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Journal of Pharmaceutical and Biomedical Analysis
33 (2003) 295–302
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Disposable electrochemical biosensor for the detection of the interaction between DNA and lycorine based on guanine and adenine signals

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Received 5 February 2003; received in revised form 18 April 2003; accepted 22 April 2003

Abstract

The interaction of lycorine (LYC) 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) and pencil graphite electrode (PGE). As a result of the interaction of LYC with DNA, the voltammetric signal of guanine and adenine greatly decreased. The changes in the experimental parameters such as the concentration of LYC, and the accumulation time of LYC were studied by using DPV with PGE. The interaction of LYC with synthetic polynucleotides, such as poly[G] was also observed. The interaction of LYC with dsDNA was also observed at PGE in solution phase. In addition, the detection limit and the reproducibility was determined by using both electrochemical transducers. The application of electrochemical methods on the interactions between DNA and DNA targeted agent were explored.

Keywords: Electrochemical DNA biosensor; Lycorine; Guanine; Adenine; DNA

1. Introduction

Nucleic acids offer the analytical chemist a powerful tool in the recognition and monitoring of many important compounds [1]. A recent active area of research is to explore the nature and dynamics of binding small molecules to biomacromolecules. The design of site-and conformation-specific reagents provide new studies for the rational drug design [2,3]. Binding of small molecules to deoxyribonucleic acid (DNA) occurs through primarily in three modes: electrostatic interactions with the negative-charged nucleic sugar-phosphate structure, binding interactions with two grooves of DNA double helix and intercalation between the stacked base pairs of native DNA [4].

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0731-7085/03/$ - see front matter
doi:10.1016/S0731-7085(03)00283-8

The study of the interactions of some anticancer agents with DNA has been employed by a variety of techniques [5–7] and there is a growing interest in the electrochemical methods [8–15]. Electrochemical DNA hybridization biosensors are mostly employed for determining early and precise diagnoses of infectious agents in various environments [16] and these devices can be used for monitoring sequence-specific hybridization events directly [17,18] or by DNA intercalators (metal coordination complexes, organic dyes, etc.) [19–23]. These studies showed that electrochemical techniques offer a very attractive route for converting the hybridization event into an electrochemical signal.

Clinically applied antitumor agent, Mitomycin C (MC)'s interaction with single stranded DNA (ssDNA) detected by changes of guanine (peak G) residues was studied by Marin et al. [9]. Marrazza et al. [24] showed the use of daunomycin as an electrochemical hybridization indicator for detecting APO E poylmorphisms from DNA fragments amplified by PCR.

Wang et al. [10] reported that the determination of interaction of daunomycin with DNA in the solution and at the surface of CPE could be observed by using a DNA biosensor. Erdem et al. [19] reported that epirubicin could be used as a promising hybridization indicator. Epirubicin was used for the detection of mismatched bases in 17-bases oligonucleotides. A bis-intercalator anticancer drug, ECH was introduced as an electrochemically active drug by Jelen et al. [25]. It was reported that the intercation of ECH with dsDNA attached to HMDE resulted in high signals of ECH. However, a strong binding of ECH to dsDNA occurred, there was almost no signal with ssDNA due to very weak binding of ECH to ssDNA in solution.

In the one study of Erdem et al. [26], the electrochemical interaction of a compound synthesized as an alkylating anticancer agent; 4,4′-dihydroxy chalcone (DHC) with DNA was investigated at CPE surface and in solution phase. It was also reported that due to the alkylation of DHC between base pairs in dsDNA, there was observed a dramatic decrease at the voltammetric signal of guanine and adenine [27].

Labuda et al. [28] developed a simple procedure for the voltammetric detection based on guanine moiety of antioxidative activity of plant extracts based on the protection from DNA damage at the electrode surface.

