg/l) and synthetic polynucleotide stock solutions (100 mg/l) were prepared with TE solution (10 mM Tris–HCl, 1 mM EDTA, pH 8.00) and kept frozen. More dilute solutions were prepared with either 0.50 M acetate buffer (pH 4.80) or 20 mM Tris–HCl buffer (pH 7.00), according to the hybridization protocol. Other chemicals were of analytical reagent grade. The in-house sterilized and deionized water was used in all solutions.

2.2.1. The synthesis of DHC

The synthesis of DHC was undertaken by Erciyas and Kucukoglu. This compound was prepared as follows by using a modification of a literature method [30]. 0.012 mole *p*-hydroxy benzaldehyde solution and 0.010 mole *p*-hydroxy acetophenon solution in ethanol were saturated with HCl. This reaction mixture was stirred at room temperature for 18–22 h. The reaction solvents were evaporated in vacuum. After having controlled that the reaction was over by using TLC controls, the residue was poured into 150 ml distilled water. Brown or yellow precipitates were obtained and recrystallized from distilled water. The standard solution stocks of DHC (1 mM) were prepared by dissolving in 0.05 M phosphate buffer solution (pH 7.40) and kept frozen. Dilute solutions were prepared with 0.05 M phosphate buffer solution (pH 7.40) before use. Other chemicals were of analytical reagent grade.

2.3. Procedure

Each measurement involved a freshly prepared CPE surface. All the experiments were performed at room temperature (25.0 ± 0.5 °C).

The electrochemical procedure for the detection of adenine, guanine and DHC oxidation signals from dsDNA and ssDNA involved two steps: DNA immobilization and voltammetric transduction.

2.3.1. DNA immobilization

The CPE was activated by applying $+1.70$ V for 1 min in 0.05 M phosphate buffer solution (pH 7.40) without stirring. The pretreated CPE was then washed with distilled and deionized water for 10 s and transferred into the DNA solution. DNA was subsequently adsorbed on CPE by applying a potential of $+0.50$ V for 5 min in the stirred 10 ppm dsDNA, ssDNA, or polynucleotide (poly[G] or poly[A]) containing 0.50 M acetate buffer

solution with 20 mM NaCl. The electrode was then rinsed with 0.50 M acetate buffer solution for 10 s.

2.3.2. DHC accumulation

The dsDNA, ssDNA or polynucleotide modified CPE was then immersed into 50 mM phosphate buffer solution (pH 7.40) containing 1 μM DHC with 200 rpm stirring for 5 min by applying a potential of +0.50 V. After the accumulation of DHC, the electrode was rinsed with 50 mM phosphate buffer solution (pH 7.40) for 10 s. The same protocol was also applied to the ssDNA modified CPE. No DHC accumulation step was employed for the detection of only the oxidation signals of guanine and adenine.

2.3.3. Voltammetric transduction

The oxidation signals of DHC, adenine and guanine were measured by using DPV in the blank 0.50 M acetate buffer (pH 4.80) with 20 mM NaCl. The raw data were treated using the Savitzky and Golay filter (level 2) of the GPES software, followed by the moving average baseline correction with a 'peak width' of 0.01 V.

2.3.4. Interaction of solution-phase DNA with DHC

CPE was pretreated by applying +1.70 V for 1 min in blank 0.05 M phosphate buffer solution (pH 7.40) without stirring. One micromole of DHC and 10 ppm dsDNA were added to 0.50 M acetate buffer solution and the mixture was left for 10 min. The constituents of the mixture was then immobilized on a pretreated CPE by applying a potential of +0.50 V for 5 min with 200 rpm stirring. The electrode was then rinsed with 0.50 M acetate buffer solution (pH 4.80) for 5 s. The oxidation signals of guanine were taken by using DPV in the blank 0.50 M acetate buffer (pH 4.80) containing 20 mM NaCl. The raw data were also treated using the Savitzky and Golay filter (level 2) of the GPES software, followed by the moving average baseline correction with a 'peak width' of 0.01 V.

Repetitive measurements were carried out by renewing the surface and repeating the above assay format.

