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ENZYMATIC SYNTHESIS OF MOLECULAR COMPLEXES OF POLYANILINE WITH DNA AND SYNTHETIC OLIGONUCLEOTIDES: THERMAL AND MORPHOLOGICAL CHARACTERIZATION

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ENZYMATIC SYNTHESIS OF MOLECULAR COMPLEXES OF POLYANILINE WITH DNA AND SYNTHETIC OLIGONUCLEOTIDES: THERMAL AND MORPHOLOGICAL CHARACTERIZATION

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This paper is dedicated to the memory of Professor Sukant K. Tripathy, our dear friend and colleague, whose inspiration, creativity, and achievements in science will always live on.

ABSTRACT

The assembly of electronic and photonic materials on biomacromolecules is of tremendous interest for the development of biofunctional nanocomplexes as well as highly selective biosensors. In the context of the use of electrically conducting polymers for sensing, polyaniline (Pani) and polypyrrole have received considerable interest because of their well-known electrical properties. Recently, we have reported an enzyme catalyzed synthetic procedure involving horseradish peroxidase (HRP) for the polymerization of aniline on a calf thymus DNA matrix. The mild reaction conditions involved in the synthesis have provided opportunities for the use of more delicate biomacromolecules as templates. The complexation of Pani with DNA has been found to induce reversible changes in the secondary structure of DNA leading to the

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formation of an over-wound polymorph. The thermal characterization (melting behavior) of the DNA in the complex and the morphological properties of the complex have provided corroborative evidence for the wrapping of Pani around the DNA matrix. Scanning probe and electron microscopy studies have indicated that the formation of Pani causes the DNA-Pani strands to agglomerate, presumably due to the neutralization of charge on the phosphate groups by the partially charged Pani. We also report the synthesis of Pani on a synthetic oligonucleotide (Poly[dA-dC].poly[dG-dT]). Demonstration of the use of a new biomimetic catalyst, polyethylene glycol modified hematin (PEG-hematin), in these reactions will also be presented. These results indicate that this biocatalytic synthetic approach is generic, versatile and can be adopted for both genomic and synthetic nucleic acids.

Key Words: DNA; Polyaniline; HRP; Modified hematin; Oligonucleotides

INTRODUCTION

In the past decade, optically responsive entities have been bound to biological polyelectrolytes, such as DNA in order to probe the fundamental properties of the DNA double helix. The electronic transport properties of DNA have attracted significant interest [1] and, until recently, it has been heavily debated [2, 3] as to whether the unique secondary structure (base pairs that create a stack of π electrons) could provide the possibility of DNA forming a "molecular conduit" [4]. After a tumultuous half-decade, femto-second studies have indicated that DNA does not exhibit efficient wire-like behavior [5, 6]. However, the polyelectrolyte behavior and the macroscopic order of nucleic acids can be exploited for the assembling and complexation of electrically conducting polymers to the unique DNA double helix structure. With this in mind, the development of a generic method for the complexation of a conducting polymer (Pani) with nucleic acids would aid in the development of electro-responsive bionanocomposites and DNA-based detection schemes for genosensors.

Polyaniline has received considerable interest because of its reversible redox and electro-chromic properties. The focus on improvement of the electrical properties and solubility of Pani has led to the development of numerous chemical [7-9] and electrochemical [10] methods to directly synthesize Pani in a conducting and processable form. The more recent chemical and electrochemical synthetic methods involve the use of an anionic polyelectrolyte as a template [11, 12]. The polyelectrolyte provides the counter-ion for doping Pani and improves the solubility of the complex. Although these methods were useful in synthesizing water-soluble complexes of Pani and polyelectrolytes, there have been severe limitations in the choice of more fragile biological templates, such as DNA, due to the use of low pH conditions involved in the synthesis.

An alternative biochemical approach towards the synthesis of polyaniline in near neutral pH conditions involves the use of a polyelectrolyte template and the

enzyme, horseradish peroxidase (HRP) as the biological catalyst [13]. More recently, the HRP catalyzed synthesis and complete characterization of Pani with the template, sulfonated polystyrene, has also been reported [14]. One of the major roles of the anionic polyelectrolyte in these enzymatic reactions is to provide a localized low pH environment (created by the high proton concentration in the vicinity of the anionic group) that promotes the head to tail coupling of the aniline monomers at a much higher pH than the conventional chemical methods of synthesis [15]. An extensive study of various anionic templates and their efficacy in acting as a suitable matrix for the synthesis of Pani has provided guidelines for the selection of templates [15].

To extend this approach to biological templates, a phosphate containing polyelectrolyte, poly(vinylphosphonic acid), was first studied for the synthesis of Pani as a proof-of-concept matrix [16]. The advantages of the mild reactions conditions in this enzymatic approach were then applied to the use of a biological polyelectrolyte (DNA) for the synthesis of Pani [17]. Circular dichroism studies showed that Pani not only forms and complexes to the DNA template, but also causes the conformation of the DNA to change from a loosely wound 'B' form to a more tightly wound polymorph [18, 19]. More recently we reported that the secondary structure of the DNA in the DNA-Pani complex might be reversibly controlled from the native form to an over-wound polymorph by changing the redox-state of the polyaniline that is bound to the DNA. In addition, the polyaniline formed around the DNA, mimics the helicity of the DNA and adopts a preferred handedness of its own [19].

Here, we report the thermal, morphological and electrochemical characterization of the Calf thymus DNA-Pani complex. The influence of counter-ions and polyhydric alcohols on the melting behavior of DNA has been previously investigated [20, 21]. The thermal results in the present work indicate that the positively charged Pani formed around DNA stabilizes the DNA in a similar fashion as the metal counter-ions reported earlier. We have also explored the possibility of extending this approach to synthetic oligonucleotides such as poly[dA-dC]. poly[dG-dT]. The synthesis and characterization of Pani on the oligonucleotide has confirmed the versatility of this approach for possible extension to other synthetic nucleotides. We will also present the use of modified-hematin as an alternative cost effective and extremely versatile biomimetic catalyst for the synthesis of DNA/Pani complexes.

