Electrochemical Monitoring of the Reversible Folding of Surface-Immobilized DNA i-Motifs

Catherine Adam,† José Manuel Olmos,‡ and Thomas Doneux*†

†Chimie Analytique et Chimie des Interfaces, Université libre de Bruxelles (ULB), Boulevard du Triomphe, 2, CP255, B-1050 Bruxelles, Belgium
‡Departamento de Química Física, Facultad de Química, Regional Campus of International Excellence “Campus Mare Nostrum”, Universidad de Murcia, 30100 Murcia, Spain

ABSTRACT: Two cytosine (C) rich DNA sequences folding in i-motif upon protonation of C at low pH have been immobilized at gold electrodes to study the impact of the electrodelectrolyte interface on the stability of the noncanonical DNA secondary structure. The effects of the molecular composition and environment on the melting and folding of the structures immobilized at the gold surface have been compared to the properties of the DNA strands in solution. The DNA folding into i-motif upon protonation, both at the surface and in solution, results in a significant variation of the charge density which is monitored electrochemically through the electrostatic interactions between the DNA strand and the electroactive hexaammineruthenium(III). This method is shown to be sufficiently sensitive to distinguish hemiprotonated folded state and single strand unfolded state of i-motif. The pH of melting has been determined for both sequences in the bulk and at the gold|electrolyte interface. The results evidence a stabilizing effect of the interface on i-motif structure, whereby the pH of melting is higher for the sequences immobilized at the surface. The reversibility and precision of the electrochemical model described here allows a clear and simple characterization of DNA structures and does not require any labeling of the sequence.

INTRODUCTION

Some highly repetitive DNA sequences, such as the human telomeric sequence, are known to form secondary structures that differ from the classical Watson and Crick double helix.1 These so-called noncanonical DNA structures, such as G-quadruplexes and i-motifs, have attracted a lot of interest in the recent decades.2,3 In addition to their biological relevance, their robustness and reversibility are well adapted to the development of nanotechnologies, for example, in the design of responsive materials or nanomachines.4,5 The stability of noncanonical structures strongly depends on the molecular environment and slight changes (pH, temperature, ionic strength, or ionic composition) lead to the formation (folding) or disruption (melting) of the structure. Their stability with respect to the physicochemical environment of the solution is being intensively studied.3,6–8 However, the behavior of DNA noncanonical structures at solid|liquid interfaces is poorly discussed9 in comparison to classical DNA double helix,10–12 but represents a critical feature for the conception of sensors or nanodevices, including electrochemical DNA sensors. In such platforms, where single-strand DNA is immobilized on the electrode surface, the structure of the interface and the effect of the electric field need to be well understood and controlled in order to design accurate analytical systems. The present work is focused on i-motifs (Scheme 1), which are noncanonical DNA structures resulting from the protonation of cytosines (C) and the formation of intercalated C−H+−C hemiprotonated base pairs.

These structures can be formed between separated DNA strands or through intramolecular bonding in C-rich sequences (e.g., human telomeric sequence) leading to the folding of the strand on itself. The stability of i-motifs is directly linked to the protonation of cytosines and thus strongly depends on the pH. The pH of melting (pHm) is defined as the pH at the midpoint of the proton induced single strand to i-motif transition and is a measure of the proneness of a given sequence to fold into an i-motif. The pHm, together with the temperature of melting (Tm), are key indicators to characterize the stability of the i-motif structure. The effect of the interface on the pHm is discussed...
herein by comparing the values determined in bulk solution ($pH_{m, surf}$) and those measured for i-motifs immobilized at the surface ($pH_{m, surf}$). The pH of melting $pH_{m, surf}$ is herein defined as the pH value, in the bulk solution, at which 50% of the surface-immobilized DNA is in the folded state. To determine the ($pH_{m, surf}$) values a method using the electrostatic interaction between the negatively charged DNA-backbone and hexaammineruthenium(III) is proposed. This method, well documented in the literature,13–17 is shown to be sensitive enough to allow the electrochemical monitoring of charge density upon protonation and folding of the DNA strand. Results presented here aim at differentiating DNA structures (folded vs unfolded) using electrochemistry without labeling and highlight the importance of considering interfacial phenomena in the use of immobilized oligonucleotides on electrode surfaces.