Lycorine (3,12-didehydro-9,10-[methylenbis(oxy)]-galanthan-1α,2β-diol; in Fig. 1) (LYC) is one of the most widespread alkaloids in the Amaryllidaceae family and has already been reported in species of several genera [29]. LYC exhibits a pronounced antiviral effect on poliomyelitis, coxsackie and herpes type 1 viruses along with cytotoxic and antimalarial activities. [30–34]. It has been documented in literature that LYC inhibited the in vivo growth of a murine ascites tumor and reduced the viability of in vitro grown tumor cells. Moreover, it was reported to inhibit the synthesis of DNA and proteins in murine cells [35]. It was also confirmed that LYC specifically inhibited protein synthesis in cultured K-ras-NRK cells, thus LYC was shown to be an effective inhibitor of protein synthesis in mammalian cells [36]. There have been some reports concerning the interaction between LYC and DNA and/or RNA in the literatures. Schmeda–Hirschmann et al. [37] assessed the DNA binding activity of twenty Amarylidaceae alkaloids, belonging to different skeletal types, including LYC by using an HPLC method. Recently, an assay performed for interactions with DNA and RNA revealed that the antiproliferative effect of LYC results from its complex formation with RNA [38].

As yet there has been no literature report for the electrochemical detection of the interaction be-

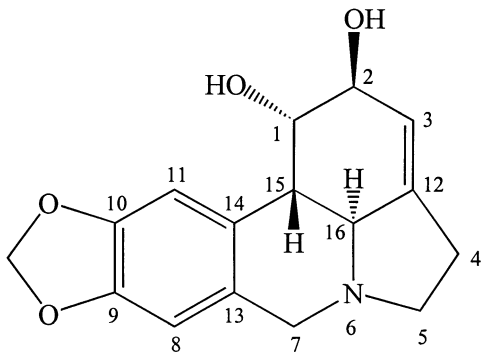

Fig. 1. Chemical structure of LYC.

tween LYC and DNA by using differential pulse voltammetry (DPV) in combination with the disposable pencil graphite electrode (PGE) based on the differences in the guanine and adenine oxidation signals. The features of this process are discussed and compared with those methods previously reported for the other type of DNA-targeted agents in the literature.

2. Experimental section

2.1. Apparatus

The oxidation signals of guanine were investigated by using DPV with an AUTOLAB-PGSTAT 30 electrochemical analysis system and GPES 4.8 software package (Eco Chemie, The Netherlands). 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.

The three-electrode system consisted of the carbon graphite working electrode, an Ag/AgCl reference electrode (Model RE-1, BAS, W. Lafayette, USA) and a platinum wire as the auxiliary electrode.

2.2. Electrode preparation

The body of in-house made carbon paste electrode (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.

The renewable PGE that was described in study of Ozsoz et al. [15], was used in voltammetric measurements for the electrochemical detection of DNA interactions. A Noki pencil Model 2000 (Japan) was used as a holder for the graphite lead. Electrical contact with the lead was obtained by soldering a metallic wire to the metallic part. The pencil was hold vertically with 12 mm of the lead extruded outside (10 mm of which was immersed in the solution).

2.3. Chemicals

The calf-thymus DNA (as lyophilized powder) and polyguanylic acid (5') poly[G] were obtained from Sigma (Germany). dsDNA stock solution (100 mg/l) and synthetic polynucleotide stock solution (100 mg/l) were prepared with TE solution (10 mM Tris–HCl, 1 mM EDTA, pH 8.00) and kept frozen. More dilute solutions of DNA were prepared with either ultrapure distilled water. Other chemicals were of analytical reagent grade.

2.3.1. The isolation and characterization of LYC

The isolation and characterization of LYC were performed in the department of Pharmacognosy at Ege University. LYC used in this study, was isolated from *Sternbergia sicula* Tineo ex Guss. and *Galanthus elwesii* Hook., wildly growing in Turkey, as previously described [39,40]. The spectroscopically pure free base was treated with HCl to obtain the hydrochloride salt. The water-soluble hydrochloride was used for the experiments.

2.4. Procedure

Each measurement involved the immobilization of the nucleic acid/interaction with LYC/detection cycle at a fresh CPE or PGE surface. All the experiments were performed at room temperature $(25.0 \pm 0.5)\ ^{\circ}C$.

