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Voltammetric Determination of Underivatized Oligonucleotides on Graphite Electrodes Based on Their Oxidation Products

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A new electrochemical method to determine underivatized oligonucleotides is developed. The electro-oxidation of the adenine moieties of adsorbed oligonucleotides at elevated potentials on pyrolytic graphite electrodes (PGE) in neutral or alkaline media gives rise to electroactive products strongly adsorbed on the electrode surface. The extent of the redox processes of these products, with $E^{\circ\prime}$ close to 0 V (vs Ag |AgCl) at pH 10, correlates well with the amount of parent oligonucleotide. Various electrochemical techniques have been compared and applied to the detection of specific DNA sequences and synthetic homopolynucleotides. Detection limits of 2 and 10 ng for $(dA)_{20}$ and a 21-mer sequence of HIV-1, respectively, have been achieved using sample volumes of 10 μL. Moreover, the adsorbed oxidized oligonucleotide shows electrocatalytic activity toward the oxidation of NADH. The capability of the new method to detect DNA hybridization is discussed.

INTRODUCTION

Nucleic acid analysis presently encompasses a wide range of fields, from molecular pathology to environmental or forensic sciences.[1] This fact has encouraged chemists to develop faster, easier, and more sensitive methods to meet the different demands in these areas.[2]

Electrochemical devices have been revealed as promising tools for these purposes.[3] Although some works are focused on the use of covalently attached redox active labels to target DNA, the analysis of underivatized nucleic acids is highly desirable if contamination derived from sample handling and cumbersome labeling procedures are to be avoided. Within this aim, two major approaches involving direct and indirect methods have been developed.

Indirect methods are based on the detection of electroactive indicators that intercalate or otherwise associate with double-stranded DNA.[4−7] Direct methods mostly rely on the intrinsic electroactivity of the nucleobases[8] pioneered by Palecek in 1960,[9] although a recently published method makes use of the electrocatalytic oxidation of the sugar moieties at copper microelectrodes.[10] Nucleobases undergo reduction at mercury electrodes and oxidation at carbon electrodes; however, the use of these processes as analytical signals is limited, because they occur at such extreme potentials that the peaks merge into the background discharge current at low concentration, resulting in detection limits close to 10 ng when combined with an adsorptive accumulation step.[8,11] A number of articles have been published to overcome this drawback. Sophisticated background-subtraction programs have been used to obtain well-defined oxidation peaks on a flat baseline in combination with constant-current chronopotentiometric analysis[12,13] or square wave voltammetry (SWV).[14] Adsorptive transfer protocols aimed to adapt electrochemical techniques to microliter sample volumes, as required to detect small amounts of DNA[15,16] have been developed. A different approach has been proposed by Palecek et al.[17] on the basis of the anodic signal, attributed to a product of the reduction of guanine residues, which appears far enough from the discharge current on a hanging mercury drop electrode. In this way, these authors[17] detected at least 0.1 $\mu g \cdot \mu L^{-1}$ of denatured DNA. Although the sample volume reported was 1.2 mL,[17] volumes of 5 μL have already been used in connection with adsorptive transfer experiments and HDME.[8] The electrocatalytic oxidation of guanine moieties within DNA or RNA mediated by $Ru(bpy)_3^{3+}$ has been used as a new scheme to

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improve the detection limits, although the high applied potential is not reduced.[18,19]

Our research group recently reported that the electro-oxidation of the adenine moiety within the cofactor nicotinamide adenine dinucleotide (NAD^+) and other adenine-containing nucleotides[20,21] on carbon electrodes yields strongly adsorbed electroactive products that show high electrocatalytic activity toward NADH oxidation. Furthermore, to find an NADH oxidation catalyst from the electro-oxidation of adenine-containing oligonucleotides, we started a project based on the idea that the polymeric nature of the oxidation products would lead to a more stable catalyst-modified electrode. In the course of this research, we found that the oxidation of different oligonucleotides in alkaline solutions at high applied potentials produces a redox system that leads to the development of a very useful electrochemical method for DNA detection.