**EXPERIMENTAL SECTION**

**Material.** All solutions were prepared using ultrapure water (Milli-Q from Millipore). DNA sequences 5′-(CCC TAA), CCC T′3 (human telomeric repeat, sequence A), 5′-(CCC TTA)3, CCC T′3 (sequence B), 5′-TTT ATT ATT ATT TTA TTA T′3 (control sequence showing no folding into i-motif structure) and their thiolated equivalent 5′-HS-(CH2)6-(CCC TAA), CCC T′3, 5′-HS-(CH2)6-(CCC TTA), CCC T′3 and 5′-HS-(CH2)6-(TTT ATT ATT ATT TTA TTA T′3) were purchased from Eurogentec (Belgium) at a HPLC-purified quality. DNA stock solutions were prepared in phosphate buffer pH 7.4 (10 mM) and stored at experimental medium (8.3 mM) for comparison. At pH around 6, spectra presented here aim at discriminating DNA structures (folded vs unfolded) using electrochemistry without labeling and highlight the importance of considering interfacial phenomena in the use of immobilized oligonucleotides on electrode surfaces.

**RESULTS AND DISCUSSION**

**Folding in Solution.** Intramolecular i-motifs result from the folding of particular DNA single strands upon the protonation of cytosines in sequence leading to the formation of hemi-protonated C–H+–C pairs.7 Their stability strongly depends on the molecular environment (pH, temperature, ionic strength, or ionic composition). Two i-motif forming sequences have been investigated: sequence A (5′-(CCC TAA), CCC T′3) is the human telomeric sequence and sequence B (5′-(CCC TTA), CCC T′3) is used as comparison, since the presence of different nucleotides in the loop is known to influence the stability of i-motifs.7 Spectroscopic studies of the two sequences have been carried out under pH and temperature control. Thermal and pH differential UV-spectra of both sequences (see Figure S1) present the typical i-motif signature with two positive peaks at 240 and 260 nm and one negative peak at 295 nm.21 This confirms that the two investigated sequences indeed fold into an i-motif in the lower pH- and temperature-ranges, and unfold at higher pH and temperatures. The fraction of i-motif, $\theta$, as a function of pH or temperature has been calculated from the corresponding melting curves ($Abs_{240}$ vs pH or T, respectively) using eqs 1 and 2 described in the experimental section and is represented for both sequences in Figure S2. The temperature and pH of melting ($T_{m, sol}$ and $pH_{m, sol}$), corresponding to the pH and T at which half of the DNA sequences are in the folded state (assuming a two state transition) are used to describe the stability of the motif in our experimental medium.

The $pH_{m, sol}$ of sequence A and sequence B are 6.6 and 6.9, respectively. The stability of i-motif is influenced by the length and composition of the loops of the sequence.7,22 According to McKim et al.,5 sequence A ($pH_{m, sol} = 5.86$) presents a lower stability than sequence B ($pH_{m, sol} = 6.15$) because of the destabilizing effect of bulky adenine present in the loop of the motif. In our results the same trend is observed with a lower value of $pH_{m, sol}$ for sequence A than sequence B and with an equivalent difference about 0.3 pH unit between the two sequences. Our values are about 0.7 pH unit higher than those reported by McKim et al.,5 which is explained by the lower ionic strength of our experimental medium, known to stabilize i-motif structures.23 This observation might seem counterintuitive compared to dsDNA, where a higher ionic strength favors the
secondary structure. This is because in dsDNA, a high ionic strength screens the unfavorable, destabilizing electrostatic repulsions between negatively charged backbones, whereas in i-motifs it screens the favorable, electrostatic stabilization brought by the proton positive charge. The stability of the structure is thus increased at low ionic strength,23 which is consistent with the higher values of pHm obtained in our experimental conditions.

