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Interactions of surface-confined DNA with acid-activated mitomycin C

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Abstract

The anti-cancer drug mitomycin C (MC) was acid-activated and its interaction with single-stranded calf thymus DNA, immobilized at the surface of the hanging mercury drop electrode (DNA-modified HMDE) was studied by cyclic voltammetry. It was found that immersion of the DNA-modified electrode in a solution of acid-activated MC (at pH 3.9) for a short time (usually 1 min) at open current circuit, followed by transfer of the electrode in a neutral blank background electrolyte, resulted in a decrease of the anodic peak G (due to guanine residues in DNA) and in the formation of a reversible couple at approx. −0.44 V. The potential of the cathodic peak was approx. 50 mV more negative than the cathodic peak of the acid-activated MC obtained under the same conditions in the absence of DNA. No changes of peak G occurred and only a very small cathodic peak appeared if the DNA-modified electrode was immersed in an MC solution not exposed to acid pH. On the basis of these results and additional experiments, including dependence on concentration, time and pH during the interaction of MC with DNA at the electrode surface, we concluded that acid-activated MC is covalently bound to guanine residues in DNA immobilized at the electrode surface and that the quinone group in the DNA-MC adduct is reversibly reduced at the electrode.

Keywords: Cyclic voltammetry; Anti-cancer drug; Hanging mercury drop electrode; Acid-activated mitomycin C; DNA-modified electrode; Mitomycin C–DNA interaction

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1. Introduction

Mitomycin C (MC) is a clinically applied antitumor agent that binds covalently to DNA in vitro and in vivo after specific activation processes [1]. In living cells MC is activated by flavoenzymes; in vitro reductive and acid activations (Fig. 1) have been described. It was shown that MC can be activated in vitro in the absence of any reducing agent by lowering the pH of the MC solution to < 4.5 [1,2]. Due to this acid activation both mono- and bifunctional binding of MC to DNA may occur. In the same way as the reductively activated MC, acid-activated MC also binds to guanine residues in DNA.

It has been shown [3] that MC can undergo an ECE reduction at the mercury and solid electrodes. The first electrochemical step, responsible for the voltammetric peak designated Ic, is a reduction of the quinone structure to the hydroquinone. The chemical step is a reaction involving demethoxylation, aziridine opening and reorganization to obtain the quinone group again. The second electrochemical process, responsible for the peak called IIc, is the reduction of the ring-opened and demethoxylated quinone to the corresponding hydroquinone. Other peaks are obtained at more negative potentials, however, our study is focused on peaks Ic and IIc.

In voltammetric [4,5] and chronopotentiometric [6] cyclic modes, DNA produces an anodic peak G which is due to guanine residues. It was shown that chemical modifications of guanine residues in DNA can be detected by changes of peak G [7]. Using cyclic voltammetry (CV) we have recently found [8] that addition of DNA to an MC solution resulted in a strong decrease of the MC peaks in the first cycle. In the second cycle a new reversible couple appeared; both the cathodic and the anodic peaks increased with the number of cycles, and a simultaneous decrease of peak G was observed. We tentatively attributed these results to the reductive activation of MC at the electrode and binding of the reductively activated MC to DNA.

The DNA-modified electrode can be easily prepared by immersing the HMDE [9,10] or carbon electrodes [10] in a DNA solution. In this paper we use the DNA-modified HMDE to study interactions of the surface-confined DNA with acid-activated MC in solution. Our results suggest that acid-activated MC binds to DNA at the electrode surface as detected by the decrease of the CV peak G and appearance of a redox couple at approx. -0.4 V. Immersion of the DNA-modified electrode in non-activated MC produces no decrease of this peak. To our knowledge no other studies of interactions of MC in solution with DNA adsorbed at surfaces have been performed. However, such studies may be important as the interactions of MC with DNA in vivo may involve DNA attached to various cell surfaces.

