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DNA-Directed Synthesis of Aniline and 4-Aminobiphenyl Oligomers: Programmed Transfer of Sequence Information to a Conjoined Polymer Nanowire

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Abstract: A new class of materials was prepared from aniline-containing oligomers that are covalently linked to the nucleobases of duplex DNA. Oligomers composed of repeating aniline (PANI) or 4-aminobiphenyl (PAB) units having the properties of conducting polymers conjoined to the DNA were prepared by the reaction of horseradish peroxidase and H_2O_2 with DNA having the appropriate monomers aligned within the major groove. These oligomers exhibit the spectral and chemical properties typical of *para*-linked polyanilines. This method of preparation enables utilization of the unique self-recognizing properties and sequence programmability of DNA to create tailored oligomers. This ability was demonstrated experimentally by preparation of PAB oligomers from alternating benzene and aniline monomers. Conjoined conducting polymers carrying the sequence information of DNA may have applicability as nanowires.

Introduction

There is a rapidly growing awareness that the self-recognizing and self-organizing properties of biomolecules, particularly nucleic acids, can be exploited for the creation of new nanoscale materials with unique properties and capabilities. In a recent example, Seeman has cleverly exploited the highly selective nucleobase recognition properties that are inherent in DNA to create a nanomechanical device that operates as a fully functional robot arm.[1]

DNA is also being used as a template for the fabrication of nanowires. These are one-dimensional structures that are anticipated to be suitable for forming self-organizing connections between functional electronic components.[2] A popular method for the creation of these nanowires is DNA-templated metallization. In this approach, the chemical reduction of metal ions bound electrostatically to DNA creates nanometer-sized metallic aggregates that act as catalysts for further metal deposition along the path defined by the DNA.[3] A recent refinement of this method allows for directed metallization of selected DNA segments.[4] This modification offers the prospect of sequence selective metallization, which could enable the creation of uniquely alloyed nanowires.

Nanowires may also be created from conducting organic polymers such as polyaniline (PANI) by a self-assembly process in the presence of surfactants or DNA, which act as "soft" templates that control formation and structure.[5] Polyanilines have attracted enormous interest as components of nanometer scale devices because of their wide range of conductivity, their stability, and the ease of synthesis. In particular, PANI can be prepared enzymatically under mild conditions in aqueous solution using DNA as a template.[6] In this process, anilinium ions bound electrostatically to the negatively charged phosphate groups of the DNA are converted to PANI by reaction with horseradish peroxidase and H_2O_2. This procedure is limited to producing only aniline homopolymers that do not take advantage of the sequence information inherent in DNA.

We recently reported a fusion of the templating and scaffolding roles of DNA in the DNA-directed assembly of PANI.[7] In this approach, cytosine nucleotides are covalently linked to aniline moieties that are subsequently polymerized to create DNA-conjoined PANI oligomers. Significantly, this process provides a method to utilize the sequence programmability of DNA to prepare any number of unique conducting polymers tailored to have desired structural or electronic properties.

In this work, we report the further development of conjoined PANI−DNA oligomers and demonstrate the sequence programmability of these polymers by preparing conjoined poly(4-aminobiphenyl)−DNA oligomers (PAB−DNA). In comparison with PANI, the PAB oligomers possess distinct structural and electronic properties, which affect their state of oxidation. However, irrespective of the structural difference in the monomers, our results show that the covalently linked PANI and PAB oligomers are formed predominantly by a para-directed, head-to-tail process. Finally, we used this approach

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DNA	Sequence
(1)	5′-TAGCACACCCCCCGTCGAACCT-3′
(2)	5′-ATACTAGCTAGGTTCATGAG--AGAGTCTACTACTACGGAGTC-3′
(X2)	5′-TAGCACACC**XX**CCGTCGAACCT-3′
(X6)	5′-TAGCACA**XXXXXX**GTCGAACCT-3′
(Y4)	5′-TAGCACA**Y**T**Y**T**Y**T**Y**GCGAACCT-3′
(Y3)	5′-TAGCACA**Y**C**Y**C**Y**CGTCGAACCT-3′
(Y3′)	5′-GACTCGTAGTAGATGACT**Y**T-3′
(Y3″)	5′-**Y**T**Y**ATGAACCTAGCTGTGAT-3′
(Y2)	5′-TAGCACACT**Y**T**Y**TCGCGAACCT-3′
(Y1)	5′-TAGCACACTCT**Y**TCGCGAACCT-3′
(X′6)	5′-TAGCACA**X′X′X′X′X′X′**GTCGAACCT-3′
(Y′4)	5′-TAGCACA**Y′**T**Y′**T**Y′**T**Y′**GCGAACCT-3′
(XZ)	5′-TACCACA**XZXZXZXZ**GGAACCT-3′
(ZX)	5′-TACCACA**ZXZXZXZX**GGAACCT-3′

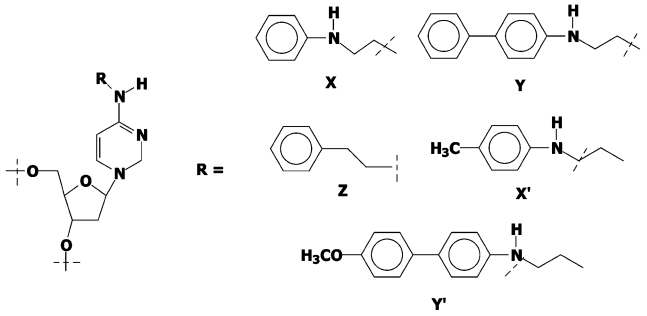

Figure 1. DNA sequences used in this work. The structural modifications incorporate modified nucleotides X,Y, Z, X′, and Y′, as indicated. The DNA oligomers used in these experiments are duplexes formed from appropriate complementary strands.

to prepare conjoined copolymers of aniline and benzene monomers positioned on alternating cytosine residues of DNA duplexes. These results demonstrate the potential of using variously modified nucleotides in preparing conjoined polymers with non-recurring, irregular structures.

Experimental Section

Materials. All reagents were used as received without further purification. 4-Bromobiphenyl, *p*-bromotoluene, phenylethylamine, ethylenediamine, sodium *t*-butoxide, ammonium persulfate, phenylhydrazine, and 1,4-dioxane were obtained from Acros Organics, Morris Plains, NJ. 1,1′-Bis(diphenylphosphino)ferrocene and 1,1′-bis(diphenylphosphino)ferrocene-palladium(II) dichloride were obtained from Strem Chemicals, Inc., Newburyport, MA. The O4-Triazolyl-deoxyuridine phosphoramidite along with the PAC phosphoramidites (phenoxyacetyl-dA, isopropyl-phenoxyacetyl-dG and acetyl-dC) were purchased from Glen Research Corporation, Sterling, VA.