The protonation of the cytosines and the folding into an i-motif is expected to change not only the net charge of the DNA strand, but also the charge density. Therefore, the interaction of the DNA strand with positively charged species should be affected, and it should be possible to monitor electrochemically the folding with the use of the electroactive hexaammineruthenium(III) cation. The electrostatic interaction between hexaammineruthenium(III) and DNA sequences has been widely investigated in the literature14–17,19 and can be described by eq 3:

$$K = \frac{\left[\text{DNA}^-\cdot [\text{Ru(NH}_3)_6]^{3+}\right]^{(z-3)^-}}{[\text{DNA}]^{-z}[\text{Ru(NH}_3)_6]^{3+}} + 3zM^+$$

(3)

where M' are the monovalent cations displaced by the ruthenium complex, s corresponds to the stoichiometry of interaction between hexaammineruthenium and the DNA strand, z is the total charge of the DNA strand, and K is the intrinsic (or site) binding constant, under the assumption that each binding site in the DNA sequence is equivalent and independent. Under proper experimental conditions, the equilibrium can be driven to the right-hand side of eq 3, which corresponds to a full compensation of charge between the phosphate groups and the complex.24

To extract values of K and s in the folded and unfolded state, titration of [Ru(NH$_3$)$_6$]$^{3+}$ with successive additions of the DNA i-motif sequences has been performed at two different pH chosen from the UV–vis melting experiment. At pH = 8.4 (>pH$_{m,\text{sol}}$), the DNA strand is unprotonated and in the unfolded state, whereas at pH = 5.3 (<pH$_{m,\text{sol}}$) it is in the hemiprotonated folded state of the i-motif sequence. The ionic strength was identical at both pHs, and kept at a low value (8.3 mM) to favor complete compensation between DNA and hexaammineruthenium(III). The titration was followed using differential pulse voltammetry (DPV), cyclic voltammetry (CV), and cyclic voltammetry under convection (CVC). Results of DPV for sequence A are presented in Figure 1 and the complete set of titration data is enclosed in the Supporting Information (Figures S3–S8).

From all methodologies, a clear decrease of the current intensity of the reduction peak of [Ru(NH$_3$)$_6$]$^{3+}$ upon addition of DNA is observed (Figures 1 and S3–S8). This variation is linked to a decrease of the effective diffusion coefficient ($D_{\text{eff}}$) of the electroactive probe following its binding to DNA. A theoretical model is used to determine the stoichiometry of interaction (s) and the binding constant (K) between [Ru(NH$_3$)$_6$]$^{3+}$ and DNA as described in eq 3. The method used here has been comprehensively described elsewhere.19 Briefly, in the case of this study, a “dynamic equilibrium”, where the association and disruption between the two entities are fast on the time scale of the experiment, is suggested. This is confirmed by the single peak observed in DPV measurement, where the peak potential is gradually moving with addition of DNA showing an average signal of the bound and unbound species, whereas a “static equilibrium” would show two separate peaks for the bound and free species.19 In this case, the equations used as model to describe our experimental data and corresponding to the dynamic equilibrium are detailed in eqs 4–6:

$$i_{\text{tot}} = \Omega D_{\text{eff}}^{-1/2}$$

(4)

$$D_{\text{eff}} = D_{\text{Ru}} + \frac{c - c_b}{c} + D_{\text{Ru,b}} \frac{c}{c}$$

(5)

$$a_b = \frac{K(\ell + s_{\text{DNA}}) + 1 - \sqrt{K(\ell + s_{\text{DNA}}) + 1}^2 - 4K^2s_{\text{DNA}}}{2K}$$

(6)
Table 1. Values of Parameters Extracted from the Fitting Procedures, Dynamic Model (from DPV Data in Figures 1 and S5)