2. Material and methods

MC was from Merck. Calf thymus DNA was isolated as previously described [9]. Single stranded DNA was prepared by denaturing double stranded DNA by heating at 100°C for 6 min with subsequent cooling in an ice-bath. The con-

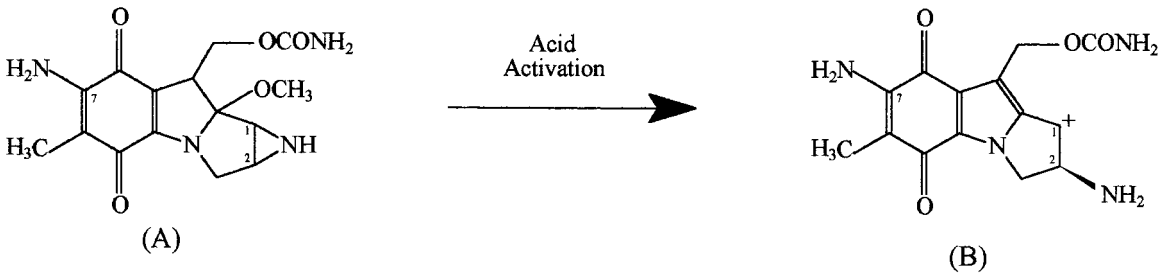

Fig. 1. Structures of mitomycin C (A) and acid-activated mitomycin C (B).

centrations of the stock DNA solutions were calculated from their absorbance at 258 nm. Other chemicals were of analytical grade.

The measurements were carried out with an Amel 473 multipolarograph analyzer connected to an Amel 863 XY recorder with time-base. All measurements were taken in a nitrogen atmosphere at room temperature. As a working electrode, a Metrohm (6.0335.000) hanging mercury drop electrode (HMDE) was used with a drop area of 1.39 mm^2. A saturated calomel electrode (SCE) and a platinum electrode, both from Ingold, were used as reference and auxiliary electrodes, respectively. The pH values were measured with a Radiometer pH meter model 62. For UV spectra measurements, a Kontron 941-plus with a data processing system was used.

The background electrolyte, 0.3 M ammonium formate, 50 mM sodium phosphate buffer, pH 6.9 (AFP), was used because this medium is suitable for measurements of the anodic response of DNA (peak G) [11,12] and also for measurements of the cathodic response of MC (peak IIc) [8]. If not stated otherwise, 0.2 M acetate buffer (pH = 3.9) was used to produce MC acid activation. MC was dissolved in this medium 1 day prior to its use in experiments to produce the complete acid activation which is not instantaneous. In the pH dependence study, Britton–Robinson and acetate buffers were used. All measurements were carried out in a nitrogen atmosphere at room temperature.

In transfer stripping cyclic voltammetry (TSCV) with the DNA-modified HMDE (Fig. 2), the DNA-modified electrode was prepared by immersing the HMDE in an 8-μl drop of DNA solution at open circuit for the accumulation time t_A (usually 60 s). The electrode was then removed from the solution, washed with distilled water and transferred to the MC solution at the given accumulation potential E_B for the accumulation time t_B. The electrode was washed again with distilled water followed by washing with the background electrolyte solution. The modified electrode was then placed in a voltammetric cell with a previously deoxygenated background electrolyte (AFP, not containing any dissolved DNA nor MC). Then the solution was purged for 2 min with nitrogen,

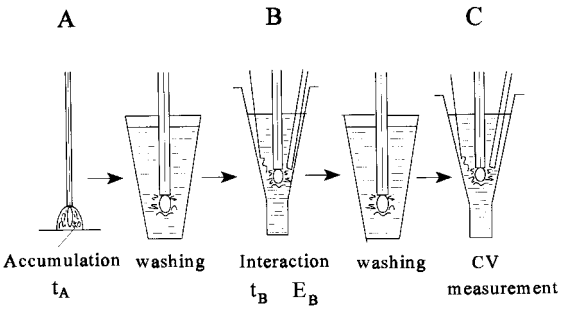

Fig. 2. Schematic representation of an experiment on the interaction of acid-activated mitomycin C with DNA at the surface of HMDE. (A) A DNA-modified electrode was prepared by immersing the hanging mercury drop electrode in a drop (8 μl) of a DNA solution for the accumulation time t_A (1 min) at open current circuit. The electrode was removed from the DNA drop, washed and (B) transferred to an electrolytic cell containing the solution of mitomycin C for the time t_B (between 30 and 600 s; without stirring), usually either at open current circuit or at the potential E_B given in the text. The electrode was washed again and (C) transferred in an electrolytic cell containing the blank background electrolyte (AFP). The cyclic voltammetric measurements were performed under nitrogen at a scan rate of 200 mV/s.

after which the initial potential E_i was applied for 15 s and the voltammetric measurement was taken at the following settings (if not stated otherwise): initial potential $E_i = 0.0$ V, switching potential $E_{sw} = -1.85$ V and scan rate = 200 mV/s. The highly negative switching potential, $E_{sw} = -1.85$ V, was used in order to visualize the anodic peak G (due to oxidation of the guanine reduction product in DNA) [4,5] which may serve as an indicator of the binding of activated MC to guanine residues.

