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Electrocatalytic intercalator-induced winding of double-stranded DNA with polyaniline

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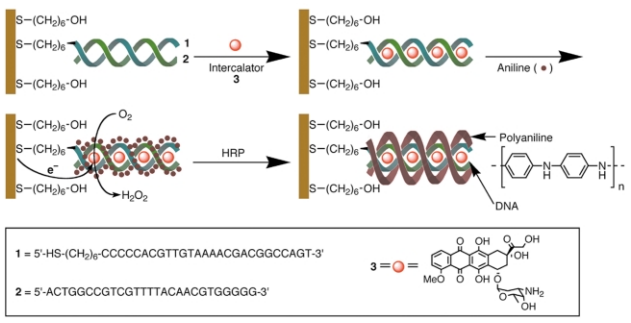
The intercalation of doxorubicin into double-stranded DNA stimulates the electocatalyzed oxidation of aniline to polyaniline and its winding on the DNA template.

The availability of enzymes for the manipulation of DNA such as ligase, endonuclease or polymerase, and the versatile synthetic methods of preparing nucleic acids, enable the preparation of ingenious DNA structures of predesigned shapes and compositions.[1,2] Thus, the binding of ions to phosphate units of DNA, or the intercalation of units into the DNA, followed by the chemical manipulation of the DNA-associated units, enables the use of the DNA as a template for the assembly of DNA-based functional structures.[3,4] Previous studies[5–7] have demonstrated the association of metal ions to DNA and their chemical reduction and generation of metallic wires. Also, the incorporation of Au-nanoparticle-functionalized intercalators into double-stranded DNA[8] or the assembly of semiconductor nanoclusters on DNA[9] have been reported. Hybrid conductive polymer–DNA systems were generated by inter-polymer electrostatic interactions to yield aggregated structures in solutions[10] or on surfaces.[11] Here we report on the electrochemically addressable biocatalyzed synthesis of polyaniline (PAn) chains on double-stranded DNA, (ds-DNA). We demonstrate that the PAn chains are redox-active at neutral pH, consistent with the formation of a PAn–DNA hybrid system.

The synthesis of the PAn chain wound on the ds-DNA template is schematically depicted in Scheme 1. The thiolated nucleic acid primer (**1**) is assembled on the Au-electrode. The surface is then treated with 1×10^{-3} M mercaptohexanol to block any bare gold defects on the surface and hybridized with the complementary nucleic acid (**2**). Doxorubicin, (**3**), is then intercalated into the double-stranded DNA, and the resulting functionalized surface is interacted with the aniline solution (0.3 M). This results in the association of anilinium units to the DNA by electrostatic and π–π interactions. The resulting interface is then rinsed and further introduced into an electrolyte solution that lacks aniline, but includes horseradish peroxidase, HRP, as a biocatalyst. Electrochemical reduction of doxorubicin, (**3**), under oxygen yields[12] H_2O_2, and the latter product, generated in the vicinity of the double-stranded DNA, activates the HRP-mediated oxidation of the aniline units to PAn.[13,14] Figure 1, curve (a), shows the cyclic voltammogram of the doxorubicin incorporated in the ds-DNA prior to the association of the aniline units. Coulometric analysis of the redox-wave indicates a surface coverage of (**3**) that corresponds to 6.6×10^{-11} mole·cm^{-2}. Figure 1, curve (b), shows the cyclic voltammogram of the PAn generated upon the application of a potential corresponding to −0.65 V *vs*. SCE at pH = 6.0 for 3 minutes in the presence of HRP. Upon the application of the potential for additional time-intervals, the redox-wave of the PAn is almost unaltered, indicating that all of the aniline units associated with the DNA were electropolymerized in the first 3 minutes.

Coulometric analysis of the reduction wave of the oxidized PAn indicates a surface coverage of aniline equivalents that corresponds to $(3.1 \pm 0.3) \times 10^{-10}$ mole·cm^{-2}. Control experiments reveal that no PAn is electrochemically generated upon the application of the negative potential on an Au-electrode that is modified with mercaptohexanol but lacks the nucleic acid (**1**), in the presence of aniline (0.3 M) in the solution. Also, no PAn is formed in the presence of an Au-electrode modified with (**1**) and treated with aniline in the presence or absence of doxorubicin, or upon the application of the negative potential on the double-stranded DNA system treated with aniline in the absence of doxorubicin, (**3**). These control experiments imply that the direct electroreduction of O_2 to H_2O_2 is inhibited in the absence of doxorubicin, and that the intercalation of doxorubicin is essential to effect the biocatalyzed formation of PAn on the DNA template. Since (**3**) binds only to the ds-assembly, no PAn is formed on the nonhybridized nucleic acid.