Here, we describe a new method to detect unlabeled oligonucleotides at low potentials using conventional electrochemical instrumentation, that is, without using background-subtraction programs. The applicability of this methodology to random sequences of nucleobases and its capability to detect specific sequences of pathogens are demonstrated. The ability of the oxidation products to electrocatalize the oxidation of NADH and the possibility of using this catalytic activity to enhance the detection of oligonucleotides are discussed in detail.

EXPERIMENTAL SECTION

Chemicals. The deoxyadenylic acid icosanucleotide, $(dA)_{20}$, the deoxyguanylic acid icosanucleotide, $(dG)_{20}$, the 21-mer synthetic oligonucleotides corresponding to a portion of the U5 region of the long terminal repeat of immunodeficiency virus type I DNA segment (HIV-1) and to the pathogen *Chlamydia trachomatis* (*C. trachomatis*) were purchased as desalted products from Sigma-Genosys (London, UK). Polydeoxyadenylic acid (Poly(dA), Catalogue no. P0887), polythymidylic acid (Poly(dT), Catalogue no. P1012), polydeoxycytidylic acid (Poly(dC), Catalogue no. P5444), polydeoxyadenylic acid·polythymidylic acid (Poly(dA)·Poly(dT), Catalogue no. P9764) were purchased as sodium salts from Sigma. Concentrated saline sodium phosphate EDTA (20 × SSPE; 0.2 M sodium phosphate, 3 M NaCl, 0.02 M EDTA) buffer solution (Catalogue no. S2015) and NaCl (Catalogue no. S3014), both reactants DNase- and RNase-free, were acquired from Sigma. The base sequences are as below:

HIV-B: 5′-ATG-TGG-AAA-ATC-TCT-AGC-AGT-3′

HIV-A: 5′-ACT-GCT-AGA-GAT-TTT-CCA-CAT-3′

C. trachomatis-A: 5′-CAC-AGG-CAG-AGC-TTG-CAA-GGA-3′

C. trachomatis-B: 5′-TCC-TTG-CAA-GCT-CTG-CCT-GTG-3′

Stock solutions of 1 $g \cdot L^{-1}$ oligo- or polynucleotides were stored at 4 °C and diluted with 2× SSPE buffer solution (dilution 1:10 of 20× SSPE solution) prior to use as needed for the specific experiment. All of the solutions were prepared with water purified with a Milli-Q (Millipore) system. The cofactor nicotinamide adenine dinucleotide (reduced form, NADH, Catalogue no. N8129) was acquired from Sigma (Spain). Other chemicals employed were of analytical grade.

Apparatus. Voltammetric and chronoamperometric experiments were performed using an Autolab PGSTAT10 electrochemical analyzer (Eco Chemie B. V., Utrecht, The Netherlands). All of the measurements were made using a conventional three-electrode system comprising a pyrolytic graphite electrode (PGE) as the working electrode, a platinum wire as the auxiliary electrode, and a silver/silver chloride/saturated potassium chloride electrode as the reference electrode, which all the potentials were referred to.

When potentials lower than −0.3 V were applied, deaeration of the solution was carried out by passage of a stream of nitrogen (N-45, Air Liquide, Spain) for at least 10 min. The inert atmosphere was maintained during the subsequent experiment.

The working electrode was homemade. We used a 3-mm-diameter pyrolytic graphite rod (Goodfellow, U.K.) sealed into a Teflon holder with epoxy resin. The electrical contact was a brass rod welded to graphite with a silver-loaded epoxy resin (RS, U.K.). The renewal of the graphite surface was achieved by polishing with wet fine sandpaper, washing in a ultrasonic bath for few minutes, and smoothing with a clean filter paper.

Procedure. The smoothed graphite surface was pretreated prior to each measurement by six cyclic potential scans from −0.2 to +1.4 V at 0.1 $V \cdot s^{-1}$ and two additional scans from −0.2 to +0.5 V at 0.05 $V \cdot s^{-1}$ in a 0.1 M phosphate solution, pH 9.