EXPERIMENTAL

Materials and Methods

Calf Thymus DNA (lyophilized), molecular weight of the order of 50 million daltons, was obtained from Worthington Biochemical Corporation, Freehold, New Jersey. Oligonucleotide, Poly [dA-dC].poly[dG-dT], and horseradish peroxidase

(HRP), type II, (200 Units/mg) were purchased from Sigma Chemical Co., St. Louis, MO. The nucleotide/DNA solutions were checked for protein contamination prior to use by measuring the UV-vis absorbance at 260 nm as well as 280 nm. Sterile glassware as well as buffers (10 mM sodium phosphate or sodium citrate) were used for the preparation of all DNA solutions. Aniline monomer (purity 99.5%) and hydrogen peroxide (30% by weight) were purchased from Aldrich Chemicals Inc., Milwaukee, WI, and were used as received. Aniline was distilled when any coloration was observed. Hydrogen peroxide (30%) was also obtained from Aldrich Chemicals Inc. and was diluted to 3% (in deionized water) and this stock solution was used for polymerization.

Synthesis of Water-Soluble DNA-Pani [DNA-Pani-S]

The polymerization of aniline in the presence of Calf Thymus DNA was carried out in sterile 10 mM citrate or phosphate buffers. 200 milligrams of lyophilized Calf Thymus DNA was dissolved in 400 ml of buffer at pH 4.3. The solution was stored in the refrigerator. The concentration of the DNA in solution as measured by UV absorbance at 260 nm after 48 hours of dissolution was determined to be 1.15 mM. To this DNA solution, 42.2 μl (1.15 mM) of aniline was added. A solution of HRP (10 mg in 2 ml) was then added to this and continuously stirred. The reaction was then initiated with the addition of hydrogen peroxide (H_2O_2). After optimization of the polymerization reaction, it was found that the addition of 95 μl of the 3% H_2O_2 solution was sufficient to both polymerize the aniline and form a water-soluble DNA complex. The water-soluble complex was then transferred to individual regenerated natural cellulose membrane bags (molecular weight cut-off 1000 D) and was dialyzed against 5000 ml of acidified deionized water maintained at pH 4.3. Dialysis was carried out for 72 hours with fresh acidified deionized water being added every 12 hours to expedite the removal of oligomers and unreacted monomer. This solution was used for doping-dedoping and conformational switching studies. The dry solid DNA-Pani-S complex was obtained by the evaporation of solvent and drying at 40°C under vacuum for 72 hours.

Synthesis of Water-Insoluble DNA-Pani [DNA-Pani-I]

The polymerization of aniline in the presence of Calf Thymus DNA was carried out either in sterile 10 mM citrate or phosphate buffer. 200 mg of lyophilized Calf Thymus DNA was dissolved in 400 ml of citrate buffer at pH 4.3. The solution was stored in the refrigerator. The concentration of the DNA in solution as measured by UV absorbance at 260 nm after 48 hours of dissolution was determined to be 1.15 mM. To this DNA solution 42.2 μl (1.15 mM) of aniline was added. The pH of the solution was checked again and adjusted to 4.3; a solution of

HRP (10 mg in 2 ml) was added to this and mixed continuously. To this solution, 475 μl (1.15 mM) of hydrogen peroxide (3% solution) was added dropwise. The addition of hydrogen peroxide was carried out in small aliquots providing sufficient time for the solution to turn green (prevent over oxidation) between successive additions. The reaction mixture was stirred for 90 minutes and the green DNA-Pani complex precipitated out of solution. The DNA-Pani complex was filtered out and washed with acidified chloroform in order to remove unreacted monomer and oligomers. The precipitate was finally washed with acidified deionized water and dried under vacuum at 40°C for 72 hours. The gravimetric yield was approximately 75%.

Synthesis of DNA-Pani Complex Using Modified Hematin

The use of hematin as a catalyst for the synthesis of polyaromatic compounds has been recently demonstrated [22]. Hematin, however, is water soluble only at very high pH and thus is not effective, as is, for the low to neutral pH aqueous conditions required for this template assisted polyaniline synthesis. To address this, a chemically modified hematin with tethered poly(ethylene glycol) (PEG) groups was synthesized. The procedure for the synthesis has been reported elsewhere [23].

A 1.0 mM calf thymus DNA solution was prepared by dissolving the required amount of DNA in 10 mL of sterilized sodium phosphate buffer maintained at pH 4. The concentration of DNA was determined by the UV absorbance at 258 nm. To this solution, 4.5 μl (5 mM) aniline and 5 mg of PEG-hematin were added. The polymerization was carried out by the drop-wise addition of hydrogen peroxide (0.3% w/v).

Synthesis of [Poly(dA-dC).Poly(dG-dT)]-Pani Complex

The oligonucleotide, [Poly(dA-dC).Poly(dG-dT)], was dissolved in 1 ml of sodium citrate buffer and left in the refrigerator for 12 hours. The concentration of the oligonucleotide was determined to be 0.5 mM. The molar extinction coefficient for the oligonucleotide was taken as 7100 liter mol^{-1} cm^{-1} from an earlier report [24]. 10 μl of aniline solution (1% solution in citrate buffer) was added to the oligonucleotide solution. 20 μl of the stock HRP solution (1 mg/ml) was added to the reaction mixture and the polymerization was initiated by the addition of 5 μl of (0.03% hydrogen peroxide solution). In order to prevent the precipitation of the oligonucleotide-Pani complex, the total amount of hydrogen peroxide added was restricted to 35 μl, (30% of the stoichiometric amount of hydrogen peroxide required for the complete (100%) polymerization). The UV-vis circular dichroism characterization of this complex was carried out with the reaction mixture, 120 minutes after the initiation of polymerization.