Microgravimetric quartz crystal microbalance (QCM) experiments were performed to characterize the system. The association of (**1**) to an Au-quartz piezoelectric crystal (AT-cut, 9 MHz) results in a frequency change of −80 Hz, indicating a surface coverage[15] of 2.7×10^{-11} mole·cm^{-2}. Thus, only *ca*. 36% of the nucleic acids associated with the surface are hybridized with (**2**). The incorporation of the intercalator (**3**) into the DNA results in a frequency change of $\Delta f = -16$ Hz, that translates to a surface coverage of 7.0×10^{-11} mole·cm^{-2}. This value is very similar to the surface coverage obtained from the coulometric assay of the redox-wave of (**3**). Knowing the surface coverage of the ds-DNA, and realizing that (**3**)



Scheme 1 Assembly of the PAn chains wound on a double-stranded DNA template associated with an Au-electrode.

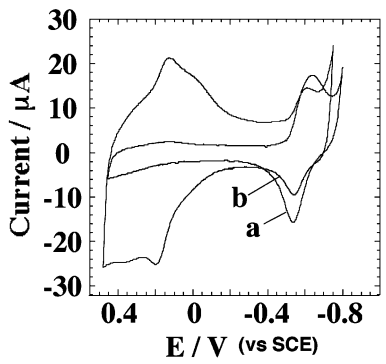


Fig. 1 Cyclic voltammograms corresponding to: (a) doxorubicin, (**3**), intercalated into the double-stranded DNA associated with the electrode surface. (b) The PAn generated on the DNA template. Data recorded in 0.1 M phosphate buffer solution, pH = 6.0, under Ar, scan rate 100 mV·s^{-1}.

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intercalates only with the ds-DNA, we estimate that *ca*. 11 doxorubicin units are associated with each ds-DNA. The association of the aniline units to the DNA yields a frequency change of $\Delta f = -12$ Hz, indicating a surface coverage of 3.2×10^{-10} mole·cm^{-2}. Knowing the surface coverage of the double-stranded DNA, we calculated that *ca*. 50 aniline units are associated with each double-stranded DNA.

Since the double-strand includes 27 base pairs, the calculation leads to the conclusion that each of the phosphate sites bind one aniline unit. Since the distance between two adjacent phosphate groups in each DNA strand is *ca*. 3.2 Å, whereas the distance between adjacent phosphate units on the two opposite strands of DNA is *ca*. 20 Å, we conclude that the polymerization processes of PAn proceeds along each strand of the double-stranded DNA to yield two polymer chains on the DNA template. Coulometric assay of the redox response of the PAn leads to a similar coverage of the aniline equivalents in the resulting PAn. We assume that the PAn chains are formed along each of the strands of the double-stranded DNA template. Microgravimetric QCM experiments indicate that the aniline monomer does not bind tightly to single-stranded DNA and it is washed off from the interface. A possible explanation for the specific tight association of the aniline monomer to the ds-DNA may be the substantially higher surface potential of ds-DNA[16] that binds by tight electrostatic interactions and synergistic π–π interaction the aniline monomer.

In situ electrochemical surface plasmon resonance (SPR) spectroscopy experiments were used to follow the redox features of PAn films on electrode surfaces.[17,18] The build-up of the PAn film was followed by SPR spectroscopy. Figure 2 shows the *in situ* electrochemical SPR spectra of the reduced and oxidized states of PAn, curves (a) and (b), respectively. The oxidation of the PAn chains associated with the ds-DNA template at 0.4 V results in a shift in the minimum incidence angle that corresponds to $\Delta\theta = 0.17°$ and the reflectivity measured at fixed angle of incidence of 68.1° increases by *ca*. 2%. By the reversible oxidation and reduction of the PAn chains, the intensity of reflected light is switched between low and high values for the two states, respectively, Figure 2, inset. Thus, the PAn chains associated with the DNA represent an optoelectronic assembly, where the redox states of the polymer are transduced by the SPR read-out signals.

