A Promising Bioelectrode Based on Gene of *Mycobacterium leprae* Immobilized onto Poly(4-aminophenol)

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ABSTRACT: A new bioelectrode for gene detection of *Mycobacterium leprae*, also known as *Hansen's bacillus*, was produced by immobilizing of single-stranded DNA (ssDNA) with 78 bases long (specific gene related to *Mycobacterium leprae*) on graphite electrode modified with poly(4-aminophenol). This biosensing platform was able to recognize complementary DNA molecules via hybridization process. Hybridization between probe and target was monitored by voltammetry, using ferrocenecarboxyaldehyde as electrochemical DNA hybridization *indicator*. The hybridization of nucleic acid probe with the DNA target resulted in significant decrease in the oxidation peak current of ferrocenecarboxyaldehyde, indicating greater affinity of this compound for ssDNA than for double-strand DNA (dsDNA). The linear range of detection for the DNA target was found to be 0.35 – 35 ng/μL. ssDNA hybridization with the DNA target was also investigated by electrochemical impedance spectroscopy (EIS), showing significant modification in Nyquist plot, by modification in electrode surface after addition of the complementary target. The effective immobilization of specific gene of *Mycobacterium leprae* onto graphite electrode modified with poly(4-aminophenol) and the detection of the hybridization process with the DNA target, monitored by voltammetry and EIS indicate that this is a new and promising biosensing platform to gene detection of *Hansen's bacillus*.
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Key words: biological applications of polymers; coatings; electrochemistry; molecular recognition; sensors

INTRODUCTION

Leprosy is a chronic granulomatous infection, mainly affecting the skin and peripheral nerves, caused by the obligate intracellular organism *Mycobacterium leprae*, also known as *Hansen's bacillus*.[1] It is a very worrying health problem worldwide, most prevalent in Asia, Africa, and Latin America, with 218,605 new cases being detected in 2008.[2]

Leprosy is object of state intervention because, when untreated, it causes disability and deformity in the economically active population, due the secondary complications of the neuropathy.

Tools of molecular biology and immunology have been of great value for the diagnosis of leprosy and for the epidemiological research.[3,4] The techniques of polymerase chain reaction (PCR) and enzyme immunoassay (ELISA) enable the detection of *Mycobacterium leprae* with high sensitivity and specificity.[5] However, these techniques have an obvious drawback due to demanded time, qualified people, and high cost.[6] Such problems can be circumvented by using low cost tools, simple, and sensitive handling as biosensors.

DNA electrochemical biosensor consists of single-strand DNA (ssDNA) immobilized on the surface of the transducer, to recognize its complementary sequence. The double-strand DNA (dsDNA) formed on the surface of the electrode is known as hybrid. The analytical signal produced reflects the amount of captured DNA.[7]

The electrochemical detection of DNA hybridization can be performed through direct oxidation of DNA bases or through hybridization indicators, which are: mediators, intercalators, enzymes, or nanoparticles. The monitoring is carried out through oxidation-reduction indicators, or direct oxidation of guanosine in the double-strand DNA.[8]

An important parameter that ensures the sensibility of the biosensor is the signal response. To

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improve the DNA hybridization signal, the electrodes can be subjected to chemical modification of the surface to increase the reactivity of the sensor. These characteristics can be obtained with polymers generated electrochemically on different surfaces.[9–12]

The modification of surfaces with polymer films has been used in the development of biosensors to protect the surface of the electrodes from impurities, block interfering, incorporating mediators, and provide biocompatibility.[13]

The stage of the immobilization of biomolecules and indicators on the surface of the electrode plays an important role in obtaining of sensitivity of the biosensor. This is achieved by means of chemical control and coverage of the area, ensuring stability, accessibility, and reactivity to the immobilized biomolecule.[14]

The use of conducting polymer films is suitable to immobilize DNA probes.[14] This feature is due to easy processing of electrodes and conductivity, combined with the increase in the area of the biomolecules in contact with the electrode, which allows greater accommodation of the molecule, simulating their natural environment, favoring the conversion of the biological signal into measurable analytical signal, fast turnaround time and high stability.[15]

Our group has carried out studies aiming the development of electrodes modified with polymers derived of phenols and ethers, to immobilization of biomolecules.[10–12,16–18]

