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DNA-modified carbon paste electrode applied to the study of interaction between Rifampicin (RIF) and DNA in solution and at the electrode surface

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Abstract

Rifampicin (RIF) is an antibiotic widely used against tuberculosis and a DNA intercalator. The interaction of RIF with double-stranded (ds) and single-stranded (ss) calf thymus DNA was studied in solution as well as at the electrode surface by means of transfer voltammetry using differential pulse as stripping mode with carbon paste electrode (CPE) at 0.2 M acetate buffer at pH 5.0 and at 0.2 M phosphate buffer pH 7.4. Differentiations in the above-mentioned interaction at different pH values are presented and compared in order to optimize the detection of Rifampicin (RIF).
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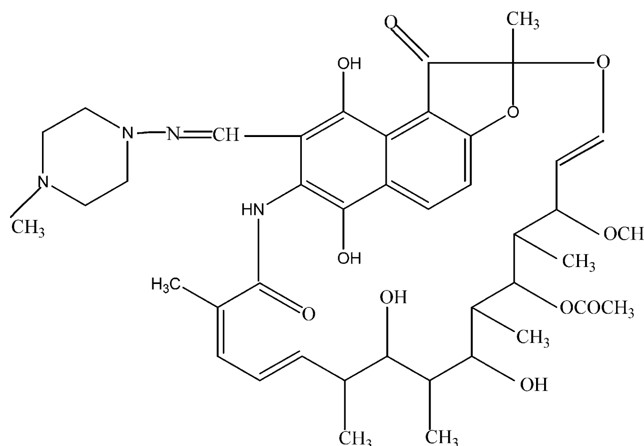
Keywords: Rifampicin; Antibiotic; DNA; Electrochemical biosensors; Carbon paste electrode

1. Introduction

Rifampicin along with isoniazid, ethambutol and pyrazinamide has been classified as a first line drug characterized as highly effective, orally administered, well tolerated and non-toxic. Analytical techniques including high-performance liquid chromatography (HPLC), spectrophotometry UV–vis, thermospray and electrospray mass spectrometry and gas chromatography have been used for the detection of RIF or the simultaneous detection of RIF and the other antituberculosis drugs [1–6].



Chemical Structure of Rifampicin

In recent years, biotechnology related to the manipulation of solid-phase DNA has expanded dramatically. These developments have paralleled the increased demands of human genome sequencing and large-scale analysis of gene expres-

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sion using DNA chips. The development of efficient methods [7] for immobilizing DNA on a solid support is essential for generating DNA-array based instruments.

Under this perspective, there has been a growing interest in the development of a simple, rapid and user-friendly method for routine, centralized testing of DNA to allow early and precise diagnosis of infectious agents in various environmental matrices [8] and for detecting the presence of genes or mutant genes associated with inherited human diseases [9].

Recent trends in nucleic acid electrochemistry are focused on the development of electrochemical biodetectors or biosensors based on specific DNA interactions, including DNA hybridization [10–13], DNA interactions with drugs or carcinogens [14–19], and DNA damage [20]. These investigations are usually carried out with solid electrodes, which are more practical and easier to handle compared to mercury electrodes, which are more sensitive for small changes or damage in DNA structure [21].

In this paper, the interaction between DNA and Rifampicin, a known antibiotic used against tuberculosis, is studied by applying transfer voltammetry with differential pulse as stripping mode with a carbon paste electrode (CPE) in solution and at the electrode surface at pH 5.0 and pH 7.4. The objective of this work is to apply DNA sensors for the detection of Rifampicin due to its affinity for DNA and examine the parameters (pH, interaction time, potential) influencing their interaction.

Furthermore, a quantitative understanding of such factors that determine recognition of DNA sites would be valuable in the rational design of new DNA targeted molecules for application in chemotherapy and in the development of tools for biotechnology based on DNA hybridization [22].

2. Experimental

2.1. Reagents

Double-stranded calf thymus DNA (Catalog No. D-1501, highly polymerized) and Rifampicin (R-3501), 95% p.a., were purchased from Sigma. The supporting electrolyte of differential pulse voltammetric experiments was acetate buffer solution 0.2 M (pH 5.0) and 0.2 M phosphate buffer (pH 7.4).

Single-stranded DNA was prepared by boiling a solution of double-stranded DNA (1 g/L) for 15 min and left at 4 °C for 10 min. The stock solution of dsDNA (1 g/L) was prepared with a solution of 10 mM Tris–HCl and 1 mM EDTA at pH 8.0.

