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Electrochemically Induced Oxidative Damage to Double-Stranded Calf Thymus DNA Adsorbed on Gold Electrodes

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Abstract—Electrochemically induced oxidative damage to DNA was studied with double-stranded calf thymus DNA immobilized directly on a gold electrode surface. Pre-polarization of the DNA-modified electrodes at +0.5 V versus Ag/AgCl reference electrode, in a free from DNA blank buffer solution, pH 7.4, allowed for subsequent detection of direct electrochemical oxidation of adsorbed on gold DNA, in the potential range from +0.7 to +0.8 V. The redox potential of the process corresponded to the potentials of the oxidation of guanine bases in DNA. It is shown that with increasing potential scan rate, v, the mechanism of electrochemical oxidation of DNA changes from the irreversible $4e^-$ oxidative damage of DNA at low v to reversible $1e^-$ oxidation at high v, keeping the electrochemical activity of the adsorbed DNA layer virtually the same.

Key words: oxidative DNA damage, guanine, gold electrode

Oxidation of nucleic acids by various types of oxidants and free radicals [1], ionizing radiation [2-6], or by photo oxidants [7-13] results in irreversible changes—damage to DNA molecules. The physical and chemical mechanisms responsible for the degree, localization, and chemical impact of oxidative damage to DNA are still not completely understood [1-13], factors affecting the rate and efficiency of the intramolecular propagation of the oxidative damage being of particular interest. The possibility of direct electrochemical oxidation of DNA molecules enables one to study the mechanisms of heterogeneous electron transfer between the biomolecule and the electrode surface. In turn, it allows one to relate and compare heterogeneous and intramolecular mechanisms of charge transfer. Hitherto electrochemistry of DNA was limited to elucidation of the effect of the electric field on the adsorption and conformation of the DNA molecules at the electrode/electrolyte interface [14-18]. Studies performed at mercury (electroreduction) [15-18] and graphite/carbon electrodes (electrooxidation of nucleic acids) [18-20] were restricted mainly to the detection of micro-quantities of nucleic acids in samples [16-19] and to the development of biosensor systems for determination of gene-toxicity of various types of compounds [20-22]. Therewith, interactions between the compounds involved and the DNA molecules adsorbed at the electrode produced changes in electrochemical activity of the DNA layer. This enabled the determination, for example, of genotoxic or intercalating properties of the samples [20, 22]. To the best of our knowledge, detailed inquiries into mechanisms involved in direct oxidation of DNA at solid electrode surfaces have not yet been carried out.

Evidence has been reported from X-ray photoelectron spectroscopy [23], quartz crystal microbalance (QCM) microgravimetry [24], and electric double layer capacity measurements [14] that DNA is adsorbed on gold surfaces in a monolayer stable at positive surface charges. However, in spite of a wide double-layer potential range convenient for the observation of any redox transformations, to the present day no electrochemical activity of DNA on gold was detected (up to the potentials of surface oxide formation) [14, 25]. Thus, the electrochemistry of nucleic acids on gold was restricted either to the detection of electrochemically active compounds, capable of binding with the adsorbed DNA layer [26, 27], or to observation of hybridization events with redox labeled complementary sequences [21]. In the present work the possibility of direct electrochemical oxidation of DNA on gold is shown for the first time, which allowed us to study the mechanism of electrochemically induced oxidative damage to DNA.

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MATERIALS AND METHODS

Reagents. Activated calf thymus double stranded DNA (commercially available sample purchased from Sigma (USA), product No. D 4522, prepared by the method of Aposhian and Kronberg using calf thymus DNA and deoxyribonuclease type I [28]) was used. The mean molecular size of the DNA was 50 kb. Buffer solution components from Merck (Germany) were of analytical grade and used as received. All solutions were prepared with Milli-Q deionized water (Millipore, USA).

Instrumentation. Electrochemical measurements with gold polycrystalline disk electrodes (CH-Instruments, USA; surface area 0.031 cm^2) were performed in a standard three-electrode cell. A silver–silver chloride (saturated NaCl) electrode was the reference electrode; the auxiliary electrode was a platinum wire. A three-electrode potentiostat μAUTOLAB (Eco Chemie, The Netherlands) equipped with GPES 4.7 software (Eco Chemie) was used. All experiments were performed at ambient temperature (22 ± 1°C).

