Fc–ssDNA conjugate: electrochemical properties in a borate buffer and adsorption on gold electrode surfaces

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Abstract

By using an electroactive species such as ferrocene (Fc), the electrochemical properties of bioorganic molecules were indirectly made accessible. 5'-Fc-NH-ssDNAs have a redox potential of 430–440 mV (vs. Ag/AgCl) in a borate buffer (pH 9), which is characteristic of ferrocene/ferrocenium (Fc/Fc⁺) substituted by an amide group. The diffusion coefficient of Fc–ssDNA conjugates in a buffer solution was found to be 10⁻⁷ cm² s⁻¹. 5'-Fc-NH-ssDNA-S-S-R (3) was adsorbed onto a gold microelectrode surface to form a self-assembled monolayer (SAM) via a disulfide linker with a high packing density. The Fc-electrophore allows the characterization of the SAM by cyclic voltammetry (CV). The rate constant of electron transfer through DNA was estimated to be 12 s⁻¹.

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1. Introduction

The adsorption of bioorganic molecules such as peptides and oligonucleotides on electrode surfaces has attracted the attention of many scientists in the last decade because of its widespread application in medical devices [1,2] and biosensors [3–5]. By incorporating an electroactive motif into oligonucleotides, the electrochemical detection and subsequent understanding of the properties of a single stranded, hybridized and mismatched DNA is possible [6–14]. We are interested in probing DNA via a ferrocene moiety, which offers the advantage of being chemically stable, easily functionalized and electrochemically reversible. In addition, the redox potential of ferrocene does not coincide with that of DNA bases. We have recently synthesized 5'-ferrocene-glucosamides and 5'-ferrocene-amido-adenosine (1) under mild conditions [15]. This reaction was extended to long chain oligonucleotides (20 bases) for recognition purposes. In this study, we present a one step synthesis of two ssDNAs labeled by ferrocene-amide in position 5' (2, 3).

The Fc–ssDNA conjugate carries a disulfide linker at the 3' position for the immobilization of the construct on gold surfaces. Furthermore, we report the
electrochemical properties of 2, 3 in a borate buffer at pH 9. The adsorption of compound 3 via sulfur–sulfur bond cleavage to afford self-assembled monolayers (SAMs) on the gold surface microelectrodes was studied. The heterogeneous rate constant of the electron transfer through DNA was determined.

2. Experimental

2.1. Reagents

5'-Amino-GTCACGATGGCCAGTAGTT-3’-C6-S2 and 5'-amino GTCACGATGGCCAGTAGTT were purchased from the University of Calgary. The concentration of DNA (20 bases) was determined by UV–Vis spectroscopy (Varian 100 Caryan) using 8000 M⁻¹ cm⁻¹ as the average molar absorption coefficient at 260 nm. THF was freshly distilled before use. FcCOOBt (H₃BO₃ + NaOH). For the CV studies in the case of 2, platinum wire counter electrode was used. Potentials are reported versus an Ag|AgCl. The oxidation peak potential (Epa) and oxidation peak current (Ipa) are given by Eqs. (1) and (2), respectively [17]:

\[ E_{pa} = E_{1/2} + 1.11RT/F, \]  
\[ I_{pa} = 0.4463nA(nF/RT)^{1/2}D_0^{1/2}v^{1/2}c^0, \]  

where \( A \) is the electrode surface area (cm²), \( D_0 \) the diffusion coefficient (cm² s⁻¹), \( v \) the scan rate (V s⁻¹), \( c^0 \) the concentration (M) and \( n \) the number of electrons. \( \Delta E = E_{pa} - E_{pc}, E_{1/2} = (E_{pa} + E_{pc})/2. \)

2.2. Electrochemical measurement in borate buffer solution

The electrochemical experiments were carried out using a CV-50W voltammetric analyzer (BAS) at room temperature. The CV studies were performed using a three-electrode configuration. A glassy carbon electrode (diameter 3 mm) served as the working electrode and a platinum wire counter electrode was used. The solvent was a borate buffer (1:1) at room temperature. The CV studies were performed using a CV-50W voltammetric analyzer (BAS) at room temperature, overnight, leading to a ferrocene-amide bond cleavage to afford self-assembled monolayers (Scheme 1). The same procedure was repeated LSV scans. The microelectrodes were then tested, using a ferrocene or a potassium ferrocyanide solution, before polishing them with alumina (0.5, 0.3 and 0.05 mesh). The roughness of the microelectrode was determined using the underpotential deposition (upd) of copper, which is generally close to 1. For a Nernstian adsorbate layer, the peak current, the full width at half-maximum of the anodic voltammetric wave (\( \Delta E_{fwhm} \)) and the charge are represented by Eqs. (3)–(5) [19]:

\[ I_p = (n^2F^2/4RT)\Gamma G = (9.39 \times 10^5)n^2A\Gamma v, \]  
\[ \Delta E_{fwhm}/mV = 90.3/n, \]  
\[ Q = nF\Gamma, \]  

where \( \Gamma \) is the coverage (mol cm⁻²) and \( Q \) the charge (C). The oxidation peak current varies linearly versus the scan rate and this shows that the voltammetric responses stem from molecules successfully immobilized on the gold surface electrode (in solution the peak current varies linearly with \( v^{1/2} \)).

3. Results and discussion

Cyclic voltammetry was used to examine the electrochemical behavior of compounds 2 and 3 in aqueous solution (borate buffer at pH 9) and to observe the adsorption of compound 3 on the gold electrode surface. Compounds 3 and 4 exhibit a one-electron oxidation peak with \( \Delta E = 66 \) mV and with a peak current ratio of

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Scheme 1. Synthesis of 5'-ferrocene-amido-DNA (2) from FeCOOBt and 5'-amino-DNA.
approximately one. Fig. 1 shows a CV of compound 3 with an $E_{1/2}$ of 440 mV. This value is characteristic of a ferrocene linked to an amide group as previously reported [20] and similar to ferrocene-glucosamines and ferrocene-adenosine reported recently [15]. The redox potential of Fe/Fc$^+$ linked by an amide group in a borate buffer at pH 9 was approximately between 400 and 440 mV (vs. Ag/AgCl). From the slope of Eq. (2) ($I = f(v)$), the diffusion coefficients ($D_0$) of compounds 2 and 3 were evaluated (Table 1). The $D_0$ values for compounds 2 and 3 are: (i) an order of magnitude smaller than that of ferrocene carboxylic acid and (ii) lower by half than the $D_0$ of a ferrocene-adenosine reported recently [15]. This is expected because of strong interactions between the hydroxyl groups of sugar and water molecules, and also due to the length of the DNA linker, which thereby reduces the mobility of ferrocene.

The gold microelectrodes were incubated in a solution of 3 ($10^{-5}$ M) for 4 days, resulting in the chemisorption of the Fe-DNA conjugate on the gold surface presumably via an Au–S linkage as shown in Scheme 2.

![Scheme 2. Schematic representation of a self-assembled monolayer of the Fc–ssDNA conjugate 3 on a gold microelectrode.](image)

Table 1

<table>
<thead>
<tr>
<th>Product</th>
<th>$E_{1/2}$ (mV)</th>
<th>$\Delta E$ (mV)</th>
<th>$10^7 D_0$ (cm$^2$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeCO$_2$H</td>
<td>300 ($\pm 10$)</td>
<td>66</td>
<td>40</td>
</tr>
<tr>
<td>1</td>
<td>412 ($\pm 5$)</td>
<td>69</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>430 ($\pm 10$)</td>
<td>66</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>440 ($\pm 10$)</td>
<td>66</td>
<td>1</td>
</tr>
</tbody>
</table>

![Fig. 1. Cyclic voltammogram of the Fe-DNA conjugate 3 ($10^{-4}$ M) in borate buffer at pH 9 at a scan rate of 100 mV s$^{-1}$ using a glassy carbon electrode versus Ag/AgCl.](image)

![Fig. 2. CVs of [Fe(CN)$_6$]$^{3-/4-}$ (1 M) in water (1 M KCl) at 1 V s$^{-1}$. (a) Bare gold microelectrode. (b) SAM of compound 3 on a gold microelectrode.](image)
Table 2
Redox potential of 3 in a self-assembled monolayer at different scan rates

<table>
<thead>
<tr>
<th>Scan rate (V s(^{-1}))</th>
<th>(E_{pa}) (mV)</th>
<th>(E_{pc}) (mV)</th>
<th>(\Delta E) (mV)</th>
<th>(E_{1/2}) (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>442</td>
<td>387</td>
<td>55</td>
<td>415 (+10)</td>
</tr>
<tr>
<td>50</td>
<td>442</td>
<td>384</td>
<td>58</td>
<td>413 (+10)</td>
</tr>
<tr>
<td>100</td>
<td>467</td>
<td>367</td>
<td>100</td>
<td>417 (+5)</td>
</tr>
<tr>
<td>200</td>
<td>475</td>
<td>351</td>
<td>127</td>
<td>415 (+8)</td>
</tr>
<tr>
<td>400</td>
<td>495</td>
<td>330</td>
<td>165</td>
<td>413 (+10)</td>
</tr>
</tbody>
</table>