Characterization

UV-vis spectroscopy of the DNA and DNA-Pani solutions was obtained with a HP diode array detector photometer (type HP8452A) and a Perkin-Elmer Lambda 9 spectrophotometer. The temperature dependent UV-vis studies were performed on the Lambda-9 UV/Vis/near-IR spectrophotometer equipped with a double-walled sample holder connected to a water bath. The temperature of the solution was measured using a thermocouple immersed in the solution. Circular dichroism (CD) spectra were obtained using a Jasco CD spectrometer J-720 equipped with a peltier type attachment for controlling the temperature of the sample cell. The CD measurements were done in either 1 or 10 mm quartz cuvettes and the data was normalized to represent measurements made with 1 mm cuvettes.

The electrochemical characterization of Pani-polylectrolyte complexes was carried out on an EG&G potentiostat/galvanostat model 263. The cyclic voltammograms were recorded by using a three-electrode cell with a platinum wire counter electrode, a Ag/AgCl reference electrode, and a platinum foil (1×1 cm^2) with a cast film of the DNA-Pani complex as the working electrode. Cyclic voltammograms were recorded at room temperature in a 1.0 M HCl electrolyte solution and scanned between –0.2V and 1.2V at 100 mV/min.

The morphology of the Calf thymus DNA and DNA-Pani complex was studied using AFM. A Park Scientific Instruments (Thermomicroscopes) Autoprobe CP instrument with a multitask head and 100 μm scanner was used for imaging the DNA and DNA-Pani complexes. The conventional method for imaging individual strands of DNA usually involves the use of divalent cation salts to bind the DNA to a mica substrate [25]. However, the presence of salt would shield the charge on the phosphate groups in the DNA molecule and render it unsuitable to act as a template for the polymerization of aniline. In order to retain the polyelectrolyte nature of the DNA and to prevent agglomeration of DNA strands, the samples for AFM were prepared by freeze-drying. The samples were first deposited from very dilute solutions (nanomolar) on to a freshly cleaved mica substrate and were then immersed into liquid nitrogen. The mica substrate was placed on a cold metal surface and brought to room temperature in a vacuum oven. The imaging was done under ambient conditions and in non-contact mode with a scan speed of 1 Hz. Bright field TEM images were obtained using a Philips EM400T microscope on samples deposited on formvar/carbon supported 200-mesh film grids. The DNA and DNA-Pani samples were deposited from a micromolar solution.

For electrical conductivity measurements, the DNA-Pani-I complexes were pressed into pellets using a standard KBr pellet die and a laboratory press under a load of 10 tons. The electrical conductivity of the Pani complexes was measured with a Cascade Microtech linear 4-point probe connected to a HP 62188 DC current source and Keithley electrometer. The probe spacing (S) was 0.0625 inch and radii of 0.010 inch and made of tungsten carbide. The probes are also spring loaded and are designed to retract under a load of 40-70 grams. A constant load of

50 grams was placed on the probe in all cases in order to exert uniform pressure and maintain optimal electrical contact.

RESULTS AND DISCUSSION

It has been shown that the phosphate groups on the DNA template are of sufficient ionic charge to provide the requisite lower local pH environment and facilitate a predominately para-directed coupling of the aniline monomer in this enzymatic approach [18]. However, if the polymerization is allowed to proceed too far and a critical chain length is reached, the DNA-Pani complex precipitates out of solution. Therefore, it was necessary to optimize the reaction such that the complex could be maintained in a water-soluble form for spectroscopic characterization. The optimization of the reaction conditions for the synthesis of water-soluble DNA-Pani complex has been reported earlier and was used in the present work without modification [19]. The formation of the emeraldine salt form of Pani was confirmed by the signature polaron absorption bands of polyaniline at 420 nm and 750 nm in the UV-vis spectrum as shown in Figure 1. The formation of Pani also induces the characteristic change in the conformation of the DNA leading to the formation of an over-wound polymorph. This is shown in Figure 2, which is a comparison of the CD spectra of DNA and DNA-Pani after 80 minutes of reaction. In addition, there are two broad, positive peaks in the visible region at 365 nm and 445 nm that are due to the Pani absorption. The presence of these new

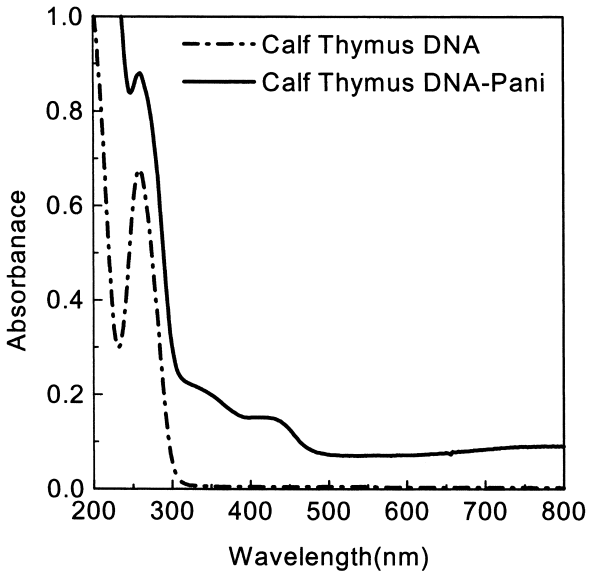

Figure 1. UV-vis spectra of calf thymus DNA and DNA-Pani.