Immobilization of DNA and measurements. The surface of gold electrodes was successively treated with fine emery paper (P1200), polished to a mirror luster with a soft polishing Microcloth (Buehler, USA) in alumna suspension (Stuers, Denmark) (size of the particles was 0.1 μm) in water, rinsed with de-ionized water, and finally electrochemically polished in 1 M H_2SO_4 by scanning the potential between –0.2 and +1.7 V with a scan rate of 0.3 V/sec. Then the electrodes were rinsed with deionized water and 5 μl of a DNA solution (0.5 mg/ml) in 0.05 M phosphate buffer solution, pH 7.4, was dropped onto the electrode surface. After 6 h immobilization, the DNA-modified electrodes were gently rinsed with water and placed in the electrochemical cell. Cyclic voltammograms were recorded in 0.05 M phosphate buffer solution, pH 7.4, containing 0.3 M NaCl (PBS), at potential scan rates ranging from 10 to 0.05 V/sec. All potentials in this work are referred to the Ag/AgCl reference electrode. Reproducibility of the data was verified by measurements with at least eight equivalently prepared electrodes.

RESULTS AND DISCUSSION

DNA was immobilized on the surface of gold electrodes. In the course of electrochemical measurements with the DNA-modified electrodes, no electrochemical signals correlating with redox transformations of DNA were obtained in the potential range from –1 to +0.9 V (Fig. 1, curve *2*). As was previously shown, the absence of any signal in the negative potential range correlates with desorption of the DNA molecules from the gold electrode surface [14, 24]. When scanning the potential in the positive potential range (from 0 to +0.9 V and back) the appearance and amplification of an electrooxidation peak at +0.7/+0.8 V was observed in cyclic voltammograms measured with the DNA-modified electrodes. Experiments were performed in PBS free from DNA. Similar results were obtained upon preliminary 30-40 min polarization of the DNA-modified electrodes at +0.5 V (this time providing the maximum intensity of the signal, Fig. 1, curve *3*). The signal was retained when the electrode was transferred from the working solution to air (storage up to 52 h) and back. Upon polarization at negative potentials the height of the oxidation peak decreased to zero, evidently correlating with the desorption of DNA from the gold surface. The oxidation process, observed at +0.7/+0.8 V, was supposedly ascribable to electrochemical oxidation of guanine bases of DNA. Guanine is the most easily oxidized of all the nucleoside bases of DNA [1-13], since this nucleoside has the least (among the other bases) value of the redox potential (E^0) [1, 29, 30]. For irreversible $4e^-$ electrochemical oxidation of guanine the potential of the oxidation peak is E_{peak} (V) = 1.115-0.065 pH (+0.634 V versus Ag/AgCl electrode, at pH 7.4) [29]. Undoubtedly, the reactivity of the folded DNA structure can be materially different from its individual monomer components. For $1e^-$ oxidation through the formation of the guanine radical cation the potential E^0 at pH 7.0 is +1.09 V [1, 30] and even less, from +0.65 to +1.0 V [1, 31], depending on the nucleotide environment of oxidized guanine in DNA [1, 6, 32]. Thus, the potential range of the electrochemical oxidation of DNA, observed in Fig. 1, corresponds to the potentials of oxidation of the guanine bases of DNA. Pre-polarization of the DNA-modified electrodes, at +0.5 V, which is necessary for the appearance of the anodic signal at +0.7/+0.8 V (Fig. 1, curve *3*), is supposed, first, to enhance the electrostatic interactions between the negatively charged DNA molecule and gold either facilitating further efficient oxidation or initiating the oxidation of the guanine bases of DNA at the electrode surface, second, to rearrange and/or align the DNA molecules on the gold surface in a manner which results in the efficient oxidation of the guanine bases inside the DNA molecule through the intramolecular transfer of the oxidative damage initiated at the electrode surface. Therewith, the system of successive base stacking interactions is responsible for the fast intramolecular charge transfer [4, 7-12].

The kinetics of the electrochemical oxidation was studied. Cyclic voltammograms for adsorbed on gold DNA, measured at different scan rates of potential, are presented in Fig. 2. The intensity of the DNA oxidation peak I_p depends linearly on the potential scan rate v (Fig. 3), corresponding to the oxidation process in the adsorbed layer [33]. Therewith, two regions of linear dependence between the intensity of the DNA oxidation and v are observed. For v higher than 1 V/sec the slope of the I–v dependence changes, indicating a variation in the reaction mechanism. Along with this, the appearance of the reduction peak is observed in cyclic voltammograms, which correlates with the oxidation process involved and