Most experiments consisted of three steps: oligonucleotide adsorption, oxidation of the immobilized oligonucleotide, and measurement of the resulting products. The adsorption was performed by placing a 10-μL droplet of oligonucleotide in 2× SSPE solution onto the inverted electrode and evaporating it to dryness in a stream of warm air. Then the electrode was dipped into the stirred and free oxygen oxidation solution (phosphate 0.1 M, pH 12). The electrode potential was held at −0.4 V for 30 s. Upon this short conditioning period, the stirring was stopped, and the adsorbed oligonucleotide was oxidized either by cyclic voltammetry or by applying a constant potential of +1.1 V for 5 s. When cyclic voltammetry was used, 40 potential sweeps at 7 $V \cdot s^{-1}$ were scanned from −0.45 to +1.3 V. The electrode was, then, briefly rinsed with water and placed in a stirred measurement solution, typically phosphate 0.1 M, pH 7, for 2 min. After that, cyclic voltammograms were recorded at 0.05 $V \cdot s^{-1}$ from −0.2 to +0.5 V. Surface coverage, Γ, is a measure of the efficiency of the previous treatment. Γ was always determined by cyclic voltammetry (CV) from the integrated charge under the anodic wave, assuming a two-electron process per adenine unit, according to the conclusions of our previous report on other adenine-containing nucleotides.[20,21] Successive measurements were carried out by renewing the surface and repeating the above assay.

RESULTS AND DISCUSSION

Single-Stranded Oligonucleotides. Since the late 1970s, it has been established that the adenine and guanine residues in nucleic acids can be oxidized at carbon electrodes.[22,23] The oxidizability of both bases depends, predominantly, on the

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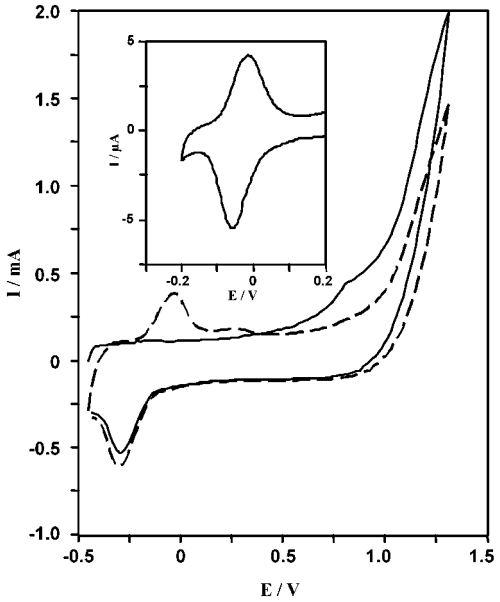

Figure 1. Cyclic voltammograms obtained in 0.1 M phosphate, pH 12, for 700 ng of $(dA)_{20}$ adsorbed on a PGE: solid line, first potential scan; dashed line, second scan; scan rate, 7 V·s^{-1}. (Inset) Cyclic voltammogram obtained afterward. Background solution, phosphate 0.1 M pH 10; scan rate, 0.05 V·s^{-1}.

secondary structure of the polynucleotide. Single-stranded nucleic acids give rise to greater oxidation signals than do double-stranded ones because of their flexibility and better accessibility to the electrode surface. These studies pointed out that polynucleotides are firmly adsorbed at pyrolytic graphite surfaces and so are their electro-oxidation products.