Electrodes modified with poly (4-aminophenol) were indicated to be effective for the immobilization of the guanosine triphosphate and adenosine triphosphate biomolecules.[10] The signal amplitude for the detection of these bases was twenty-four times greater when compared to the bare graphite electrode. 4-Aminophenol was used as monomer for electrodeposition of poly(4-aminophenol) due to the fact that it presents two oxidizable groups (NH_2 and OH) unlike the phenol and aniline. The presence of these functional groups is appropriate for biomolecule immobilization.[19]

The aim of this work was to develop a biolectrode based in the immobilization of the single stranded DNA (specific DNA fragment to *M. leprae*, 78 mer) on poly(4-aminophenol) matrix, providing a recognition surface for hybridization with the nucleic acid probe, being detected by voltammetry and EIS.

This is, to the best of our knowledge, the first report in the literature concerning to immobilization and detection in modified electrodes of specific DNA sequence to *M. leprae*.

EXPERIMENTAL

Materials and reagents

The electrochemical studies were carried using a graphite disk (6 mm diameter) as working electrode, cut from a graphite rod (99.9995%, Alfa Aesar). Platinum and a saturated calomel electrode (SCE) were used as counter and reference electrode, respectively.

A three-compartment electrode cell was used throughout the study. A potentiostat (420A model, CH Instruments, USA) was used for the electrochemical measurements with the exception of the impedance measurements which were performed with an Autolab PGSTAT20 (Eco Chemie, Holland) equipped with a FRA (Frequency Response Analyzer) module. The frequency interval covered extended from 10^{-3} to 10^5 Hz using signal amplitude of 5 mV (p/p).

All reagents were of analytical grade and used without further purification. All experiments were conducted at room temperature (25 ± 1°C).

Ultra high purity water (Master System, Gehaka, Brazil) was used for preparation of the 4-aminophenol (Acros Organics) solutions. Monomer solutions (2.5×10^{-3} mol L^{-1}) were prepared in 0.5 mol L^{-1} $HClO_4$ solution, immediately before use. For impedance measurements, standard solutions containing the redox pair $[Fe(CN)_6]^{4-}/Fe(CN)_6]^{3-}$ were prepared using $K_4Fe(CN)_6$ (5×10^{-3} mol L^{-1})/$K_3Fe(CN)_6$ (5×10^{-3} mol L^{-1}) in KNO_3 solution (0.1 mol L^{-1}).

Phosphate or acetate buffer solutions were prepared at pH 7.4 and 4.5, respectively.

The DNA hybridization buffer (SSC 6X) was prepared with sodium citrate 9×10^{-2} mol L^{-1}, pH 7.0, containing NaCl 0.9 mol L^{-1}.

For the DNA detection, 15 μL of ferrocenecarboxyaldehyde in methanol (4.8×10^{-3} mol L^{-1}) was added on the surface of the electrodes.

The PCR was performed in a final volume of 25 uL, containing 200 umol L^{-1} of dNTPs, 1.6 mmol L^{-1} of $MgCl_2$, 1X PCR buffer (Tris-HCl 10 mM, pH 9.0), 10 pmol of primers sense: 5′-GCA GTG GGC AGT AGG GTG AT-3′ and anti-sense: 5′-CAC CGA AAG CTC ATG GCC AC-3′, 1 U of Platinum Taq, 400 ng of genomic DNA. All reagents to this reaction were obtained from Invitrogen Life Technology.

After preparation of the mixture for PCR reaction, the reaction of sequencing was performed at the following PCR conditions: 95°C (1 min), 58°C (20 sec), 72°C (30 sec) - 36 cycles, 72°C (10 min), 4°C (10 mins). At the end of the cycling, samples were analyzed in 0.8% agarose gel stained with ethidium bromide under UV light. The size of the PCR product was 78 bp. Subsequently, the concentration of dsDNA was measured through the ultraviolet absorption at 260 nm ($\mu = 6600$ L mol^{-1} cm^{-1}). A tota of 15 μL of the PCR product (RLEP3) 3.93×10^{-8} mol L^{-1} was added onto the modified electrode surface.

The product obtained from PCR, sequenced below, was used as probe for the steps of immobilization and detection of the analyte.