3. Results and discussion

The influence of experimental parameters on the interaction of DHC with DNA including ionic strength, temperature and pH were obtained for optimum analytical performance in DNA biosensor [33]. The interaction of DHC with dsDNA and ssDNA was monitored based on the signal of DHC in connection with DPV and cyclic voltammetry. The effect of DHC concentration on the voltammetric signals was also observed. In both bare and dsDNA modified CPEs, the response of DHC increased sharply with concentration up to 1 μM, above which it started to level off till 5 μM (not shown). The highest difference between these signals was obtained at 1 μM, so that 1 μM DHC was employed for further experiments.

Fig. 1 shows the DPV signals of DHC at dsDNA modified CPE. In this medium, DHC at a concentration of 1 μM produced a signal at about +0.84 V. The oxidation signals of DHC obtained with the bare CPE (Fig. 1-a) and guanine and adenine obtained with the dsDNA modified electrode without the interaction of DHC (Fig. 1-b_G and b_A) was higher than the ones obtained after the alkylation process (Fig. 1-c_D, c_G and c_A). The oxidation signal of DHC was attributed to the strong alkylation of DHC to the dsDNA at the CPE surface. A series of three repetitive DPV measurements of the interaction of DHC at dsDNA modified CPE resulted in reproducible results with a relative standard deviation (RSD) of 8.32% was obtained. The detection limits estimated from $S/N = 3$, correspond to 63 nM for DHC at dsDNA modified CPE in 5 min accumulation time.

Fig. 2 shows the changes in the DPV signals of DHC at ssDNA modified CPE. The oxidation signal of DHC was decreased due to the strong alkylation of DHC to the ssDNA at the CPE surface. The oxidation signals of DHC obtained with the bare CPE (Fig. 2-a) and guanine and adenine obtained with the ssDNA modified CPE without the interaction of DHC (Fig. 2-b_G and b_A) was higher than the signals of DHC, guanine and adenine obtained after the alkylation process (Fig. 2-c_D, c_G and c_A). A series of three repetitive DPV measurements of the interaction of DHC at

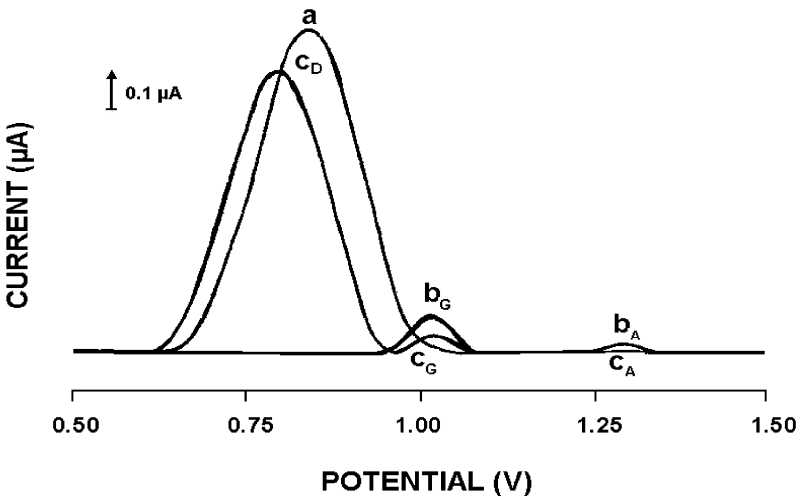

Fig. 1. Differential pulse voltammograms for the interaction of DHC with dsDNA. Oxidation signals of (a) DHC at bare CPE; (b) guanine, (b_G) and adenine (b_A) at dsDNA modified CPE; (c) DHC (c_D), guanine (c_G) and adenine (c_A) after the alkylation of 1 μM DHC at dsDNA modified CPE in 0.50 M acetate buffer (pH 4.80).