In our preliminary studies, 700 ng of $(dA)_{20}$ was adsorbed onto the graphite electrode surface. Then the electrode was subjected to the previously described cyclic oxidation procedure (Experimental Section). The processes taking place (Figure 1) are analogous to those previously described for the oxidation of different monomeric adenine nucleotides.[20,21] In the first anodic scan, an oxidation peak appears that is attributed to the oxidation of the adenine residues. The oxidation products form a quasireversible redox system at potentials near 0 V. It can hardly be observed when the oxidation step is performed at low scan rates (typically 0.05 V·s^{-1}), but it is perfectly defined if the oxidation step is conducted at scan rates of 1 V·s^{-1} or higher. If the anodic vertex of the cyclic scan is lower than +0.8 V at pH 12 or if it is carried out in the absence of the oligonucleotide, this reversible process is not observed at all, so it can only be attributed to products of the electro-oxidation of the oligonucleotide. As expected, these products are strongly adsorbed on the graphite surface. This result was verified by transferring the electrode to a free-oligonucleotide 0.1 M phosphate solution, pH 10 (Figure 1, inset). As shown in this figure, a well-defined redox system with a formal potential (E°′) of −0.036 V appears. This value is in good agreement with those previously reported for different adenine-containing nucleotides.[20,21] The voltammogram shape is that expected for a surface-immobilized redox couple (ΔE = 15 mV at pH 7, 45 mV at pH 10). The existence of a linear relationship between the peak currents of the reversible system and the scan rate (from 2 to 300 mV·s^{-1}) also confirms the adsorption nature of the process.

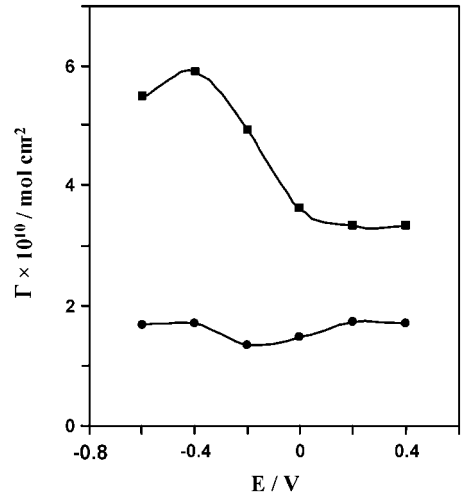

Figure 2. Effect of conditioning potential in a phosphate (■) 0.1 M and (●) 0.005 M solution, pH 12, on the amount of oxidized oligonucleotide obtained with a modified PGE prepared from 700 ng of $(dA)_{20}$. Conditioning time, 30 s; oxidation step at +1.1 V for 5 s in the same solution; measurement solution, 0.1 M phosphate, pH 7.

The formal potential (E°′) of the surface redox couple is pH-dependent in the pH range tested ($E^{\circ\prime}$ = 0.51−0.055 pH; r = 0.9991; n = 7; 7 ≤ pH ≤ 12). The experimental slope, 55 mV/pH unit, is consistent with the anticipated Nernstian value for a process involving the same number of electrons as protons.

To obtain the maximum efficiency in the adenine-moiety oxidation process, the influence of the H^+ concentration in the oxidation solution should be examined. The same redox system was obtained in all the oxidation media tested (0.1 M phosphate media of pH 7−12 and NaOH solutions between 0.032 and 0.1 M). A sharp increase in the measured surface coverage takes place for oxidation from pH 10 up to pH 12, where the amount of adenine oxidation products reaches the maximum value. A phosphate solution of pH 12 was used as optimum medium to generate the oxidation products henceforth.

Ideally, nucleic acid tests should be simple, low-cost, and easy to automatize.[3] Using double-step chronoamperometry instead of CV during the oxidation step should better fulfill these criteria. After a conditioning step, a symmetrical double potential step format from −0.45 V was applied in order to maintain the oligonucleotide or its oxidation product in their corresponding reduced form. The pulse amplitude and width were optimized. Pulse amplitude must be high enough to allow the oligonucleotide oxidation and, at the same time, low enough to avoid the background being oxidized in a large extent.

The products of this undesirable process might contribute to desorption of the product of interest, so an optimum value should be found. The optimum amplitude value was found to be 1.55 V for a 5-s period.

Before performing the oxidation step, the electrode with the adsorbed oligonucleotides should be conditioned in the phosphate 0.1 M pH 12 oxidation solution. The conditioning potential E_c and length of this step was found to affect the efficiency of the following oxidation. Figure 2 shows the influence of E_c. A maximum Γ is achieved when E_c = −0.4 V. Less negative conditioning potentials produce smaller Γ values, and a steady regime appears from E_c between 0 and +0.4 V, as in previous works.[13,16] These results

are against those expected from the pure electrostatic attraction corresponding to the polyanionic nature of oligonucleotides. In fact, electrostatics predicts that at such negative potentials, rejection of the oligonucleotides from the negatively charged surface would occur. However, the observed phenomenon can be explained if oligonucleotides ionically shielded by ion-pairing between buffer cations and phosphate group anions in a single strand become the adsorbed species.[24] This point was confirmed using a 0.005 M phosphate buffer, pH 12, as the background solution (Figure 2). In this case, and for all the potentials tested, smaller amounts of oxidation products were found in comparison to those obtained in 0.1 M phosphate solution. This might be due to the lesser screening capability of the dilute solution.