5′-GCAGGCGTGAGTGTGAGGATAGTTGTTAGC
GCCGCGGGGTAGGGGCGTTTTAGTGTGCATGTC
ATGGCCTTGAGGTGT- 3′
3′-CGTCCGCACTCACACTCCTATCAACAATCG
CGGCGCCCCATCCCCGCAAAATCACACGTACA
GTACGGGAACTCCACT- 5′

Electrode surface modification

Prior to electropolymerization, the bare graphite electrode was mechanically polished with alumina (0.3 μm) slurry, ultrasonicated, washed with deionized water and dried in the air. 4-Aminophenol solution was deoxygenated through bubbling with N_2, prior to electropolymerization. The monomer 4-aminophenol was electropolymerized on graphite electrode through continuous cycling of the potential, according to Franco et al.[12]. After electropolymerization, the modified electrode was rinsed with deionized water to remove unreacted monomer.

Immobilization and hybridization of the PCR product (RLEP3)

RLEP3 was denatured at 98°C for 5 min. a total of 15 μL of denatured sample (3.93×10^{-8} mol $L^{-1)}$ was added onto the surface of the modified electrode. The electrode was kept at 98°C for 3 min, to assure the maintenance of ssDNA. After that, the modified electrode containing the probe (ssDNA) was washed by immersion in phosphate buffer, pH 7.4 during 5 sec and dried with ultra-pure N_2. A total of 15 μL of complementary target (ssDNA) 3.93×10^{-8} mol L^{-1} was added onto the surface of the modified electrode containing the probe. The hybridization was carried out at 42°C, at different times. According to the literature,[8] high sensitivity can be achieved to the modified electrode, using the procedure of hybridization at 42°C, washing in phosphate buffer and drying, as described previously. Ferrocenecarboxyaldehyde was added onto electrode surface. It was kept during 5 min and after that, the modified electrode was washed with phosphate buffer.

The electrochemical detection was made through voltammetry or pulse differential voltammetry using phosphate buffer solution (pH 7.4) as electrolyte.

Molecular modeling studies

The calculations were performed using Gaussian 03.[20] The geometry of all species was obtained through gradient minimization at the DFT (B3LYP) level of theory.[21,22] For the *ab initio* DFT B3LYP calculations, the 6-31G(d) atomic basis set was used. The geometry optimizations were considered complete always when a stationary point was reached.

Methodology of calculation of thermochemical values (variation of the Gibbs free energy) of the interaction ferrocenecarboxaldehyde/ssDNA and ferrocenecarboxaldehyde/dsDNA was based on the material available in http://www.gaussian.com/g_whitepap/thermo.htm.[23]

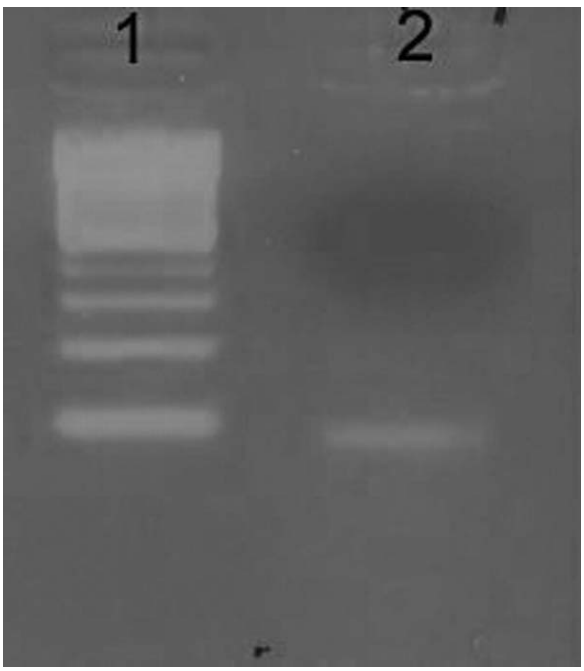

Figure 1 Agarose gel electrophoresis of PCR-amplified DNA, RLEP3. Line 1, DNA marker, 100 pb; line 2, 3 μl of the PCR-amplified product from *M. leprae*.

RESULTS AND DISCUSSION

Analysis, immobilization, and detection of the PCR product

Analysis of the PCR product

The analysis of the PCR product (RLEP3) by gel electrophoresis is shown in Figure 1.