Rifampicin was purchased from Sigma. Stock solutions of RIF (0.01 M) were prepared with water, while dilute solutions were prepared by successive dilutions just before use. The water used was doubly distilled and sterilized. Trichloroacetic acid (TCA) 20% was purchased from Fluka, while bovine serum was purchased from Sigma.

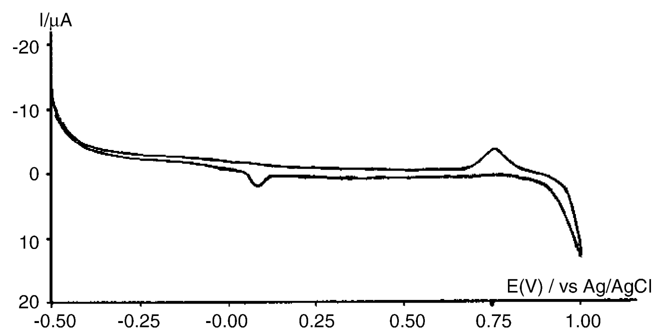

Fig. 1. Cyclic voltammogram of Rifampicin (34×10^{-9} M, pH 5.0, preconcentration step at +0.2 V for 180 s).

2.2. Apparatus

Differential pulse voltammetric measurements were performed with a Metrohm 647 VA-Stand controlled by a 646 VA-Processor. The working electrode was a carbon paste electrode of 6 mm diameter, the reference electrode was a saturated Ag/AgCl and the counter electrode was a platinum wire electrode. The carbon paste was prepared in the usual way by hand-mixing graphite powder and nujol oil. The ratio of graphite powder to nujol oil was 75:25. The resulting paste was packed tightly into a Teflon sleeve. Electrical contact was established with a stainless steel screw. The surface was polished to a smooth finish before use. The electrode was pretreated by applying a potential at +1.7 V for 1 min without stirring prior to the accumulation step. The electrochemical pretreatment produces a more hydrophilic surface state and a concomitant removal of organic layers [23].

All water and pipette tips were sterilized by autoclaving for 20 min. The electrochemical cells were cleaned with diluted nitric acid, rinsed with water and sterilized for 20 min. Ultrapure nitrogen was used to deaerate the solutions for 5 min before each experiment.

2.2.1. Procedures [24–27]

2.2.1.1. Interaction of surface-confined DNA with Rifampicin. The procedure consists of DNA immobilization, interaction of RIF with immobilized DNA and transduction by transfer voltammetry with differential pulse mode. Prior to each medium exchange, the electrode was rinsed carefully with water for 5 s. After the pretreatment of the electrode, the

Table 1
The effect of interaction time between the immobilized dsDNA (0.1 g/L) and a constant concentration of RIF (5×10^{-12} M) into the solution at a potential of +0.2 V at pH 7.4

Interaction time (s)	I(nA) dsDNA peak at +0.918 V	I(nA) dsDNA peak at +1.195 V
0	1290	2000
120	179.66	239.13
240	220.17	293.07
300	199.4	223.84
420	185.17	201.75
600	181.1	182.92

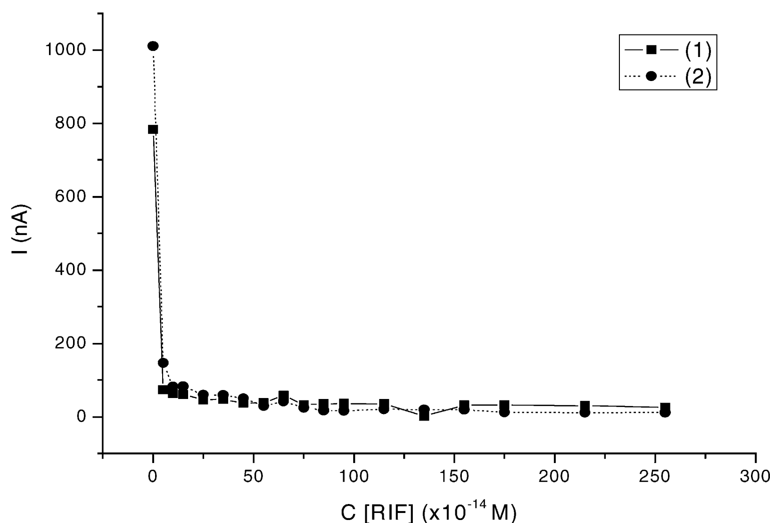

Fig. 2. Dependence of peak current of oxidation of: (1) guanine residues in dsDNA immobilized on the electrode surface on increasing concentrations of RIF at pH 7.4 (the interaction time prior to each scan was 120 s at +0.2 V) and (2) adenine residues in dsDNA immobilized on the electrode surface on increasing concentrations of RIF under the above conditions.

