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Characterization of DNA Immobilized on Electrochemically Prepared Conducting Polypyrrole–Polyvinyl Sulfonate Films

Anamika Gambhir,* Manju Gerard, Shailesh K. Jain, and B. D. Malhotra

Biomolecular Electronics and Conducting Polymer Research Group, National Physical Laboratory, New Delhi 110012, India, E-mail: anamikagulati@yahoo.com

Abstract

The article describes the adsorption characteristics of DNA onto electrochemically generated polypyrrole–polyvinyl sulfonate (PPY-PVS) films obtained as a function of pH. Adsorption on PPY doped with an anion proceeds by anion exchange, and since DNA possesses a fixed negative charge owing to PO_4^-, it favors a very strong binding displacing PVS with favorable energetic interactions. Characterization of adsorbed DNA onto the PPY-PVS films was carried out by ultraviolet-visible, Fourier transform infrared spectroscopy, and cyclic voltammetric studies.

Index Entries: DNA biosensor; immobilization; conducting polymers; polypyrrole/polyvinyl sulfonate.

Introduction

DNA biosensors hold an enormous promise for the clinical diagnosis of inherited diseases, rapid detection of pathogenic infections, and screening of cDNA colonies required in the field of molecular biology research. Present methods of genetic analysis are dependent on the ability to detect specific DNA sequences in a heterogeneous mixture. The immobilization of DNA on a solid support has been used to separate complementary DNAs from solution by procedures such as affinity capture methods *(1)* using radioisotope-labeled or hapten-labeled DNA. However, these methods require relatively longer time and cannot quantitatively detect the absolute amount and the time course of hybridization. In addition, pre- and post-treatments are required to modify DNAs with probes or proteins and to analyze hybridization, respectively.

*Author to whom all correspondence and reprint requests should be addressed.

Fink and Schonenberger *(2)* have recently reported electrical conduction through DNA molecules by direct measurement of electrical current as a function of the potential applied across a few DNA molecules grouped into single ropes at least 600 nm long. Other experiments addressing DNA conductivity involve a large number of DNA strands doped with intercalated donor and acceptor molecules, and the conductivity has been estimated from electron transfer rates obtained as a function of the distance between the donor and acceptor sites.

More recently, DNA-integrated electroactive polymers (thin films or two-dimensional Langmuir-Blodgett monolayers) have created a novel class of intelligent materials that possess superior intelligent material properties of, e.g., self-assembly, self-multiplication, self-repair, self-degradation, redundancy, and self-diagnosis *(3,4)*.

A full range of optical, electrochemical, and piezoelectric transduction modes aimed at detecting the base-pair hybridization between the immobilized cDNA probe and the target DNA have been developed. Mediated electron transfer reactions of DNA for detecting polymerase chain reaction–amplified genomic DNA and ferrocene-mediated oligonucleotides for sandwich-based electrochemical detection of DNA hybridization have been described. Immobilization of DNA onto glass carbon and gold electrodes has also been reported *(5)*.

The interaction between DNA and other molecules is an important fundamental issue in life sciences that is related to the replication and transcription of DNA in vivo, the mutation of genes and related variation of species in character, the action mechanisms of some DNA-targeted drugs, the origin of some diseases, the action mechanisms of some synthetic chemical nucleases, and so on. Very few studies on interaction of DNA with conducting polymers have been reported *(6,7)*. Livache et al. *(8)* have described one-step electrodeposition of a polypyrrole (PPY) film functionalized by a covalently linked oligonucleotide. Earlier they had described electrochemically directed copolymerization of pyrrole and oligonucleotides bearing on their 5' end a pyrrole moiety introduced by phosphoramidite chemistry *(9)*. Most of these moieties rely on measuring changes in the peak current of a redox active marker that preferentially binds to the duplex formed in the hybridization *(10)*. Marrazza et al. *(11)* have described detection by differential pulse voltammetry and chronopotentiometric stripping analysis by the use of an electroactive indicator, daunomycin hydrochloride, that interacts with the double-stranded DNA. Doping of nucleic acid (DNA) probes within electropolymerized PPY films and monitoring of the current changes provoked by the hybridization event is another possibility described by Wang et al. *(12)*.

