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3′-Ferrocene-Labeled Oligonucleotide Chains End-Tethered to Gold Electrode Surfaces: Novel Model Systems for Exploring Flexibility of Short DNA Using Cyclic Voltammetry

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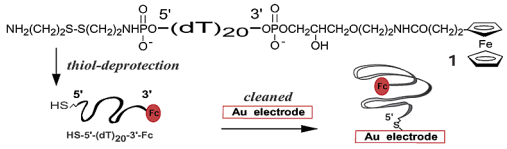
Nucleic acid probes tethered by one end to a solid surface are to date more than just unique tools for detecting targets in solution. They are also emerging as critical model systems for directly assessing the basic physical properties of DNA polymer chains as well as their specific interactions with molecules.[1,2] Particularly relevant examples for these studies are those experimental single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) probe systems that feature at the free end or at a precise location along its length a reporter molecule or a force sensor. For instance, short dsDNA-grafted electrodes containing guanine residues labeled with a redox intercalator able to act as an electron acceptor (e.g., anthracycline) have proven to be useful systems for exploring on the nanometer-length scale, long-range electron transport through densely packed duplex DNA monolayer films. However, the results of these studies strongly suggested that the flexibility (deformability) of DNA polymer strands may play an important role in modulating the rate and efficiency of charge transport. Clearly, this interesting possibility stresses the need for further refined experimental tools that would allow for a detailed understanding of the flexibility of short DNA chains at surfaces. In contrast, progress in manipulating DNA-grafted systems on the micrometer-scale currently enables the direct accurate determination of both the simple elasticity properties and the structural transitions of individual end-tethered DNA molecules.[1b,c] However, the need to use rather large force sensors in such experiments typically limits their applicability to studying deformations of long kilobase ss and dsDNAs.

Elucidating the flexibility of the DNA duplex at the local level of a few turns of duplex (a few 10−12 bases) is now regarded as a key issue to understanding many biological processes involving DNA, such as chromatin packaging and protein recognition.[2b,4,5] In this context, considerable work has been directed toward characterizing the sequence-dependent flexibility from intrinsic curvature of a DNA chain. However, most of these attempts have focused so far on peculiar DNA constructs expressly tailored with anomalous flexibility. Clearly, the problem for methodologies capable of evaluating the deformability of a short "natural" DNA strand of any arbitrary sequence remains open, as very recently pointed out also by several research groups.[5]

We have found that pure synthetic ssDNA oligonucleotides (typically 20-base) terminally attached by the 5′-end to a gold electrode surface via a short carbon atom-linker, and bearing at the free 3′-end a small redox-active label, endowed with the low redox potential of a alkylferrocene unit (Fc), are valuable low-density models for investigating the flexibility of tethered-ssDNA chains, using cyclic voltammetry as the excitation/measurement technique. Here, we report the creation and full characterization of the first system of this type. In a further aspect, specific hybridization of the grafted-ssDNA chains with unlabeled complementary target in solution is demonstrated to induce an unprecedented perturbation of the electrochemical response of the ferrocene heads that may ultimately provide quantitative information on the deformability of a short tethered dsDNA.

Scheme 1



In the present study, a presynthesized 3′-ferrocenylated-$(dT)_{20}$ oligonucleotide-5′-cystaminyl disulfide derivative **1** (Scheme 1)[6a−c] was reduced efficiently to its 5′-ethylthiol form by treatment with TCEP at pH 7.0. After purification by RP-HPLC, an ∼5 μM solution of the thiol sample was reacted from aqueous acetate with a cleaned gold surface for ∼16 h, to effect the direct attachment of a highly stable, nonblocking redox-labeled ssDNA monolayer (see Supporting Information for full details).[3c,6d,e]

The 3′-Fc$(dT)_{20}$-modified gold electrode was characterized in an electrolyte solution of high ionic strength (∼1 M) so that direct intramolecular electrostatic interactions are screened. Typical cyclic voltammograms are given in Figure 1. At a low enough scan rate, $v \leq 1$ V/s, a remarkable symmetrical signal is recorded as expected for a surface confined one-electron redox system (Fc/Fc^+) exhibiting an ideal Nernstian behavior:[8] the peak heights are proportional to v, the peak-to-peak separation ΔEp is less than 5 mV, and the width of each peak at mid-peak height is ca. 95 mV.[6f] Such results ascertain that all of the Fc heads are allowed to reach the electrode surface and proceed to reversible heterogeneous electron transfer. The total amount of bound Fc heads can then be deduced quantitatively from the area under either the anodic or the cathodic peak corrected from the background current, thus yielding $\Gamma = (3-5) \times 10^{-12}$ mol/cm^2 per effective area in end-bound $(dT)_{20}$-Fc chains. This low value (less than 10% of that for a monolayer of

