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The oxidation double peak of reduced guanine residues in short oligodeoxynucleotides: A study of its origin

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ABSTRACT

The aim of this study is to find a deeper understanding of the long-neglected oxidation double-peak of reduced guanines (G_{red}ODP) produced at a mercury electrode (ME) by the reverse oxidation of the guanine reduction product (G_{red}). Although the reduction processes of G moieties at an ME are hidden in the cathodic currents of the background solvent decomposition, and the G reduction process thus cannot be examined directly, the G reduction product (G_{red}) can be oxidized back to G, yielding an oxidation signal at about −0.2 V (vs. an Ag/AgCl/3 M KCl electrode). Depending on the measurement conditions, the oxidation signal of G_{red} may assume the form of a single-peak or a double-peak with GI and GII. The cause of the double-peak generation remains unknown. However, the novel approach based on combining elements of chronoamperometry and voltammetry proposes that the G_{red}ODP originates from the oxidation of DNA fragments in two different adsorption conformations. The results indicate that the orientation of G residues is controlled by the repulsive interaction after their reduction and from the difference of GI and GII potentials the repulsion energy was estimated. The new findings may find application as a tool to indicate the structural and surface properties of short oligonucleotides with G bases.

1. Introduction

Nucleic acids are widely known to be biomolecules of enormous importance. Their function is linked with both the transfer and the storage of genetic information for all life forms. For that reason, it is important to study not only their composition but also physicochemical properties, such as the catalytic activity, assembling, or interaction with interphase. A convenient tool to enable the research into the given properties consists in electrochemistry, which allows us to simulate the charged phase boundary with the electrode double-layer and to detect the compositional and structural changes during that interaction.

Although the electrochemical investigation of nucleic acids has been an object of scientific interest for almost sixty years [1–6], multiple questions are still left unanswered. One of these unknowns lies in the origin of the oxidation double-peak (G_{red}ODP), which is obtainable via cyclic voltammetry after previous reduction of the G moiety and may be the after-effect of the specific interaction between the nucleic acid and the electrode interphase.

Nucleic acids are electroactive at a mercury electrode (ME) thanks to adenine (A), cytosine (C), and guanine (G) bases. While the A and C bases yield cathodic peaks at MEs [1–6], the potentially more negative reduction signal of G is hidden under the electrolyte decomposition; nevertheless, the reduction product of G (G_{red}) can be reversibly oxidized, accompanied by the formation of an anodic signal (Fig. 1) [7–13]. To date, only one paper has demonstrated experimentally, using potentiostatic macro-electrolysis and NMR, that the reduction of the G product corresponds to 7,8-dihydroguanine [11].

Depending on the measurement conditions, the resulting G_{red} oxidation signal at an ME may have the form of a single or a double-peak. An anodic double-peak is achievable via measuring short DNA fragments, such as d(GCGAAGC), using an acetate [14] or a phosphate-acetate buffer [15–17] as the supporting electrolyte.

Ammonium formate is often added to the supporting electrolyte because it enhances the intensity of the analytical signal; however, the cyclic voltammetry performed in the solution then offers only a G_{red} oxidation single peak, regardless of the DNA length or scan rate [9,10,12,13].

The electrochemical reduction of G (preceding its oxidation) takes place at very negative potentials, simultaneously with hydrogen evolution. The relationship between hydrogen evolution and the electrochemical reduction of G moieties was discussed in several recent papers [17,18]. The referenced paper [17] examines both the impact of hydrogen evolution on the production of 7,8-dihydroguanine and the catalytic effect of DNA heptamers with different central triplets of

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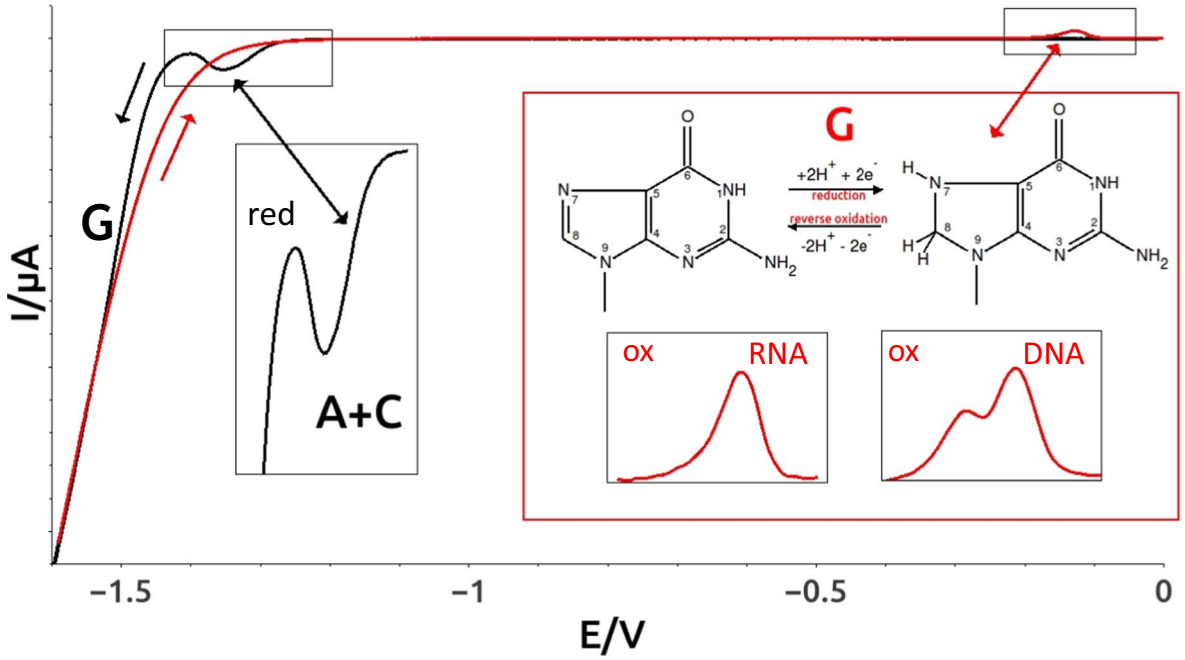