<table>
<thead>
<tr>
<th>pH</th>
<th>sequence</th>
<th>$D_{\text{eff}}$ ($10^{-6}$ cm$^2$ s$^{-1}$)</th>
<th>$D_{\text{eff}}$ ($10^{-6}$ cm$^2$ s$^{-1}$)</th>
<th>$K^*$ ($10^{4}$ L mol$^{-1}$)</th>
<th>$s$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.4</td>
<td>A</td>
<td>6.51</td>
<td>1.88</td>
<td>3.7 ± 1.1</td>
<td>73.7 ± 0.5</td>
<td>99.3</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>6.51</td>
<td>1.52</td>
<td>3.5 ± 1.6</td>
<td>73.7 ± 0.7</td>
<td>99.5</td>
</tr>
<tr>
<td>5.3</td>
<td>A</td>
<td>6.51</td>
<td>1.43</td>
<td>3.0 ± 0.7</td>
<td>3.9 ± 0.2</td>
<td>99.8</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>6.51</td>
<td>1.27</td>
<td>3.2 ± 0.5</td>
<td>4.4 ± 0.1</td>
<td>99.9</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>6.51</td>
<td>1.41</td>
<td>3.7 ± 0.6</td>
<td>6.2 ± 0.2</td>
<td>99.9</td>
</tr>
</tbody>
</table>

The value was fixed during fitting and was calculated in our experimental conditions from CV at different scan rates (data not shown). These values were fixed during fitting and calculated from data at the end of titration. $K^*$ is the value of the intrinsic binding constant and $s$ is the stoichiometry of interaction, and those values are extracted from the fitting procedures. $R^2$ is the correlation coefficient, as provided by the non-linear fit tool of the software. The deviations are those associated with the fitting.

where $i_{\text{tot}}$ is the peak current for DPV and CV measurements and the plateau current in CVC experiments, $\Omega$ is a constant coefficient depending on the employed electrochemical method and is detailed in our previous work. $c$ is the total concentration of $\text{[Ru(NH}_3\text{)}_6\text{]}^{3+}$, $\zeta = 1/2$ for CV and DPV, $\zeta = 2/3$ for CVC and $D_{\text{eff}}$ is the effective diffusion coefficient of hexaammineruthenium corresponding to the weighed contributions of bound ($D_{\text{eff,b}}$) and free ($D_{\text{eff,f}}$) species and is described in eq 5. The concentration of bound $\text{[Ru(NH}_3\text{)}_6\text{]}^{3+}$, $c_b$, is calculated using eq 6, where $c_{\text{DNA}}$ is the total concentration of DNA added to the electrolyte. Results of the model for DPV measurement are contained in Table 1 (see SI, Tables S1 and S2, for other methods).

The diffusion coefficient of free and bound $\text{[Ru(NH}_3\text{)}_6\text{]}^{3+}$ are estimated from cyclic voltammetry at different scan rates at the beginning and at the end of the titration respectively (data not shown). Using those values (see Table 1), the experimental data were fitted and values of $K$ and $s$ were extracted. The value of the intrinsic binding constant $K$ does not vary appreciably between sequences or with pH. For both sequences, the stoichiometry of interaction is close to $7\text{[Ru(NH}_3\text{)}_6\text{]}^{3+}$ per DNA strand at high pH (pH 8.4), which points reasonably to a total compensation of charges when the motif is unfolded (1 $\text{[Ru(NH}_3\text{)}_6\text{]}^{3+}$ compensates for 3 phosphate groups, and the sequences are 22-mer). A good correlation between the number of bases and the quantity of bound redox probe, which is a typical behavior for ssDNA, can thus be attested in our experimental medium at pH 8.4. At low pH (pH 5.3), a clear decrease of $s$ (≈4) is observed for both sequences, indicating a lower amount of $\text{[Ru(NH}_3\text{)}_6\text{]}^{3+}$ interacting with the DNA strand. Such a significant decrease does not occur for the negative control sequence which is not able to fold into an i-motif. The partial compensation of charge of the DNA strand, linked to the protonation of C and the formation of C−H−C bond leads to the release of $\text{[Ru(NH}_3\text{)}_6\text{]}^{3+}$. The folding of i-motif in our sequence leads to the formation of 6 C−H−C bonds and hence to the compensation of six negative charges or, equivalently, a difference of two $\text{[Ru(NH}_3\text{)}_6\text{]}^{3+}$ per strand. Accordingly, a total charge compensation should ideally correspond to a stoichiometry of 5.3 hexaammineruthenium(III) per strand at the low pH. The extracted stoichiometries are slightly smaller, a fact that has also been observed with other DNA secondary structures such as G-quadruplexes and could possibly be linked to steric hindrance or charge densities issues possibly be linked to steric hindrance or charge densities issues that would not occur for single strand, unfolded sequences. In spite of this slight deviation from the total charge compensation hypothesis, the method presented here is sensitive enough in order to follow the change in charge distribution occurring with the folding/unfolding of the i-motifs.