PPY as a conducting polymer is attracting interest because it can be easily prepared as a flexible free-standing film having conductivity (10^{-3}–10^{3} Ω cm^{-1}). There have been several reports on the incorporation of biomolecules (e.g., enzymes) on PPY *(13–15)*. A recent study has shown polycationic histone protein interaction with poly(styrene sulfonate)-

doped PPY films *(16)*. This assumes significance because histones are the proteins that interact very strongly with DNA, which is a large and highly structured biomacromolecule, to pack it in the cell nucleus.

The electrically conducting PPY polymer provides a unique surface for DNA binding in that its delocalized electronic structure allows mobility to positively charged groups along the chain axis. It has been established that PPY doped with a negatively charged group such as polyvinyl sulfonate (PVS) can exchange its negatively charged dopant ions easily with other negatively charged molecules including biomolecules. Since DNA possesses a fixed negative charge owing to PO_4^-, it favors a very strong binding that displaces PVS with favorable energetic interactions. Hydrogen bonding of PO_4^- oxygens to PPY ring nitrogen atoms in the DNA backbone can enhance binding of DNA and PPY.

Immobilization of DNA on a conducting polymer matrix facilitates the detection of a signal (amperometric/potentiometric) generated as a result of the interaction of proteins or drugs with DNA. Possibilities for detecting signal generated as a result of hybridization of DNA strands can also be explored.

In the present article, we report the results of our studies relating to the characterization of physically adsorbed DNA (calf thymus) on conducting PPY/PVS films.

Materials and Methods

All the chemicals—sodium phosphate, Tris (Merck), HCl (BDH), calf thymus DNA (Sigma)—were of analytical grade. Pyrrole was distilled before use. Water from a Millipore water-purifying system was used.

PPY-PVS films were electrochemically deposited from an aqueous solution of predistilled pyrrole (0.1 *M*) and PVS (25% by weight) on an indium-tin-oxide (ITO) glass plate serving as the working electrode, platinum as the counterelectrode, and saturated calomel electrode (SCE) as the reference electrode at a constant current of 2 mA *(17)*. The electrical conductivity of these films, measured by four-points-probe technique, was found to be about 10^{-3} S/cm.

The buffers used were 0.05 *M* Tris (pH 8.0) and 0.1 *M* sodium phosphate buffer (pH 6.0–8.0). Calf thymus DNA (6 µg/mL) was dissolved in 0.05 *M* Tris buffer (pH 8.0) containing 0.01 *M* EDTA and sonicated in an ice bath for 1 min followed by 60 s of nitrogen bubbling to purge off the dissolved oxygen. This procedure was repeated 15 times. Two 50-µL aliquots were adsorbed on a 1×1 cm^2 surface of PPY/PVS-coated ITO glass plates for cyclic voltammetric and Fourier transform infrared (FTIR) studies. For studying the time kinetics, the film-coated ITO glass plate was suspended in a 3-mL cuvet containing DNA dissolved in Tris buffer (6 µg/mL) processed prior to use as described earlier.

The cyclic voltammetric studies of the DNA electrodes were carried out using an electrochemical interface (Schlumberger SI 1286) in the range

of –0.8 to 1.4 V vs SCE at a scan rate of 50 mV/s. FTIR spectroscopy studies were carried out using a Nicolet 510 P spectrophotometer from 400 to 4800 cm^{-1} using KBr powder (spectroscopic grade). Spectrophotometric studies were carried out on an ultraviolet-visible Schimadzu (160A) spectrophotometer.