In TSCV with the bare HMDE, the electrode was immersed in the MC solution at the given accumulation potential, E_A for the accumulation time t_A, removed from the solution, washed and transferred to the voltammetric cell containing AFP where the measurements were carried out as indicated above.

3. Results

Conventional CV of MC in acid medium yields two cathodic peaks and we have observed an increase of the more negative peak (IIc) current

and a decrease of the first peak (Ic) current during the time of incubation, at room temperature, reaching a limit value (at pH 3.9 the equilibrium is reached after 3 h of incubation). In the experiments below with MC at acid pH, we used acid MC solutions (if not stated otherwise) prepared 1 day before measurements to avoid unwanted changes in the peak currents.

3.1. Transfer stripping CV of acid-activated MC

In a previous paper [8] we have shown that reduced MC is firmly attached to HMDE from a neutral solution. TSCV with accumulation at potential −0.35 V resulted in a voltammogram (showing the peak IIc and its anodic counterpart) almost identical to that obtained without transfer (Fig. 3A). However, accumulation at open circuit or at a potential more positive than −0.2 V resulted in a voltammogram with a negligible peak (IIc).

When MC was accumulated from an acid medium (sodium acetate buffer, pH 3.9) at E_A = 0.0 V and the electrode transferred to AFP (Fig. 3B), peak IIc was much smaller than that obtained at $E_A = -0.3$ V (Fig. 3C). These results suggest that the electroreductive activation of MC, but not the acid activation alone, is necessary for the strong immobilization of MC at the electrode surface.

MC peaks on the second and following cycles decreased strongly (Fig. 3B,C), however, this decrease was lower with accumulation at neutral pH (Fig. 3A) and a very small decrease was observed by reversing the scan at −0.8 V (Fig. 3D), suggesting that, at highly negative potentials, deeper changes (desorption included) occur in acid-activated than in neutral MC solutions.

3.2. Interactions of DNA immobilized at the electrode surface with MC in solution

Conventional CV of a DNA-modified electrode (Fig. 4A) shows the anodic peak of DNA (peak G). The interaction of the surface-confined DNA layer with non-activated MC in solution resulted in a DNA voltammogram with a small peak (Ic-D′) at −0.42 V in the first cycle; this peak disappeared at further cycles (Fig. 4B). The height of peak G on the first cycle did not differ from that of DNA alone (compare Fig. 4A and B). Gradual decreases of peak G at the second and following cycles agree with previous results obtained with mixtures of DNA and MC at neutral pH in solution [8]. This suggests that the layer of single-stranded DNA formed at the electrode surface is inadequately permeable to non-activated MC, almost completely preventing its redox processes at the electrode surface during the first cycle. The small cathodic peak on the first cycle (Ep = −0.42 V) might be due to non-specific binding of non-activated MC described by Crothers et al. [13].

3.3. Acid-activated MC binds to DNA at the surface

Immersion of the DNA-modified electrode into the solution of acid-activated MC produced a voltammogram (Fig. 4C) very different from that obtained after immersion in non-activated MC (Fig. 4B); the peak G of DNA was substantially diminished and high MC peaks, IIc-D and IIa-D were formed.[1] The MC peaks decreased on subsequent cycles, but at the fourth cycle their heights were much larger than that of peak IIc-D observed on the first cycle in the experiment with non-activated MC (Fig. 4B). In addition to the IIc-D and IIa-D peaks, interaction of acid-activated MC with DNA resulted in the IIIc-D peak at −0.9 V on the first scan. The appearance of peaks IIc-D and IIa-D could likely be explained as the result of the binding of acid-activated MC to DNA. MC covalently bound to DNA immobilized at the surface is held in the close neighborhood of the electrode where it can undergo the redox processes responsible for peaks IIc-D and IIa-D. Strong decrease of peak G supports this assumption as it is known that MC binds preferentially to guanine residues [1].