Preparation of Modified DNA Oligomers. Aniline, 4-aminobiphenyl, 4-methylaniline, and 4′-methoxy-4-aminobiphenyl modified DNA oligomers were synthesized by the convertible nucleotide approach[8] using the O4-triazolyl-deoxyuridine phosphoramidite for coupling the desired monomer. For this purpose, *N*-phenylethylenediamine, *N*-[(1,1′-biphenyl)-4-yl]ethane-1,2-diamine, *N*-(4-methylphenyl)-ethane-1,2-diamine and *N*-[4′-methoxy-(1,1′-biphenyl)-4-yl]ethane-1,2-diamine were synthesized following a previously published report.[9] The resin-bound oligonucleotides containing the convertible nucleotide were treated with 500−750 μL of 5 M (in CH_3CN) solutions of the appropriate amines for 24 h at 60 °C. The yield of modified oligomers is similar to that of regular unmodified DNA sequences based upon data from the trityl monitor during automated DNA synthesis. The oligonucleotides were deprotected by treatment with 4 mL of concentrated aqueous ammonium hydroxide at room temperature for 8−10 h. The ammonium hydroxide solution was dried on a Speed Vac and the samples reconstituted with water for purification by HPLC. The oligomers were purified by reversed phase HPLC on a Hitachi preparative HPLC system using a Dynamax C18 column. Purified DNA sequences were desalted using Waters Sep-Pak cartridges and characterized by ESI−Mass spectrometry.

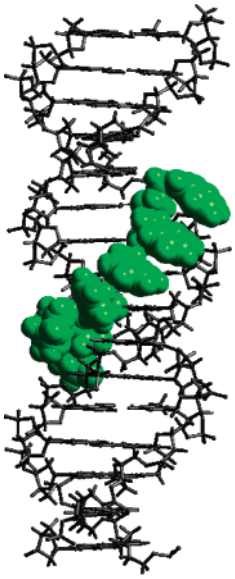

Figure 2. A structural model showing aniline modifications on cytosines extending into the major groove of a DNA duplex.

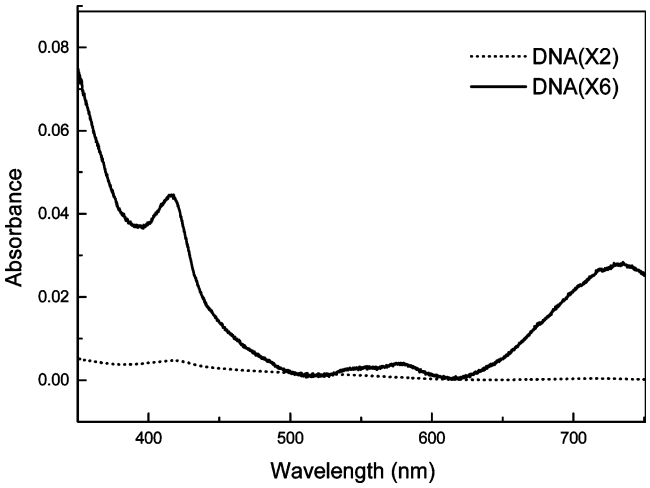

Figure 3. UV−vis absorption spectra of duplexes of DNA(X2) and DNA-(X6) after treatment with HRP and H_2O_2.

Synthesis of Oligoanilines and Oligo(4-aminobiphenyl). Horseradish Peroxidase (HRP), type II (200 units/mg) was purchased from Sigma Aldrich Chemical Co., St. Louis MO. A solution of HRP was prepared (2 mg in 2 mL) and used as a stock solution for the polymerization. Hydrogen Peroxide (H_2O_2; 30%) was purchased from Fisher Scientific, Pittsburgh, PA and diluted to 0.15% in deionized water for use. The 5 μM DNA duplexes were prepared in 10 mM citrate buffer (pH 4.5) containing 500 mM of NaCl. These conditions were used and maintained consistently after ensuring the stability of the corresponding DNA duplexes. After addition of HRP to DNA samples, polymerization was initiated by the addition of 2−5 μL of H_2O_2. UV−vis spectra were recorded 30 min after addition of H_2O_2 and at 10 min intervals thereafter to confirm the completion of reaction. For mass spectral characterization, samples were prepared at a concentration of ∼100 uM. Addition

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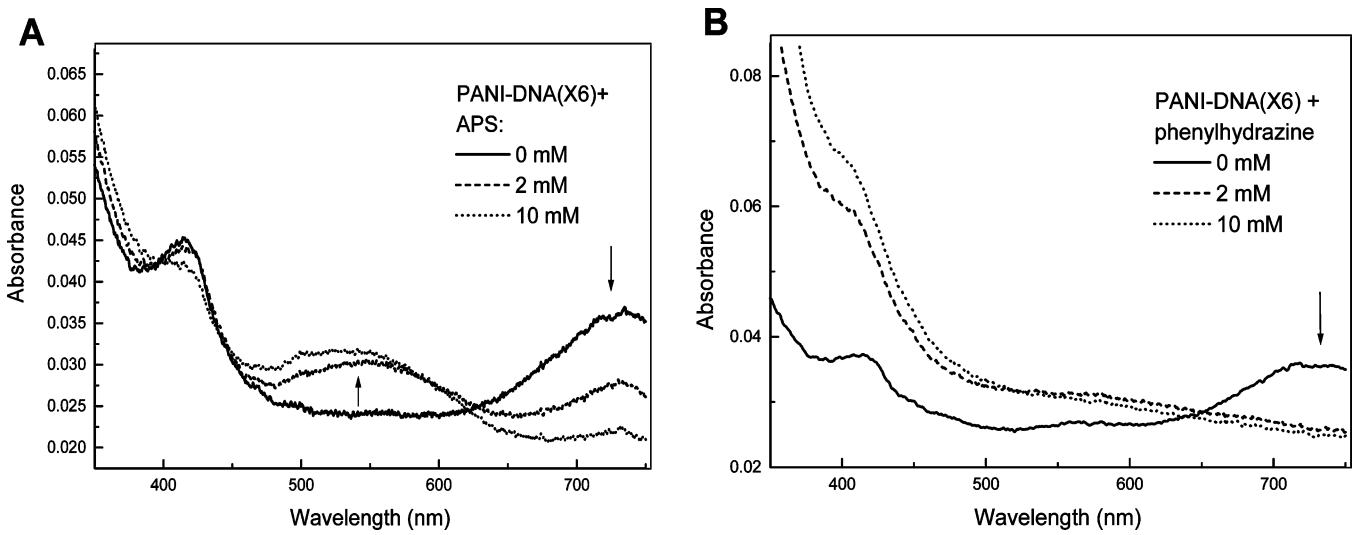

Figure 4. UV−vis absorption spectra of PANI−DNA(X6) after treatment with different amounts of (A) APS and (B) phenylhydrazine. Arrows indicate loss or gain of absorbance.

of H_2O_2 was followed by raising the pH of the solution to 7.0 (by addition of triethylamine). Ethanol precipitation of the samples was then performed followed by desalting using Waters Sep Pak cartridges and the samples were subjected to Electrospray Mass spectrometry.