2.4.1. Interaction of surface-confined DNA with LYC by using CPE

CPE was pretreated by applying +1.70 V for 1 min in blank 0.05 M acetate buffer solution (pH 4.80) without stirring. The dsDNA was immobilized on a pretreated CPE by applying a potential of +0.50 V for 5 min in 10 μg/ml DNA in 0.50 M acetate buffer solution containing 20 mM NaCl with 200 rpm stirring. The electrode was then rinsed with 0.50 M acetate buffer solution (pH 4.80) for 10 s. The dsDNA-modified CPE was then immersed into 0.50 M acetate buffer solution (pH 4.80) containing different concentrations of LYC

with 200 rpm stirring for 5 min at open circuit system. After the interaction of LYC, the electrode was rinsed with 0.50 M acetate buffer solution (pH 4.80) for 10 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.

2.4.2. Interaction of surface-confined DNA with LYC by using PGE

PGE was pretreated by applying +1.40 V for 30 s in blank 0.05 M acetate buffer solution (pH 4.80) without stirring. The dsDNA was immobilized on a pretreated PGE by using wet-adsorption during 7.5 min in 16 μg/ml DNA in 0.50 M acetate buffer solution containing 20 mM NaCl. 7.5 min as the immobilization time of dsDNA on PGE and 16 μg/ml as dsDNA concentration for a novel wet adsorption method on PGE surface was chosen as the optimum conditions regarding to the results obtained from the one of our studies (unpublished results). The electrode was then rinsed with 0.50 M acetate buffer solution (pH 4.80) for 10 s. The dsDNA-modified PGE was then immersed into 0.50 M acetate buffer solution (pH 4.80) containing different concentrations of LYC with 200 rpm stirring for 5 min at open circuit system. The same protocol at above by using CPE was also applied to the dsDNA modified PGE based on both guanine and adenine signals.

Repetitive measurements were carried out by renewing the surface and repeating the above assay formats by using both electrochemical transducers. The analytical signals represent the differences in guanine peak height magnitudes obtained with dsDNA modified electrodes.

2.4.3. Interaction of solution-phase DNA with LYC

PGE was pretreated by applying +1.40 V for 30 s in blank 0.05 M acetate buffer solution (pH 4.80) without stirring. The dsDNA was immobilized on a pretreated PGE by applying potential at +0.5 V during 5 min in 10 μg/ml DNA in 0.50 M acetate buffer solution containing 20 mM NaCl. After transfer of dsDNA-modified PGE into blank solution, 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.

Various concentrations of LYC and 10 μg/ml dsDNA were added to 0.50 M acetate buffer solution and the mixture was left for 5 min. The constituents of the mixture was then immobilized on a pretreated PGE by applying a potential of + 0.50 V for 5 min in different concentrations of LYC between 25 and 100 μg/ml 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.

2.4.4. Interaction of surface-confined poly[G] with LYC

PGE was pretreated by applying +1.40 V for 30 s in blank 0.05 M acetate buffer solution (pH 4.80) without stirring. The poly[G] was immobilized on a pretreated PGE by applying potential at +0.5 V during 5 min in 10 μg/ml poly[G] in 0.50 M acetate buffer solution containing 20 mM NaCl. After transfer of poly[G] modified PGE into blank solution, the oxidation signal of guanine were taken by using DPV in the blank 0.50 M acetate buffer (pH 4.80) containing 20 mM NaCl.

The poly[G] modified PGE was then immersed into 0.50 M acetate buffer solution (pH 4.80) containing different concentrations of LYC with 200 rpm stirring for 5 min at open circuit system. The same protocol at above was also applied after interaction with LYC in different concentrations at the poly[G] modified PGE surface based on guanine signal. Repetitive measurements were carried out by renewing the surface and repeating the above assay formats by using the poly[G] modified PGE.

