Communication
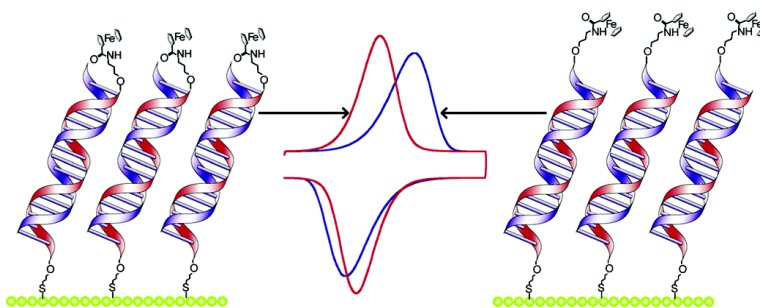
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A Comparison of Electron-Transfer Rates of Ferrocenoyl-Linked DNA

Yi-Tao Long,[†,‡] Chen-Zhong Li,[†,‡] Todd C. Sutherland,[†,‡] M'hamed Chahma,[†,‡] Jeremy S. Lee,*,[†] and Heinz-Bernhard Kraatz*,[‡]

Department of Biochemistry, University of Saskatchewan, 107 Wiggins Road, Saskatoon, S7N 5E5, Canada, and Department of Chemistry, University of Saskatchewan, 110 Science Place, Saskatoon, S7N 5C9, Canada

Received February 14, 2003; E-mail: kraatz@sask.usask.ca

Electron transfer through DNA has become a popular area of research over the past decade, while the mechanistic details remain elusive.[1] Selected references have addressed mechanistic details concerning tunneling, hopping, hole transfer, and combinations therein.[2] The innate ability of DNA to recognize sequences both specifically and reversibly can be exploited under the proper condition to give valuable environmental information. In this vein, coupling of redox-active groups to DNA has recently received much interest.[3] Self-assembled monolayers (SAMs) lend themselves well to developing a bridge between solution and solid-state chemistry, and extensive literature has shown that SAMs are suitable for the immobilization of biomolecules on transducer surfaces.[4] The thrust for this type of research comes from the potential application of DNA as a self-assembling molecular wire of defined geometry. Before DNA can be considered as a scaffold for molecular wire structures, the electron-transfer properties must be very well understood. Particularly, ferrocenoyl (Fc)-labeled DNA has emerged as an effective strategy to investigate DNA, for example, as a probe for DNA hybridization.[5] We have synthesized two configurations of DNA disulfide derivatives coupled to Fc moieties at the terminus of the DNA strand, as follows: **1**, Fc-NH-(CH₂)₃-5′-AAC-TACTGGGCCATCGTGAC-3′-(CH₂)₃-S-S-(CH₂)₃-OH; **2**, 3′-T-TGATGACCCGGTAGCACTG-5′; **3**, 5′-AACTACTGGGCCAT-CGTGAC-3′-(CH₂)₃-S-S-(CH₂)₃-OH; **4**, FcNH-(CH₂)₃-3′-TTG-ATGACCCGGTAGCACTG-5′.

The purpose of this Communication is to compare and contrast the electron transport properties of the two ds(double stranded)-DNA configurations, **1:2** and **3:4**.

This paper characterizes ds-DNA bound to a gold surface and its associated electron-transfer properties. More importantly, a comparison is made between two configurations, which differ only in the Fc attachment point: the first is on the same strand as the thiolate linkage, and the second is on the complementary strand. The two configurations were synthesized from the 5′(**1**) or 3′(**4**) amino-labeled ss-DNA. FcOBt[6] was reacted with amino-labeled DNA (**1** or **3**) to give the final products.