3.4. Dependence on MC concentration, pH and incubation time t_B

The effect of the MC concentration on the

[1] Affix-D is used to denominate MC peaks obtained with the DNA-modified electrode.

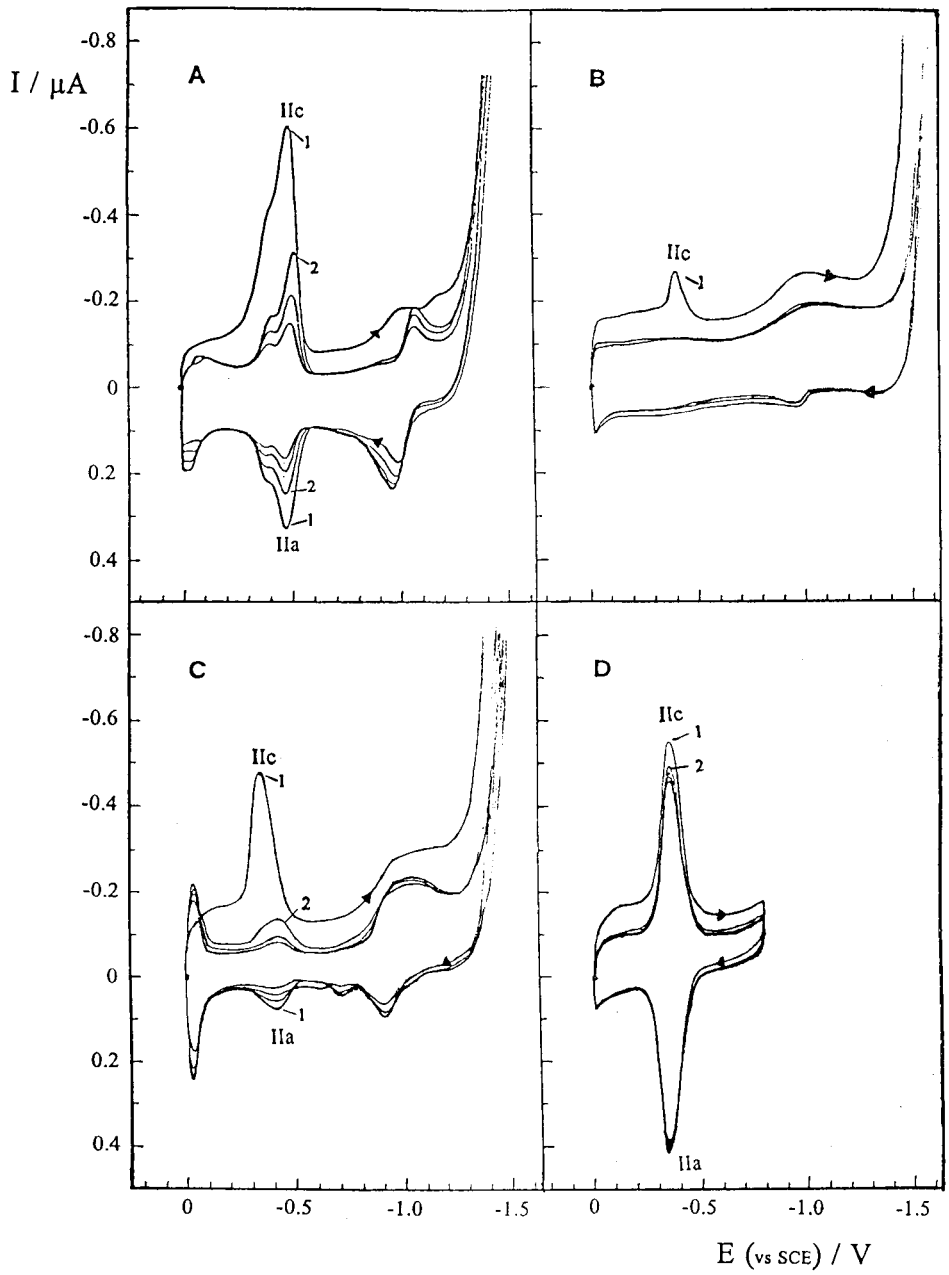

Fig. 3. Transfer stripping cyclic voltammograms of 10 μM MC accumulated at the HMDE from: AFP pH 6.9 (A), acetate buffer pH 3.9 (B–D) at E_A: −0.30 V (A, C, D), 0.00 V (B), for $t_A = 2$ min. After accumulation the HMDE was washed and transferred to the electrolytic cell containing blank AFP to perform the CV measurements: $E_i = 0.0$ V; $E_{sw} = -1.85$ V (A, B, C), −0.80 V (D); scan rate = 200 mV/s.

MC–DNA interaction was tested by immersing the DNA-modified electrode in acid-activated MC solutions at different MC concentrations for 5 min at open current circuit, followed by transfer and measured in AFP. Concentrations under 10^{-4} M did not produce any decrease in the peak G

