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Electrochemical oxidation of the antitumor antibiotic mitomycin C and *in situ* evaluation of its interaction with DNA using a DNA-electrochemical biosensor

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ABSTRACT

The electrochemical behaviour of the antitumor drug mitomycin C (MMC) was investigated on carbon paste electrode over a wide pH range and using voltammetric techniques. The MMC undergoes diffusion-controlled irreversible oxidation in two different processes, one pH-independent for 2.2 ≤ pH ≤ 4.5 and one pH-dependent for 4.5 ≤ pH ≤ 12.0, and does not involve the formation of any electroactive oxidation product. Upon incubation in different pH electrolytes, chemical degradation of MMC was electrochemically detected by the appearance of a new oxidation peak at a lower potential. The chemically degraded MMC undergoes an irreversible, pH-dependent oxidation for 3.4 ≤ pH ≤ 5.4, and its redox products are reversibly oxidised. The spontaneous degradation of MMC in aqueous solution was confirmed by UV–Vis spectrophotometry. Moreover, a multilayer dsDNA-electrochemical biosensor was used to evaluate the interaction between MMC and DNA. The results have clearly proven that MMC interacts and binds to dsDNA strands immobilized onto a glassy carbon electrode surface and its metabolite(s) cause oxidative damage to DNA.

1. Introduction

The mitomycins are a group of highly potent antibiotics, produced by the microorganism *Streptomyces caespitosus*, as discovered in Japan in the 1950s [1]. The prototype, most studied member of this group is mitomycin C (MMC), Scheme 1. Due to its broad-spectrum antitumor activity, such as breast, stomach, esophagus and bladder tumors [2], MMC has been widely used in clinical cancer chemotherapy [3].

MMC is comprised of quinone (a), carbamate (b) and aziridine (c) moieties arranged in a compact pyrrolo-[1,2-a] indole structure, Scheme 1, and considered a bioreducible antineoplastic, which becomes active after an enzymatic reduction process that occurs preferentially in the absence of oxygen. This activation results in the formation of reactive intermediates which bind to DNA *via* cross-links between two complementary strands with high efficiency and specificity to the GC base pairs of the double helix, avoiding its replication and proliferation of cancer cells [4–6]. MMC has been recognized as a classical DNA damaging agent, due to its monofunctional and bifunctional DNA alkylating activity [7].

MMC is a prodrug that needs to be metabolized *in vivo* to its active therapeutic forms in order to exert its biological activity. Such activation generates highly reactive electrophiles that react with several nucleophilic molecules, especially DNA [8]. Several enzymes have been shown to catalyse the *in vivo* bioreduction of MMC, ultimately resulting in the formation of MMC metabolites. These included one-electron reductases, such as NADPH: cytochrome P-450 reductase and xanthine oxidase and NADPH: cytochrome b_5 reductase, and the two-electron reductases DT-diaphorase [NAD(P)H: (quinone acceptor) oxidoreductase, EC 1.6.99.21] and xanthine dehydrogenase [9].

The MMC reductive activation generates some active primary metabolites such as 2,7-diaminomitosene (2,7-DAM), which is the major metabolite isolated from cells and tissues treated with MMC, and 1,2-cis- and 1,2-trans-1-hydroxy-2,7-diaminomitosene [8,10].

Several studies have demonstrated that MMC can generate *in vitro* the same metabolites observed *in vivo* using a suitable acid treatment [11], electrochemical reduction [12] or chemical reduction of the drug using reducing agents such as sodium dithionite [13], sodium borohydride [4] and ascorbic acid [14].

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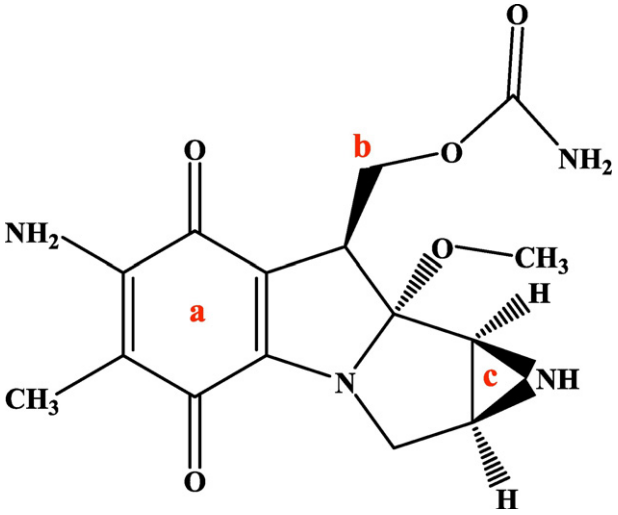

Scheme 1. Chemical structure of mitomycin C.

MMC is not stable in solution but achieves maximum stability in neutral solutions [15], whereas in acidic and alkaline solutions it undergoes spontaneous degradation [11,16], which degradation rate is dependent of temperature, buffer pH and composition, ionic strength and storage conditions.