The characterization and purification of the DNA derivatization was performed by RP-HPLC, MALDI-TOF MS, and UV–vis (see Supporting Information). The ds-DNA monolayers were formed in two steps: the first step is DNA hybridization in 20 mM Tris buffer (pH 8.7) for 24 h at room temperature; the second step involves the incubation of the Au microelectrode (50 μm diameter) in a 0.05 mM ds-DNA solution containing the same hybridization buffer at room temperature for 5 days. The surface was characterized by XPS and electrochemical measurements. The S₂ₚ peaks at 162 and 163 eV, in a 2:1 ratio, respectively, are indicative of a thiolate bound to Au,[7] thus proving that DNA is linked to the surface in both configurations. By monitoring the attenuation of the Au₄f

† Department of Biochemistry.
‡ Department of Chemistry.
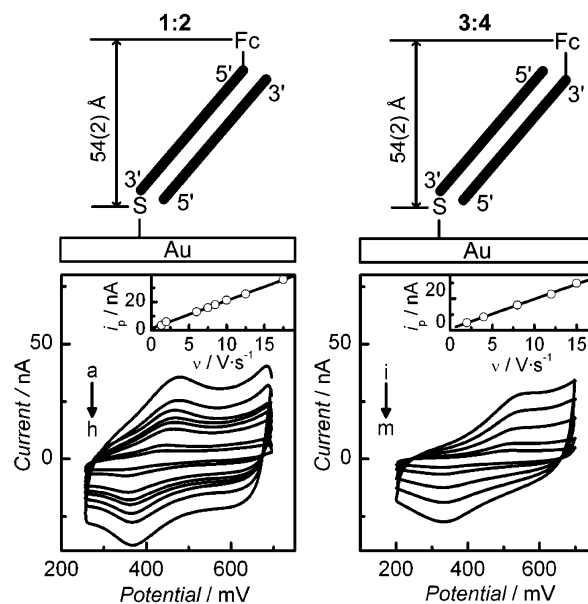
Figure 1. Model of **1:2** Fc-labeled DNA bound to a Au surface. **1:2**, the Fc is on the same strand as the thiolate; **3:4**, the Fc is on the opposite strand of the thiolate. CVs: (a) 17.5 V s⁻¹, (b) 12.5 V s⁻¹, (c) 10 V s⁻¹, (d) 8.5 V s⁻¹, (e) 7.5 V s⁻¹, (f) 6 V s⁻¹, (g) 2 V s⁻¹, (h) 1.36 V s⁻¹, (i) 15 V s⁻¹, (j) 12 V s⁻¹, (k) 8 V s⁻¹, (l) 4 V s⁻¹, (m) 2 V s⁻¹. Insets: Linear relationship between scan rate and peak current.

peaks, the film thickness was calculated to 54(2) Å for both configurations,[8] which agrees with the same ds-DNA sequence measured by ellipsometry (data not shown). Electrochemical measurements, by way of cyclic voltammetry (CV) shown in Figure 1, confirmed a linear relationship between peak current and scan rate (see insets of Figure 1), indicating a surface bound Fc-DNA. The CVs of the two configurations exhibit quasi-reversible redox reactions as the anodic to cathodic peak current ratios are near unity. In addition, the CV of **1:2** has a peak width at half-maximum of 102(10) mV, which is close to ideal redox behavior of 90 mV, while **3:4** has a peak width at half-maximum of 125(7) mV. These results could mean that the Fc groups in the **1:2** hybrid act as independent redox centers in the same, yet isolated, environments, whereas, in the **3:4** hybrid, the redox centers may have some lateral interactions with each other or are located in several different microenvironments. Integration of the background-subtracted peak currents provided a coverage of 5(4) × 10¹² molecules cm⁻², which is in agreement with results reported by others.[9] The coverage value is a realistic density and eliminates the likelihood of multilayer formation; however, it is lower than the theoretical packing density of DNA of ∼3 × 10¹³ molecules cm⁻². In summary, the surface structural characterization shows that the two configurations form well-defined SAMs on Au microelectrodes and that both mono-layers are essentially identical.
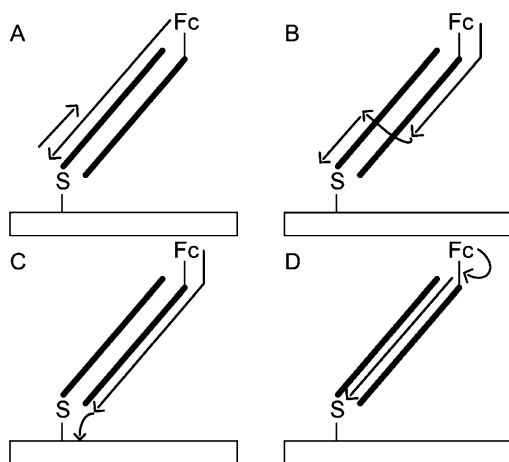
Figure 2. **3:4** models of ET through DNA. (A) 5′-3′ strand preference, (B) barrier to interstrand ET, (C) barrier through space to Au from terminal base pair, (D) specific Fc orientation effect.

