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Electrode potential-controlled DNA damage in the presence of copper ions and their complexes

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Abstract

Supercoiled (sc) DNA immobilized at the surface of a hanging mercury drop electrode was cleaved by reactive oxygen species generated by an electrochemically modulated reaction of copper ions, hydrogen peroxide and/or oxygen. The cleavage was observed in a certain potential region where redox cycling of DNA-bound Cu(II)/Cu(I) took place. In the presence of 1,10-phenanthroline the maximum efficiency of DNA cleavage was shifted to more negative potentials and the effect was enhanced.

Keywords: DNA damage; Mercury electrode; Copper ions; Phenanthroline

1. Introduction

Ions of transition metals are involved in the processes resulting in damage to DNA, which may be followed by the mutations and/or malignant transformation of the cells [1]. Metal ions that can undergo one-electron redox reactions (such as iron, copper or manganese) react with oxygen producing reactive oxygen species (ROS); the latter then damage DNA, frequently yielding strand breaks [2]. By measuring changes in DNA electrochemical behavior at mercury [3–7] or carbon [8,9] electrodes, DNA damage can be detected. A mercury electrode modified with supercoiled (sc) DNA can serve as a highly sensitive tool for detecting DNA cleaving species, including chemical [3,5,7] or enzymatic [5] nucleases. Recently, we have shown that supercoiled DNA immobilized at the surface of the HMDE was cleaved in the presence of iron/EDTA and hydrogen peroxide if electrode potential was sufficiently negative to reduce Fe(III) to Fe(II) [6]. Under these conditions, hydroxyl radicals (cleaving the surface-attached DNA) were produced through a Fenton reaction mediated by electrochemically regenerated iron(II)/EDTA complex. Electrode potential-dependent DNA cleavage was observed also in the absence of transition metals if oxygen was present in the solution, suggesting a role of radical intermediates of oxygen electroreduction at mercury [6] or platinum [10] electrodes. In this report, we show distinct potential-dependent effects of copper ions and of copper–1,10-phenanthroline complex on DNA damage at the HMDE surface.

2. Experimental

2.1. Preparation of scDNA-modified HMDE

Supercoiled (sc) $pBSK_{(-)}$ DNA (prepared as described previously [3]) was adsorbed at the electrode surface from a 3–5 μl drop of solution containing 50 μg/ml scDNA in 0.2 M NaCl for 60 s. The electrode was thereafter washed by distilled water and transferred into the solution of the DNA damaging reagent. Details about the cleavage reaction are given in Results and discussion.

2.2. Apparatus and measurements

AC voltammetric measurements were performed with an Autolab analyzer (Eco Chemie, The Netherlands) in connection with VA-Stand 663 (Metrohm, Switzerland) in HMDE mode. The three-electrode system (with Ag/AgCl/3 M KCl electrode as a reference and platinum wire as an auxiliary electrode) was used. 0.3 M NaCl and 0.05 M

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Na_2HPO_4 were used as a background electrolyte. The following parameters for measurement were chosen: initial potential, −0.6 V; frequency, 230 Hz; amplitude, 10 mV; scan rate, 20 mV s^{-1}. In phase component was measured.

3. Results and discussion

The scDNA-modified HMDE was incubated in 0.2 M sodium perchlorate, 10 mM Tris–HCl (pH 7.5) containing 10 μM cupric sulfate. Potentials E_C (potential facilitating the cleavage reaction) (Fig. 1) were then adjusted and the cleavage reaction was started by an addition of hydrogen peroxide. After 60 s, the circuit was opened and the electrode was transferred into a background electrolyte, followed by the measurement of DNA AC voltammetric peak 3, which is sensitive to DNA strand breaks [3]. In the presence of 1 mM H_2O_2, the heights of peak 3 displayed a potential dependence with a sharp maximum at $E_C = -0.1$ V. At more negative potentials, heights of peak 3 declined, reaching a plateau at −0.3 V corresponding to about 20% of the maximum peak 3 height obtained at −0.1 V. In the absence of hydrogen peroxide or in the presence of 10-fold excess of EDTA, no DNA damage was observed around $E_C = -0.1$ V. These observations suggest that (i) DNA damage in this system requires the presence of hydrogen peroxide and EDTA-unchelated copper ions and (ii) a production of ROS takes place around a potential of −0.1 V, corresponding to the reduction peak of Cu^{2+} in the same medium (not shown). Such a behavior markedly differs from the previously observed [6] potential dependence of DNA damage yielded by iron/EDTA-mediated Fenton reaction, displaying an inflection point at a potential corresponding to the iron(II)/EDTA reduction peak and continuously increasing towards negative potentials. The limitation of the DNA damage to this potential region can be explained by the requirement for Cu^+ in the processes leading to DNA damage. At bare HMDE, copper yields one reversible pair of peaks related to a two-electron reaction (Cu^{2+}/Cu^0). Nonetheless, some ligands (including cytosine or purines) stabilize Cu^+. This phenomenon results in the splitting of the reduction/oxidation of coordinated copper at HMDE in two one-electron steps [11]. We propose that under our conditions, certain fraction of copper ions may be "trapped" as DNA-bound Cu^+. These Cu^+ ions may then undergo redox cycling in the presence of hydrogen peroxide, thus producing ROS [12]. This stabilization of Cu^+ in the DNA layer was impossible at too negative potentials and copper was rapidly reduced to Cu^0. On the contrary, iron in the EDTA complex is further reduced to Fe^0 only at highly negative potentials (outside the range examined in Ref. [6]), and thus, no decline of the DNA cleavage at potentials more negative than the reduction peak of iron/EDTA was observed.