Folding of i-Motif at the Surface. Interaction of $\text{[Ru(NH}_3\text{)}_6\text{]}^{3+}$ with DNA has also been widely exploited to study monolayers immobilized on gold electrodes and presents great advantages since the interaction is quantitative, reversible, and does not require any labeling of the DNA sequence. It is thus possible to extend the method presented here to study the folding of DNA i-motif structures immobilized on gold surfaces, which is valuable for the design of sensors. Similarly to the characterization of the DNA sequences in solution presented above, electrochemical methods based on the reduction of hexaammineruthenium(III) have been used to monitor the folding of DNA i-motifs at the interface. In Figure 2, cyclic voltammetry and ac voltammetry of the immobilized DNA i-motif of sequence B in the presence of hexaammineruthenium at two pH are presented (see Figure S9 for sequence A). The two pH, where the DNA strand is assumed to be folded (pH 5.5) or unfolded (pH 8.4), were chosen far enough from the pH$_{\text{mool}}$ determined from the results obtained in solution.

In cyclic voltammetry (Figure 2a), two cathodic peaks are observed. The first peak at $-0.13$ V versus Ag/AgCl corresponds to the diffusion limited reduction of hexaammineruthenium(III) dissolved in the bulk solution. The second peak is observed at lower potential ($-0.29$ V) and is characteristic of a surface confined redox probe, corresponding to $\text{[Ru(NH}_3\text{)}_6\text{]}^{3+}$ adsorbed on the DNA backbone. In ac voltammetry (Figure 2b), the imaginary part of admittance is plotted in order to minimize the contribution of dissolved $\text{[Ru(NH}_3\text{)}_6\text{]}^{3+}$ versus the contribution...
of electrostatically adsorbed \([\text{Ru(NH}_3\text{)}_6]^{3+}\), allowing thus a more precise characterization of the surface phenomena. In both measurements the signal of the adsorbed hexaammineruthenium is consequently diminished at low \(pH\) corresponding to the decrease of the amount of the redox probe interacting with DNA backbone and attributed to the formation of the i-motif structure. 

The decrease of the height of the adsorption peak between the two \(pH\)s is observed for both i-motif sequences but is not observed for the control sequence (see Figure S10). The electrochemical monitoring of the interaction between \([\text{Ru(NH}_3\text{)}_6]^{3+}\) and the DNA backbone is thus sensitive enough to differentiate the two states (folded and unfolded) of the motif immobilized at the gold electrode. Figure 3 represents the height of the ac voltammetric peak \((Y_{el,p})\) measured in different solutions alternating the \(pH\) between pH 6 (folded state) and pH 9 (unfolded state). The conservation of the intensity after various cycles is a good indication of the stability of the monolayer and of the reversibility of the formation of the i-motifs at the interface, which are essential features for the use of DNA in biosensing application or nanotechnologies.