The gel electrophoresis confirmed the amplification of PCR product with 78-bp, and the integrity of the DNA sequence.

Analysis of the nucleotides sequence (Table I) was performed using the Blast program (Basic Local Alignment Search Tool).

Immobilization of the PCR product

RLEP3 was immobilized on graphite electrode modified with poly(4-aminophenol), and the voltammetric behavior was studied in phosphate buffer (pH 7.4) and in acetate buffer (pH 4.5) (see Fig. 2).

TABLE I
The Sequence of PCR Product (RLEP3) has Identity with *M. leprae*

Analysis type/Access number	Organism	TBLASTn or BLASTp analysis E-value	Bit score
TBLASTn			
U00010.1	*M. leprae* cosmid B1170	1 E^{-12}	74.9
U00021.1	*M. leprae* cosmid L247	1 E^{-12}	74.9
M14341.1	*M. leprae* 65 kd antigen	1 E^{-12}	74.9
BLASTp			
CAB09904.1	Hypothetical protein MLCL383.30c [*M. leprae*]	1 E^{-9}	65.5
CAB11401.1	Hypothetical protein MLCB22.36c [*M. leprae*]	1 E^{-9}	65.5
CAA22930.1	Hypothetical protein MLCB2533.16 [*M. leprae*]	1 E^{-9}	65.5

TBLASTn: *M. leprae* amino acid sequence when compared with the translated genomic nucleotide sequence. BLASTp: *M. leprae* amino acid sequence when compared with the predicted open reading frame (ORFs) of fully sequenced genomes; E-value (Expectation value): number of different alignments with scores equivalent to or better than score that are expected to occur in a database search by chance. The lower the E value, the more significant the score; Bit score: percent sequence similarity used to compare alignment scores from different searches.

An important dependence is observed for the different buffers used. The peaks observed in Figure 2 are attributed to the oxidation of the bases guanosine monophosphate (I) and adenosine monophosphate (II). Figure 2 shows that the oxidation potentials of the purine bases are located at lower values in phosphate buffer when compared with acetate buffer (guanosine monophosphate: +1.15 V and +1.40 V; adenosine monophosphate: +1.36 V and +1.54 V), indicating that the detection of the purine bases in phosphate buffer is more adequate if the analytical detection of these bases is of concern, because lower potentials values are important to minimize the presence of interferents. Also, it was observed that the purine bases are easier to be oxidized than the pyrimidine bases, in agreement with the literature for the DNA nitrogenated bases.[10,24]

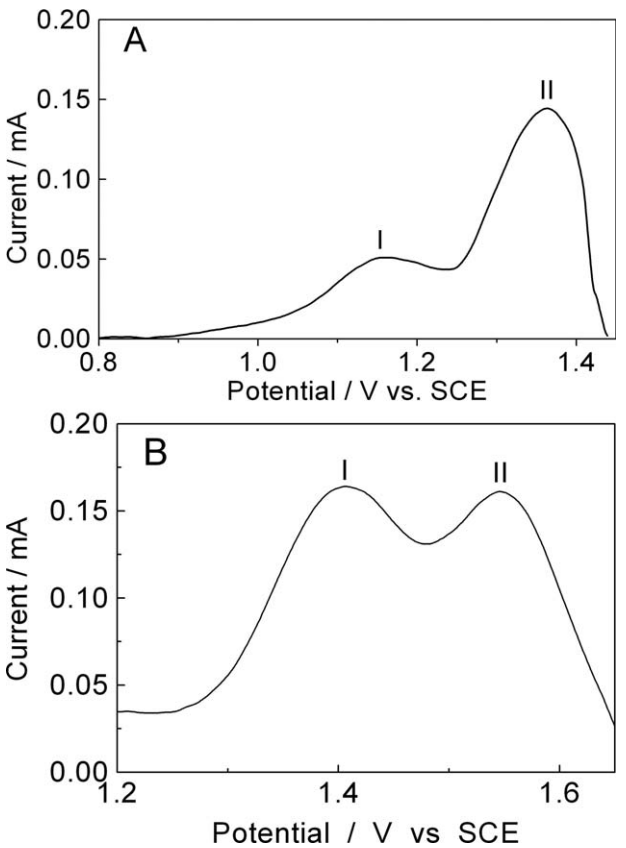

Figure 2 Differential pulse voltammetry of graphite electrode modified with poly(4-aminophenol) containing dsDNA, 78pb, of specific DNA fragment to M. leprae (—), in: (A) phosphate buffer, pH 7.4 or (B) acetate buffer, pH 4.5. Oxidation signal of guanosine (I) and adenosine (II); modulation amplitude: 0.05 mV; pulse interval: 0.2 s; 5 mVs^{-1}. The experimental curves were corrected for the background current.