nucleic acid was subsequently immobilized onto the electrode surface by adsorptive accumulation for 5 min at +0.2 V. The dsDNA-coated electrode was transferred to the stirred sample solution (analyte plus buffer solution) for the optimal interaction time, while holding a potential of +0.2 V. The transduction was performed in the blank acetate buffer or the blank phosphate buffer solution. The same procedure was followed for the immobilization of ssDNA and the study of the ssDNA–sensor interaction with RIF.

2.2.1.2. Interaction of solution-phase DNA with Rifampicin. The analysis of solution-phase DNA with RIF consisted of mixing the two components, followed by accumulation and transduction by transfer voltammetry with differential pulse mode. The electrode was rinsed with water for 5 s prior to each medium exchange. Stock DNA (1 g/L) and RIF solutions were added to 0.2 M acetate buffer or 0.2 M phosphate buffer to produce the required concentrations and the mixture was left to stand for the optimal time. A freshly polished carbon paste electrode was first pretreated as described above and subsequently immersed into the mixture solution. The accumulation of the mixture was performed by applying a potential of +0.2 V for 5 min. The transduction was carried out in the blank acetate buffer or the blank phosphate buffer

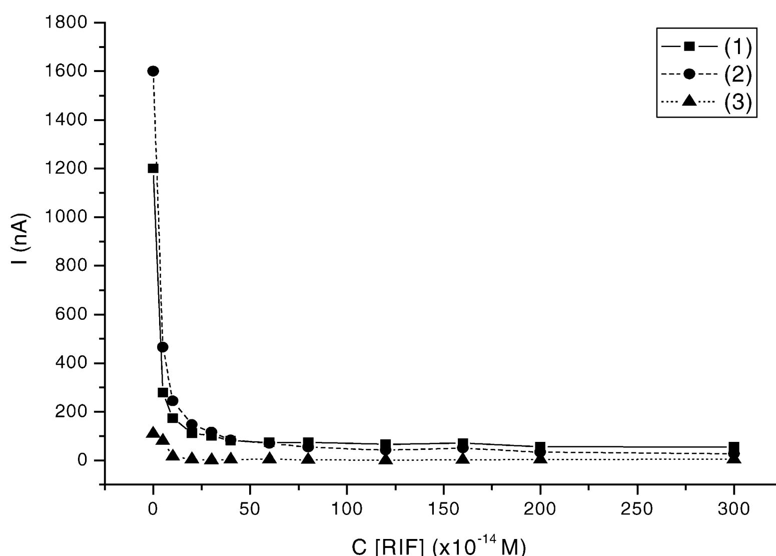

Fig. 3. Dependence of peak current of oxidation of: (1) guanine residues in dsDNA immobilized on the electrode surface on increasing concentrations of RIF at pH 7.4 (the interaction time prior to each scan was 420 s min at +0.2 V) and (2) adenine residues in dsDNA immobilised on increasing concentrations of RIF under the above conditions. Dependence of the characteristic peak current probably due to the amount of RIF in excess on increasing concentrations of RIF under the above conditions.