At such negative potentials, rearrangements in the adsorbed layer of oligonucleotide driven by electrostatic stabilization cannot be ruled out. In this way, the hydrophobic bases might more easily adsorb flat onto the electrode surface and would be closer to it. This effect might contribute to the enhancement of their oxidizability. In fact, a conditioning period of at least 30 s is needed to achieve the largest surface coverage. Additionally, longer times tested (up to 300 s) do not imply a decrease in Γ, suggesting that the adsorption of the oligonucleotide is very strong, even at this negative potential. Consequently, a conditioning step of 30 s at −0.4 V was finally selected.

The bioanalytical utility of the present methodology will be based on the correlation between the voltammetric response of the generated oxidation product and the amount of parent oligonucleotide used. To evaluate this correlation, we prepared electrodes from different amounts of $(dA)_{20}$, as was established. The charge obtained from the integrated CV anodic peak of the oxidized oligonucleotide at pH 7 was plotted versus the amount of parent oligonucleotide and the linear relationship, $Q(C) = 40 \cdot [g\ (dA)_{20}] - 2 \times 10^{-8}$; ($r = 0.9991$; $n = 9$), was found between 2 and 80 ng of $(dA)_{20}$. From the calibration slope, the percentage of oxidized adenine moieties that yields the electroactive product can be roughly estimated. Taking, as previously, 2 electrons/unit of oxidized adenine, only 6.4% of the adenine moieties was estimated to be effectively oxidized and, at the same time, to exhibit redox activity.

Different voltammetric techniques using nonlinear potential scans, such as differential-pulse voltammetry (DPV), square wave voltammetry (SWV), and alternating current voltammetry (a.c.V) were employed in the measurement step. Instrumental parameters were optimized. Optimal values and signals for the oxidized $(dA)_{20}$ obtained with each technique are shown in Figure 3.

Linear relationships between peak height and the initial amount of $(dA)_{20}$ were found with all techniques investigated. The resulting calibration data are shown in Table 1. The detection limits were estimated as the concentration giving a signal three times the standard deviation of the blank signal, calculated from the calibration slope. These results are higher than those obtained using advanced baseline corrections and similar to those obtained using the direct anodic process of guanine.[11] Repetitive measurements of 20 ng of parent $(dA)_{20}$, requiring a new 10-μL sample drop for each evaporation/oxidation/measurement cycle, resulted in a mean peak height (DPV) of (1.8 ± 0.3) μA with a relative standard deviation (RSD) of 15.8%.