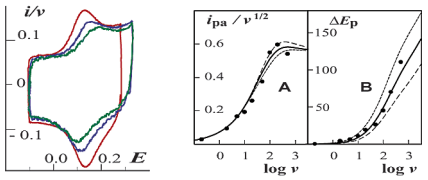


Figure 1. Cyclic voltammetry at various scan rates (v) of a 3′-Fc$(dT)_{20}$-modified gold disk electrode. Supporting electrolyte 1 M $NaClO_4$ + 25 mM KH_2PO_4 (pH 7.0), surface coverage $\Gamma = 3.9 \times 10^{-12}$ mol/cm^2, effective electrode area $A = 0.024$ cm^2. v (V/s) = 0.2 (red line), $v = 50$ (blue line), $v = 200$ (green line). The current (i/μA) is normalized versus v. The potential (E/V) is referred to SCE. Insets: (A) v dependence of the anodic peak current (i_{pa}/μA), normalized versus $v^{1/2}$. (B) v dependence of the peak-to-peak separation ΔEp (in mV). The continuous, dotted, and dashed lines are computed for $\Lambda^* = 0.8$, 0.4, and 1.6 (see Supporting Information).

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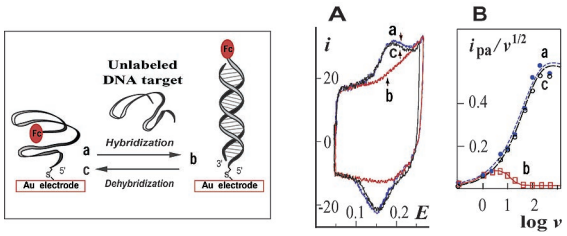

Figure 2. Hybridization between a Fc-$(dT)_{20}$-modified gold electrode and its full $(dA)_{20}$ complementary target in solution. Left: Schematic representation of the redox layers. Right: (A) Cyclic voltammetry, $v = 200$ V/s. (B) v dependence of the anodic peak current (i_{pa}/μA), normalized versus $v^{1/2}$. (a) Before and (b) after ex situ hybridization and (c) after dehybridization. The red curve in (A) is superimposed to the background. In (B), the continuous line is meant only as a visual guide; other lines are computed for $\Lambda^* = 0.8$. Same experimental conditions as in Figure 1.

close-packed ~20-mer ssDNA in an extended configuration)[3c,9] indicates that the chains are sparsely grafted on the electrode surface as noninterpenetrating polymer coils.[6g] At $v > 1$ V/s, ΔEp increases with increasing v (inset B, Figure 1), while the anodic peak current i_{pa} is no longer proportional to v. Actually, as shown in inset A, Figure 1, the ratio $i_{pa}/v^{1/2}$ increases and tends toward a plateau at $v > 100$ V/s, reflecting a diffusion-like motion of the Fc probes.[7,10] The observed changes are quite satisfactorily accounted for by a model of bounded-diffusion we elaborated earlier, allowing for an elastic penalty.[7] The limiting value of $i_{pa}/v^{1/2}$ thus gives access to the response time of the thin film system:[6h] $t_r = 2RT/\pi Dk_{spr} \approx 10^{-3}$ s, D being the diffusion coefficient of the redox head, and k_{spr}, the elastic constant of the coil chain, entropic in nature. The best fits between the experimental and computed data are depicted in Figure 1 (see Supporting Information for details). That gives individually $k_{spr} = 0.02 \pm 0.01$ pN/nm and $D = (7 \pm 3) \times 10^{-10}$ cm^2/s for a single $(dT)_{20}$ chain molecule in 1 M monovalent salt.[6h]

As a linear chain of nucleotides with thin diameter (~0.65 nm) and high flexibility, ssDNA is a more contractile polymer than is dsDNA β-helix (2.0 nm in diameter).[3,9a] Hybridization of Fc-ssDNA coils with a perfect unlabeled complement was thus anticipated to slow the chain mobility as well as to induce morphological changes of the film, positioning at rest the Fc heads borne by the solution-side termini of the helices away from the electrode surface at some distance (20-mer ~6.8 nm in contour length) perpendicular or tilted relative to normal (Figure 2). The net effect is predicted to severely decrease the system's response time.[7b] This is indeed what was observed experimentally.