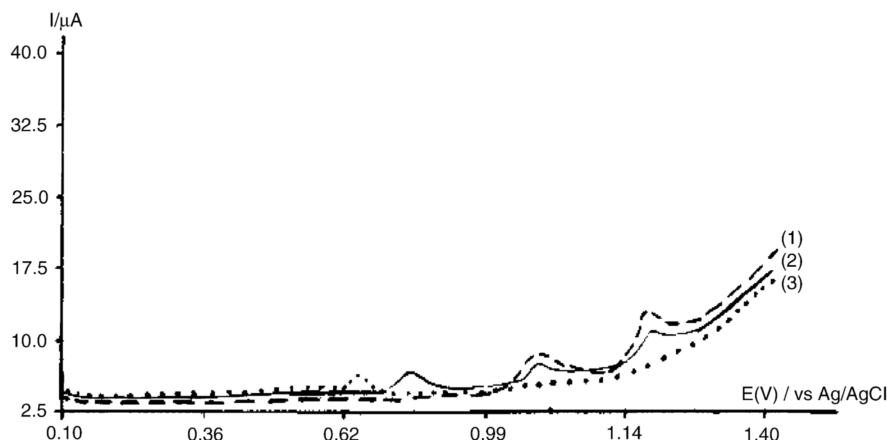

Fig. 4. Differential pulse voltammogram of: (1) dsDNA (0.1 g/L) immobilized on the CPE surface at pH 7.4 and (2) surface confined dsDNA (0.1 g/L) and RIF (5×10^{-12} M) in solution, where a new peak at +0.7 V appears, probably due to the formation of a complex, (3) RIF (34×10^{-9} M) at pH 7.4 (preconcentration step: at +0.2 V for 240 s).

solution, with an initial potential of +0.1 V and a scan rate of 50 mV/s.

2.2.1.3. Interaction of surface-confined analyte with DNA. Rifampicin was first accumulated at the surface of CPE and then the resulting RIF-coated electrode was immersed into the stirred sample solution containing DNA plus 0.2 M acetate buffer solution pH 5.0 or 0.2 M phosphate buffer solution pH 7.4. The surface of the electrode was renewed prior to each assay. All the experiments were conducted at room temperature (20 °C).

2.2.1.4. Preparation of samples. An amount of 1.5 mL of TCA was added to 1 ml of bovine serum and the mixture was subsequently centrifuged for 5 min in 3000 rpm. The 40 μL of the supernatant was added into the electrochemical cell, which contained 10 ml of 0.2 M acetate buffer solution pH 5.0. The solution was dearated for 5 min before each measurement.

3. Results and discussion

3.1. Transfer voltammetry using differential pulse mode of Rifampicin and DNA at carbon paste electrodes

For our studies we used 0.2 M sodium acetate (pH 5.0) or 0.2 M sodium phosphate (pH 7.4) buffer as a background electrolyte. Native double-stranded (ds) DNA yielded a positive peak at +0.99 V at pH 5.0 due to the oxidation of guanine residues, and two positive peaks at pH 7.4 due to the oxidation of guanine and adenine residues. Thermally denatured (single-stranded) DNA, yielded two higher peaks at both buffer solutions, a higher peak at +1.016 V due to the oxidation of adenine residues and another fable one at +0.86 V due to the oxidation of guanine residues. The nature of these peaks at the above conditions and the optimal accumulation conditions (potential and time) were studied elesewhere [28].

Rifampicin produces a well-developed peak at +0.75 V with a preconcentration step at +0.2 V for 180 s at pH 5.0 as presented in Fig. 1. Linearity is observed in the range $(0–360) \times 10^{-9}$ M ($y = 0.178x + 0.5$, sensitivity 0.178 nA/nM, $r = 0.99$). At pH 7.4, RIF yields an oxidation peak at +0.65 V with a preconcentration step at +0.2 V for 240 s. Linearity is observed in the range $(20–300) \times 10^{-9}$ M ($y = 0.5312x + 55.154$, sensitivity 0.5312 nA/nM, $r = 0.97$).

3.2. Interaction of surface-confined DNA with Rifampicin in solution

The DNA-modified electrode was prepared by immersing the CPE in a solution of dsDNA at a concentration of 0.1 g/L in 0.2 M acetate buffer (pH 5.0) for 2 min at +0.5 V. In the case of 0.2 M sodium phosphate buffer pH 7.4, the electrode was washed and immersed in RIF solutions of different concentrations between 0 and 12×10^{-12} M. In the case of 0.2 M sodium acetate buffer pH 5.0, the electrode was immersed in RIF solutions of very low concentrations in the range of 5×10^{-18} M, where the peak of dsDNA disappeared immediately. The interaction time was selected according to the potential changes caused to the characteristic oxidation peak of DNA and the appearance of a new peak declaring the formation of a complex. Table 1 indicates the changes at the peak current of the guanine residues at pH 7.4, while the

Table 2
The effect of incubation time between dsDNA (0.1 g/L) and a constant concentration of RIF (10^{-7} M) into the solution at pH 5.0

Incubation time (min)	I(nA) dsDNA peak at +1.035 V	I(nA) dsDNA peak at +1.195 V
5	92.59	68.01
15	7.95	64.6
30	1.2	61.51
50	0.95	49.67
80	0.85	45.51
125	0.65	50.94

The incubated mixture was immobilized on the CPE as previously described.