DNA hybridization detection using ferrocenecarboxyaldehyde

For the preconditioning and polarization of the surface of graphite electrode modified with poly(4-aminophenol), it was submitted to successive potential scans from 0 V to +0.8 V vs. SCE in phosphate buffer, until the voltammograms remained constant.

An aliquot of sample diluted in hybridization buffer (SSC 6X) was subjected to thermal denaturation and dropped on surface of the graphite electrode modified with polymer film. The hybridization was observed through the oxidation potential of ferrocenecarboxyaldehyde (Fig. 3).

As shown on Figure 3 (curve b), the oxidation current of ferrocenecarboxyaldehyde decreases after the addition of the complementary strand. This decrease is associated to the lower concentration of

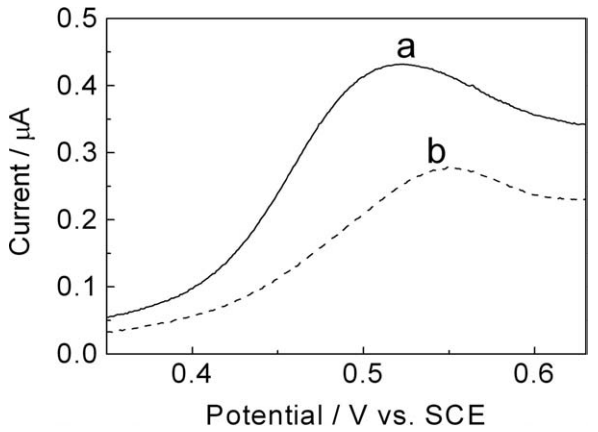

Figure 3 Linear voltammetry of ferrocenecarboxyaldehyde added onto graphite electrode modified with poly(4-aminophenol), containing: (a) ssDNA specific of *M. leprae* and (b) after hybridization with the target complementary, for 15 min. Eletrolyte: phosphate buffer (0.1 mol L^{-1}), pH 7.4, 10 mVs^{-1}.

ferrocenecarboxyaldehyde on electrode surface, in presence of the complementary target, indicating occurrence of hybridization.

Figure 3 shows that, after hybridization of the DNA, the oxidation potential of ferrocenecarboxyaldehyde shifted from 30 mV to more positive value. This is justified by helix formation of the double strand DNA, with a significant change in the flexibility and size of the DNA, increasing the distance of the double strand formed with the electrode surface, due to the lower conformational flexibility, resulting in decrease of the charge transfer. Similar effect was observed in assays with synthetic oligonucleotides, using methylene blue as indicator.[18]

Molecular simulations of the interaction between ferrocenecarboxaldehyde and DNA

To explore this process of interaction between ferrocenecarboxaldehyde/ssDNA and ferrocenecarboxaldehyde/dsDNA, we have investigated this interaction with molecular simulations and free energy analyses (Fig. 4).

Figure 4(A) shows the formation of hydrogen bonding between the carbonyl groups of the mediator ferrocenecarboxaldehyde and N-H atoms of the ssDNA. Figure 4(B) shows that hydrogen bonding is made preferentially between the base pairs of the double strand. This figure also suggests that the proximity between ferrocenecarboxaldehyde and dsDNA does not compromise the double strand. These molecular simulations indicate that the ssDNA has a greater affinity by ferrocenecarboxaldehyde, and they are consistent with the voltammetry results (see Fig. 3).