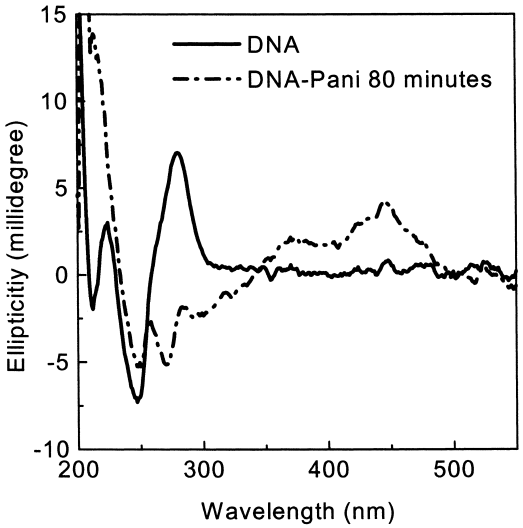

Figure 2. CD spectra of calf thymus DNA and DNA-Pani.

peaks upon polymerization indicates a threading of the polyaniline along the helical DNA template and its adoption of a preferred handedness.

Melting Studies of the DNA-Pani Complex

The thermal denaturation or melting of DNA results in the separation of the double stranded DNA into single strands. This process can be monitored experimentally by observing the UV absorption of the DNA in solution. As a solution of DNA is heated, the strands separate and the solution exhibits hyperchromicity at 260 nm. This change in absorbance after melting is attributed to the change in the nature of interactions of the base pairs when they are unstacked. To determine if the complexation of Pani had an effect on the melting behavior of DNA, experiments were carried out by heating a solution of DNA or DNA-Pani at the rate of 1°C per minute in a UV-vis spectrometer. The following parameter was evaluated from the measured melting profile at 260 nm. The fractional change 'f' is defined as $(A- A_n)/ (A_d – A_n)$, where A_n is the absorbance of the native DNA sample, A_d is the absorbance of the heat-denatured DNA sample and A is the absorbance at a given temperature. The melting temperature T_m is defined as the temperature at which f = 0.5. The plot of the parameter 'f' versus temperature is shown in Figure 3. The melting temperatures for DNA and DNA-Pani were calculated to be 43.5 °C and 54.7°C, respectively. This 11.2°C increase in the T_m of the DNA-Pani complex can be explained by stabilization of the helical structure of the DNA by the Pani. It has been previously reported that divalent cations like magnesium can also stabilize the helical structure and cause the T_m to increase by approximately 30°C [20].

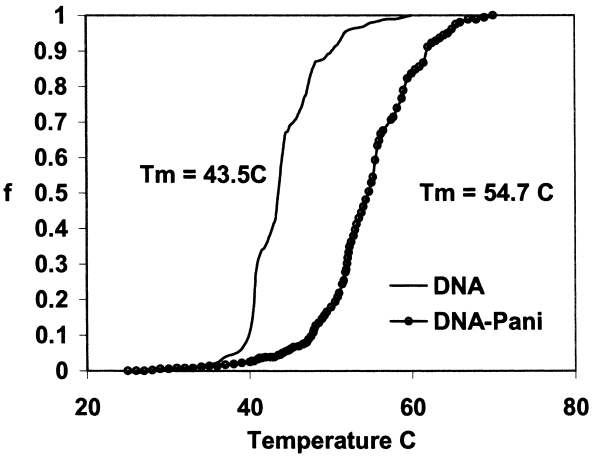

Figure 3. Melting study of DNA and DNA-Pani.

The melting of DNA and DNA-Pani was also monitored using CD polarimetry. Figures 4 and 5 show the changes observed by circular dichroism polarimetry and UV-vis spectroscopy, respectively for the DNA-Pani complex. It can be seen from the CD spectra that as the DNA in the DNA-Pani is heated, it undergoes a transition back to the more loosely wound 'B' form prior to the melting. This is indicated by the recovery of the CD bands at 275 and 285 nm. It is also noticed that the CD bands at 365 nm and 445 nm reduce in intensity with heating and are

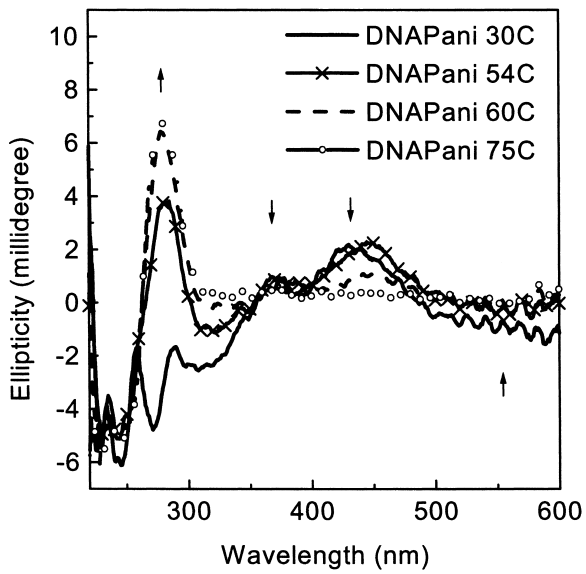

Figure 4. CD spectra of DNA-Pani in the temperature range of 30°C to 75°C.

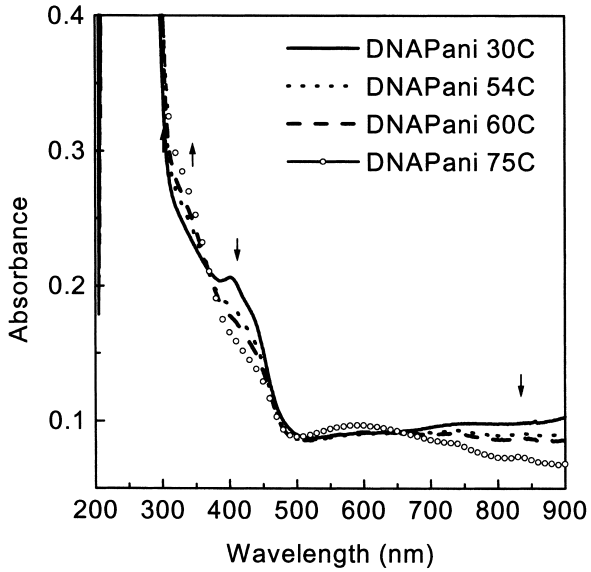

Figure 5. UV-vis spectra of DNA-Pani in the temperature range of 30°C to 75°C.