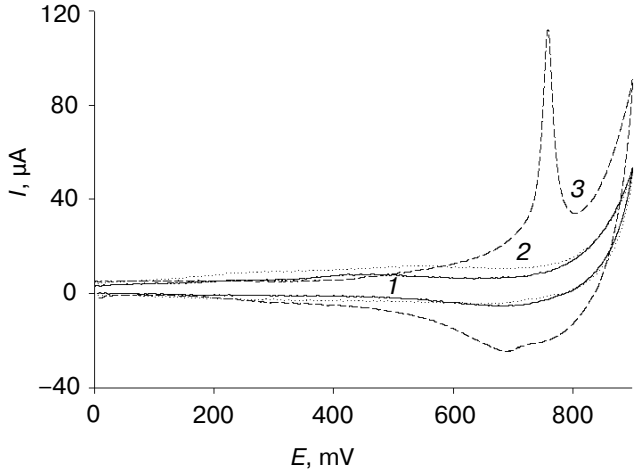

Fig. 1. Cyclic voltammograms measured in a DNA-free PBS, pH 7.4, for: *1*) the gold electrode upon 30 min polarization at +0.5 V; *2*) the DNA-modified gold electrode; *3*) the DNA-modified gold electrode upon 30 min polarization at +0.5 V. Potential scan rate was 1 V/sec.

suggests the reversibility of the electrode process (Fig. 2). Redox potential E^0 determined as an average between the potentials of the oxidation and reduction peaks was +0.725 ± 0.005 V. The number of electrons n participating in the process and the amount of the oxidized species Γ (mol/cm^2) were calculated according to the Laviron equations for the adsorbed layer [34]:

$$I_p = n^2F^2A\Gamma v/4RT = nFQv/4RT, \quad (1)$$

where I_p is the height of the electrooxidation peak (in amperes), A is the electrode surface area (0.031 cm^2), Q is the amount of electricity consumed in the process (in coulombs) and calculated from the area of the electrooxidation peak, and F, R, and T are the Faraday constant, the universal gas constant, and the Kelvin temperature, respectively. The number of electrons n, calculated from the slopes of the I_p–v dependence (Fig. 3), corresponds to 3.75 ± 0.35 for v less than 1 V/sec, and to 0.76 ± 0.14 for v greater than 1 V/sec. The values of Γ were 152 ± 34 and 447 ± 68 pmol/cm^2 for 4e$^-$ and 1e$^-$ processes, correspondingly. The relationship between base pairs in calf thymus DNA, AT/GC, is 0.58 : 0.42 [14]. The value of Γ was supposed to correspond to the amount of the oxidized guanine bases of DNA. Considering this, the amount of electrooxidized DNA was estimated according to the approximated formula:

$$\text{mass of DNA} = (265 + 225 + 2\cdot63)\cdot\Gamma + (249 + 240 + 2\cdot63)\cdot(\Gamma\cdot58/42), \quad (2)$$

where the figures are the molecular weights of the bases and phosphate groups. It was obtained for the 4e$^-$ and 1e$^-$ processes that the guanine bases are oxidized corresponding to 223 and 655 ng/cm^2 of adsorbed DNA. Therewith the total amount of DNA adsorbed at the electrode surface, determined with QCM, varies from 645 ng/cm^2 for highly polymerized DNA (molecular weight 10-15,000 kb) [24] to 1500 ng/cm^2 for activated DNA (unpublished authors' data). Consequently, electrochemically oxidized DNA (or the fraction of the oxidized guanine sites in the individual DNA molecule) constitutes from 15 to 35% (4e$^-$ process) and from 44 to 100% (1e$^-$ process) of the total amount of DNA adsorbed on gold (or of the total amount of the guanine bases in the individual DNA molecule, respectively).

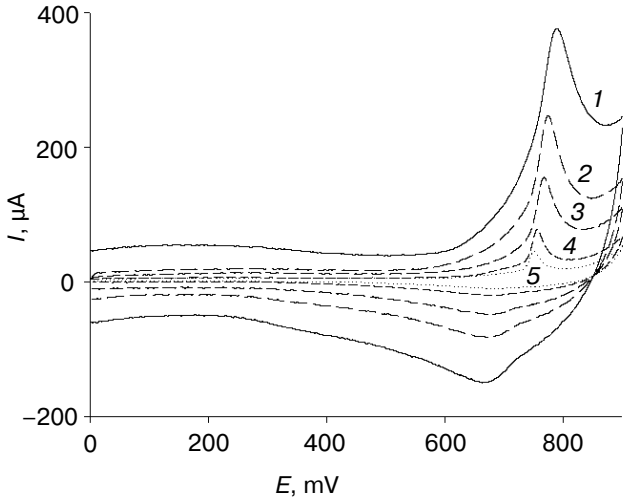

Fig. 2. Cyclic voltammograms for the DNA-modified gold electrode measured in DNA-free PBS, pH 7.4, at potential scan rates 10 (*1*), 5 (*2*), 3 (*3*), 1 (*4*), 0.5 (*5*) V/sec. Prior to measurements the electrode was pre-polarized for 30 min at +0.5 V.