Table 3
The effect of incubation time between dsDNA (0.1 g/L) and a constant concentration of RIF (10^{-7} M) into the solution at pH 7.4

Incubation time (min)	I(nA) dsDNA peak at + 0.952 V	I(nA) dsDNA peak a +1.231 V	I (nA) peak at +0.677 V
5	300.87	471.09	273.87
15	27.56	49.89	118.15
30	11.87	1.8	77.99
50	1.88	-	56.02
80	3.66	-	34.43
125	7.08	-	19.9

The incubated mixture was immobilized on the CPE as previously described.

concentration of RIF was stable and equal to 5×10^{-12} M. The interaction times selected were 120 s at +0.2 V and 420 s at +0.2 V [29].

The interaction at pH 5.0 is very strong and the characteristic peak of dsDNA disappears, while at pH 7.4 the characteristic peaks of dsDNA are gradually decreased. The effect of the interaction time is shown in Figs. 2 and 3, where the two oxidation peaks of dsDNA decrease in both cases, but at an interaction time of 420 s a new peak appears at +0.7 V declaring the intercalated RIF (Fig. 4).

A decrease was also observed in the case of the use of thermally denatured (single-stranded) DNA at pH 5.0, where the characteristic peak current due to the oxidation of adenine residues disappears at a RIF concentration equal to 5×10^{-18} M and the peak current due to the oxidation of guanine residues fluctuates. The same interaction was studied at pH 7.4 which resulted in a fluctuation of the peak current values of the two characteristic oxidation peaks. This different behavior between ssDNA and RIF can be used as a criterion for the characterization of the intercalation phenomenon.

3.3. Interaction of Rifampicin and DNA in solution

The incubation time of the two components is a very important factor affecting the DPV response. Table 2 shows the peak current response at pH 5.0 of the immobilized mixture after incubation of DNA with a constant concentration of RIF equal to 10^{-7} M. The incubation times selected were 5 min and 10 min, since the characteristic oxidation peak of dsDNA disappears and a new one appears at +0.799 V. Table 3 presents the peak current response at pH 7.4 of the immobilized mixture under the same conditions as previously described. The incubation time selected was 10 min, since a new peak appears at +0.677 V [30,31].

Double-stranded DNA solution of 0.1 g/L (final concentration) was left to react for 5 min with different concentrations of RIF from 0 until 1.795×10^{-6} M at pH 5.0. The peak current due to the oxidation of guanine residues disappears and a new peak at +0.799 V appears as shown in Fig. 5, which increases and then decreases gradually. In addition, Fig. 6 shows the dependence of the characteristic peak current of DNA on the increasing amounts of RIF after incubation for 10 min in solution, where the characteristic peak of dsDNA decreases and the new peak at +0.77 V appears. At pH 7.4, the behavior is different; the two characteristic peaks of dsDNA decrease, while a peak at +0.675 V appears. Fig. 7 presents the dependence of the peak current of the three peaks in relation to increasing concentrations of RIF into the incubated mixture.

The same experimental procedure was performed by using single-stranded DNA. Table 4 presents the peak current response at pH 5.0 of the immobilized mixture after incubation of ssDNA with a constant concentration of RIF equal to 10^{-7} in relation to different incubation periods. Fig. 8 de-

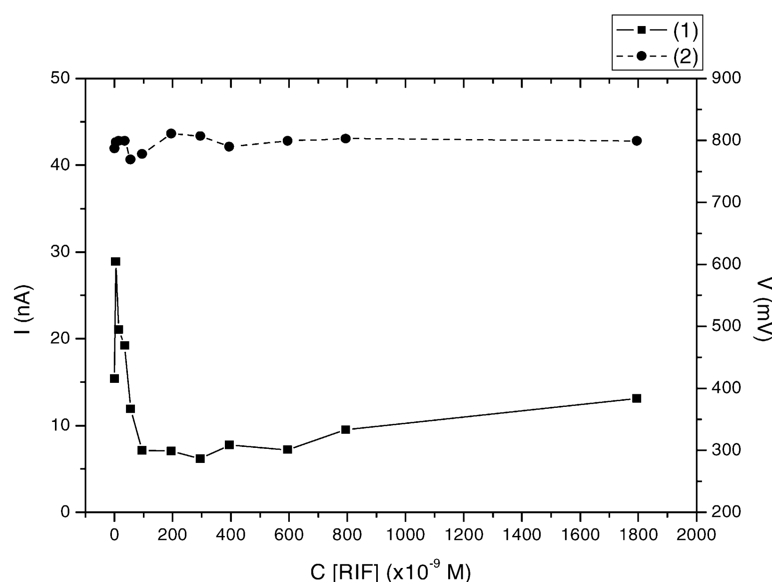

Fig. 5. (1) Dependence of peak current of oxidation of guanine residues in dsDNA on increasing concentrations of RIF after incubation of stock dsDNA with the drug to 0.2 M acetate buffer pH 5.0 for 5 min. (2) Dependence of the characteristic oxidation potential at +0.799 V in relation to increasing concentrations of RIF under the above conditions.