3. Results and discussion

Fig. 2 shows the DPV signals of LYC at dsDNA modified electrode as the one with CPE (A) and PGE (B). When LYC concentration was increased in different concentration as 25, 50 and 100 μg/ml, there was observed a gradually decrease at guanine oxidation signal by using dsDNA modified electrode: CPE or PGE. No oxidation signal of LYC was obtained with the bare CPE or PGE at the

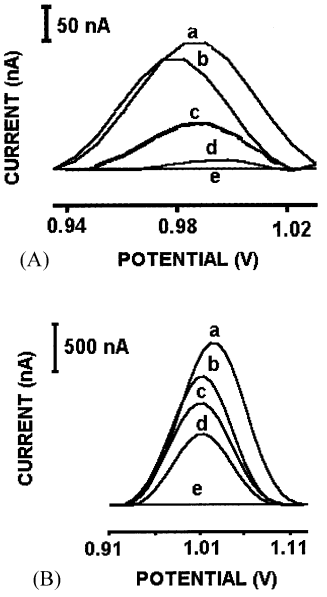

Fig. 2. (A) Differential pulse voltammograms for the interaction of LYC with 10 μg/ml concentration level of dsDNA at CPE surface: oxidation signals of guanine (a) at bare CPE; after interaction LYC at concentration level of (b) 25 μg/ml, (c) 50 μg/ml, (d) 100 μg/ml, (e) LYC alone at concentration level of 25 μg/ml at dsDNA modified CPE in 0.05 M acetate buffer (pH 4.80); (B) differential pulse voltammograms for the interaction of LYC with 16 μg/ml concentration level of dsDNA at PGE surface: oxidation signals of guanine (a) at dsDNA modified PGE; after interaction LYC at concentration level of (b) 25 μg/ml, (c) 50 μg/ml, (d) 100 μg/ml, (e) LYC alone at concentration level of 25 μg/ml at dsDNA modified PGE in 0.05 M acetate buffer (pH 4.80). dsDNA immobilization on CPE surface, +0.5 V during 5 min and dsDNA immobilization on PGE surface by using wet adsorption method during 7.5 min; LYC accumulation at open circuit system during 5 min; measurement, scanning between +0.80 and +1.40 V in blank 0.50 M acetate buffer (pH 4.80) with 20 mM NaCl.

potential range between +0.80 and +1.4 V. Thus the changes at oxidation signals of guanine and adenine obtained with the dsDNA modified electrode with the interaction of LYC at CPE or PGE surface was obtained.

A series of three repetitive DPV measurements of the interaction at 25 μg/ml concentration level of LYC with 10 μg/ml concentration level of dsDNA at CPE surface resulted in reproducible results such as a mean response of 166.1 nA with a relative standard deviation of 11.3% was obtained. At this concentration level of LYC and dsDNA, the detection limit estimated from S/N = 3, corresponds to 225 ng/ml for LYC at dsDNA modified CPE in 5 min accumulation time.

A series of three repetitive DPV measurements of the interaction at 25 μg/ml concentration level of LYC with 16 μg/ml concentration level of dsDNA immobilized by using wet-adsorption method on PGE surface resulted in reproducible results such as a mean response of 1242 nA with a relative standard deviation of 4.6% was obtained. At this concentration level of LYC and dsDNA, the detection limit estimated from S/N = 3, corresponds to 30.2 ng/ml for LYC at dsDNA modified PGE in 5 min accumulation time.

CPE was not found to be the less suitable electrode for this study because of its reproducibility effects and sensitivity. PGE was chosen as the detecting electrode for the remaining parts like that in use as a disposable electrode.

It was shown in Fig. 3, the possibility of a similar binding event between adenine at dsDNA and LYC. The adenine oxidation signal obtained from the dsDNA modified PGE gradually decreased after interaction with LYC in different concentration as 25, 50 and 100 μg/ml.