Ammonium Persulfate and Phenylhydrazine Titrations. Aniline and 4-aminobiphenyl modified DNA duplexes were first treated with HRP and H_2O_2 to prepare DNA-conjoined oligomers. Then, 2 μL aliquots of 1 M ammonium persulfate or phenylhydrazine, respectively were added to 1 mL solutions of the DNA duplexes (5 μM) and the UV−vis absorption spectra were recorded. The additions were continued until no further change in absorbance was observed. Treatment of an unmodified DNA duplex with ammonium persulfate or phenylhydrazine under similar conditions did not show any changes in absorbance at 260 nm.

Equipment. UV−visible absorption spectra were recorded on a Cary 1E spectrophotometer, UV melting and cooling curves were recorded using a multicell block and temperature controller on the spectrophotometer. CD spectra were recorded on a JASCO J-715 spectropolarimeter. ESI−MS spectra were recorded on a Applied Biosystems QSTAR-XL in negative ion mode.

Results

DNA-Conjoined PANI Oligomers. A series of DNA oligomers, see Figure 1, comprising cytosines bearing covalently linked aniline groups was prepared to assess the possibility of forming conducting PANI oligomers that maintain the sequence programmability inherent in DNA. The cytosines were modified by replacement of the 4-amino group by N-(2-aminoethyl)aniline giving a nucleobase that is abbreviated as X in Figure 1.

DNA(1) is an unmodified 22-mer strand that forms duplex DNA with its complement (not shown). DNA(X2) and DNA(X6) are related to DNA(1) except that they are constructed with two and six contiguous aniline bearing cytosines (X), respectively. Molecular modeling[10] of the duplex formed from DNA(X6) and its complement reveals that the covalently linked aniline groups are positioned in the major groove of the duplex, see Figure 2. Although the presence of six aniline groups in the major groove of DNA(X6) results in a melting temperature that is 18 °C below that of DNA(1), the circular dichroism (CD) spectrum of DNA(X6) is similar to that of DNA(1), and this indicates that it maintains an overall B-form structure.

The treatment of duplex DNA(X6) with HRP and H_2O_2 under conditions known to cause aniline polymerization leads to the formation of a conjoined PANI oligomer characterized by absorption bands at ~420 and 730 nm, see Figure 3.[7] This absorption spectrum shows that the conjoined PANI−DNA oligomer is formed primarily in the "pseudo-proton doped" emeraldine oxidation state. Melting temperature data and CD spectroscopy show that the PANI−DNA oligomer maintains a duplex structure. However, molecular modeling indicates that the DNA duplex is severely distorted in the region of the PANI.[7] As expected, the conjoined PANI oligomer has a circular dichroism spectrum with an apparent peak at 680 nm.[7]

Significantly, ESI mass spectrometric analysis of DNA(X6), before reaction of the duplex [DNA(X6)/DNA(1)] with HRP and H_2O_2, reveals that it has the expected mass to charge ratio (m/e, $e = 1$) of 7300 amu (see Supporting Information). The complete oligomerization of DNA(X6) should result in conversion of its six covalently linked aniline monomers to PANI−DNA(X6) with the formation of five new carbon-nitrogen bonds and a concomitant reduction in mass of 10 amu. Mass spectrometric analysis of the PANI−DNA resulting from this reaction confirms this expectation. After treatment with HRP and H_2O_2, the m/e ratio for the PANI−DNA is found to be 7291 $\pm$ 1 (see Supporting Information), which confirms oligomerization. Moreover, careful analysis of the mass spectrum of PANI−DNA(X6) shows that there is no detectable residual monomer-containing strand and there are no significant partially oligomerized compounds. That is, based on the mass spectrometric data, reaction of duplex DNA(X6)/DNA(1) with HRP and H_2O_2 results in its essentially complete conversion to PANI-DNA in which all monomers have reacted. There is no detectable PANI formation when a DNA oligomer (up to a concentration of 60 μM) having only one covalently linked aniline group is treated with HRP/H_2O_2, which shows that intermolecular oligomerization does not occur under these conditions.

The redox properties of the conjoined PANI−DNA oligomer formed from polymerization of DNA(X6) were probed further

(10) Geometry optimizations were performed in HyperChem 7.5 using standard molecular mechanics methods based on the amber94 force field and a conjugate gradient method with a termination value of the RMS gradient of 0.01 kcal/mol/Å.

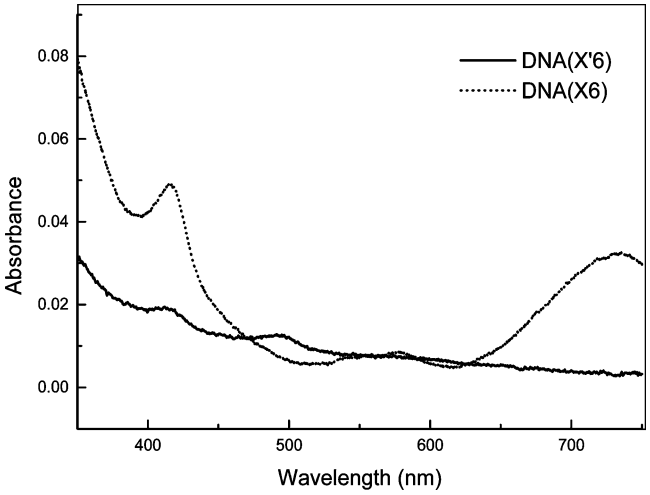

Figure 5. UV−vis absorption spectra of 4-methyl-*N*-(2-aminoethyl)aniline containing DNA (DNA(X′6)) and DNA(X6) upon treatment with HRP/H_2O_2.