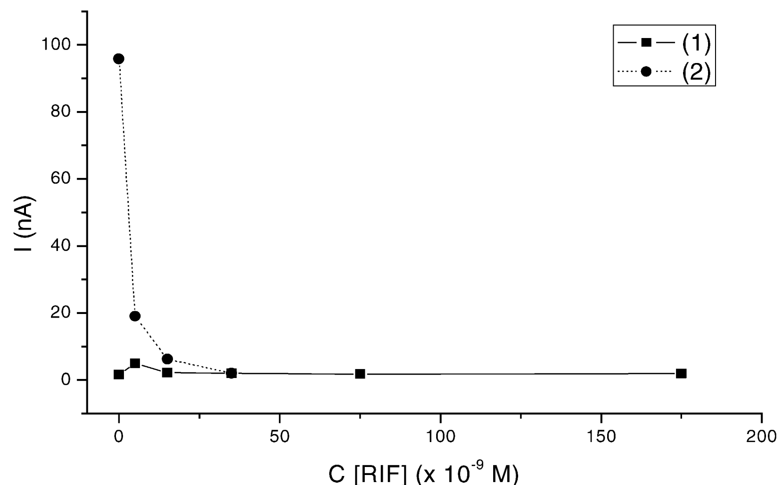

Fig. 6. (1) Dependence of peak current of oxidation of guanine residues in dsDNA on increasing concentrations of RIF after incubation of stock dsDNA with the drug to 0.2 M acetate buffer pH 5.0 for 10 min. (2) Dependence of the characteristic peak current probably due to the amount of RIF in excess on increasing concentrations of RIF under the above conditions.

picts the dependence of the peak current after the incubation of ssDNA with RIF in relation to increasing concentrations of RIF into the incubated solution.

3.4. Detection of RIF in serum samples

The two different electrodes, carbon paste and dsDNA-modified carbon paste, were applied in order to detect RIF in bovine serum samples. The measurements were performed at pH 5.0. The electrochemical behavior of the blank was also examined in order to compare any differentiations of the baseline before and after the addition of the sample into the buffer solution.

In the first case, the CPE was first pretreated as described previously, was subsequently immersed into the buffer solution containing the sample and differential pulse voltammetry was performed. The detection limit obtained equals the concentration of 0.25×10^{-7} M. In the second case, the dsDNA-modified electrode was prepared as already described and was immersed into the buffer solution containing the sam-

Table 4
The effect of incubation time between ssDNA (0.1 g/L) and a constant concentration of RIF (10^{-7} M) into the solution at pH 5.0

Incubation time (min)	I(nA) ssDNA peak at + 1.035 V	I(nA) peak at +0.713 V
5	205.96	251.27
15	20.58	204.18
30	1.76	148.87
50	0.85	111.6
80	0.75	81.48
125	0.63	61.7

The incubated mixture was immobilized on the CPE as previously described.