The higher affinity of ssDNA to ferrocenecarboxaldehyde, when compared with dsDNA (see Fig. 3), suggests that the formation of hydrogen bonding between the carbonyl groups of the mediator ferrocenecarboxaldehyde and N-H atoms is larger with ssDNA. The formation of the helix double (hybridization) diminishes the interaction of the ferrocenecarboxaldehyde with the nitrogenated bases of the DNA because hydrogen bondings are made between the base pairs of the probe strand and the complementary strand, producing a decrease in the incorporation of ferrocenecarboxaldehyde (see Fig. 4(A,B)). Moreover, the hybridization increases the distance between the dsDNA/mediator and the electrode, decreasing the electron transfer.[18,25]

These effects are in agreement with the interaction observed between oligonucleotides and another mediator, methylene blue.[18]

The values of the variation of Gibbs free energy, calculated for the interaction of the mediator and DNA were −660.96 kcal mol^{-1} (ferrocenecarboxaldehyde/ssDNA) and +6.28 kcal mol^{-1} (ferrocenecarboxaldehyde/dsDNA), indicating that the interaction between ferrocenecarboxaldehyde/dsDNA is not favored. These results are in accordance with the voltammetry experiments (see Fig. 3).

Hybridization time and complementary target concentration

Hybridization time and complementary target concentration were studied, using the graphite electrode

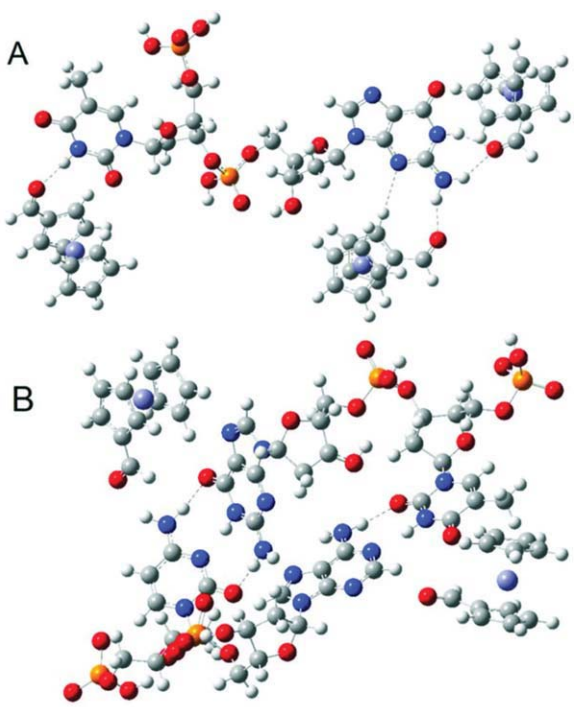

Figure 4 Structures proposed for interaction between: (A) ssDNA/ferrocenecarboxyaldehyde and (B) dsDNA/ferrocenecarboxyaldehyde. Gray: carbon; white: hydrogen; blue: nitrogen; red: oxygen; purple: iron; orange: phosphorous. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

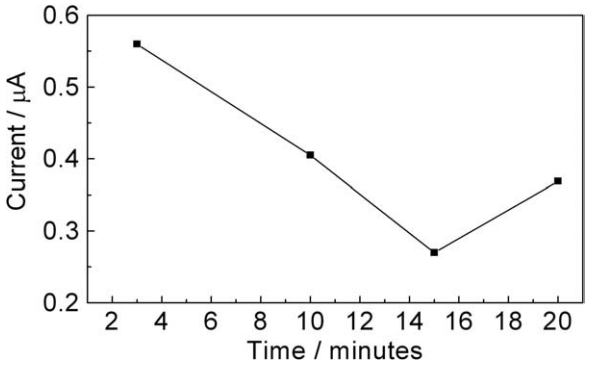

Figure 5 Oxidation current of ferrocenecarboxyaldehyde in function of the hybridization time. The indicator was added onto graphite electrode modified with poly(4-aminophenol) containing ssDNA, after hybridization with the complementary target. Eletrolyte: phosphate buffer (0.1 mol L^{-1}), pH 7.4, 10 mVs^{-1}.

modified with poly(4-aminophenol)/ssDNA, for electrochemical detection of DNA hybridization. The influence of the hybridization time is represented in Figure 5.

Figure 5 shows that the variation in the oxidation current of ferrocenecarboxyaldehyde is higher after hybridization during 15 min. After this hybridization time, the oxidation current increases, suggesting nonspecific accumulation of the DNA target on electrode surface.