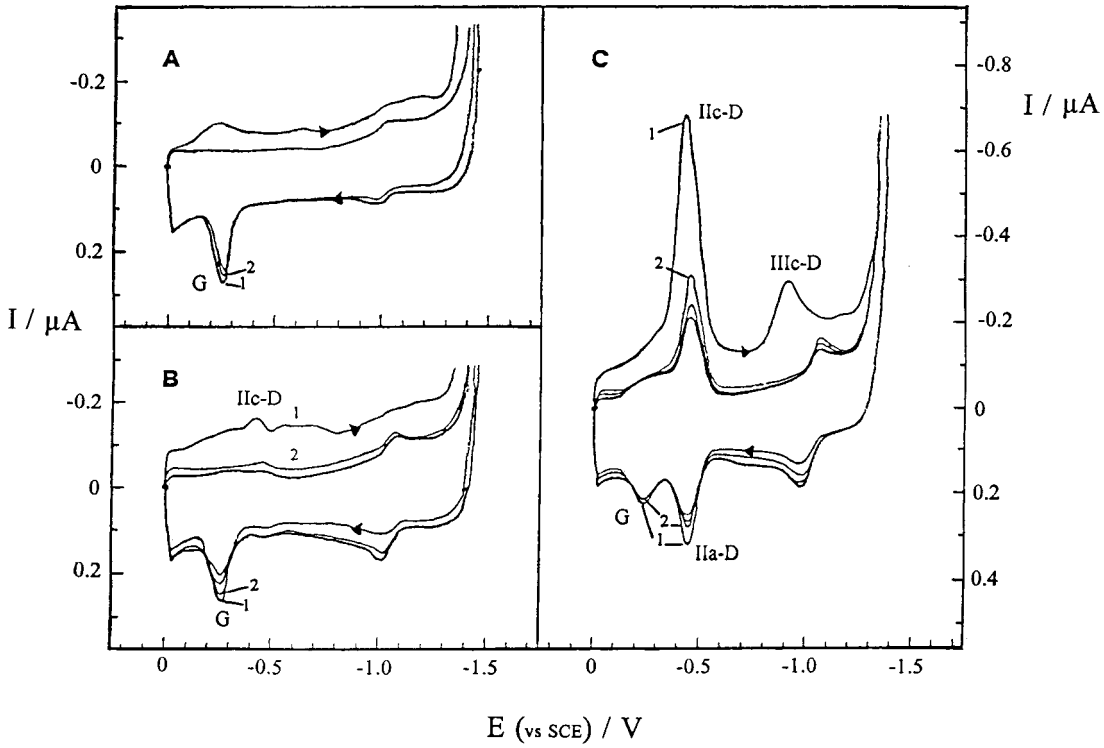

Fig. 4. Transfer stripping cyclic voltammograms in AFP of (A) single-stranded (ss) DNA without interaction with mitomycin C and (B,C) after interaction of the DNA-modified electrode with 1 mM MC in Britton–Robinson buffer, (B) at pH 6.5 and (C) at pH 3.1. ssDNA (35 μg/ml in AFP) was accumulated at HMDE at open circuit for 1 min. The DNA-modified HMDE was washed and transferred either (A) in blank AFP to record the CV or (B,C) in the MC solutions for 5 min at open circuit, with subsequent washing and immersion in the AFP for CV measurements. Other settings as in Fig. 3.

current; higher concentrations led to decreases in peak G in a sigmoidal curve (Fig. 5B). Peak G currents obtained after DNA-modified electrode immersion in blank buffers of the same pH were taken as 100%. An intermediate value (1 mM) was chosen as MC concentration for the whole work, if not stated otherwise.