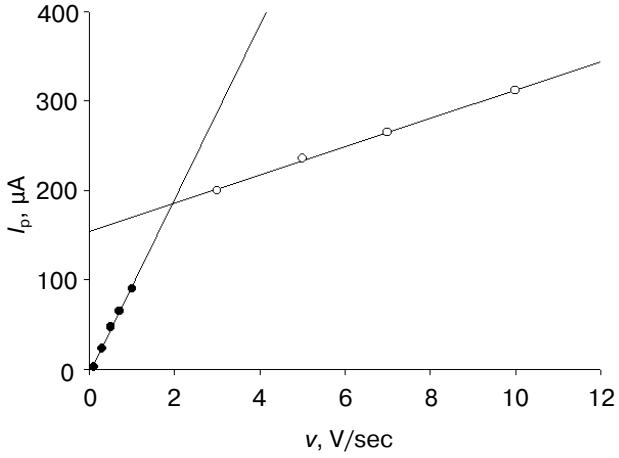

Fig. 3. Dependence of the peak current of electrochemical oxidation of DNA I_p on the potential scan rate.

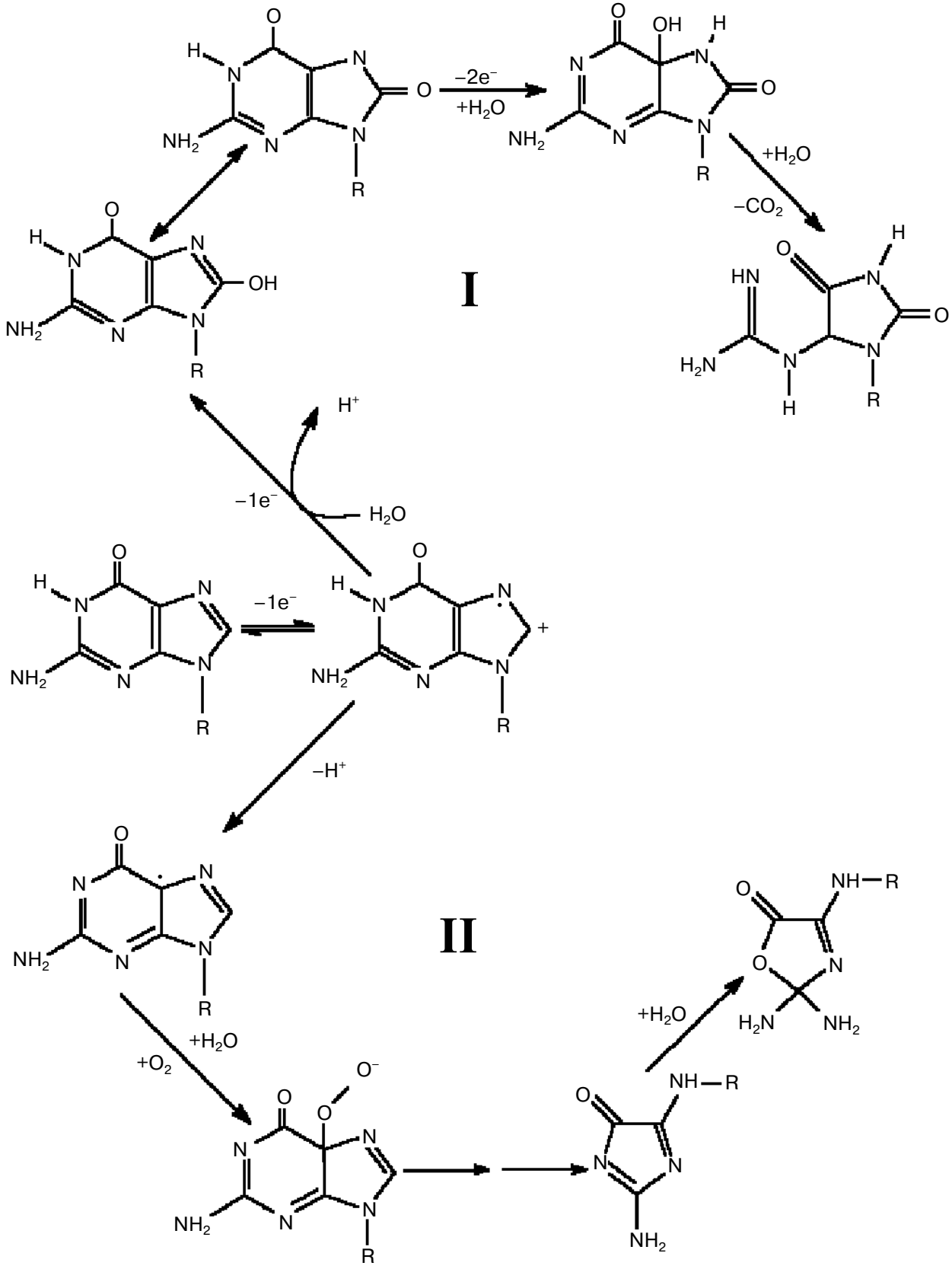

Fig. 4. Proposed scheme of oxidation of the guanine bases of DNA: I) electrochemical oxidation of DNA through the formation of the guanine radical cation; II) one of the possible pathways for the reaction of homogeneous oxidation of DNA.

Thus, it can be assumed that at low potential scan rates the guanine bases of DNA undergo an extensive four-electron oxidation. The mechanism of this reaction is likely to correspond to the mechanism of electrochemical oxidation of pure guanine [29] in accordance with the overall reaction pathway I schematically presented in Fig. 4 (a limiting case extended to the guanine bases of DNA). Therewith, the process of DNA electrochemical oxidation is most probably accompanied by the hydrolysis of intermediate products in the same manner as was earlier shown for guanine [29]. This contributes to irreversibility of the oxidation process: at v less than 0.3 V/sec the intensity of electrochemical oxidation decreases, upon scanning potential with a scan rate of 0.05 V/sec the electrooxidation peak disappears and is not recovered as one returns to higher values of v. The values of v from 0.3 to 1 V/sec, apparently, determine a multimode mechanism of the reaction, when with a varying efficiency the processes involving $4e^-$, $2e^-$, and $1e^-$ can proceed simultaneously (Fig. 4), in going to the higher potential scan rates (from 0.3 to 10 V/sec and back) the area of the oxidation peak, and, as a consequence, the amount of the oxidized sites of DNA (Eq. (1)) remaining unchanged. At high rates of the potential scanning, the electrooxidation of guanine in DNA proceeds predominantly through the reversible $1e^-$ formation of the guanine radical cation [1-4, 6, 11]. This radical cation, as discussed in [1-3, 32], can spontaneously (depending on the time of relaxation and nucleotide environment) convert back to the reduced state or undergo hydration followed by the oxidation process (reaction pathway II, Fig. 4). In this case, under conditions of fast electrochemical $1e^-$ oxidation–reduction of DNA, the stability of the signal for repeated measurements and calculated