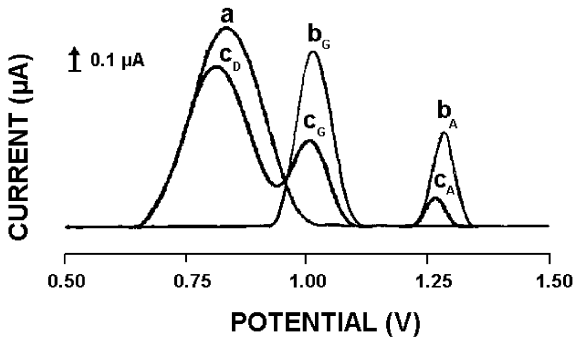

Fig. 2. Differential pulse voltammograms for the interaction of DHC with ssDNA. Oxidation signals of (a) DHC at bare CPE; (b) guanine (b_G) and adenine (b_A) at ssDNA modified CPE; (c) DHC (c_D), guanine (c_G) and adenine (c_A) after the alkylation of 1 μM DHC at ssDNA modified CPE in 0.50 M acetate buffer (pH 4.80).

ssDNA modified CPE resulted in reproducible results with a RSD of 9.45% was obtained. The detection limit estimated from $S/N = 3$, correspond to 42 nM for DHC at ssDNA modified CPE in 5 min accumulation time.

According to the reference method proposed by Millan and Mikkelsen [36], calibration data obtained from dsDNA and ssDNA were used to estimate the partition coefficient of DHC in the microenvironment near the CPE surface as in the equation below;

$$DHC_{bound}/DHC_{free} = (i_{bound} - i_{free})/i_{free}$$

Where DHC_{bound} and DHC_{free} are the concentrations of bound and free DHC complexes, respectively, i_{bound} is the voltammetric peak current of guanine obtained at the dsDNA or ssDNA modified CPE after interaction with DHC as described in the Section 2 and i_{free} is the peak current of guanine obtained at the dsDNA or ssDNA modified CPE without DHC. The validity of this equation depended on the following assumptions that were reported by Millan and Mikkelsen [36]; (a) the equilibration of the free and bound forms of DHC occurred rapidly on the voltammetric time scale, (b) the diffusion coefficient of DHC was the same in dsDNA as in the bulk solution. The partition coefficients of DHC at dsDNA and ssDNA modified CPEs were found as 0.86 and 0.52, respectively, by comparing the voltammetric peak currents obtained under the same conditions at dsDNA or ssDNA modified CPEs. These results indicated that DHC partitions more into the dsDNA microenvironment than the one of ssDNA as a result of the alkylating process between the double helix of dsDNA.

In order to prove that DHC significantly interacts with guanine and adenine bases, the experi-

ments were also performed by using polynucleotides of guanine (poly[G] and adenine (poly[A]). Fig. 3 shows the interaction of DHC with poly[G] at the CPE surface. The DHC oxidation signal obtained from the bare CPE (Fig. 3-a) remarkably decreased after interaction with poly[G] (Fig. 3-c_D). The decrease in the signal of guanine from poly[G] modified CPE (Fig. 3-b) was attributed to the alkylation of DHC to the guanine bases (Fig. 3-c_G).

Fig. 4 arose the possibility of a similar binding event between adenine and DHC. The DHC oxidation signal obtained from the bare CPE (Fig. 4-a) decreased after interaction with poly[A] (Fig. 4-c_D). The adenine signal also decreased to a great extent after interaction with DHC at the CPE surface (Fig. 4-c_A), when compared with the adenine signal obtained from the poly[A] modified CPE without DHC (Fig. 4-b). The decrease in the signal of adenine from poly[A] modified CPE was attributed to the alkylation of DHC to the adenine bases.

A series of three repetitive DPV measurements of the interaction of DHC at poly[G] modified CPE resulted in reproducible results with a RSD of 9.82% was obtained. A series of three repetitive DPV measurements of the interaction of DHC at poly[A] modified CPE resulted in reproducible results with a RSD of 8.36% was obtained.

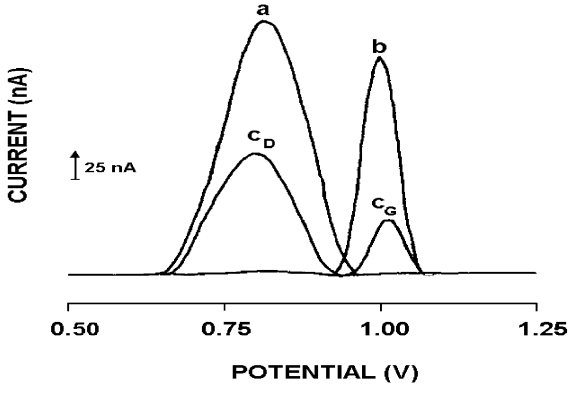

Fig. 3. Differential pulse voltammograms for the interaction of DHC with poly[G]. Oxidation signals of (a) DHC at bare CPE; (b) guanine at poly[G] modified CPE; (c) DHC (c_D) and guanine (c_G) after the alkylation of 1 μM DHC at poly[G] modified CPE in 0.50 M acetate buffer (pH 4.80).