completely lost at temperatures above the melting temperature. This is evidence that the DNA induced macroasymmetry in the Pani is lost during the melting process. The UV-vis spectrum of DNA-Pani in Figure 5 provides evidence for partial dedoping of the Pani when the temperature of the solution is increased. This is indicated by the decrease in the polaron bands at 440 nm and 800 nm with a small increase in the exciton transition bands of the quinoid rings in the range of 560-600 nm. The increase in the intensity of the bands in the range of 200-300 nm is consistent with the hyperchromicity expected during the melting of DNA. There are minor changes in the CD bands in the 245 and 275 nm range, which is consistent with earlier reports [26]. It can be concluded that in the case of DNA-Pani, the melting process causes disruption of the macroasymmetry due to the thermal distortion and/or partial removal of Pani that is loosely bound to the DNA. The changes observed in the CD as well as the UV spectra are irreversible.

Conductivity

The electrical conductivity of the DNA and the DNA-Pani-I was measured by forming pellets of each and using a four-point probe apparatus. The appropriate corrections for the sample thickness and diameter were applied. Both pellets were also doped using HCl vapor and the conductivity was measured on the doped samples. The results are presented in Table 1. The conductivity of the DNA pellet is comparable to that of cast films of DNA reported earlier and is of the order of 10^{-7}-10^{-8} S/cm [27]. The complexation of Pani to the DNA increases the conductivity marginally. The Pani being bound to the non-conducting DNA matrix may explain

Table 1. Conductity of DNA and DNA-Pani Before and After Doping

Sample	Conductivity Before Doping (S/cm)	Conductivity After Doping (S/cm)	Change in Conductivity (%)
DNA	2.92×10^{-7}	4.58×10^{-7}	56.8
DNA-Pani	4.98×10^{-7}	40.9×10^{-7}	796.6

this small increase in conductivity. However, the conductivity of the DNA-Pani complex increases almost by an order of magnitude upon doping with HCl vapors while the conductivity of DNA increases only marginally. This result confirms the presence of the electrically conducting form of Pani in the DNA-Pani complex. However, the low values of conductivity in the DNA-Pani complex may be explained by discontinuity of the Pani along the DNA template and/or incomplete polymerization of the Pani due to precipitation during the reactions.

Redox Reversibility

The electrochemical behavior of the DNA-Pani complex was studied using cyclic voltammetry. The cyclic voltammogram of DNA-Pani obtained at a scan rate of 20 mV/sec, over a potential window of 0 mV to 1000 mV versus Ag/AgCl is shown in Figure 6. DNA-Pani shows two anodic peaks at 0.21 V and 0.54 V. The corresponding cathodic peaks appear at 0.64 V and 0.38 V, respectively. The peak in the region of 0.2 –0.5 V has been assigned to the first redox process wave in the Pani. Electrochemically synthesized Pani shows a peak at 0.51 V [28]. The redox

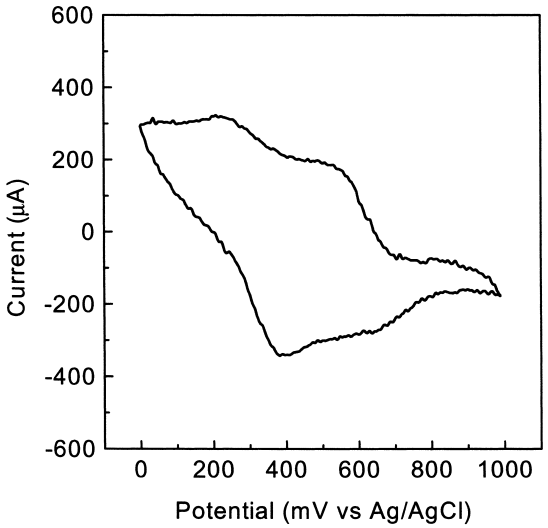

Figure 6. Cyclic voltammogram of a solution cast film of DNA-Pani.

peaks are not very sharp compared to the peaks observed from pure Pani and this may also be explained in terms of a weakly conducting form of Pani. However, these results do confirm the presence of an electrochemically redox reversible form of Pani in the DNA-Pani complex.

AFM and TEM Characterization of DNA-Pani

A few drops of dilute solutions (nanomolar concentration) of DNA and DNA-Pani were deposited on mica substrates and freeze-dried before being imaged in the AFM. A typical image of a DNA strand is shown in Figure 7. The 'Z' scale (height) resolution of the AFM is several orders of magnitude higher than the lateral resolution and was used for the identification of the strands. A typical image obtained from a DNA sample [Figure 7] indicates the presence of individual strands with diameter in the range of 20-22 Å (measured from strand height), as expected for a double stranded DNA. The image obtained from the DNA-Pani complex [Figure 8] indicates the presence of globules, 0.4–0.6 μm in