The redox potential of Fc is known to be sensitive to its environment and as such has been employed in several sensor development schemes. Based on the monolayer structural evidence, the Fc environment in both situations should be very similar and should not result in different formal potential ($E^{0\prime}$) values. Any local differences were expected to be "averaged-out" by a flexible C_3 linker to the Fc. Surprisingly, our results show a difference in redox potential for the two configurations of 29(14) mV. **1:2** has an $E^{0\prime}$ value of 408(8) mV ($n = 7$), whereas **3:4** has an $E^{0\prime}$ of 437(11) mV ($n = 5$) which corresponds to a $\Delta\Delta G$ value of 2.8(1.4) kJ mol^{-1}. The difference in $E^{0\prime}$ values shows that the Fc groups are in different environments such that Fc is less accessible to electron transfer in **3:4**. The implications of these results suggest that electron transport to the Fc is more facile when the redox probe is positioned 5′ as compared to 3′. The difference in $E^{0\prime}$ values could be attributed to geometry and orientation of the Fc due to 5′ or 3′ linkage leading to a different interaction with the base-pair stack.

Not only are the $E^{0\prime}$ values different, but also the ΔE_p values increase when moving from **1:2** to **3:4**, which manifests in a lower k_{ET} for **3:4**. Electron-transfer rates of the two configurations were calculated by CV using the Butler−Volmer methodology.[10] The calculated ET rate constants for **1:2** and **3:4** are 115(15) and 25(9) s^{-1}, respectively. Therefore, not only are the thermodynamics in favor of electron transport through the same strand as the thiolate, but also the kinetics favor **1:2** for electron transfer. Note that the 3′-thiolate linkage is the same for both configurations.

A close examination of the results provides more clues toward solving the electron transfer in DNA debate. Both configurations should give indistinguishable results if ET proceeds entirely in a through-space model. Recent literature[11] has shown ET is favored in the 5′ to 3′ direction (Figure 2A); however, this is unlikely to be the case here. If there were a strand orientation preference for ET, the CVs for **1:2** would be different (as estimated from the peak shape). For example, **1:2** would have a very fast oxidation wave, as ET through the 5′ to 3′ direction to the thiolate does not involve any strand jumping. The return reduction wave would be much broader as the electron must jump strands at the first base pair, to follow the 5′ to 3′ orientation, and then jump strands again to reduce the ferricinium cation. Following the same reasoning, **3:4** would yield a CV that is symmetrical in both anodic and cathodic peaks. Therefore, model 2A can be discarded. Three other potential mechanisms are shown in Figure 2B, 2C, and 2D. Figure 2B assumes that there is no directional preference (5′-3′ = 3′-5′) for DNA electron transfer. Thus, the only additional barrier to electron

transfer is interstrand jumping, which results in **3:4** having a lower k_{ET} and symmetrical CVs. Figure 2C assumes that interstrand jumping is forbidden and, as such, the rate-limiting step occurs when the electron must tunnel to and from the base pair proximal to the Au surface. Figure 2D assumes that ET is not confined to one strand, but rather the rate-determining step is the feeding in of the electron into the first base pair. It is possible that there is orientation or geometric effects that predispose the 5′-labeled Fc to be more accessible to the base pairs than the 3′-labeled Fc. This is supported by the different $E^{0\prime}$ values observed, as was discussed previously. Of course, a combination of strand jumping and Fc orientation could be operating simultaneously.

In summary, this Communication has shown that Fc-labeled ds-DNA can form reproducible monolayers on Au surfaces. There is a difference in k_{ET} when comparing **1:2** versus **3:4** under the same conditions. The results suggest a model of ET in DNA that does not show strand orientation preference, but does exhibit either an energetic barrier to interstrand crossing or orientation effects due to 5′ or 3′ linkage of the Fc.

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