Fig. 3. CV of 3 attached to a gold microelectrode surface at 100 V s\(^{-1}\) in borate buffer at pH 9.

electrode which corresponds to \(2.40 \times 10^{13}\) mol cm\(^{-2}\).
The maximum value of the theoretical packing density of the ssDNA has been reported by Steel et al. [25] to be \(8 \times 10^{13}\) molecules cm\(^{-2}\), which was overestimated because it did not take into account the additional solvation and counter ion effects. On the other hand, they found that the maximum value of the experimental packing density of ssDNA (shorter than 24 nucleotides) was between 3 and \(4 \times 10^{13}\) mol cm\(^{-2}\) [25]. Moreover, the difference observed between the experimental and theoretical coverages may due to the alkyl fragment of the disulfide in compound 3, which competes with the adsorption of Fe-ssDNA on the electrode surface after cleavage of the disulfide bond. Our experimental result is in agreement with that of Steel et al. [25] and values reported elsewhere [26–28]. This is an indication of the non-formation of a multilayer.

The organization of SAMs depends on many parameters such as (i) the solvent [29], (ii) the supporting electrolyte [30], (iii) the length of the monolayer [31,32] and (iv) the applied potential [33]. All these parameters affect the electron transfer between the ferrocene probe and gold microelectrode through DNA. Our preliminary investigations involve the calculation of the electron transfer rate constant using the Laviron equation [34]. For modified electrodes, and when \(n\Delta E < 200\) mV, the electron transfer rate constant is determined using the following equations:

\[
E_{pc} = E_{1/2} - (RT/(znF)) \ln(znFv/RTk),
\]

where \(z\) is the charge electron transfer coefficient, \(v\) the scan rate (V s\(^{-1}\)), \(n\) the number of electrons and \(k\) the electron transfer rate constant (s\(^{-1}\)). \(\Delta E = E_{pa} - E_{pc}\). From the anodic voltammetric wave \(\Delta E_{fwhm}\) is 100 mV, which implies \(n\) is 1.1.

From the linear portion of the plot \(E_p - E_{1/2} \sim f(\ln(v))\) in Fig. 4, the electron transfer rate constant \((k)\) through the ssDNA bonds and the electron transfer coefficient \((z)\) are estimated to be \(12(\pm 1)\) s\(^{-1}\) and 0.49 (±0.1), respectively. This value is higher than the electron transfer rate constant of \([\text{Ru}(\text{NH}_3)_6]^{3+}\) on DNA modified electrodes [35]. This is understandable since the ferrocene is attached directly to DNA through a carbon–carbon bond formation. This bond connection facilitates the electron transfer between the electroactive form and DNA. For the \([\text{Ru}(\text{NH}_3)_6]^{3+}\) complex, the electron transfer rate constant depends mainly on the binding constant of the complex to the DNA. For other electrophores linked to DNA such as 2,6-disulphonic acid anthraquinone and pyrrolino–quinoline–quinone, the electron transfer rate constants have been estimated to be 1.5 and \(< 1\) s\(^{-1}\) respectively [36,26], which is an order of magnitude lower than that of Fe-ssDNA. In comparison, we recently reported that the electron transfer constant in the case of double stranded Fe-DNA (with a short linker –(CH\(_2\))\(_3\)– between Fc and DNA) was 115 s\(^{-1}\) [37]. This is understandable since the electron transport was facilitated by hydrogen bonding through ds-DNA and by the shortening of the linker. The resulting difference in the electron transfer rate constant of Fe-ssDNA and Fe-dsDNA is promising for the discrimination of mismatched DNA as reported by Yu et al. [38].
4. Summary

DNA (20 bases) was successfully labeled by ferrocene amide under mild conditions. No significant difference was observed in the diffusion coefficient of the ferrocene substituted either by one adenosine or by a long chain of DNA (20 bases). The packing density of DNA adsorbed onto the gold microelectrodes was close to the maximum experimental value of the packing density reported by Steel et al. The specific area of each molecule was $\frac{400}{C_23}$ $\AA^2$. The electron transfer rate constant through DNA-SAMs was calculated, which holds promise towards future efforts and investigations for the recognition of double stranded DNA and detection of base mismatches.

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References