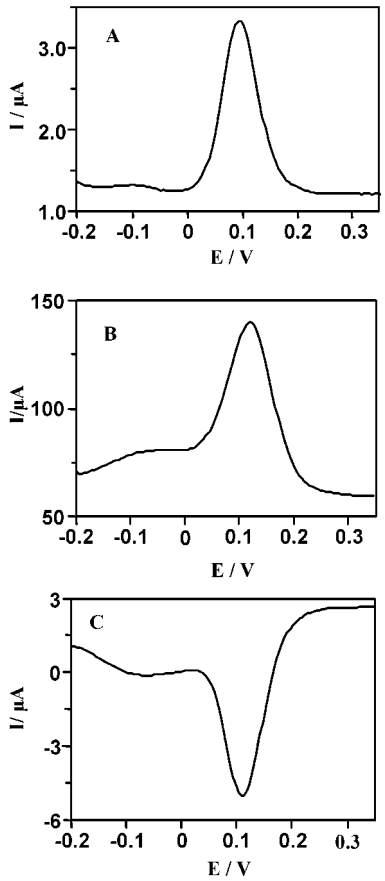

Figure 3. Voltammetric signals obtained in 0.1 M phosphate, pH 7, with a PGE modified with an adsorbed and oxidized amount of 20 ng of $(dA)_{20}$: (A) DPV, (B) SWV, and (C) a.c.V. Instrumental parameters: (DPV) pulse amplitude (ΔE), 0.050 V; modulation time, 0.08 s; interval time, 0.8 s. (SWV) ΔE, 0.050 V; frequency, 30 Hz. (a.c.V) ΔE, 0.01 V; frequency, 60 Hz; phase angle (φ), +60°.

Table 1. Calibration Data for the Determination of $(dA)_{20}$

technique	a[a]	b[b]	r	n	linear range, ng	LOD, ng
DPV	$-2.1 \cdot 10^{-7}$	110	0.9993	9	2−100	2
SWV	$-2 \cdot 10^{-6}$	2930	0.9992	6	2−40	1.9
a.c.V	$-5 \cdot 10^{-7}$	330	0.999	5	2−20	1.9

[a] Intercept expressed in A. [b] Slope expressed in $A \cdot g^{-1}$.

The detection scheme was also applied to determine specific 21-mer sequences (HIV-1 U5 LTR DNA segment and *C. trachomatis*) to test its applicability to oligonucleotides containing the four most common randomly distributed nucleobases. Linear calibration plots are achieved for both oligonucleotides (Table 2). The obtained detection limits are a bit higher than those for $(dA)_{20}$, as expected, as a result of the fewer adenine units per molecule (7 and 20, respectively).

It is well-known that at carbon electrodes, guanine undergoes oxidation at lower potentials than adenine.[22] Nonetheless, under the experimental conditions used in this work, $(dG)_{20}$ did not yield any oxidation product (Figure 4B). It was necessary to raise by 1 order of magnitude the largest amounts of $(dA)_{20}$ employed here and to work under different conditions to observe, upon the oxidation of $(dG)_{20}$, a small redox system at potentials around 100

Table 2. Calibration Data for the Determination of VIH-B Sequence (above) and *C. trachomatis*-A (below)

technique	a[a]	b[b]	r	n	linear range, ng	LOD, ng
DPV	$-1.0\cdot10^{-7}$	14.2	0.9991	6	10−200	10
SWV	$-9\cdot10^{-7}$	482	0.9995	5	10−100	10
a.c.V	$-3\cdot10^{-8}$	32.6	0.9992	5	10−100	10
DPV	$-5\cdot10^{-8}$	15.8	0.9992	7	10−120	10
SWV	$-4\cdot10^{-7}$	680	0.998	5	10−100	10
a.c.V	$-2\cdot10^{-6}$	72	0.9996	4	20−100	20

[a] Intercept expressed in A. [b] Slope expressed in $A\cdot g^{-1}$.

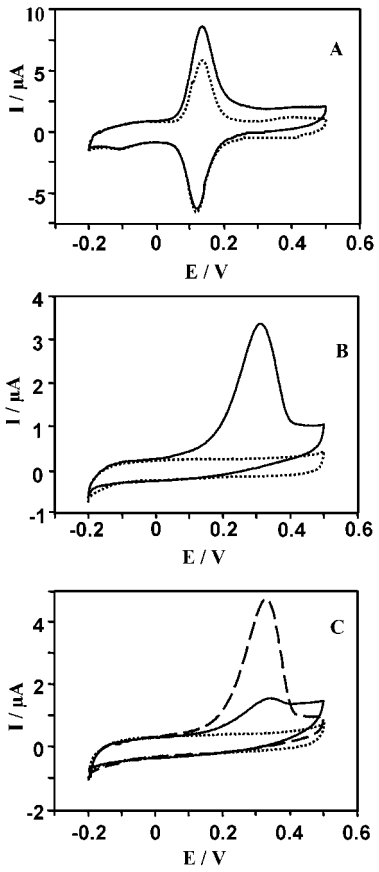

Figure 4. Cyclic voltammograms registered with (A) PGE modified with oxidized $(dA)_{20}$ (700 ng); (B) PGE modified with oxidized $(dG)_{20}$ (700 ng); (C) dotted and solid lines, PGE modified with non-oxidized $(dA)_{20}$ (700 ng); dashed line, bare PGE. Dotted lines, background; solid and dashed lines, $1\cdot10^{-4}$ M NADH; scan rate, 0.05 $V\cdot s^{-1}$; background, 0.1 M phosphate, pH 7.