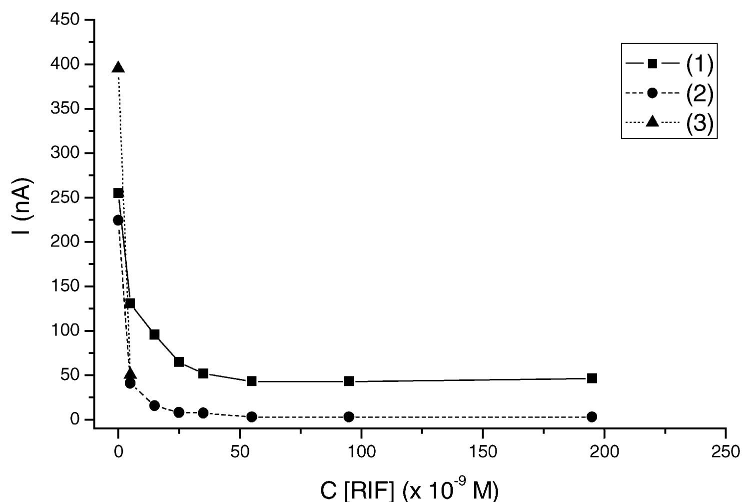

Fig. 7. (1) Dependence of the characteristic peak current probably due to the amount of RIF in excess on increasing concentrations of RIF after incubation of stock dsDNA with the drug to 0.2 M phosphate buffer pH 7.4 for 10 min. (2) Dependence of peak current of oxidation of: guanine residues in dsDNA on increasing concentrations of RIF under the above conditions and (3) adenine residues in dsDNA on increasing concentrations of RIF under the above conditions.

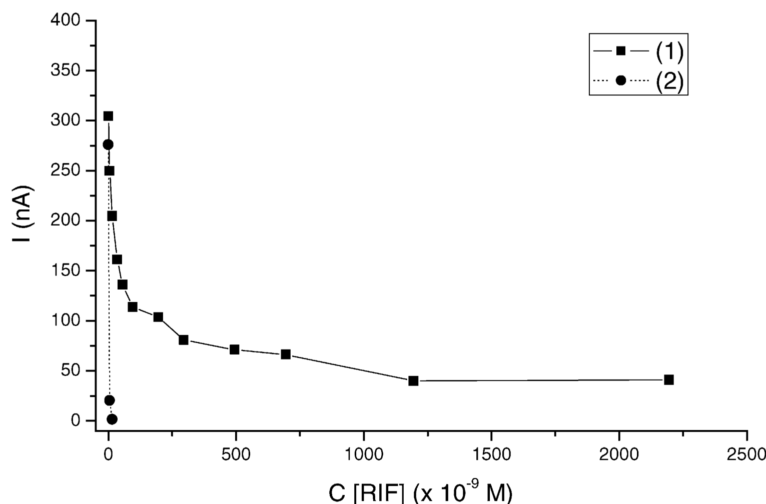

Fig. 8. Dependence of peak current of oxidation of: (1) guanine residues in ssDNA on increasing concentrations of RIF after incubation of stock ssDNA with the drug to 0.2 M acetate buffer pH 5.0 for 5 min and (2) adenine residues in ssDNA on increasing concentrations of RIF under the above conditions.

ple and differential pulse voltammetry was performed. The detection limit equals the concentration of 8×10^{-15} M.

As shown by the above values, the modified electrode is more sensitive and thus suitable for the detection of smaller amounts of RIF [32].

4. Conclusions

In this paper, we have shown that RIF can be detected using a DNA-modified carbon paste electrode by applying differential pulse adsorptive transfer stripping voltammetry. The differences occurred to the electrochemical signals of the two forms of DNA can be used in order to monitor the interaction between DNA and RIF. Although RIF is used as a drug against antituberculosis, it is proved that its function is closely related to its complexation with DNA. This electrochemical system can be used not only to detect very low concentrations of RIF, but in order to clarify its reaction mechanism concerning its role into the cellular function. In addition, it can be used as model system in order to compare the behavior of newly synthesized drugs against tuberculosis.

In conclusion, DNA-sensor technology has become a powerful tool applicable to the detection of compounds presenting affinity for DNA [33], the clarification of their reaction mechanism and the comparison of model compounds with compounds, such as drugs, polymers used in drug delivery and any kind of chemical substance having potent effect into the DNA structure.