Results and Discussion

Electrochemical methods can be utilized to study the interactions of DNA in solution with other molecules. Methods such as cyclic voltammetry, differential pulse voltammetry, and chronocoulometry have been used to study the interactions of Co(III) *(17)*, Fe(II), Ru(II), and Os(II) chelates with 1,10-phenenthroline and 2,2'-bipyridyl bound to DNA. Pang and Hector *(18)* have carried out a micromethod for the investigation of the interaction between DNA and redox active molecules. In our experiments, a shift of 23 mV in the negative direction was observed in PPY-PVS/DNA as compared to PPY-PVS electrodes. This suggests that the interaction of DNA with polymer molecules is electrostatic in nature, which is in agreement with the literature.

Molecular vibrations lead to the absorption bands observed in the IR spectral region. IR spectroscopy is a tool to obtain information on the conformations of macromolecules since vibrations within three molecules may couple with each other. In polynucleotides, with the double-helix formation, the transition moments of C=O groups become oriented antiparallel. Thus, the C=O stretching vibrations split, and the double-bond structure is observed, indicating a double-helix formation.

By considering the changes in the NH stretching vibrations with hydrogen bond formation, the association of nucleic acid bases can be studied. The peaks obtained at 3482 and 3416 cm^{-1} show the NH stretching vibrations of the adenine derivatives. The peak at 2750 cm^{-1} is caused by the NH stretching vibrations in the NH—N hydrogen bonds formed by semiprotonated poly(C). Figure 1 shows the characteristic peaks for pyrrole at 1690 (C=N), 1360 (C-N), (O=N) vibrations at the 1020 and 1160 cm^{-1} PPY ring nitrogen atoms, which are in agreement with the literature *(19)*. The 2950 cm^{-1} vibration band has been ascribed to the aromatic C-H vibration. The 1600 cm^{-1} vibration band is owing to the C=C bond associated with the stretching and bending vibrations of C-N. The vibrations at 1170 cm^{-1} are owing to the presence of the C=N double bond, and the peak at 810 cm^{-1} results because of the C-H vibration band–linked phenyl ring.

Calf thymus DNA dissolved in buffer solutions of pH 6.0, 6.5, 7.0, and 8.0 were used to study the effect of pH on rate of adsorption. Figure 2 depicts the characteristics of adsorption on PPY-PVS electrodes at indicated pH values. As evident from Fig. 2, pH significantly affected the adsorption of DNA. At pH 6.0, the maximum amount of DNA adsorbed on PPY/PVS was 3.5 $\mu g/cm^2$, which was 22% of the total used. An overview of Fig. 2 suggests that adsorption decreased with a rise in pH. No significant

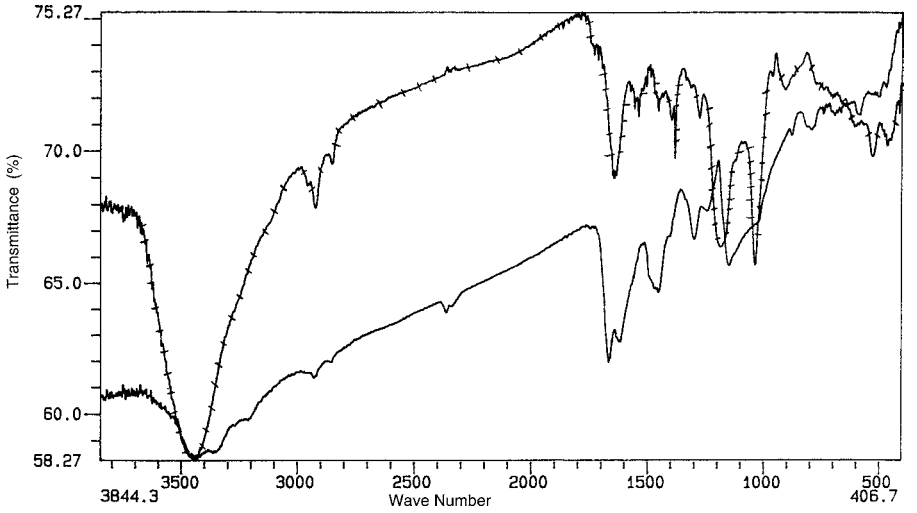

Fig. 1. FTIR spectrum of PPY-PVS composites (top curve) and physically adsorbed DNA on PPY-PVS composites (bottom curve) in KBr disc.