The Fc$(dT)_{20}$-modified electrode was exposed to an ~5 μM solution of the fully complementary $(dA)_{20}$ target in 0.1 M $NaClO_4$ (pH 7.0) at 20 °C for at least 2 h. Complete hybridization was clearly evidenced above a critical high $v = 100$ V/s by a remarkable switch-off of the diffusion controlled current response of the Fc probes (see cyclic voltammetry in Figure 2A).[6i,j] Actually, this experimental cutoff does mean that the motion of the loose solution-side ends of the bound-$(dT\text{-}dA)_{20}$ duplex chains to reach the electrode surface is "frozen" out on the short millisecond time window. Consistently, decreasing sufficiently v allowed us to detect and measure a new faradaic peak current reflecting the chains' motion. At a low enough $v \leq 1$ V/s, an ideal surface Nernstian signal is recovered.[6f,7b] The measured coverage in Fc heads indicates that the hybridization process occurs with no significant loss in redox grafted material (~10%) and produces a moderately packed duplex layer on the gold surface.[3,6g] Interestingly, these Fc-dsDNA-electrodes were easily amenable to full dehybridization in 0.1 M $NaClO_4$ (pH 7.0) at 55 °C for 0.5 h, the main voltammetric features being reversed after the process (see Figure 2A, B). Figure 2B compares the $i_{pa}/v^{1/2}$ versus v plots before and after hybridization. The existence of a striking reliable maximum for the Fc-$(dT\text{-}dA)_{20}$ duplex layer (curve b), which is not observed when simple diffusion takes place within thin film systems (curve a),[7b] suggests a fundamental novel parameter pertaining to end-tethered semirigid polymers. Work is underway to further quantitatively analyze these unprecedented results.

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Supporting Information Available: MALDI-TOF MS and analytical HPLC of **1**. Experimental details. Electrochemical characterizations (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(6) (a) The homopolymer $(dT)_{20}$ was selected as a sequence because of its known little ordered structure. Importantly, also, the (dA-dT) base-pair runs are interesting tracts because they have many unusual properties, including a biological functional role that may be rigidity/length dependent.[4c,5b,6b,c] (b) Nelson, H. C. M.; Finch, J. T.; Luisi, B. F.; Klug, A. *Nature* **1987**, *330*, 221−226. (c) Riley, M.; Maling, B.; Chamberlin, M. J. *J. Mol. Biol.* **1966**, *20*, 359−389. (d) This novel redox DNA probe underwent a stringent quality control process (see Supporting Information); its synthesis will be reported elsewhere. (e) Control experiments showed that disulfide **1** does not adsorb on cleaned gold surfaces. (f) Additionally, the midpoint peak potential, of 135 mV/*ECS*, is equal to the standard potential $E°$ of the Fc/Fc^+ redox couple measured for Fc-$(dT)_{20}$ (or (dT-$dA)_{20}$ duplex) chains dispersed in solution. The present ideal behavior indicates a homogeneous environment around the redox centers and an absence of interactions between them. (g) See Supporting Information for discussion and comparison to literature data. (h) The pertinence of these values which indicate that the surface-$(dT)_{20}$ chain is a rather rigid system will be discussed in future work. (i) Control experiments with a fully uncomplementary $(dC)_{20}$ target showed no interaction with immobilized $(dT)_{20}$ under identical experimental conditions. (j) It should be emphasized that hybridization of thiolated **1** with its complementary $(dA)_{20}$ target in solution, followed by deposition onto a cleaned gold surface, gave in our hands no Fc voltammetric signal even at a v as slow as 10 mV/s; there was also no evidence of a cathodic desorption signal of a alkanethiolate monolayer chemisorbed at gold,[6k] evidencing the absence of attached dsDNAs onto the surface. (k) Zhong, C. J.; Porter, M. D. *J. Electroanal. Chem.* **1997**, *425*, 147−153.

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