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References

[1] K. Hashimoto, K. Ito, Y. Ishimori, Anal. Chim. Acta 286 (1994) 219–224.
[2] Ullmann's Encyclopedia of Industrial Chemistry, vol A2, VCH, 1985, pp. 496–497.
[3] A. Walubo, P. Smith, P.I. Fold, J. Chromatogr. B 658 (1994) 391–396.
[4] F.A. Benetton, E.R.M. Kedor-Hackmann, M.I.R.M. Santoro, V.M. Borges, Talanta 47 (1998) 639–643.
[5] A. Espinoza-Mansilla, M.I. Acedo Valenzuela, A. Munoz DelaPena, F. Salinas, F. Canada Canada, Anal. Chim. Acta 427 (2001) 129–136.
[6] M.A. Alonso Lomilo, O. Domínguez Renedo, N.J. Arcos Martinez, Anal. Chim. Acta 449 (2001) 167–177.
[7] I. Moser, T. Schalkhammer, F. Pittner, G. Urban, Biosens. Bioelectron. 12 (1997) 729–737.
[8] F. Yen, A. Erdem, B. Meric, K. Kerman, M. Oszoz, O.A. Sadik, Electrochem. Commun. 3 (2001) 224–228.
[9] J. Wang, Nucl. Acids Res. 28 (2000) 3011–3016.
[10] A. Erdem, K. Kerman, B. Meric, U.S. Akarca, M. Ozsoz, Anal. Chim. Acta 422 (2000) 139–149.
[11] J. Wang, G. Rivas, J.R. Fernandes, J.L. Lopez Paz, M. Jiang, R. Waymire, Anal. Chim. Acta (1998) 375.
[12] G. Marrazza, G. Chiti, M. Mascini, Clin. Chem. 46 (2000) 31–37.
[13] D. Ozkan, H. Karadeniz, A. Erdem, M. Mascini, M. Ozsoz, J. Pharm. Biomed. Anal. 35 (2004) 905–912.
[14] A.M. Oliveira Brett, T.R.A. Macedo, D. Raimundo, M.H. Marques, S.H.P. Serrano, Biosens. Bioelectron. 13 (1998) 861–867.
[15] J. Wang, G. Rivas, X. Cai, H. Shirashi, P. Farias, N. Dontha, D. Luo, Anal. Chim. Acta (1996) 332.
[16] V. Brabec, Electrochim. Acta 45 (2000) 2929–2932.
[17] M.A. La-Scalea, S.H.P. Serrano, E.I. Ferreira, A.M. Oliveira Brett, J. Pharm. Biomed. Anal. 29 (2002) 561–568.
[18] B. Meric, K. Kerman, D. Ozkan, P. Kara, A. Erdem, O. Kucukoglu, E. Erciyas, M. Ozsoz, J. Pharm. Biomed. Anal 30 (2002) 1339–1346.
[19] H. Karadeniz, B. Gulmez, F. Sahinci, A. Erdem, G. Irem Kaya, N. Unver, B. Kivcak, M. Ozsoz, J. Pharm. Biomed. Anal. 33 (2003) 295–302.

[20] E. Palecek, Electroanalysis 8 (1996) 7–14.
[21] M. Fojta, L. Havran, J. Fulneckova, T. Kubicarova, Electroanalysis 12 (2000) 926–934.
[22] E. Palecek, M. Fojta, F. Jelen, Bioelectrochemistry 56 (2002) 85–90.
[23] M. Rice, Z. Galus, R.N. Adams, J. Electroanal. Chem. 143 (1983) 89–102.
[24] J. Wang, M. Chicharro, G. Rivas, X. Cai, N. Dontha, P.A.M. Farias, H. Shiraishi, Anal. Chem. 68 (1996) 2251–2254.
[25] J. Wang, G. Rivas, D. Luo, X. Cai, F.S. Valera, N. Dontha, Anal. Chem. 68 (1996) 4365–4369.
[26] A.M.O. Brett, S.H.P. Serrano, T.A. Macedo, D. Raimundo, M.H. Marques, M.A. La-Scalea, Electroanalysis 8 (1996) 992–995.
[27] A.J. Bard, M.T. Carter, M. Rodríguez, J. Am. Chem. Soc. 111 (1989) 8901–8911.
[28] I.Ch. Gherghi, S.Th. Girousi, A. Pantazaki, A.N. Voulgaropoulos, R. Tzimou-Tsitouridou, Intern. J. Environ. Anal. Chem. 83 (2003) 693–700.
[29] I.Ch. Gherghi, S.T. Girousi, A.N. Voulgaropoulos, R. Tzimou-Tsitouridou, J. Pharm. Biomed. Anal. 31 (2003) 1065–1078.
[30] I.Ch. Gherghi, S.Th. Girousi, A.N. Voulgaropoulos, R. Tzimou-Tsitouridou, Talanta 61 (2003) 103–112.
[31] I.Ch. Gherghi, S.Th. Girousi, A.N. Voulgaropoulos, R. Tzimou-Tsitouridou, Anal. Chim. Acta 505 (1) (2004) 135–144.
[32] I.Ch. Gherghi, S.Th. Girousi, A.N. Voulgaropoulos, R. Tzimou-Tsitouridou, Intern. J. Environ. Anal. Chem., in press.
[33] I.Ch. Gherghi, S.Th. Girousi, A.N. Voulgaropoulos, R. Tzimou-Tsitouridou, Chem. Anal. (Warsaw), in press.