Fig. 1. A typical cyclic voltammogram of hetero-oligodeoxynucleotides at a mercury electrode (black – reduction and red – oxidation). The left-hand inset displays the magnified reduction peaks of adenine and cytosine (A + C), while the right-hand one shows the magnified oxidation peaks of reduced guanine moieties (G_{red}OP). The latter inset also includes the guanine reversible chemical reduction and presents an example of a single peak for RNA (left) and a double peak for DNA fragment (right). The experimental conditions for CV of d(GCGAAGC) and r(GCGAAGC) heptamers (2 μM) in an acetate buffer (pH 4.5); the scan rate of 0.4 V/s; the potential step of 2 mV; adsorption at 0 V for 60 s. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

nucleotides on hydrogen evolution. The related research report [18] proposes that hydrogen evolution catalyzed by the electrochemical reduction of cisplatin markedly contributes to the reduction of G moieties, concluding that both processes, i.e., the reduction of G and hydrogen evolution are linked together.

Although the origin of the oxidation double-peak (G_{red}ODP) at MEs remains unknown, some articles have attempted at a viable description in recent years [15–17]. The first published paper dealing with the G_{red}ODP embodied a voltammetric study of d(GCGAAGC) [14], where the appearance of the G_{red}ODP was rather surprising and discussed only within the effect of the scan rate, revealing changes in the peak ratio with increasing scan rates. The subsequent reports targeting the G_{red}ODP monitored the relationship between heptamers with various sequences and relevant G_{red}ODPs, summarizing that a small variation in the central trinucleotide sequence substantially alters the structure as well as the formation of a G_{red}ODP and suggesting a possible relationship between the structure and corresponding oxidation signals [15–17].

In the given context, the aim of this paper is to introduce the hitherto unknown characteristics of the G_{red}ODP. To discover the character of the G_{red}ODP, we decided to employ a methodical approach different from the commonly used cyclic voltammetry (CV), mainly because CV does not allow us to evaluate the effects of time and reduction potential separately, and to observe the characteristics of both anodic signals as a possible indirect evidence of the structural changes of ONs with G residues based on the interaction with an electrode surface.

2. Experiment

The lyophilized samples were purchased from Thermo Scientific, Ulm, Germany and Integrated DNA Technologies, Inc., USA. A sample of d(GGG) was used in the majority of the measurements as it includes only G bases and thus excludes the effect of others; simultaneously, there is probably no intramolecular interaction of the d(GGG) in prepared solutions (low concentrations, the neutral form of G at pH 5 because pKa is about 3.3), which could otherwise also affect the results. The exact concentration of ONs was determined by means of UV/VIS spectroscopy using a NanoDrop 2000c Thermo scientific (M.G. P., spol. s.r.o.). All the solutions were prepared in distilled MILIQ water. An acetate buffer as the supporting electrolyte was formed as a mixture of 0.1 M acetic acid (glacial, Sigma Aldrich; ACS reagent) and 0.1 M sodium acetate. The ionic strength of the buffer was not adjusted by adding a salt (NaCl, KCl, $HCOONH_4$) and exhibited the value of 0.1 M. The ON samples were diluted to the concentration of 0.23, 0.72, or 1.00 μM.

The voltammetric experiments were performed using an electrochemical analyzer μAUTOLAB TYPE III (Metrohm, Switzerland). The solutions of ONs with the concentration of 0.72 μM were dosed into a voltammetric cell. A three-electrode set was applied, configured as follows: a hanging mercury drop electrode (HMDE with the area of 0.4 mm^2) as the working electrode; an Ag/AgCl/3 M KCl and Pt wire as the reference; and a counter electrode, respectively. The results were obtained through a combination of chronoamperometry and linear sweep voltammetry (LSV). After 60 s of adsorption at 0 V, the sample adsorbed on the surface of the ME was exposed to negative potentials (e.g. −1.45 V) for a specific time; then, following reduction, the LSV curves from different potentials (e.g., −1 V) to 0 V were measured (at the scan rate of 0.9 V/s and step 2 mV), and if the previous step had caused the G moieties to be reduced, the LSV curve showed oxidation signals around the potential of −0.2 V (Fig. 2).

The elimination voltammetric procedure was performed to conserve the diffusion current component I_d at the expense of the kinetic and charging ones using the elimination function $f(I_d)$ [19–25]:

$$f(I_d) = -11.6569\ I_{200\ mV/s} + 17.4853\ I_{400\ mV/s} - 5.8284\ I_{800\ mV/s} \qquad E\,(4)$$

Conserving the diffusion current component, the adsorbed state of the analyte is reflected in the formation of a characteristic peak-counter peak [20–23].