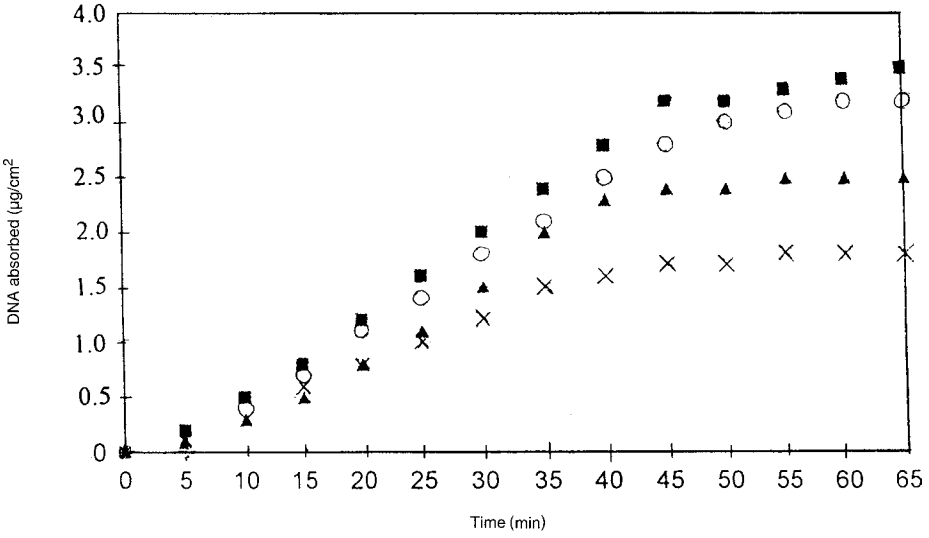

Fig. 2. Adsorption of DNA onto conducting PPY-PVS films as a function of pH 6.0 (■), 6.5 (○), 7.0 (▲), and 8.0 (×) on ITO glass plates.

variation was observed in the adsorption profiles at pH 6.0–6.5; however, as the pH approached 8.0, the rate of initial adsorption decreased significantly.

Charge on DNA was conferred only by the phosphate groups present since the bases were uncharged in the pH range used as also the pentose sugar. DNA adsorption increased as the pH was lowered to a more acidic range (0.0–5.0); however, such a pH cannot be realized with experiments on

biomolecules such as DNA, which get denatured over narrow pH ranges, and even single-stranded DNA may exhibit ionization of bases disrupting the secondary structure.

Several groups have demonstrated that PPY undergoes protonation/deprotonation and doping/dedoping that leads to a modification in the positive charge density borne on the polymer backbone *(20,21)*. Furthermore, Saoudi et al. *(22)* have shown by photoelectron spectroscopy (XPS) and ion-exchange chromatography that PPY/Cl is an anion exchanger. PVS, being a negatively charged molecule, is likely to behave similarly.

Adsorption of DNA onto PPY follows time-dependent kinetics ($t_{1/2}$), showing a straight line (Fig. 2), which gets saturated only after a period of about 45 min. Although we cannot provide any experimental evidence to explain this finding, a direct interpretation could be that the time-dependent kinetics in PPY is a result of gradual interchange (ion exchange) of PVS with DNA.

Conclusion

DNA adsorption on PPY follows time-dependent kinetics and reaches a maximum at pH 6.0. Cyclic voltammetric studies indicate a shift in potential in the PPY-PVS/DNA in the negative direction as compared to PPY-PVS electrodes. FTIR studies suggest electrostatic interactions between DNA and conducting polymers. Further studies are in progress to study the kinetics of adsorption of DNA, its stability, and the effects of pH and temperature on denaturation and annealing of immobilized DNA.

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