invariable amount of electrooxidized DNA, which is close to the total amount of DNA adsorbed at the electrode surface, can be explained on the assumption that not only do the guanine bases of the folded double-stranded DNA in the vicinity or in direct contact with the electrode surface undergo oxidation, but (and first of all) spatially remote from the electrode surface guanine bases in the DNA volume. For the times of electrooxidation in the order of 0.01 sec (the time of the DNA oxidation for the potential scan rates of the order of 10 V/sec) the oxidation process, initiated at the electrode surface through the formation of the guanine radical cation, appears to propagate further through the DNA molecule, being reversible. Under these conditions the resulting oxidative damage to DNA should be minimal. For the times of electrooxidation in the order of 1-4 sec (for the potential scan rates of the order of 0.05-0.1 V/sec) more extensive irreversible $4e^-$ oxidation of the guanine bases of DNA occurs, which prevents the reversible migration of the radical cation within the DNA volume [7-9, 11] and produces substantial oxidative damage to DNA molecules. Therewith, the oxidative damage to DNA resulting in the irreversible change in electrochemical activity of DNA (a suppress of the electrooxidation peak), constitutes approximately 5-12% of the total amount of DNA adsorbed at the electrode surface or, on the average, 5-12% of the guanine bases in each individual molecule of DNA. The calculations were performed according to Eqs. (1) and (2) using the data of cyclic voltamperograms measured at scan rates from 0.05 to 0.1 V/sec and under the conditions when the area of the DNA electrooxidation peak (peak height 1.5 ± 0.2 μA) decreased to a minimum value (0.8 ± 0.3 μC) and disappeared in the following measurements.

The complexity and ambiguity of the processes involved in oxidative DNA damage enable one to estimate the mechanisms of the electrochemically induced oxidative damage only with a certain degree of approximation, drawing the analogy to the discussed mechanisms of the DNA damage in a homogenous phase [1-13]. Nevertheless, the results obtained suggest the possibility to temporally control the degree of the oxidative damage to DNA.

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