Figure 6 shows the voltammograms of the modified electrodes containing ferrocenecarboxyaldehyde, in phosphate buffer, after hybridization between DNA probe and increasing concentration levels of the DNA target.

From Figure 6, it can be seen that the oxidation potential of ferrocenecarboxaldehyde is about +0.54 V (vs. SCE), and that the oxidation current of ferrocenecarboxaldehyde is inversely proportional to the concentration of the DNA target added. The decrease in oxidation current, after incubation with the DNA target, is attributed to hybridization process, due to lower affinity of ferrocenecarboxaldehyde by dsDNA.

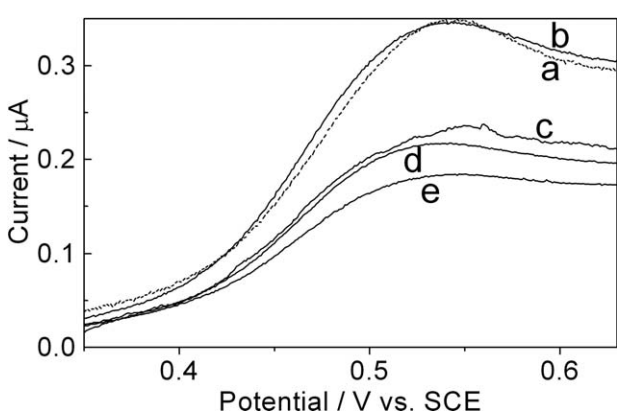

Figure 6 Linear voltammograms of ferrocenecarboxaldehyde in phosphate buffer (0.10 mol L^{-1}), pH 7.4, (baseline-corrected). The indicator was added onto graphite electrode modified with poly(4-aminophenol), containing ssDNA, before (a) or after hybridization with DNA target in different concentrations: 0.035 ng/μL (b), 0.35 ng/μL (c), 3.5 ng/μL (d), and 35 ng/μL (e); 10mVs^{-1}.

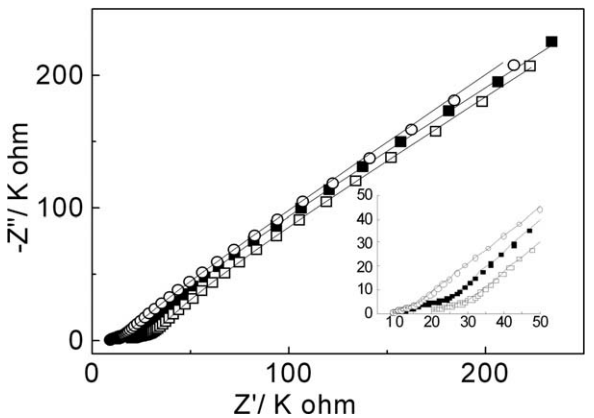

Figure 7 Nyquist diagrams for graphite electrode modified with poly(4-aminophenol), in $K_4Fe(CN)_6$ (5×10^{-3} mol L^{-1})/$K_3Fe(CN)_6$ (5×10^{-3} mol L^{-1})/KNO_3 (0.1 mol L^{-1}) solution: without DNA probe (○); with DNA probe (□); with DNA probe after hybridization with the complementary target (■). The continuous lines represent the fitting to the equivalent circuit shown in Figure 7. Inset: amplification of the low Z′ region.

The RLEP3 sequence could be quantified over the linear range from 0.35 to 35 ng/μL with linear correlation of 0.9937. Below this concentration, no difference in signal was observed in relation to complementary target (see Fig. 6, curve b).

Electrochemical impedance spectroscopy analysis

EIS was used to confirm the modification of the surface with the DNA probe and after hybridization with the complementary target. Data were represented by the Nyquist diagram (Z′ vs. -Z''), measured at the open circuit potential, for the different surfaces (Fig. 7).