The faradaic contribution of the imaginary admittance \(Y_{el,p}\) plotted in Figure 3 is directly related to the surface coverage of \([\text{Ru(NH}_3\text{)}_6]^{3+}\) adsorbed on the DNA strand immobilized at the gold electrode. The DNA surface coverage on the gold electrode in our experimental conditions was calculated from charge integration (data not shown) and is equal to \(1.0 \pm 0.3 \times 10^{-15}\) mol cm\(^{-2}\) and \(1.5 \pm 0.3 \times 10^{-11}\) mol cm\(^{-2}\) for sequence A and sequence B, respectively, which explains the lower values of \(Y_{el,p}\) on Figure 3a compared to Figure 3b. In agreement with this trend in surface coverage, the peak potential of adsorbed \([\text{Ru(NH}_3\text{)}_6]^{3+}\) is more negative for sequence B (see Figure 2) than for sequence A (see Figure S9). For both sequences, the surface coverage in hexaammineruthenium is lower in the folded state (\(pH\) 6) than in the unfolded state (\(pH\) 9), with the same relative decrease of the intensity. Assuming a total compensation of charge at \(pH\) 9 and thus a stoichiometry of interaction of 7.33 \([\text{Ru(NH}_3\text{)}_6]^{3+}\)/DNA strand (theoretically calculated for the 22-mer DNA strand), the estimated relative stoichiometry at \(pH\) 6 is equivalent to 4.2 for sequence A and 4.5 for sequence B. The trend is thus similar to that observed for the sequence in solution described in the first part of this work.

Interestingly, the variations of peak heights are very significant between the two extreme \(pH\)s, opening the possibility to monitor the evolution of the amount of adsorbed hexaammineruthenium-(III) in the intermediate \(pH\) range. Figure 4 represents the variation of the ac voltammetry signal as a function of \(pH\) for sequence B (see Figure S11 for sequence A). For the two sequences, a clear increase is observed at the peak potential corresponding to adsorbed \([\text{Ru(NH}_3\text{)}_6]^{3+}\) on the immobilized DNA strand while increasing the \(pH\). In this experiment, careful attention has been paid to maintain a constant ionic strength even though potassium hydroxide is added directly in the electrochemical cell. This was ensured by the use of the TMA buffer mixture as described in the experimental part.\(^{18}\) The gradual increase of the quantity of adsorbed \([\text{Ru(NH}_3\text{)}_6]^{3+}\) is attributed to the unfolding of the i-motif structure to single strand and allows the electrochemical monitoring of the structural change of the DNA structure.

The fraction of i-motif is calculated from the ac voltammograms of Figure 4, by analogy to what is reported in solution, using eq 7:

\[
\theta = \frac{Y_{el,p}(pH) - Y_{el,p}(pH_{melted})}{Y_{el,p}(pH_{i-motif}) - Y_{el,p}(pH_{melted})}
\]

As can be seen in Figure 5, the \(pH\) at the midpoint of the proton induced single strand to i-motif transition at the gold electrolyte interface \((pH_{surf})\), corresponding to the bulk \(pH\) at which half of the immobilized DNA strands are in a folded state is about 7.2 and 7.7 for sequences A and B, respectively.

This figure compares the data obtained in solution, using the spectroscopic methods described above, and the results obtained at the interface using our electrochemical method. The trend in the stability between sequence A and sequence B is maintained at the surface, with a higher \(pH_{surf}\) for sequence B, in line with

![Figure 3. Peak height in ac voltammogram \((Y_{el,p})\) in 50 \(\mu\)M \([\text{Ru(NH}_3\text{)}_6]^{3+}\) in TMA buffer mixture measured in two distinct solutions and alternating between pH 6 and 9 for sequences A (a) and B (b).](image)

![Figure 4. ac voltammograms of immobilized sequence B on gold electrode in 50 \(\mu\)M \([\text{Ru(NH}_3\text{)}_6]^{3+}\) in TMA buffer mixture at different \(pH\) (a). Peak height \(Y_{el,p}\) as a function of \(pH\) (b), red line is guided to the eye (see Figure S11 for sequence A).](image)
the destabilizing effect of bulky adenine contained in the loop of the motif, as observed in solution. Of more interest is the fact that for both sequences, pH_{m,surf} is higher than pH_{m,sol}. This result points to a stabilizing effect of the interface on the i-motif structure which remains folded at or close to the physiological pH. The effect of the interface thus plays an important role on the properties of adsorbed probes and needs to be carefully addressed in the development of technologies. In the field of bioanalytics, such effects are often neglected and the properties of an immobilized probe are often assumed to be similar to the properties exhibited in the bulk solution. However, the results presented here highlight the non-negligible effect of the interface on the secondary structure of DNA which will directly impact the recognition of the target analyte.