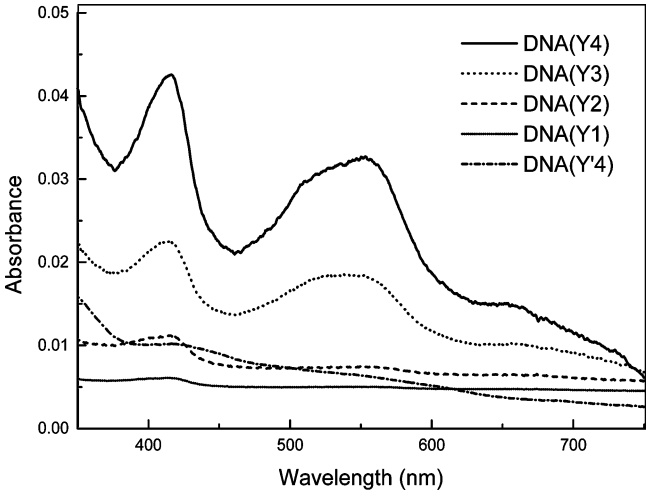

Figure 6. UV−vis absorption spectra of DNA(Y1−4) and DNA(Y′4) after treatment with HRP/H_2O_2.

by examining the effects of the chemical oxidizing and reducing agents ammonium persulfate (APS) and phenylhydrazine, respectively, on the conjoined polymer. Both APS and phenylhydrazine have been used in the preparation of the pernigraniline base and leucoemeraldine base forms of polyaniline.[11] As shown in Figure 4A, treatment of the PANI−DNA(X6) conjoined oligomer with APS results in the loss of absorbance at 730 nm and the concomitant increase in absorbance at ∼550 nm. The loss of the 730 nm band by reaction with a strong oxidizing agent such as APS is indicative of the further oxidation of the emeraldine form.[12] Alternatively, the reduction of PANI−DNA-(X6) with phenylhydrazine results in the complete disappearance of the absorbance at 730 nm without the appearance of any additional bands (Figure 4B). This is the expected result for reduction of PANI to the leucoemeraldine form of polyaniline.[12,13] These results are consistent with the conclusion that the PANI-DNA(X6) conjoined oligomer is formed primarily in the conducting emeraldine oxidation state by the reaction of duplex DNA(X6) with HRP/H_2O_2.

The absorbance spectrum of conjoined PANI−DNA(X6) suggests that the oligomer is formed by a head-to-tail reaction; that is, the oligomer is in the benzenoid-quinoid structure.[14] We assessed the role of the DNA template in enforcing the head-to-tail polymerization by incorporating 4-methyl-*N*-(2-aminoethyl)aniline (nucleobase X′, see Figure 1) in place of the *N*-(2-aminoethyl)aniline of DNA(X6). Of course, the 4-methyl substituent is expected to prevent PANI formation by the head-to-tail mechanism.[15] As shown in Figure 5, HRP/H_2O_2 treatment of DNA(X′6) does not result in significant PANI formation as revealed by the lack of characteristic spectral features in the visible region. This finding supports the conclusion that HRP/H_2O_2 treatment of duplex DNA(X6) gives predominantly the head-to-tail emeraldine-form polyaniline.

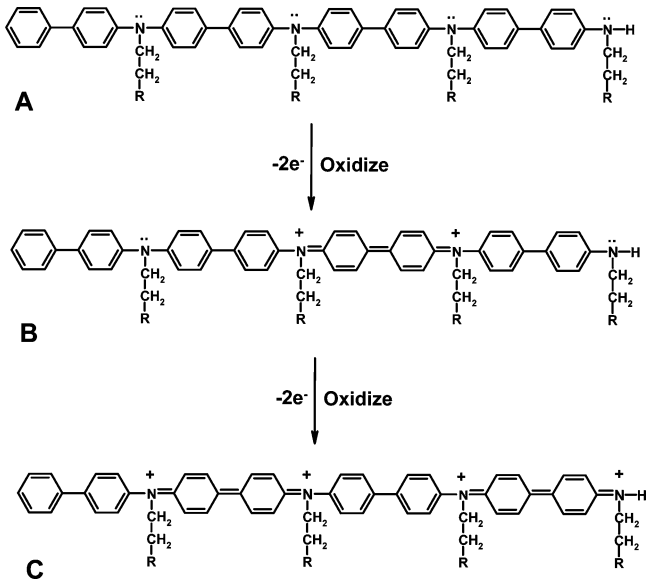

Figure 7. Structure of poly(4-aminobiphenyl) oligomers in oxidation states that correspond to (**A**) the fully reduced (leuco equivalent of PANI), (**B**) the partially oxidized (emeraldine equivalent) and (**C**) the fully oxidized (pernigraniline equivalent) state. Cytosine nucleobases connecting the oligomer to DNA are designated by R.

DNA-Conjoined 4-Aminobiphenyl Oligomers. To investigate the range of structures that will form conducting polymers by this technique, we chose 4-aminobiphenyl as a test reactant. Clearly, aniline has only one aromatic ring and the 4-aminobiphenyl has two. Molecular modeling studies indicated that the covalently linked aminobiphenyl groups would align in the major groove of the DNA duplex. However, in contrast to similarly situated aniline groups, the aminobiphenyls are properly aligned for oligomerization when they are placed on every other nucleobase in the sequence. We prepared DNA oligomers containing cytosines modified by replacement of their 4-amino groups by *N*-[(1,1′-biphenyl)-4-yl]ethane-1,2-diamine (abbreviated as nucleobase Y, see Figure 1). These oligomers contain a varying number of Y nucleobases arranged in an alternating fashion along one strand of the duplex DNA, and in the case DNA(Y3′) and DNA(Y3″) the monomers required to form a PAB oligomer are on two separate pieces of DNA that are brought together only in the presence of DNA(2) a

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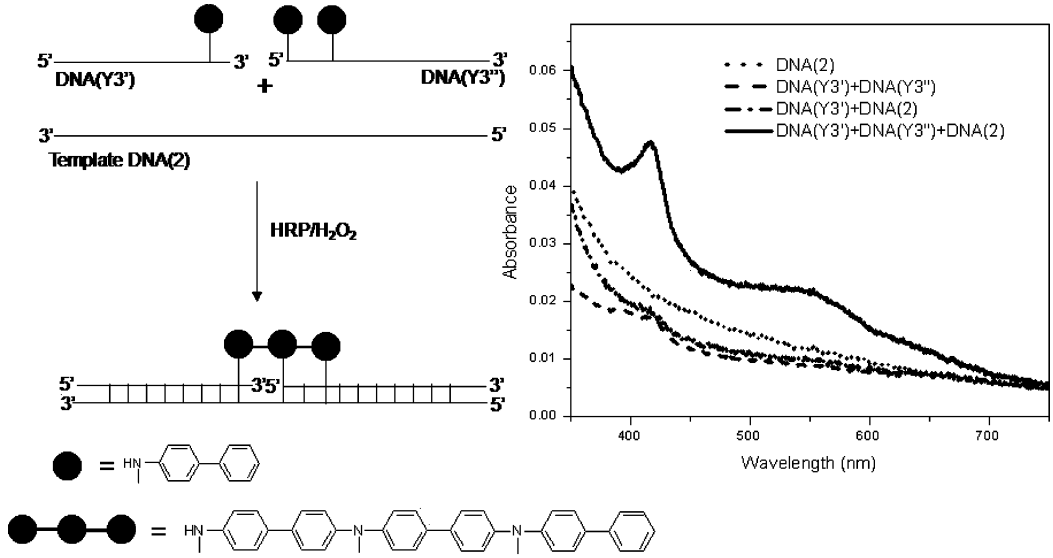