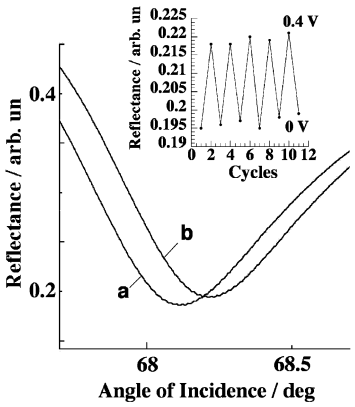

Fig. 2 *In situ* electrochemical SPR spectra of the PAn/DNA assembly: (a) The reduced PAn state associated with the DNA, generated by applying a potential corresponding to 0.0 V *vs*. SCE. (b) The oxidized PAn state associated with the DNA, generated by applying a potential corresponding to 0.4 V *vs*. SCE. **Inset:** Cyclic changes in the intensity of reflected light ($\theta = 68.1°$) upon the reversible switching of the potential on the PAn/DNA-functionalized Au-electrode between the reduced state ($E = 0.0$ V) (a) and the oxidized state ($E = 0.4$ V) (b).

In conclusion, the present study has demonstrated the addressable *in situ* bioelectrocatalytic generation of PAn on ds-DNA templates associated with electrodes. The PAn chains that wind the DNA template generate a supramolecular structure of the two oppositely charged polyelectrolytes. The resulting PAn is redox-active in neutral environments in contrast to regular PAn that reveals redox functions only in acidic solutions (ca. pH = 2.0). Previous studies have demonstrated that blends consisting of PAn and negatively charged polyelectrolytes (*e.g*. polyacrylic acid)[19,20] exhibit an electrochemical function in neutral aqueous solutions. This was attributed to the effective protonation of PAn by the polyelectrolyte in a broad pH region. Thus, the double-stranded DNA does not only act as a template for the assembly of the PAn, but it also controls the redox functions of the wound PAn chains. The new synthetic methods to assemble PAn chains on DNA paves the way to generate new wires consisting of conductive polymers, and to explore conductivity features of new nanowire circuitry.

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Notes and references

1 N. C. Seeman, *Trends Biotechnol.*, 1999, **17**, 437.
2 N. C. Seeman, *Angew. Chem. Int. Ed.*, 1998, **37**, 3220.
3 C. M. Niemeyer, *Angew. Chem. Int. Ed.*, 2001, **40**, 4128.
4 W. Shenton, S. A. Davies and S. Mann, *Adv. Mater.*, 1999, **11**, 449.
5 E. Braun, Y. Eichen, U. Sivan and G. Ben-Yoseph, *Nature*, 1998, **391**, 775.
6 J. Richter, R. Seidel, R. Kirsch, M. Mertig, W. Pompe, J. Plaschke and H. K. Schackert, *Adv. Mater.*, 2000, **12**, 507.
7 W. E. Ford, O. Harnack, A. Yasuda and J. M. Wessels, *Adv. Mater.*, 2001, **13**, 1793.
8 F. Patolsky, Y. Weizmann, O. Lioubashevski and I. Willner, *Angew. Chem. Int. Ed.*, 2002, **41**, 2323.
9 T. Torimoto, M. Yamashita, S. Kuwabata, T. Sakata, H. Mori and H. Yoneyama, *J. Phys. Chem. B*, 1999, **103**, 8799.
10 D. Fischer, A. Von Harpe, K. Kunath, H. Petersen, Y. X. Li and T. Kissel, *Bioconj. Chem.*, 2002, **13**, 1124.
11 Y. Shao, Y. Jin and S. J. Dong, *Electrochem. Commun.*, 2002, **4**, 773.
12 F. Patolsky, E. Katz and I. Willner, *Angew. Chem. Int. Ed.*, 2002, **41**, 3398.
13 W. Liu, J. Kumar, S. Tripathy, K. J. Senecal and L. Samuelson, *J. Am. Chem. Soc.*, 1999, **121**, 71.
14 R. Nagarajan, S. Tripathy, J. Kumar, F. F. Bruno and L. Samuelson, *Macromolecules*, 2000, **33**, 9542.
15 The surface coverage was confirmed by complementary electrochemical measurements using $[Ru(NH_3)_6]^{3+}$ as a label. (*Cf*. A. B. Steel, T. M. Herne and M. J. Tarlov, *Bioconj. Chem.*, 1999, **10**, 419).
16 J. Wang and A. J. Bard, *Anal. Chem.*, 2001, **73**, 2207.
17 A. Baba, M. K. Park, R. C. Advincula and W. Knoll, *Langmuir*, 2002, **18**, 4648.
18 A. Baba, R. C. Advincula and W. Knoll, *J. Phys. Chem. B*, 2002, **106**, 1581.
19 P. N. Bartlett and E. Simon, *Phys. Chem. Chem. Phys.*, 2000, **2**, 2599.
20 O. A. Raitman, E. Katz, A. F. Bückmann and I. Willner, *J. Am. Chem. Soc.*, 2002, **124**, 6487.