We studied the dependence of the height of peak G (I_G) on pH of the MC solution in which the DNA-modified electrode was immersed for the time $t_B = 5$ min. We observed an S-shaped curve (Fig. 5A) (showing low I_G at lower pHs) with a midpoint at a pH of approx. 4.3. Immersion of the DNA-modified electrode in buffer solutions not containing MC resulted in no changes in peak G in the studied pH range. The height of peak IIc-D also displayed an S-shaped curve with about the same midpoint. In contrast to changes in peak G, high IIc-D peaks were observed at lower pHs in good agreement with the assumption that acid-activated MC was bound to DNA at these pHs.

In a similar experiment the effect of time t_B was studied at pH 3.9. Peak G decreased strongly with t_B up to 5 min (reaching 50% of its original value) (Fig. 5C). At longer t_B its further decrease was very small showing 48% of its original value at $t_B = 10$ min. The strong decrease of peak G at $t_B = 5$ min was observed only with the acid-activated MC; if freshly prepared acid MC solution was used, peak G decreased at $t_B = 5$ min only by 5%, and at $t_B = 10$ min it reached 93% of its original value.

We mixed DNA with acid-activated MC in so-

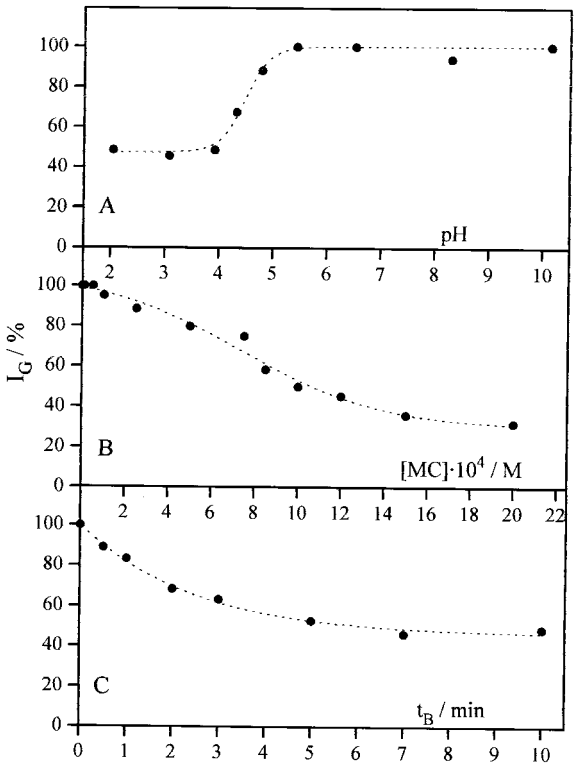

Fig. 5. Dependence of the height of the DNA peak G: (A) on pH of MC solution; (B) on the concentration of MC solution; and (C) on the time t_B in/for which the DNA-modified electrode was immersed at open current circuit. Acetate buffer pH 3.9 (B, C), 1 mM MC (A, C) and t_B 5 min (A, B) were used as general conditions. Peak G currents obtained after DNA-modified electrode immersion in blank buffers of the same pH were taken as 100%.

lution at pH 3.9 and withdrew the samples at different times between 0 and 30 min. HMDE was immersed in each sample for the time $t_A = 60$ s at open current circuit, the electrode was washed and transferred to the electrolytic cell containing AFP. Compared to the previously mentioned measurements with DNA immobilized at the electrode, we observed a much smaller decrease of peak G; after 30 min of incubation peak G decreased to 87% of its original value. Our results suggest that under the conditions of our experiment, the interaction of MC with DNA adsorbed at the surface is much faster than in solution.

4. Discussion

This work shows that acid-activated MC in solution binds to DNA immobilized at the surface as indicated by changes in the MC redox CV peaks and by a decrease of the DNA anodic peak G due to guanine residues (Figs. 4 and 5). In addition this paper provides some new information about the properties of acid-activated MC alone (in the absence of DNA) (Fig. 3).