In our previous work [6], we showed that hydrogen peroxide which is converted into hydroxyl radicals in the Fenton type reactions can be generated electrochemically at the HMDE by dioxygen reduction. In the following experiment, we incubated scDNA-modified HMDE in air-saturated solution containing 1 μM Cu^{2+} at different potentials

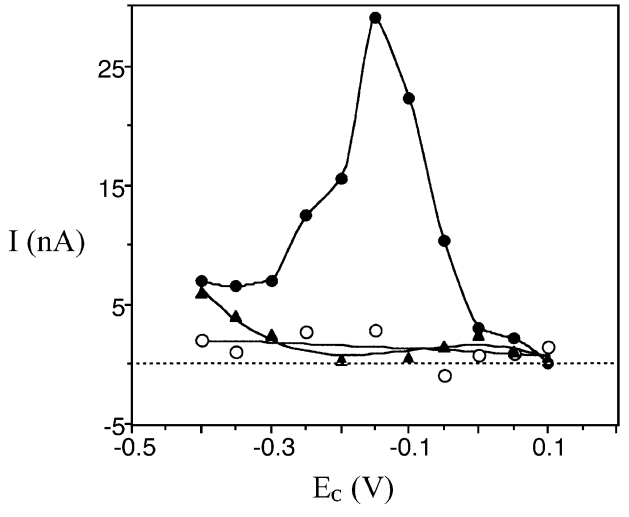

Fig. 1. The dependencies of copper-mediated DNA cleavage (height of DNA peak 3) on the potential E_C. (○), 10 μM Cu^{2+}, deaerated solution; (●), 1 mM H_2O_2, 10 μM Cu^{2+}; (▲), 1 mM H_2O_2, 10 μM Cu^{2+}, 100 μM EDTA. DNA-modified electrodes were prepared by incubation of HMDE in a 4-μl drop of 50 μg ml^{-1} scDNA in 0.2 M NaCl, 10 mM Tris–HCl (pH 7.4) for 60 s. After washing (at the open current circuit), the electrode was immersed into the solution of the reactants (except H_2O_2) which was always deaerated. Then, potential E_C was adjusted and the reaction was started by the addition of H_2O_2 into the stirred solution. After 60 s, the reaction was stopped (by opening the current circuit) and the electrode was washed and transferred into blank background electrolyte to record the voltammogram.

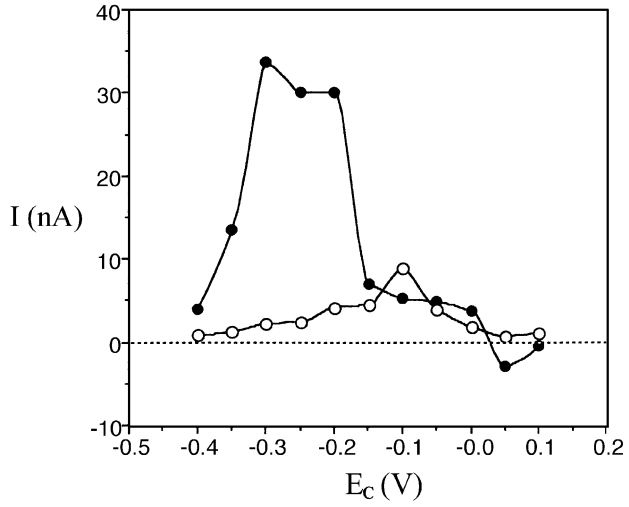

Fig. 2. Dependence of scDNA cleavage at the HMDE surface on the electrode potential in the presence of (○), 1 μM Cu^{2+}, air-saturated solution; (●), 1 μM $[Cu(phen)_2]^{2+}$, air-saturated solution. For other details, see Fig. 1.

(Fig. 2). A dependence of DNA damage on the potential E_C similar to Fig. 1 was obtained. The maximum I_3 value at -0.1 V reached about 30% of the value measured under the conditions shown in Fig. 1.

Copper complex with 1,10-phenanthroline (phen) has been applied as a chemical nuclease in DNA footprinting [13]. We tested the behavior of the $[Cu(phen)_2]^{2+}$ complex in potential-modulated DNA damage at the HMDE. Upon addition of 2 μM phen into air-saturated solution of 1 μM Cu^{2+}, the DNA cleavage at the electrode was three- to four-fold enhanced and the potential region where DNA damage occurred was shifted in the negative direction and was wider (Fig. 2). These observations can be explained by a stabilization of the Cu^{+} in the phen complex where it can undergo one-electron redox cycling, yielding ROS in the presence of dioxygen. Moreover, the $[Cu(phen)_2]^{2+}$ complex is accumulated in DNA (immobilized at the electrode surface) by the intercalation of the phen moieties into the DNA double helix, facilitating formation of ROS in the close vicinity of the DNA molecules.

In conclusion, our results demonstrate that scDNA-modified mercury electrodes can be used not only as sensitive biosensors for the detection of DNA damaging species, but also in basic studies of redox processes resulting in DNA damage, including reactions of transition metal ions and their complexes. More details will be published elsewhere.

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