Figure 8. Schematic representation of PAB formation in a ternary complex comprised of DNA(2)−DNA(Y3′)−DNA(Y3″). Analysis by absorption spectroscopy after reaction with H_2O_2/HRP indicates that oligomerization occurs only when all three components are present. UV−vis spectra: (...) DNA(2); (− − −) 1:1 mixture of DNA(Y3′) + DNA(Y3″); (− · −)1:1 mixture of DNA(Y3′) + DNA(Y2); (—)1:1:1 mixture of with DNA(Y3′) + DNA(Y3″) + DNA(2).

templating strand that has adjacent sequences complementary to DNA(Y3′) and DNA(Y3″), see Figures 1 and 8. The structure of the DNA oligomers was confirmed by ESI mass spectrometry and the corresponding duplexes were characterized by their UV melting (T_m) and CD spectral features (see Supporting Information). Duplexes containing 4-aminobiphenyl residues show an overall B-form structure and display cooperative melting behavior, albeit at lower T_m compared with the corresponding unmodified duplexes (see Supporting Information).

In a first set of experiments, modified duplexes containing 4-aminobiphenyl groups were treated with HRP and H_2O_2 to initiate polymerization, and the reactions were monitored by absorption spectroscopy. No significant changes are observed in the absorption spectra of DNA(Y1) or DNA(Y2), which contain one and two Y-nucleobases, respectively. However, treatment of DNA(Y3) or DNA(Y4) duplexes with HRP/H_2O_2 results in the appearance of new absorption bands at ∼415 and 550 nm with a shoulder at 520 nm, see Figure 6. These bands are indicative of the formation of poly(4-aminobiphenyl).[16]

Poly(4-aminobiphenyl) prepared by electrochemical oxidation of the monomer has been shown previously to have unique absorption bands at 450 and 550 nm.[16] These bands correspond to radical cations and diimine species, respectively, see Figure 7. We showed that similar absorption bands are observed upon treatment of 4-aminobiphenyl or *N*-[(1,1′-biphenyl)-4-yl]ethane-1,2-diamine with HRP/H_2O_2 in presence of calf thymus DNA using the procedure shown previously to give polyanilines.[17] The similar optical absorption spectra of the conjoined PAB−DNA oligomers and those of PAB prepared by conventional techniques is strong evidence for formation of the PAB−DNA conjoined conducting polymer. CD and melting experiments reveal that the PAB−DNA maintains a DNA duplex structure albeit with reduced thermal stability (see Supporting Information).

The reactions of DNA(2) with complementary components DNA(Y3′) and DNA(Y3″) were examined to demonstrate that formation of these oligomers is an intramolecular process that requires alignment of the monomers by DNA. DNA(Y3′) contains one 4-aminobiphenyl monomer attached covalently at a position one nucleobase from its 3′-terminus. Similarly, DNA(Y3″) has two 4-aminobiphenyl monomers; one attached at its 5′-terminus and the other one base removed. The nucleobases of DNA(Y3′) and DNA(Y3″) are complementary to adjacent sections of DNA(2), which is referred to as the template strand, see Figure 8. The combination of these three oligomers forms a ternary complex (T_m = 70.1 °C, see Supporting Information) that organizes the 4-aminobiphenyl monomers so that the two on DNA(Y3′) and the one on DNA(Y3″) are adjacent to each other. A series of experiments was carried out on this system to show that oligomerization of the 4-aminobiphenyl monomers occurs only when the template strand is present.

Treatment of a 1:1 mixture of DNA(Y3′) and DNA(Y3″) with HRP and H_2O_2 does not result in the detectable formation of oligomers as revealed by the absorption spectrum of the reaction products (see Figure 8). Similarly, treatment of a 1:1 mixture of DNA(2) and DNA(Y3″) or DNA(2) with DNA(Y3′) with HRP/H_2O_2 does not lead to oligoaniline formation. However, the reaction of the ternary complex consisting of a 1:1:1 mixture of DNA(2), DNA(Y3′), and DNA(Y3″) clearly forms the PAB oligomer as revealed by its characteristic absorption bands. These experiments show that oligomer formation is an intramolecular reaction that requires pre-alignment of the aniline monomers by the self-recognition properties of DNA.

To confirm the state of oxidation of the conjoined PAB oligomers, the PAB−DNA(Y4) oligomeric duplex was treated separately with APS and with phenylhydrazine. As shown in Figure 9A, treatment of the PAB−DNA(Y4) with APS leads to an increase in intensity of the 520 nm absorption band without causing any other significant spectral changes. The ∼30 nm blue-shift in the diimine transition upon treatment of PAB-DNA(Y4) with APS is likely due to nominal further oxidation of the