The decrease both in the signals of guanine and adenine from dsDNA modified PGE was attributed to the binding of LYC to these bases: this phenomenon could be explained by the shielding of oxidizable groups of electroactive bases such as

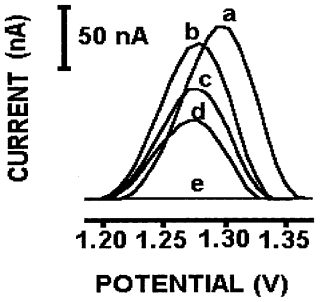

Fig. 3. Differential pulse voltammograms for the interaction of LYC with 16 μg/ml concentration level of dsDNA at PGE surface: oxidation signals of adenine (a) at bare PGE; after interaction LYC at concentration level of (b) 25 μg/ml, (c) 50 μg/ml, (d) 100 μg/ml, (e) LYC alone at concentration level of 25 μg/ml at dsDNA modified PGE in 0.05 M acetate buffer (pH 4.80). Other conditions are as in Fig. 2B.

guanine and adenine while LYC interacts with DNA at electrode surface.

When DNA concentration was increased from 8 to 32 μg/ml (not shown) as optimizing the concentration level of LYC as 25 μg/ml, there was observed no significant changes neither guanine signal nor adenine signal. All experiments at electrode surface employed a 16 ppm as optimum dsDNA concentration by using PGE.

Fig. 4 displays the accumulation time study for LYC at dsDNA-modified PGE based on the changes in the guanine signal (Fig. 4A) and adenine signal (Fig. 4B) before/after interaction with LYC at electrode surface. The electrochemical detection were assessed and obtained in the range from 1 to 10 min as accumulation time of LYC. A gradual decrease was obtained in the guanine signals (Fig. 4A) till 1 min and then sharply decreased from 1 to 3 min. After 3 min, there was observed a gradual decrease till 10 min. When the accumulation time of LYC increased up to 10 min, there was a dramatic decrease in the adenine signal (Fig. 4B) till 3 min and then it was almost leveled off till 10 min. The optimum time for interaction of LYC with dsDNA was determined as 5 min.

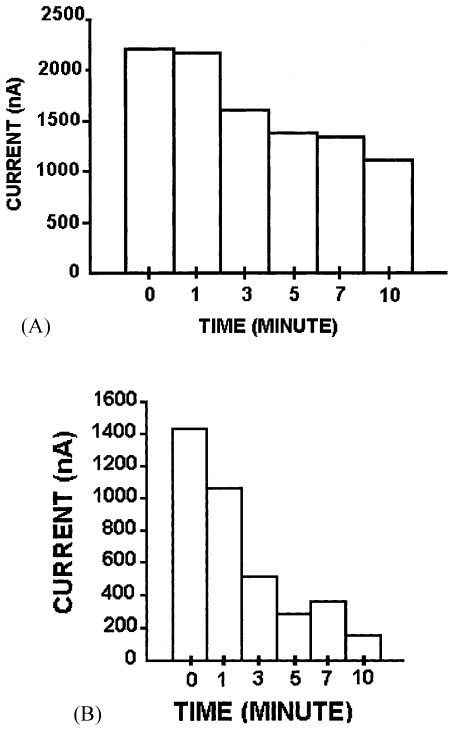

Fig. 4. The effect of different accumulation time of LYC with dsDNA modified PGE based on the changes at guanine signal (A) and adenine signal (B) after interaction with LYC. Other conditions are as in Fig. 2B.

The effect of LYC concentration on the guanine/adenine signal from dsDNA at concentration level as 10 μg/ml was also observed by using PGE in solution phase. When dsDNA interacted with the increasing concentration of LYC in solution phase, a dramatic decrease was observed as the signal of guanine with concentration up to 0.5 μg/ml of LYC, above which it started to increase till 1 μg/ml and then almost leveled off till 2 μg/ml as in Fig. 5A. The increase at the guanine signals were attributed to the cleavage and/or unwinding of the dsDNA helix. A similar increase in the oxidation signals of guanine were observed for the cleaving agent by using hanging mercury electrode by Fojta et al. [41] and by using CPE/PGE by Ozsoz et al. [15]. As LYC may cleave dsDNA, the guanine bases could become available to the oxidation. After 1 μg/ml LYC, the guanine signals remained constant indicating that all/ some guanine bases on dsDNA were exposed to damage by LYC.