Figure 7 shows that all the experimental curves present semicircles in the high frequency domain (see inset), and a straight line having a 45° slope in low frequency domain. For all modified electrodes

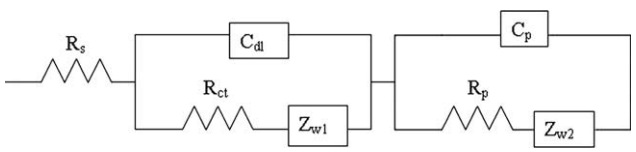

Figure 8 Equivalent circuit used for fitting the EIS data. RS: solution resistance; Rct: charge transfer resistance; Rp: polymer resistance; Cdl: Electric double layer capacitance; Cp: capacitance of the polymer; ZW1: resistance to ion transport of the electric double layer; ZW2: resistance to ion transport in the polymer.

TABLE II
Fitting Parameters Obtained

Surface	R_s	R_{ct}	R_p	C_{dl}	C_P	Z_{W1}	Z_{W2}
A	8.90	1.37	7.06	2.79×10^{-3}	1.39×10^{-5}	1.28×10^{-2}	1.38×10^{-2}
B	14.32	7.52	67.1	6.22×10^{-5}	2.05×10^{-9}	1.95×10^{-2}	1.42×10^{-2}
C	10.55	15.36	3.42	7.08×10^{-6}	4.79×10^{-5}	1.39×10^{-2}	5.18×10^{-2}

R_s: solution resistance; R_{ct}: charge transfer resistance; R_p: polymer resistance; C_{dl}: electric double layer capacitance; C_p: capacitance of the polymer; Z_{W1}: resistance to ion transport of the electric double layer; Z_{W2}: resistance to ion transport in the polymer. $R(\Omega)$ and $C(F)$.

studied, we observed a Warburg (W) behavior, characterizing a system controlled by diffusion in the high frequency domain and mass transfer control at low frequency.

Figure 8 Presents the equivalent circuit that describes the electrical properties of the system.

Table II shows that the ohmic resistances to the solution (R_s) and polymer (R_p) for the electrode modified with poly(4-aminophenol)/ssDNA are higher, when compared to electrodes modified with poly(4-aminophenol) or poly(4-aminophenol)/dsDNA. This effect is possibly due to negative charges of the phosphate groups in ssDNA, more available than in dsDNA, resulting in expulsion of counter ions ClO_4^- of the polymer structure. When ssDNA is incorporated in poly(4-aminophenol), the negative charges more available in ssDNA may cause distortions in π orbitals, increasing the energy of the conduction band[26] and decreasing the conductivity of the polymer.

It is observed that the polymer capacitance (C_p) is significantly lower to poly(4-aminophenol)/ssDNA, in accordance with the significant reduction of its conductivity.

The decrease in double layer capacitance (C_{dl}) shows that significant changes occur in the outer electrode area, with the ssDNA immobilization and after hibrydization. It is observed that the graphite electrode modified with poly(4-aminophenol) presents C_{dl} values about 45 times higher when compared to the poly(4-aminophenol)/ssDNA, and about 400 times higher, if compared with poly(4-aminophenol)/dsDNA. The changes in C_{dl} of the electrode-solution interface indicate that the target analyte is captured by probe strands (hybridization), in agreement with the DNA hybridization detection through linear voltammetry, using ferrocenecarboxyaldehyde as redox indicator (see Fig. 3).

Table II shows also that the recognition of the complementary target by DNA probe increases the charge transfer resistance (R_{ct}). A factor that justifies the change in R_{ct} is the electrostatic repulsion between the negatively-charged redox pair $[Fe(CN)_6]^{4-}/Fe(CN)_6]^{3-}$ and negative charge on the DNA due the fact of the DNA to be a polyelectrolyte.[27]

CONCLUSIONS

The potentialities of a simple method, aiming at the detection of specific DNA to *M. leprae* based in voltammetric response of affinity of ferrocenecarboxyaldehyde to ssDNA or dsDNA after hybridization, have been evaluated. The voltammetric results show that the bioelectrode presents negative current change following DNA hybridization. The approach does not require the labeling of any nucleic acid probes or targets prior to analysis, making the method advantageous in terms of speed and low cost. The biolectrode produced presents high sensitivity and response time of 15 minutes.

The DNA hybridization with the complementary target, analyzed through EIS, showed modification on Nyquist plot upon addition of the complementary target, with increase in the charge transference resistance, and decrease of the double layer capacitance. The results presented indicate that the bioelectrode reported is a promising tool for gene detection of *M. leprae*.

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