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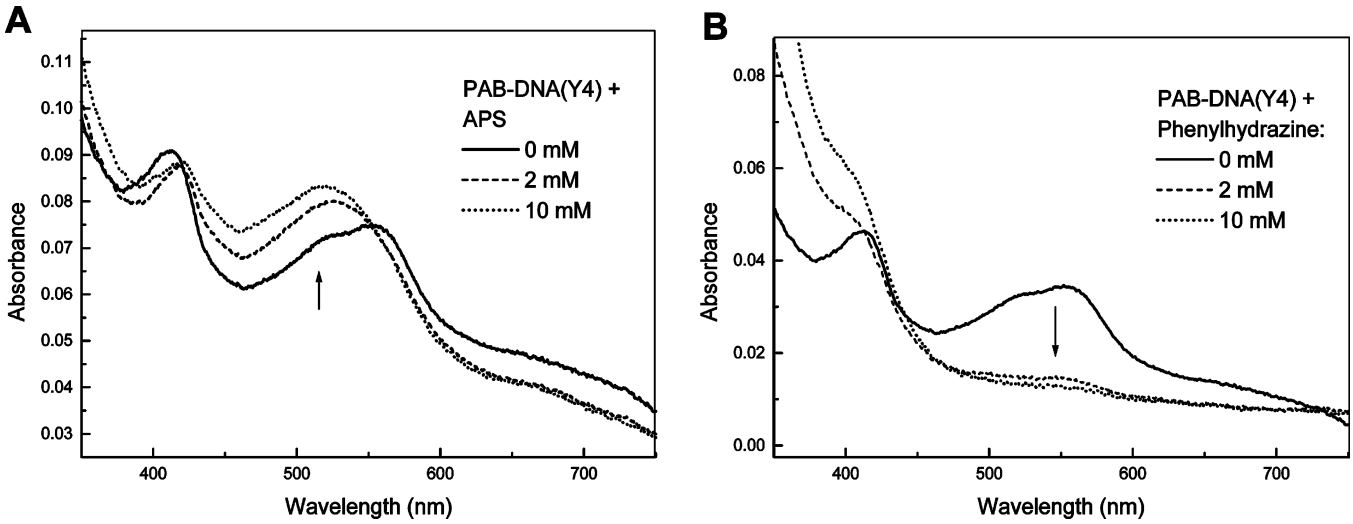

Figure 9. UV−vis absorption spectra of PAB−DNA(Y4) after treatment with (A) APS and (B) phenylhydrazine. Arrows indicate gain or loss of absorbance.

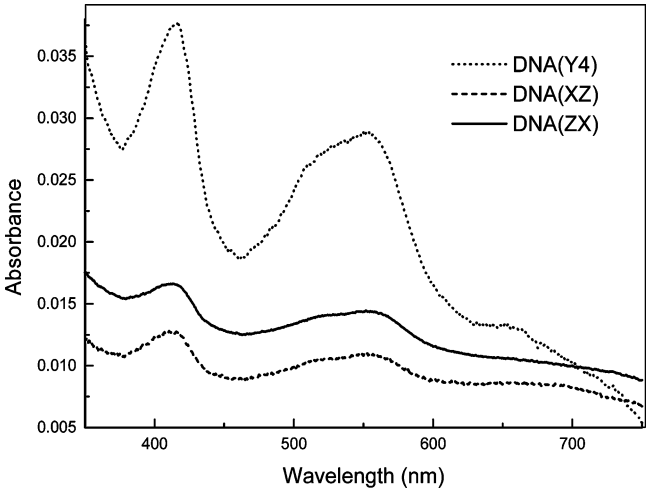

Figure 10. UV−vis absorption spectra of DNA(XZ) and DNA(ZX) after treatment with HRP/H_2O_2 and its comparison with DNA(Y4).

conjoined PAB oligomers. A comparison of these results with those obtained from similar experiments with PANI−DNA(X6) indicates that the PAB oligomer being formed by treatment with HRP/H_2O_2 is already in the highest oxidation state. In contrast, treatment of PAB−DNA(Y4) conjoined polymer with phenylhydrazine causes the disappearance of the absorbance bands at 550 and 520 nm, see Figure 9B. The reaction with phenylhydrazine, as expected, forms the fully reduced leuco form of the conjoined PAB−DNA oligomer.

We showed above that polymerization of PANI−DNA(X6) proceeds by a head-to-tail mechanism; a similar process for formation of para-directed PAB oligomers is not compulsory. The structure of the conjoined PAB-DNA oligomers was investigated by modifying the PAB monomers covalently linked to the cytosines of DNA(Y4) by incorporating a 4′-methoxy group (nucleobase Y′; 4′-methoxy-*N*-[(1,1′-biphenyl)-4-yl]ethane-1,2-diamine, see Figure 1). For obvious structural reasons, the head-to-tail para linkage of nucleobase Y′ will be inhibited compared with that of nucleobase Y. Treatment of DNA(Y′4) with HRP/H_2O_2 does not produce any significant spectral features in the visible region and the absorption bands characteristic of PAB oligomers. This result is consistent with a mechanism that requires head-to-tail addition for the formation of the conjoined PAB−DNA oligomers.

Copolymer of Aniline and Benzene. Utilizing the sequence information of DNA is a potential advantage for the creation of unique conducting polymers using the conjoined DNA template approach. Clearly, the PAB oligomers described above can be thought of as a copolymer of aniline and benzene. The applicability of this method to the formation of such DNA-conjoined copolymers was examined by investigating the reactions of compounds DNA(XZ) and DNA(ZX), see Figure 1. In these compounds, the relevant cytosines are modified by replacement of the pertinent 4-amino groups by two different groups, aniline and benzene. The cytosines that are modified by covalent linkage to *N*-phenylethylamine are abbreviated as nucleobase Z. The alternating Z and X modifications of DNA-(XZ) and DNA(ZX) have opposite directionality with respect to the DNA. It is important to note that the modifications Z and X were introduced into the DNA by different routes. This demonstrates the precise structural control possible using DNA to template creation of conjoined copolymers. In particular, the Z nucleobase was incorporated as its phosphoramidite during standard machine synthesis of DNA (see Supporting Information), whereas the X nucleobase was introduced postsynthetically using the convertible nucleotide approach as described previously.[7]

DNA(XZ) and DNA(ZX) were treated with HRP/H_2O_2 and these reactions were monitored by absorption spectroscopy. As shown in Figure 10, the absorption bands that result from this reaction at ∼415, 520, and 550 nm are essentially identical with those observed for the conjoined DNA−PAB oligomers formed from the 4-aminobiphenyl modified structures that are described above. This result demonstrates that the arrangement of aniline and benzene groups in DNA(XZ) and DNA(ZX) produces oligomers that are isostructural to the PAB oligomers obtained from DNA(Y4). Also, comparing the spectra of DNA(XZ) and DNA(ZX), indicates that this change in directionality does not affect the nature of the product. Significantly, these results demonstrate the potential of designing conjoined copolymers using the sequence information inherent in DNA to control the unique placement of monomer groups in the resulting polymer.

Discussion

There is rapidly growing interest in the preparation and investigation of nanowires.[18−20] These are structures that have a lateral dimension in the nm range, longitudinal dimension in the range from nm to μm, and electrical properties characteristic of conductors or semiconductors. Similarly, there is growing awareness that the unique properties of DNA may be exploited to fabricate nanowires and other electrical and mechanical objects of this length scale.[21−23] We describe here a novel approach applicable to the fabrication of such devices—the synthesis of conducting polymers conjoined to DNA. Specifically, designed DNA sequences with aniline-containing monomers covalently linked to the nucleobases can be used in directed self-assembly that takes advantage of the sequence programmability properties of DNA to exercise precise control on polymer formation. Treatment of these aniline-modified duplexes with H_2O_2 in the presence of HRP leads to oligoaniline formation.