After dsDNA interacted with the increasing concentration of LYC in solution phase, a dramatic decrease was observed as the signal of adenine with concentration up to 0.5 μg/ml of LYC, above which it sharply increased till 1 μg/ml and then almost leveled off till 2 μg/ml as in Fig. 5B. Our results based on adenine signals in parallel to the ones based on guanine signals. Thus, this similar increase in the adenine signals may be attributed to the cleavage and/or unwinding of the dsDNA helix. As LYC may cleave dsDNA, the adenine bases also could become available to the oxidation.

In Fig. 6, it was also shown the interaction of LYC with poly[G] at the PGE surface. The guanine oxidation signal obtained from the poly[G] modified PGE gradually decreased after interaction with LYC at concentration level of 25, 50 and 100 μg/ml, respectively. The decrease in the

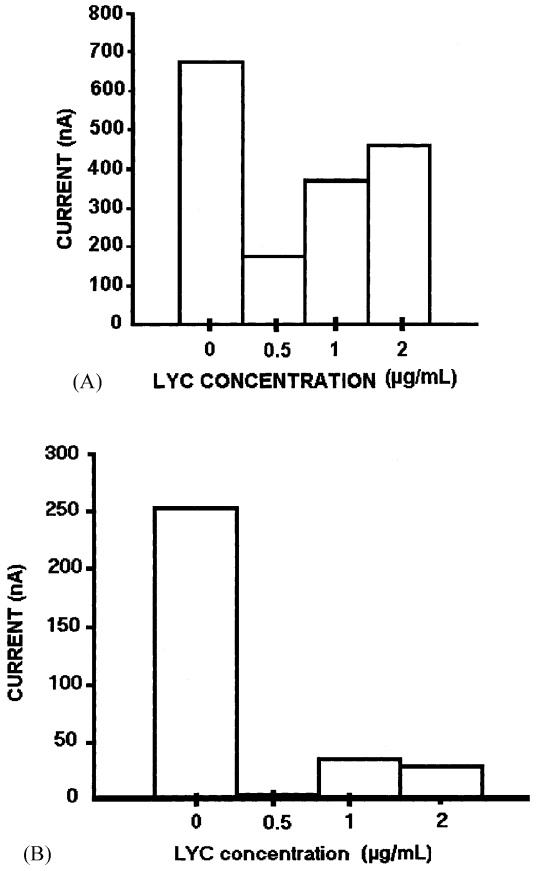

Fig. 5. The interaction of LYC with dsDNA at concentration level as 10 μg/ml in solution based on the changes at guanine signal (A) and adenine signal (B) after interaction with LYC. dsDNA immobilization on PGE surface: applying potential at +0.5 V during 5 min; LYC accumulation at open circuit system during 5 min; measurement, scanning between +0.80 and + 1.40 V in blank 0.50 M acetate buffer (pH 4.80) with 20 mM NaCl.

signal of guanine from poly[G] modifed PGE was attributed to the binding of LYC to the most electroactive base as guanine.

A series of three repetitive DPV measurements of the interaction between 25 μg/ml concentration level of LYC and 10 μg/ml concentration level of poly[G] modified PGE resulted in reproducible results such as a mean response of 1870.7 nA with a relative standard deviation of 8.12% was obtained.

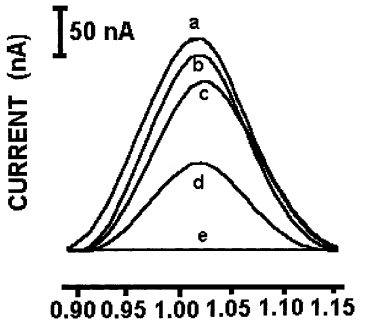

Fig. 6. Differential pulse voltammograms for the interaction of LYC with 10 μg/ml concentration level of poly[G] at PGE surface: oxidation signals of guanine (a) at bare PGE; after interaction LYC at concentration level of (b) 25 μg/ml, (c) 50 μg/ml, (d) 100 μg/ml, (e) LYC alone at concentration level of 25 μg/ml at poly[G] modified PGE in 0.05 M acetate buffer (pH 4.80). poly[G] immobilization on PGE surface: applying potential at +0.5 V during 5 min; LYC accumulation at open circuit system during 5 min; measurement, scanning between + 0.80 and +1.40 V in blank 0.50 M acetate buffer (pH 4.80) with 20 mM NaCl.