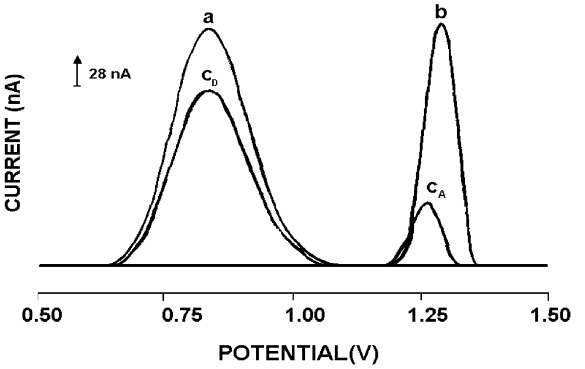

Fig. 4. Differential pulse voltammograms for the interaction of DHC with poly[A]. Oxidation signals of (a) DHC at bare CPE; (b) adenine at poly[A] modified CPE; (c) DHC (c_D) and adenine (c_A) after the alkylation of 1 μM DHC at poly[A] modified CPE in 0.50 M acetate buffer (pH 4.80).

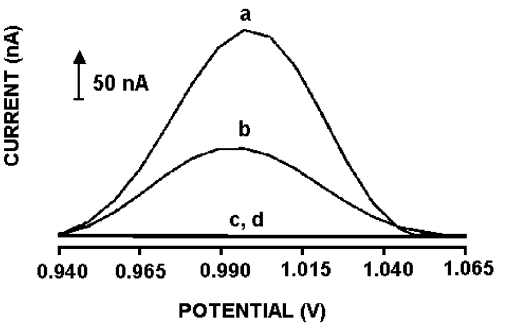

Fig. 5. Differential pulse voltammograms for the interaction of DHC with dsDNA in solution. Oxidation signals of guanine (a) before interaction of 1 μM DHC with 10 ppm dsDNA; (b) after interaction of 1 μM DHC with 10 ppm dsDNA in 0.50 M acetate buffer (pH 4.80); (c) blank 0.50 M acetate buffer (pH 4.80); (d) DHC response in blank 0.50 M acetate buffer (pH 4.80).

Fig. 5 displays the triplicate results from the oxidation signals of guanine before and after the interaction of DHC with dsDNA in solution. In parallel to the results obtained with surface immobilized dsDNA, the signal of guanine obtained before interaction with DHC (Fig. 5a) was higher than the signal obtained after interaction with DHC (Fig. 5b). DHC was alkylated into the double helix of dsDNA in the solution and the guanine signal greatly diminished. No signal of 0.50 M acetate buffer solution at bare CPE (Fig. 5c) and no signal of DHC at bare CPE were also

found in this potential range (Fig. 5d). The interaction of DHC with dsDNA modified at the electrode surface did not differ from those occurring in the solution. It is therefore probable that a similar interaction of DHC with dsDNA may take place even in vivo. The differences in the way of drug interaction with DNA in solution and at surfaces might be important for the efficiency of the administered drug. Further studies in this laboratory will focus on the in vivo determination of the anticancer potential of DHC and similar compounds.

4. Conclusion

The interaction of electroactive and non-electroactive agents with DNA can be monitored by using the procedure reported here. The intrinsic signals of DNA gives an idea about the behavior of the agent towards DNA. The DPV detection system provides lower detection limits than the other voltammetric systems [37], because trace amount of agent such as 42 nM could easily be detected. The oxidation signals of DHC, guanine and adenine were used for detecting the interaction mechanism of DHC with DNA at the electrode surface. Detecting the voltammetric behavior of several drugs that interact with DNA would be valuable in the design of sequence-specific DNA binding molecules for application in chemotherapy and in the development of biotechnological tools for the point-of-care tests based on DNA. Progress in this laboratory is towards the goal of determining the voltammetric behavior of newly synthesized drugs with DNA, thus introducing the electrochemical methods to solve the phenomenal drug—DNA interaction mechanisms.

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