This stabilizing effect of the surface is likely linked to the different physicochemical environment experienced by the DNA at the interface, although its exact origin is difficult to pinpoint owing to the complexity of the system. Nevertheless, various possible contributions to the actual value of the measured pH_{m,surf} can be identified. (i) The formation of i-motif being a protonation-driven phenomenon, the pK_a of cytosine should have a direct influence on the overall stability of the folded structure. This pK_a is not necessarily identical for DNA immobilized at the surface and for DNA dissolved in solution. It is for instance well-documented that the surface pK_a of carboxylic acids immobilized through self-assembled monolayers differs significantly from the bulk values and is affected by parameters such as the ionic strength, the electrolyte composition or even the electric field. (ii) The local proton concentration and hence the pH can vary between the bulk and the surface, or even between the bulk and the close vicinity of the DNA strands. It was very recently reported that, for i-motifs dissolved in solution, the local pH in the immediate vicinity of the nucleic acid is significantly lower than the bulk pH. This fact is rationalized by a simple Boltzmann distribution effect, whereby an enrichment in cations, including protons, occurs close to the negatively charged DNA strands due to the existence of a net negative electrostatic potential. In the present case, it is very likely that the surface potential is also negative, owing to the high density of negatively charged immobilized DNA, so that a similar Boltzmann effect would also increase the local concentration of protons. (iii) In solution, it is known that the stability of i-motifs is strongly affected by molecular crowding, which describes a situation where the volume fraction occupied by biomacromolecules, small molecules, ions, or cosolutes is very high. The interfacial environment at the surface can possibly mimic such crowding conditions: it is characterized by a very high density of DNA strands, as compared to typical dilute solutions conditions and by local concentrations of anions and cations, including protons, that can be much larger than in the bulk. Incidentally, the surface coverage of DNA in the present work is so high that intermolecular association between strands cannot be ruled out.

These various contributions are not mutually exclusive, and each of them might contribute to some extent to the observed stabilization effect at the surface. Disentangling them and providing quantitative evaluation of their impact would require extensive and systematic investigations that are beyond the scope of the present work. Nevertheless, this work demonstrates that, in the case of DNA C-rich sequences, the secondary structure can be modified at the surface of the electrode. This effect occurs around physiological pH reinforcing the idea that important considerations need to be made for the use of immobilized DNA in its different applications.

SUMMARY AND CONCLUSIONS

Two C-rich DNA sequences, including the human telomeric repeat, have been studied by spectroscopy and electrochemistry to compare the folding of i-motif structures in bulk solution and at the gold-electrolyte interface. The variations of charge density between the folded and unfolded states are large enough to impact the electrostatic interactions between the DNA strands and the electroactive hexaammineruthenium(III) cation. The electrochemical quantification of these interactions, conducted in solution and for surface-immobilized sequences, revealed a stabilizing effect of the surface on the i-motif structure around physiological pH. This work shows that the notion of molecular environment should include surfaces as an additional lever to control the stability of noncanonical DNA structures. This will be beneficial for the design of accurate DNA sensing devices and the use of oligonucleotides in nanotechnology. It also highlights the need of considering interfacial phenomena for immobilized DNA and opens the way to study the impact of an electric field on the folding/melting of noncanonical DNA structures.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.7b04088.

UV-melting experiments for sequences A and B and control sequence and data for the titration of [Ru(NH_3)_6]^3+, CV, and ACV of immobilized sequence A and control sequence (PDF).

AUTHOR INFORMATION

Corresponding Author

E-mail: tdoneux@ulb.ac.be. Tel.: +32 2 650 35 80. Fax: +32 2 650 29 34.

ORCID

Thomas Doneux: 0000-0002-9082-8826

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.
Funding
This work was supported by the Fonds de la Recherche Scientifique-FNRS under Grant No. MIS F.4542.16. JMO thanks the Ministerio de Economía y Competitividad for the grant received under the Project CTQ-2012–36700 cofunded by the European Regional Development Fund.

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS
The authors thank Dr. Eduardo Laborda (Universidad de Murcia, Spain) for the critical reading of the manuscript and helpful comments.

REFERENCES