4. Conclusion

Chemical agents with a redox active group can be observed electrochemically by using the procedure reported here. The oxidation signals of LYC, guanine and adenine were used for detecting the interaction mechanism of LYC 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 diagnosis based on DNA. It has been planned to develop a disposable electrochemical biosensor at our lab for further experiments on the plant extracts to directly detect possible interaction or even DNA damage between the compounds in plant extracts and DNA. Progress in this laboratory is towards the goal of determining the voltammetric behavior of newly synthesized compounds with DNA, thus introducing the electrochemical methods to solve the phenomenal drug–DNA interaction mechanisms.

Acknowledgements

This work has been supported by the Turkish Academy of Sciences, in the framework of the Young Scientist Award Program (KAE/TUBA-GEBIP/2001-2-8). Authors acknowledge the financial support from TUBITAK (Project number: TBAG-2161).

References

[1] L.B. Mc Gown, M. Joseph, J. Pitner, G. Vonk, C. Linn, Anal. Chem. 67 (1995) 663A–668A.
[2] Q. Feng, N.Q. Li, Y.Y. Jiang, Anal. Chim. Acta 344 (1997) 97–104.
[3] F. Qu, N.Q. Li, Y.Y. Jiang, Talanta 45 (1998) 787–793.
[4] C. Xia, S. Guoli, J. Jianhui, Y. Ruqin, Anal. Lett. 32 (1999) 717–727.
[5] J.W. Lown, C.C. Hanstock, R.D. Bradley, D.G. Scraba, Mol. Pharmacol. 25 (1984) 178–184.
[6] H. Fritzsche, A. Akhebat, E. Taillandier, K. Rippe, T.M. Jovin, Nucleic Acids Res. 21 (1993) 5085–5091.
[7] C.M. Nunn, L.V. Meervelt, S. Zhang, J. Mol. Biol. 222 (1991) 167–177.
[8] C. Teijeiro, P. Perez, D. Marin, E. Palecek, Bioelectrochem. Bioenerg. 38 (1995) 77–83.
[9] D. Marin, P. Perez, C. Teijeiro, E. Palecek, Biophys. Chem. 75 (1998) 87–95.
[10] J. Wang, M. Ozsoz, X. Cai, G. Rivas, H. Shiraishi, D.H. Grant, M. Chicarro, J.R. Fernandes, E. Palecek, Bioelectrochem. Bioenerg. 45 (1998) 33–40.
[11] J. Labuda, M. Buckova, S. Jantova, I. Stepanek, I. Surugiu, B. Danielsson, M. Mascini, Fresen. J. Anal. Chem. 367 (2000) 364–368.
[12] A. Erdem, M. Ozsoz, Electroanalysis 14 (2002) 965–974.
[13] A. Erdem, M. Ozsoz, Turk. J. Chem. 25 (2001) 469–475.
[14] P. Kara, D. Ozkan, K. Kerman, B. Meric, A. Erdem, M. Ozsoz, Anal. Bioanal. Chem. 373 (2002) 710–716.
[15] A. Erdem, P. Kara, K. Kerman, D. Ozkan, M. Ozsoz, Electroanalysis. 15 (2003) 613–619.
[16] J. Wang, G. Rivas, X. Cai, E. Palecek, P. Nielsen, H. Shiraishi, N. Dontha, D. Luo, C. Parrado, M. Chicarro, P.A.M. Farias, F.S. Valera, D.H. Grant, M. Ozsoz, M.N. Flair, Anal. Chim. Acta 347 (1997) 1–8.
[17] J. Wang, A.-N. Kawde, Anal. Chim. Acta 431 (2001) 219–224.