Forming DNA-Conjoined Oligomers of Aniline and 4-Aminobiphenyl. In contrast to the DNA templated polymerization of free anilines in solution, the covalent attachment of the aniline monomers to DNA enforces a particular structural orientation and restricts certain interactions between moieties on different DNA molecules. This determines the nature of the polymers that can be formed. In particular, the observation that characteristic oligoaniline absorption bands are formed only when all three components of the ternary complex DNA(2), DNA(Y3′), and DNA(Y3′′) are present confirms the role template and scaffold play in controlling oligomer formation by preventing the reaction of monomers attached to different DNA molecules.

The properties of the conjoined PANI oligomers that are formed in this process indicate both a close similarity to conventional conducting polymers and unique attributes resulting from their linkage to DNA. The absorbance bands of PANI−DNA(X6) at 420 and 730 nm are attributed to $\pi-\pi^*$ and diimine radical cations, respectively, and are indicative of the conducting "pseudo-proton doped" emeraldine oxidation state of PANI.[7] The PANI linked DNA(X6) oligomer maintains a duplex structure, albeit with reduced thermal stability. This is expected since the wrapping of polyaniline onto the DNA has been found to induce changes in the secondary structure of DNA leading to the formation of an over-wound polymorph.[24] Detailed molecular modeling has suggested that while the region of the DNA duplex bearing the PANI oligomer is distorted and exhibits reduced inter-base pair hydrogen bonding, the overall stability of the conjoined system is improved by incorporating longer flanking duplex DNA regions ("leads").[7] Regardless, the fate of the DNA duplex after conjoined polymer formation is of secondary importance for the creation of conducting polymer nanowires. The role of the DNA is to order the covalently linked monomers and to provide a template for formation of a PANI oligomer in the emeraldine oxidation state; once this is completed, the properties of the conducting nanowires should be largely independent of the structural fate of the conjoined DNA.

The applicability of this method for formation of DNA-conjoined polymers to monomers beyond aniline was demonstrated by showing that the DNA(Y) series, which contains covalently linked 4-aminobiphenyl groups, forms conjoined oligomers. The 4-aminobiphenyl oligomers formed in this reaction possess distinctive chemical and electronic properties compared with polyaniline, while at the same time PAB and PANI retain some common structural features such as π-conjugation and a preferred head-to-tail orientation.[16] Significantly, the presence of the additional phenyl ring in the 4-aminobiphenyl monomers requires a change to the pattern for covalent linkage from contiguous to alternating bases along one strand of the DNA because the 4-aminobiphenyl spans a larger region of the major groove. Clearly, structural constraints resulting from the covalent attachment of these monomers to DNA control the oligomerization to form poly(4-aminobiphenyl); i.e., the reaction of DNA(Y4) leads to oligomer formation, but DNA(Y2) does not. These results highlight again the templating ability of DNA in controlling the formation of conjoined oligomers.

There are meaningful differences in the properties of PAB−DNA(Y4) and PANI−DNA(Y6). The diimine transition band for poly(4-aminobiphenyl) is considerably blue-shifted compared with PANI−DNA(X6), which appears at 730 nm. This is attributed to a less delocalized character for the quinoid-imine in the structure of PAB, see Figure 7. Notably, even tetramers of aniline in the emeraldine oxidation state exhibit absorption bands at ca. 750 nm, albeit at shorter wavelengths compared with long chain polyanilines (∼850 nm).[25] In contrast, the structure of poly(4-aminobiphenyl) restricts delocalization of quinoid-imines resulting in the blue-shift of the diimine optical transition.

Structure and Oxidation States of Conjoined PANI and PAB. One advantage of polyaniline is its wide and controllable range of oxidation states. These redox states have been correlated with the electrical properties of these conducting polymers.[26] The absorption spectrum of PANI−DNA(X6) formed in the reaction with HRP/H_2O_2 is indicative of the emeraldine oxidation state. Since the emeraldine oxidation state of PANI−DNA(X6) lies between the fully reduced (leuco) and fully oxidized (pernigraniline) forms, it is possible to manipulate the redox state of these polymers with appropriate reagents.[27,28]

The addition of ammonium persulfate, a strong chemical oxidizing agent, to PANI−DNA(X6) causes its absorbance at 730 nm to diminish while causing a simultaneous increase in the absorbance at 550 nm. The absorbance at 730 nm is attributed to diimine radical cations and is related to the presence of similar numbers of reduced and oxidized units that characterize the emeraldine oxidation state.[27] Further oxidation of the PANI is expected to yield a larger number of diimine units with

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concomitantly reduced delocalization throughout the oligomer. Notably, the fully oxidized form of PANI (pernigraniline) has been previously prepared by the chemical oxidation of emeraldine, and it absorbs at ~530 nm.[29] The absence of a significant shift in absorbance of the 420 nm band when PANI−DNA(X6) is treated with APS is expected since absorptions in the 300−450 nm region are known to be relatively insensitive to the overall oxidation state of polyaniline. These findings are fully consistent with the conclusion that the conjoined oligoaniline formed by treatment of DNA(X6) with HRP/H_2O_2 is in the emeraldine oxidation state and that this material is further oxidized to the pernigraniline form by reaction with APS.

In contrast to the most oxidized form, the leuco form of polyaniline is characterized by absorption at ~330 nm, which is attributed to $\pi-\pi^*$ transitions. The leuco form has no significant features in the visible spectral range.[13] Reduction of PANI−DNA(X6) with phenylhydrazine causes an increase in absorption intensity in this near-UV region and a decrease in the absorption that is characteristic of the emeraldine form at 420 nm. These findings are consistent with the conclusion that the conjoined oligoaniline formed by treatment of DNA(X6) with HRP/H_2O_2 is in the emeraldine oxidation state and that this material is reduced to the leuco form by phenylhydrazine.

In contrast to PANI, the structure of the PAB oligomer mandates a restricted delocalization of diimines. In addition, oxidation of the PAB oligomer, especially at the ends, is unlikely. Thus the diimine delocalization is limited, which results in significantly blue-shifted absorption bands compared with PANI. The results of oxidation of PAB−DNA(Y4) with APS and reduction with phenylhydrazine are consistent with the chemical structure proposed for conjoined PAB. The absence of significant spectral features in the visible region after reduction with phenylhydrazine is reasonable since reduced PAB is characterized only by $\pi-\pi^*$ transitions at ~360 nm. Evidently, the PAB−DNA(Y4) oligomer is formed in a higher oxidation state in the reaction with HRP/H_2O_2 than is PANI−DNA(X6). This is not a consequence of being conjoined to DNA because similar results are observed with the DNA templated PAB formed from free monomers in solution (see Supporting Information).