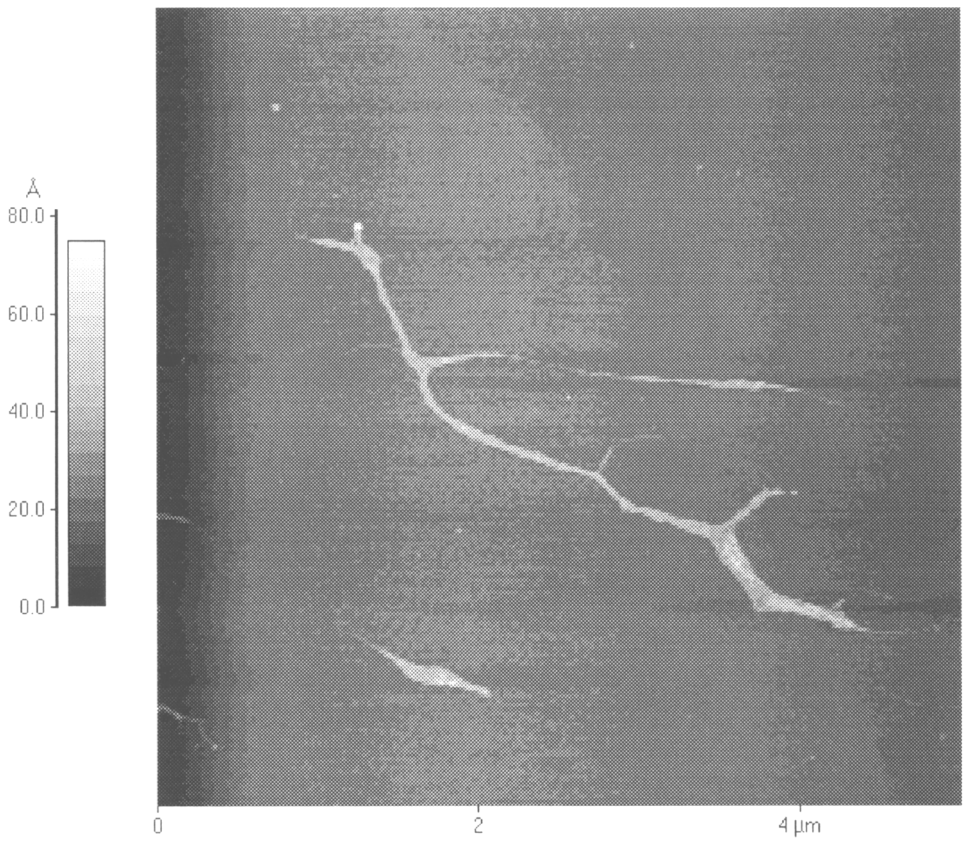

Figure 7. AFM image of freeze-dried DNA.

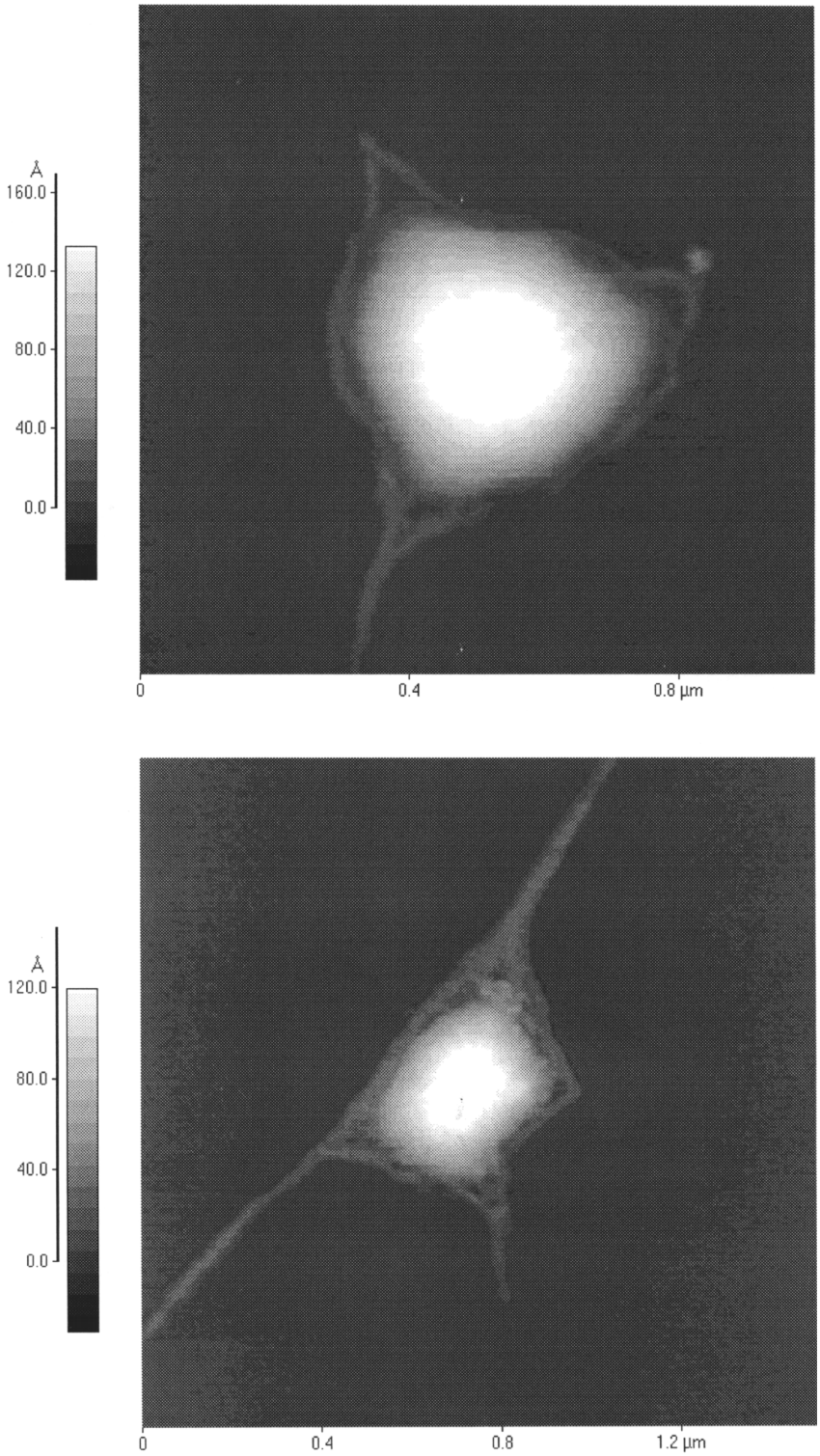

Figure 8. AFM images of freeze-dried DNA-Pani.

width and 100-150 Å in height. Individual strands of DNA of width 300-450 Å and height 20-25 Å were also observed along the outer contour of the globule. Several individual strands with agglomerates distributed randomly, were also observed in other regions of the sample [Figure 8].