mV higher than those of the adenine oxidation products. Further investigations of the oxidation products of guanine and their derivatives and their potential catalytic activity toward NADH oxidation are being completed at our laboratory and will be published elsewhere.

According to our previous work, the immobilized product on the graphite surface is expected to show catalytic activity for NADH oxidation. Figure 4A shows cyclic voltammograms for an electrode prepared by the oxidation of 700 ng of $(dA)_{20}$ in the presence and absence of NADH (10^{-4} M). An enhancement in the oxidation current at 0.135 V and a decrease in the reduction current is observed, in agreement with an electrocatalytic effect. NADH is oxidized at the same potential as in a bare PGE treated in the same way when a drop of distilled water substitutes for the oligonucleotide solution and the electrode is equally treated, as is shown in Figure 4C. This result excludes any significant contribution of surface quinone groups generated during the application of the high potential pulse. In addition, the same result is obtained when a drop containing 700 ng of $(dG)_{20}$ is submitted to the same protocol (Figure 4B). Thus, the presence of guanine moieties within DNA structure does not interfere with the oxidation or the catalytic process.

When the oxidation step was substituted for holding the potential at −0.45 V, only the uncatalyzed oxidation wave could be observed (Figure 4C, solid line), which verifies that nonoxidized $(dA)_{20}$ is not catalytically active. The lower intensity current and higher peak potential might be due to the electrostatic repulsion between NADH and the oligonucleotide, both negatively charged at pH 7. In addition, the adsorbed oligonucleotide might block the adsorption sites to NADH on the electrode surface and the subsequent oxidation.

The catalytic process might then be useful for detecting the oligonucleotide with an improved sensitivity. To prove it, some different amounts of $(dA)_{20}$ between 6 and 15 ng were adsorbed onto the electrode surface and treated as usual. Then a DPV voltammogram was recorded in 0.1 M phosphate, pH 7, containing 10^{-4} M NADH. Very good linearity was found. These preliminary data suggest that the electrocatalytic method might permit reaching lower detection limits. This new approach needs further development and optimization that are currently being explored in our laboratory.

Double-Stranded Oligonucleotides. A most interesting application for environmental or clinical purposes is the detection of specific sequences of nucleic acids. The specific recognition event is made through hybridization experiments in which the target sequence interacts specifically with its complementary sequence (probe) to form the double-helix DNA.

We explored the capability of the proposed method to detect the formation of the double-stranded oligonucleotide, taking advantage of the lower oxidizability of the ds-DNA than the ss-DNA.[14] This should yield smaller amounts of oxidized DNA and consequently smaller voltammetric responses. To ascertain this assumption, we submitted increasing amounts of the commercially available duplex model helix poly(dA)·poly(dT) to the established protocol of oxidation and compared the obtained DPV responses with those from equivalent amounts of poly(dA) in terms of moles of adenine moieties. The open symbols in Figure 5 depict the voltammetric responses resulting from oxidation of equimolar increasing amounts of adenine moieties within duplex and single-stranded forms. As expected, oxidized duplex gave signals that were smaller than those from the single-stranded species, since the latter have their adenine moieties more accessible to oxidation than the former. The decrease of the DPV response might be used to detect the hybridization event.