Analytical methods such as high-performance liquid chromatography (HPLC) and UV–Vis spectrophotometry have been used for detection, characterization and determination of MMC and its degradation products in aqueous solutions and biological fluids [11,17]. Electrochemical methods to study the electrochemical reduction of MMC have also been used [12,18]. Therefore, a direct electrochemical oxidation process for detection of MMC and its degradation products in aqueous solution has not been investigated.

The interaction of ions/molecules with DNA using DNA-electrochemical biosensors and voltammetry has been investigated successfully, in order to monitor the damage to DNA and detecting small perturbations of the double-helical structure, such as anticancer drugs [19], cyanotoxins [20], heavy metals [21], and hydroxyl free radicals [22] and comparing with other methods shows great sensitivity [23]. Until the moment, an *in situ* study of MMC direct interaction with DNA—as well as a description of conformational changes and oxidative damage after interaction—using electrochemical biosensors based on nucleic acid has not been investigated.

The development of the DNA-electrochemical biosensor has opened wide perspectives using a particularly sensitive and selective method for the detection of specific interactions. The possibility of foreseeing the damage that some compounds cause to DNA integrity arises from the pre-concentration of either the starting materials or the redox reaction products on the DNA-biosensor surface, thus permitting the electrochemical probing of the presence of short-lived intermediates and of their damage to DNA [24].

In this context, the present study is concerned with the electrochemical oxidation behaviour of MMC and its degradation in aqueous solution, using cyclic, square wave and differential pulse voltammetry techniques on a carbon paste electrode, and also with the *in situ* interaction of MMC with dsDNA immobilized on glassy carbon electrode surface, using differential pulse voltammetry.

2. Experimental

2.1. Reagents and solutions

Mitomycin C from *Streptomyces caespitosus* (MMC, ≥99.0% HPLC) and sodium salt of highly polymerized calf thymus dsDNA (length 10,000–15,000 bp) were purchased from Sigma.

A stock solution of 1200 μmol L^{-1} MMC was prepared in pure water, stored in amber glass vial to avoid the photolytic degradation and kept at 4 °C until further utilization.

Stock solutions of 450 μg mL^{-1} dsDNA were prepared in deionised water and kept at 4 °C until further utilization for 24 h to ensure the homogenization and to prevent their degradation. Its concentration was determined by measuring the absorbance at 260 nm and considering the relation between optical units and micrograms given by the Sigma-Aldrich certificate of analysis. The absorbance of the nucleic acid solutions were measured using a UV–Vis spectrophotometer. The purity level of calf thymus dsDNA samples was checked spectrophotometrically by determining their UV 260/280 nm absorbance ratio and was always higher than 1.8.

All stock solutions were diluted to the desired concentration by mixing supporting electrolyte and are mentioned throughout in the text.

The stock solution and all supporting electrolyte solutions were prepared in purified water obtained from a Millipore Milli-Q System, Millipore S.A., France, (conductivity ≤ 0.1 μS cm^{-1}) and using analytical grade reagents. Their composition and the correspondent pH values are obtained according to the literature [25]. The pH measurements were performed with a Metrohm 827 pH Lab pH-meter (Switzerland) with a Metrohm combined glass electrode.

Microvolumes were measured using VF-10, VF-100 e VF-1000 Microliter Monocanal Pippettes. All experiments were performed at room temperature (25 ± 1 °C).

Nitrogen saturated solutions were obtained by bubbling high purity N_2 for a minimum of 10 min in the solution and continuing with a flow of pure gas over the solution during some voltammetric experiments.

2.2. Instrumental

2.2.1. Voltammetric measurements

Voltammetry experiments were carried out in an Autolab PGSTAT 302 N potentiostat in combination with GPES 4.9 Software (Eco Chemie B. V., Utrecht, The Netherlands). A carbon paste electrode (CPE, Ø = 3,51 mm) was used for the MMC and degradation MMC electrochemical study and a glassy carbon electrode (GCE, Ø = 3 mm) for the MMC-DNA interaction study as working electrodes, an Ag/AgCl (3 M KCl) as reference electrode and a Pt wire as counter electrode, in a 10 mL one-compartment electrochemical cell.

Cyclic voltammetry (CV) measurements were carried out at scan rate 50 mV s^{-1} and step potential 2 mV. Differential pulse (DP) voltammetry were performed with 50 mV pulse amplitude, 70 ms pulse width and 5 mV s^{-1} scan rate. Square wave (SW) voltammetry technique was used with 10 Hz frequency and 5 mV potential increment, corresponding to an effective scan rate of 50 mV s^{-1}, and pulse amplitude of 20 mV.

2.3. UV–Vis analysis

The UV–Vis measurements were performed using a spectrophotometer UV–Vis Varian Cary 50, running with Cary Win UV. All UV–Vis spectra were measured from 200 nm to 400 nm, in a quartz glass cuvette with an optical path of 1 cm.

2.4. Data acquisition, analysis and presentation

The DP voltammograms presented were baseline-corrected using the moving average application with a step window of 2 mV included in the GPES version 4.9 software. This mathematical treatment improves the visualization and identification of peaks over the baseline without introducing any artefact, although the peak intensity is in some cases reduced (<10%) relative to that of the untreated curve. Nevertheless, this mathematical treatment of the original voltammograms was used in the presentation of all experimental voltammograms for a