Transmission electron micrographs of DNA-Pani were obtained from samples that were freeze-dried on formvar/carbon support film grids. While AFM can provide high resolution images of the surface topography of the DNA-Pani strands, aggregates of the strands cannot be resolved efficiently. The prime motive for the TEM studies was to obtain complementary information about the fine structure in the interior regions of these DNA-Pani agglomerates. The bright field images of the DNA-Pani show a collection of strands with typical dimensions of 10 nm [Figure 9]. Similar features are observed in images of DNA-Pani that have not been freeze-dried [Figure 10]. Electron diffraction from DNA-Pani has indicated a strand diameter of 34 Å, which is higher than the typical diameter expected for pure DNA. Aggregation of the strands is also observed in the TEM micrographs of native DNA deposited and freeze-dried in the same way. The images obtained from DNA and DNA-Pani are very similar and provide direct evidence for the retention of the DNA morphology even after the formation of Pani. From TEM and AFM images it can be concluded that the secondary structure of the DNA has been preserved even after the formation of Pani. The shielding of charge on the DNA by Pani has resulted in the agglomer-

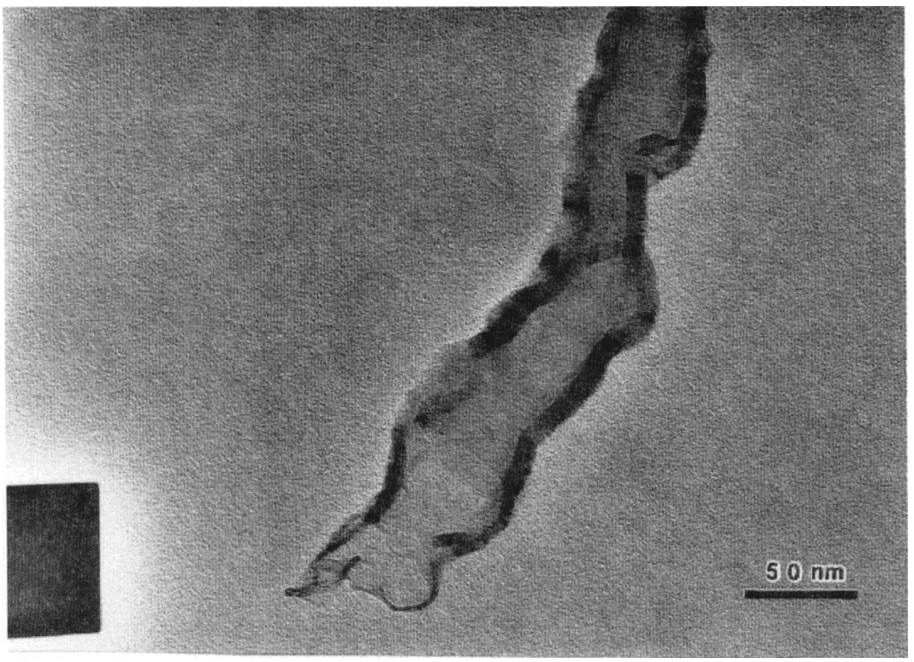

Figure 9. Bright field TEM image of freeze-dried DNA-Pani.

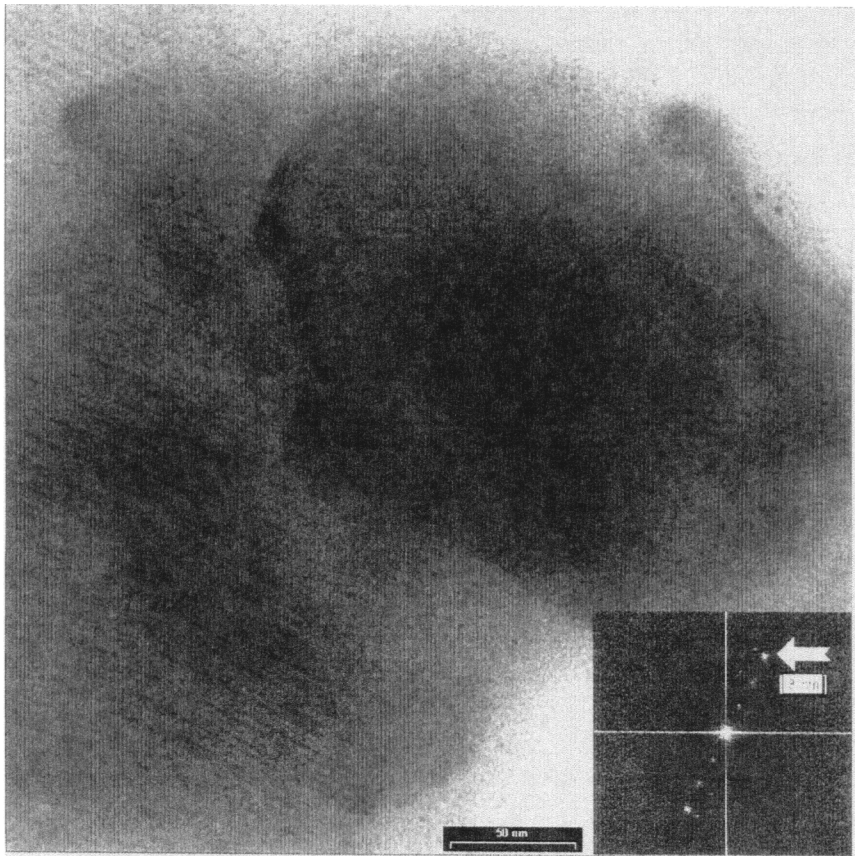

Figure 10. Bright field TEM image and electron diffraction of DNA-Pani.

ation of strands leading to the formation of globules embedded in the ensemble of DNA strands.