A hybridization step was added to the method, and an aliquot of the resulting hybridization mixture was placed on the electrode and treated as previously. The hybridization step was performed in a high ionic strength buffer (2× SSPE, 0.4 M NaCl). First, increasing amounts of poly(dA) were made to hybridize with their complementary polynucleotide poly(dT) at a 1:1 molar ratio (Figure 5, filled circles). The obtained results compared well with

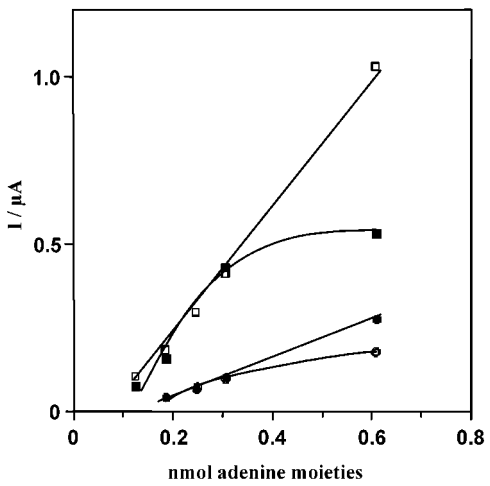

Figure 5. DPV responses obtained in 0.1 M phosphate, pH 7, with a PGE modified with increasing adsorbed and oxidized amounts of poly(dA) (□), commercial duplex poly(dA)·poly(dT) (○), hybridized poly(A)·poly(dT) (●) and poly(dA)·poly(dC) (■).

those obtained for the commercial duplex. When we tried to hybridize poly(dA) with the noncomplementary poly(dC), the voltammetric response was similar to the poly(dA) response (Figure 5, filled squares). This indicates that the presence of the other polynucleotide does not disturb, at least at the lowest amounts, the oxidation efficiency of poly(dA).

Most interesting is the application of the methodology to the detection of specific short sequences. Consequently, several parameters affecting the hybridization, such as hybridization time, temperature, and ionic strength (NaCl concentration), were optimized. The hybridization reaction was completed in <5 min. The NaCl concentration has a minor influence in the range tested (up to 1 M). High temperatures slightly improve hybridization efficiency. This process was finally allowed to develop for 5 min in a moderate concentration of NaCl, 0.4 M, at 37 °C.

Under such conditions, we hybridized 100 ng of $(dA)_{20}$ with increasing amounts of its complementary sequence $(dT)_{20}$. The oxidation current corresponding to the products of the oligonucleotides' oxidation diminishes linearly as the amount of complementary $(dT)_{20}$ is increased ($I/A = -43[\text{g } (dT)_{20}] + 1.21 \times 10^{-5}$; $r = 0.9992$; $n = 5$). The same scheme was applied to random sequences. 120 ng of *C. trachomatis*-A sequence were used as probe to detect increasing amounts of complementary *C. trachomatis*-B. Similar results were obtained.

The calibration equation is $I/A = -5.6[\text{g } / \textit{C. trachomatis}\text{-B})] + 1.22 \times 10^{-6}$ ($r = 0.996$; $n = 5$). We tried to apply this methodology to (HIV-A)·(HIV-B) hybridization but it failed. The signal decreases with increasing amounts of the complementary strand of HIV-A at first, but from a certain amount of added HIV-A, it began to increase. This is probably due to the very similar number of adenine moieties present in both sequences. The formation of the double helix produces a decrease in the signal but at the same time, more adenine moieties are added within the complementary sequence. Nevertheless, this tentative explanation requires elaboration, and further experiments are now in progress at our laboratory.

CONCLUSIONS

In this work, we show that the redox processes associated with the products of oligonucleotide electro-oxidation on graphite electrodes are good analytical signals for determining adenine-containing oligonucleotides. The greater performance of these systems for quantifying polynucleotides versus the anodic peak of the adenine moiety is related to reversibility, symmetry, and location at potential regions far away from the discharge current.

The strong adsorption of the oligonucleotides and their products makes the method very versatile. It allows for independent optimization of adsorption, oxidation, and measurement. The analysis can be made from media not suitable for conventional voltammetric measurements. Additionally, small sample volumes (10 μL) are required, as demanded in the most common applications of nucleic acids analysis, such as clinical diagnostics or environmental control.

However, adenine-free oligonucleotides could not be determined with this methodology. Better sensitivity is achieved when there is a higher percentage of adenine within the oligonucleotide.

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