4.1. Changes in the DNA peak G

Changes in peak G can be taken as a sign of covalent binding of activated MC to guanine [1,14]. In contrast to the covalent binding of activated MC to DNA, intercalation of daunomycin (anti-cancer drug containing the quinone ring system) between the DNA GC base pairs in solution did not induce any decrease of the guanine oxidation peak obtained with the carbon electrode [15].

4.2. DNA-MC adducts formed under acid conditions

Acid activation is sufficient to trigger aziridine ring opening producing the carbocation B [1] (Fig. 1) which is a hard alkylating agent and reacts preferentially with the site of highest electron density in guanine, i.e. with N-7 [1]. There exists some disagreement in the literature regarding formation of cross-links under acidic conditions: in contrast to earlier reports [16], no DNA cross-linking was indicated by Tomasz et al. [2] under acidic conditions.

4.3. Redox behavior of the quinone group in the DNA-MC adduct

By immersion of the DNA-modified electrode in the solution of acid-activated MC at open current circuit, a reversible couple (IIc-D and IIa-D) appeared (Fig. 4C). The cathodic peak IIc-D was 10-fold higher than the analogous peak obtained after the immersion of the electrode in the non-activated MC solution (Fig. 4B,C) and by 50 mV more negative than the peak IIc produced

Table 1
MC peak potentials in transfer stripping cyclic voltammetry at HMDE or at DNA-modified electrode

Electrode used	MC accumulation media	Peak IIc/V	Peak IIc-D/V
Bare HMDE	Acetate buffer/pH 3.9	−0.39	–
Bare HMDE	AFP/pH 6.9	−0.44	–
DNA-modified HMDE	Acetate buffer/pH 3.9	–	−0.44
DNA-modified HMDE	Britton–Robinson/pH 6.5	–	−0.42[a]

[a] Peak current obtained after immersion of the DNA-modified electrode in MC solution at pH 6.5 was more than 10-fold smaller than that obtained at pH 3.9.
Bare or DNA-modified HMDE were immersed in 1 mM MC in the indicated medium. After the accumulation the electrode was washed and immersed in the electrolytic cell containing blank AFP to perform the CV measurements.

by MC in the absence of DNA under what were otherwise the same conditions (Table 1). The purified adduct of calf thymus DNA with reductively activated MC (sodium dithionate was used for the reductive activation) showed a cathodic peak in AFP at −0.44 V [14] analogous to peak IIc-D (Fig. 4C). The appearance of peaks IIc-D added to the decrease of peak G (Fig. 4C) together represent strong evidence for binding of acid-activated MC to DNA immobilized at the electrode surface. This result is well in keeping with previous work [8] indicating binding of electrochemically reduced MC to DNA at the electrode surface.

Many anti-cancer drugs are electroactive [17] and a number of them bind covalently to DNA forming specific DNA adducts both in vitro and in vivo [18,19]. To the best of our knowledge, no electroactivity of any of these adducts has yet been observed. The DNA-MC adduct thus represents the first described electroactive adduct of DNA with an anti-cancer drug. It may be, however, expected also that the DNA (and RNA) adducts with other anti-cancer drugs will show electroactivity. Redox reactions involving the residues of several low-molecular mass compounds bound covalently to DNA at mercury, platinum and carbon electrodes were described [12,20–22]. These DNA adducts included osmium tetroxide, L complexes where L was pyridine, 2,2′-bipyridine or some other nitrogen ligands [20,21,23]. Such complexes have been used in chemical probing of the DNA structure, and electrochemical methods offered a very sensitive determination of their adducts with DNA [23,24]. Ferrocene–DNA conjugates have been recently prepared as redox-active probes for the DNA microanalysis [22].

The results suggest that electrochemical methods may represent an important tool in the studies of DNA–drug interactions, not only in solution but also at surfaces. Determination of various DNA–drug adducts by electrochemical methods in combination with powerful separation methods such as HPLC and capillary electrophoresis may be of special interest in the biochemical analysis. Application of these methods will be particularly interesting if the drug is reversibly reduced or oxidized as is the case with many anti-cancer drugs whose therapeutic activity is closely connected with their binding to DNA.

Acknowledgements

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