Formation of Pani on Oligonucleotides and Single Stranded DNA

Pani was also synthesized in the presence of an oligonucleotide (Poly [dA-dC].poly[dG-dT]). Similar changes in the UV-vis absorption spectra (Figure 11) and CD spectra, (Figure 12) were also observed with this system. The conformation of the oligonucleotide is observed to change in a similar way as that observed with the high molecular weight Calf thymus DNA. The CD peaks in the 365 nm and 445 nm region indicate that macroasymmetry in the organization of Pani with the oligonucleotide is also observed in this case. This indicates that the molecular weight of the nucleic acid matrix does not have a critical influence on the polymerization and organization of Pani. Conformational switching of the oligonucleotide due to the dedoping and redoping using base and acid, respectively was also observed in the oligonucleotide-Pani complex (results not shown).

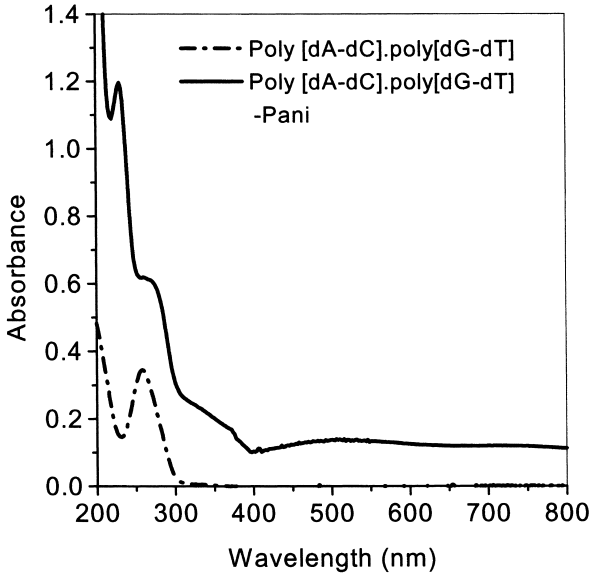

Figure 11. UV-vis spectrum of (Poly [dA-dC].poly[dG-dT])-Pani complex.

Hematin-PEG Catalyzed Synthesis of Pani

PEG modified hematin was used for the synthesis of Pani with Calf thymus DNA. The reaction yielded a green water-soluble complex of DNA-Pani. The UV-vis spectra of the DNA and DNA-Pani are shown in Figure 13. The spectra show

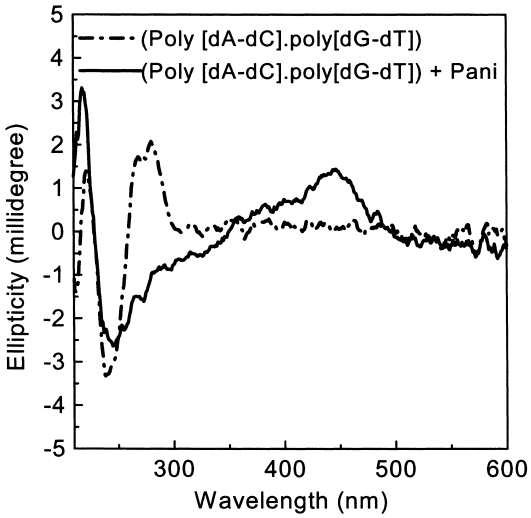

Figure 12. CD spectrum of (Poly [dA-dC].poly[dG-dT])-Pani complex.

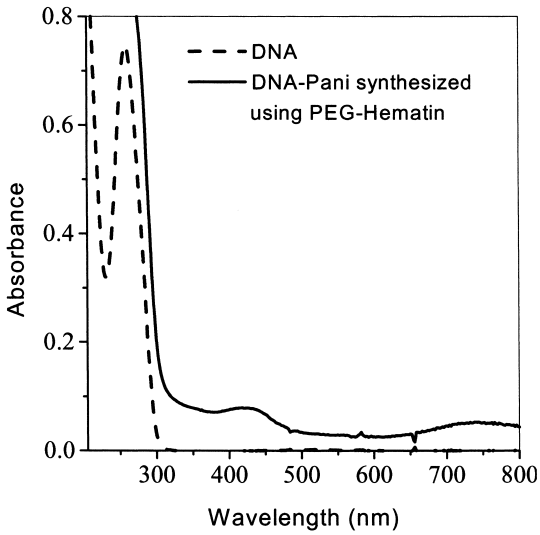

Figure 13. UV-vis spectra of DNA-Pani synthesized using PEG-Hematin.

polaron absorption bands at 420 nm and 750 nm indicating the formation of the conducting emeraldine salt form of polyaniline. The CD spectrum of the DNA-Pani complex (not shown) indicated a polymorphic transition in DNA similar to the transition observed in the case of Pani synthesized using HRP. This result proves that the new PEG-hematin complex provides sufficient catalytic activity for the polymerization of aniline in the presence of DNA and may be an inexpensive and versatile alternative catalyst to HRP for these reactions.

CONCLUSION

A generic, enzymatic approach for the synthesis of polyaniline on genomic/synthetic nucleic acid matrices has been developed. The complexation of Pani to nucleic acids has been found to induce reversible changes in the secondary structure of the nucleic acid template, often leading to the formation of an over-wound polymorph. The melting behavior of the DNA-Pani complex has provided evidence for the stabilization of the DNA by the Pani that is complexed to it. Scanning probe and electron microscopy studies have indicated that the formation of Pani, causes the DNA-Pani strands to agglomerate. However the structural integrity (secondary structure) of the individual DNA strands has been retained even after the formation of Pani. Molecular complexes of nucleic acids and Pani can also be synthesized using the inexpensive modified hematin catalyst. This development of a generic route for the binding of an electroactive polymer to a nucleic acid matrix opens new possibilities for the fabrication of biosensors and electro-responsive biomaterials.

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