Head-to-Tail Directionality and Copolymer Formation. The DNA templated enzymatic polymerization of aniline is known to promote a preference for para-directed, head-to-tail polymerization[30] thereby improving the electrical properties of the polymers compared with those formed in untemplated reactions. In comparison to the chemical or enzymatic polymerization of anilines in solution, duplexes such as DNA(X6) offer greater control of the arrangement of substituent anilines. Nevertheless, the aniline groups still enjoy some flexibility in their movement due to the alkyl tethers linking them to DNA. Thus, the possibility of branching in PANI oligomer formation cannot be precluded. Also, for the PAB oligomers, the presence of the second aromatic ring provides additional positions for reactions and branching. We wished to confirm that para-direction of oligomer formation is enforced in the conjoined oligomers reported here.

The cytosines incorporating 4-methyl-*N*-(2-aminoethyl)aniline and 4′-methoxy-*N*-[(1,1′-biphenyl)-4-yl]ethane-1,2-diamine (nucleobases X′ and Y′ respectively) sheds light on this mechanistic aspect of polymer formation. Treatment of DNA(X′6) and DNA(Y′4) with H_2O_2 and HRP does not generate the visible absorbance bands characteristic of para-linked PANI. Evidently, the "blocking" substituents on the para positions of these monomers prohibit reaction. In particular, the enzymatic synthesis of polyaniline in the absence of templates has been shown to result in branched structures that are characterized by absorption bands at ~460 nm.[31] These findings are indicative of a preferred head-to-tail reaction in the formation of the conjoined PANI and PAB oligomers.

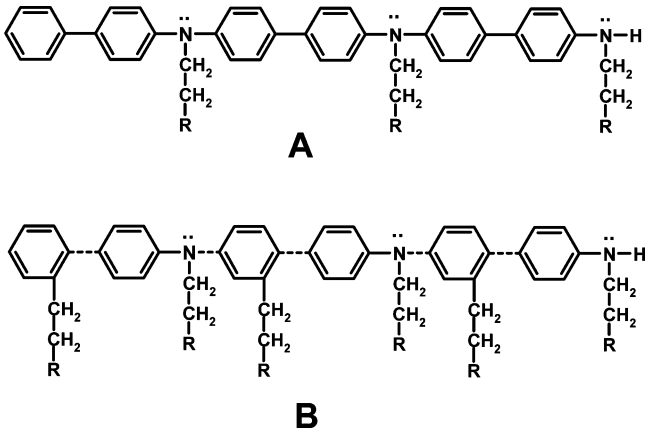

Figure 11. Structures of (**A**) homopolymer of 4-aminobiphenyl and (**B**) copolymer of aniline and benzene. Dashed lines in **B** indicate location of bond formation between aniline and benzene monomers. Cytosine nucleobases connecting the oligomer to DNA are designated by R.

DNA Sequence Programmability Applied to the Formation of Conjoined Conducting Oligomers. In the approaches discussed thus far for forming conjoined PANI oligomers, each of the modified cytosine nucleotides is identically substituted. There is no fundamental reason why this must be the case or why the covalently conjoined monomers are restricted to cytosines. To demonstrate this principle experimentally, we prepared oligomers having cytosines modified with two different moieties suitably arranged to form copolymers corresponding to PAB−DNA. This approach effectively demonstrates the power of this technique to use the sequence programmability of DNA to generate conducting copolymers having a wide range of possible, non-regular structures.

The order of the aromatic rings associated with 4-aminobiphenyl modifications in DNA(Y4) can be mimicked by an appropriate pattern of aniline and benzene substitutions. This was accomplished by preparing DNA(XZ) and DNA(ZY), which have modified cytosines carrying the two monomers in an alternating fashion. The treatment of these compounds with HRP/H_2O_2 results in the formation of oligomers that have the same absorption spectra as that of the PAB−DNA(Y4) conjoined oligomer. This is indicative of the formation of oligomers of similar structures by these two routes, see Figure 11. The oligomers formed from DNA(XZ) and DNA(ZX) have lower intensity absorbance bands compared with that from DNA(Y4), which may be due to lower yields. Finally, it should be noted that absorbance bands of similar intensity are observed independent of the directionality of the X and Z nucleobases with

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respect to the DNA since they are reversed in DNA(ZX) from that in DNA(XZ).

The important conclusion from these findings is that two distinct covalently linked monomers can be programmed by the DNA sequence to form unique oligomers with non-recurring, irregular structures. The modification of more than one nucleobase in a DNA oligomer with appropriate monomers is expected to further expand the range and complexity of polymers that can be formed by this technique.

Conclusions

Aniline monomers covalently attached to DNA nucleobases can be converted to conjoined oligoanilines having the properties of conducting polymers by treatment with HRP/H_2O_2. The DNA acts as a soft template that restricts the reaction to monomers aligned along one strand of the duplex DNA and inhibits the intermolecular reaction of monomers on separate DNA molecules. The covalent attachment of the monomers enforces a head-to-tail, para linkage that generates the more desired, higher conducting form of the polyaniline. The oligomers formed in this process are in the emeraldine oxidation state, but they can be oxidized or reduced by chemical reagents. Monomers of diverse structures can be converted to oligomers in this reaction. The oligomers that result may be tailored to have unique structures by taking advantage of the sequence programmability of the templating DNA. The oligoanilines formed in this process may be useful in the development of nanowires that take advantage of the self-recognizing and self-organizing properties of DNA to create unique structures.

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Supporting Information Available: ESI mass spectrometric characterization of modified DNA sequences, HPLC purification profile of DNA(Y4), UV-thermal melting temperatures of modified DNA duplexes and melting profile of ternary complex DNA(Y3′)-DNA(Y3″)-DNA(Y2), synthetic scheme and procedure for the preparation of the Z phosphoramidite, CD spectra of DNA(Y4) before and after HRP/H_2O_2 treatment, UV thermal melting behavior of DNA(Y4) before and after HRP/H_2O_2 treatment, comparative CD spectra of DNA(X6), DNA(Y4), DNA(X′6), and DNA(Y′4) and mass spectrometric characterization of conjoined PANI formation. This material is available free of charge via the Internet at http://pubs.acs.org.

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