[18] J. Wang, A.-N. Kawde, A. Erdem, M. Salazar, Analyst 126 (2001) 2020–2024.
[19] A. Erdem, M. Ozsoz, Anal. Chim. Acta 437 (2001) 107–114.
[20] A. Erdem, K. Kerman, B. Meric, U.S. Akarca, M. Ozsoz, Electroanalysis 10 (1999) 586–588.
[21] A. Erdem, K. Kerman, B. Meric, U.S. Akarca, M. Ozsoz, Anal. Chim. Acta 422 (2000) 139–149.
[22] A. Erdem, K. Kerman, B. Meric, M. Ozsoz, Electroanalysis 13 (2001) 219–223.
[23] D. Ozkan, A. Erdem, P. Kara, K. Kerman, B. Meric, J. Hassmann, M. Ozsoz, Anal. Chem. 74 (2002) 5931–5936.
[24] G. Marrazza, G. Chiti, M. Mascini, M. Anichini, Clin. Chem. 46 (2000) 31–37.
[25] F. Jelen, A. Erdem, E. Palecek, Bioelectrochemistry 55 (2002) 165–167.
[26] A. Erdem, K. Kerman, D. Ozkan, O. Kucukoglu, E. Erciyas, M. Ozsoz, In: M. Butler, P. Vanysek, N. Yamazoe (Eds.), Electrochem. Soc. P.V. (the 200th Meeting of the Electrochemical Society and the 52th meeting of the International Society of Electrochemistry, 2–7 September 2001, San Francisco, CA, USA), 18 (2001) 563–575.
[27] B. Meric, K. Kerman, D. Ozkan, P. Kara, A. Erdem, O. Kucukoglu, E. Erciyas, M. Ozsoz, J. Pharm. Biomed. Anal. 30 (2002) 1339–1346.
[28] J. Labuda, M. Buckova, L. Heilerova, A. Caniova-Ziakova, E. Brandsteterova, J. Mattusch, R. Wennrich, Sensors 2 (2002) 1–10.
[29] O. Hoshino, in: A.G. Cordell (Ed.), The Alkaloids. Chemistry and Pharmacology, vol. 51, Academic Press, New York, 1998, pp. 323–424.
[30] M. Ieven, A.J. Vlietinck, D.A. Vanden Berghe, J. Totte, J. Nat. Prod. 45 (5) (1982) 564–573.
[31] M. Ieven, D.A. Vanden Berghe, A.J. Vlietinck, Planta Med. 49 (1983) 109–114.
[32] R. Vrijsen, D.A. Vanden Berghe, A.J. Vlietinck, A. Boeyé, J. Biol. Chem. 261 (2) (1986) 505–507.
[33] J. Renard-Nozaki, T. Kim, Y. Imakura, M. Kihara, S. Kobayashi, Res. Virol. 140 (1989) 115–128.
[34] K. Likhitwitayawuid, C.K. Angerhofer, H. Chai, J.M. Pezzuto, G.A. Cordell, J. Nat. Prod. 56 (8) (1993) 1331–1338.
[35] S. Ghosal, K.S. Saini, S. Razdan, Phytochemistry 24 (10) (1985) 2141–2156.
[36] N. Kushida, S. Atsumi, T. Koyano, K. Umezawa, Drugs Exp. Clin. Res. 5/6 (1997) 151–155.
[37] G. Schmeda-Hirschmann, L. Astudillo, J. Bastida, F. Viladomat, C. Codina, Bo. Soc. Chil. Quim. 45 (2000) 515–518.
[38] J. Hohmann, P. Forgo, J. Molnar, K. Wolfard, A. Molnar, T. Thalhammer, I. Mathe, D. Sharples, Planta Med. 68 (2002) 454–457.
[39] B. Kivçak, T. Gözler, Ege Üniversitesi Eczacilik Fak. Derg. 1 (2) (1993) 65–71.
[40] A. Latvala, M.A. Önür, T. Gözler, A. Linden, B. Kivçak, M. Hesse, Phytochemistry 39 (5) (1995) 1229–1240.
[41] M. Fojta, L. Havran, E. Palecek, Electroanalysis 9 (1997) 1033–1034.