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In situ dsDNA-bevacizumab anticancer monoclonal antibody interaction electrochemical evaluation

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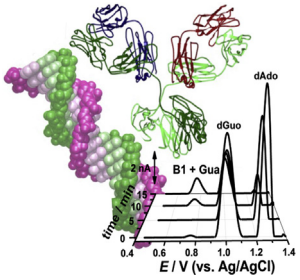
HIGHLIGHTS

- Interaction of the anticancer monoclonal antibody BEVA with dsDNA.
- BEVA binds to the dsDNA but causing no DNA oxidative damage.
- Formation of compact BEVA-dsDNA adduct.
- dsDNA-electrochemical biosensors confirmed that BEVA undergoes 3D structural modification upon binding to dsDNA.

GRAPHICAL ABSTRACT



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ABSTRACT

The interaction of the anticancer monoclonal antibody bevacizumab (BEVA) with double-stranded DNA (dsDNA) was studied by voltammetry and gel-electrophoresis in incubated samples and using the dsDNA-electrochemical biosensor. The voltammetric results revealed a decrease and disappearance of the dsDNA oxidation peaks with increasing incubation time, showing that BEVA binds to the dsDNA but no DNA oxidative damage was detected electrochemically. Non denaturing agarose gel-electrophoresis experiments were in agreement with the voltammetric results showing the formation of compact BEVA-dsDNA adduct. The dsDNA-electrochemical biosensor in incubated solutions showed that BEVA also undergoes structural modification upon binding dsDNA, and BEVA electroactive amino acid residues oxidation peaks were detected.

1. Introduction

Tumour cells, like most normal cells, have high diversity of receptors on their surfaces. Molecules on the outside of the cell can attach to these receptors, causing changes to occur within the cells. Monoclonal antibodies (mAb) have earned special attention due to their specific and effective anticancer therapeutic properties. Bevacizumab (BEVA) is a recombinant humanized monoclonal antibody (mAb) used for the treatment of certain types of metastatic cancers [1–3]. BEVA exerts its antitumoral activity by blocking vascular endothelial growth factor A (VEGF-A) [4–6], a glycoprotein considered to be the main inducer to the growth of blood vessels. As a result, the blood supply to tumour cells is reduced, therefore

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slowing or interrupting growth [5].

Due to its therapeutic efficacy and increasing use in clinical oncology [1,3,7], the understanding of the *in vivo* behaviour and physiological mechanism of action, as well as of the toxic and chemotherapeutic adverse side effects of BEVA is of utmost importance.

Studies on the interaction of anticancer drugs with DNA have enabled to elucidate their possible implications in DNA structural modifications, and the mechanistic and cytotoxic aspects of their physiological action [8–13]. Several methods such as UV–VIS, IR and Raman spectroscopy, NMR, mass spectrometry, capillary electrophoresis, surface plasmon resonance, femtosecond laser spectroscopy, HPLC, molecular modelling techniques and electrochemistry have been applied for that purpose [8,9,14–17].

Electrochemical methods offer fast response, high sensitivity and great selectivity [9,11,14–16]. A DNA-electrochemical biosensor consists of an electrode with dsDNA immobilized on the surface [9,15,16]. The extensive potential window of carbon electrodes allows electrochemical detection of both dsDNA conformational changes and oxidative damages caused to DNA [8–11,13,15,16,18–21]. AFM images have shown that a complete covering of the electrode surface is essential in order to avoid non-specific adsorption so that the DNA modifications detected are only due to the interaction with the compound [9,15,16].

The DNA-electrochemical biosensor has been used to study the interaction mechanisms of DNA with anticancer drugs [9–11] and the monoclonal antibody rituximab [13]. DNA structural modifications and oxidative damage were observed following changes in the DNA oxidation peaks of purine bases, nucleotides, nucleosides, and the oxidation products of guanine, 8-oxoguanine (8-oxoGua), and of adenine, 2,8-dyhydroxyadenine (2,8-oxoAde), which are biomarkers of DNA base oxidative damage [9,15,16].

The voltammetric behaviour of native and denatured BEVA was investigated [22]. In native BEVA, only one pH-dependent oxidation peak, corresponding to tyrosine, first tryptophan and first cysteine, amino acid residues oxidation, was observed. The interfacial behaviour and adsorption of BEVA at the glassy carbon surface were evaluated by voltammetry and electrochemical impedance spectroscopy. The unfolding of the protein 3D morphological structure upon denaturation with chemical agents denaturing agent, sodium dodecyl sulphate and the reductants tris(2-carboxyethyl)phosphine and dithiothreitol, showed additional second tryptophan and second cysteine residues oxidation peaks [22].

In this context, the aim of the present work was to elucidate the interaction mechanism between BEVA and dsDNA. Experiments have been carried out in incubated solutions and using the dsDNA-electrochemical biosensor. Gel-electrophoresis was also applied to support the BEVA-dsDNA interaction mechanism.

2. Experimental

2.1. Materials, reagents and solutions

Sodium salt double stranded calf thymus DNA (dsDNA), polyadenylic (poly[A]) and polyguanylic (poly[G]) acids from Sigma–Aldrich and bevacizumab (BEVA, 25 mg mL^{-1}) from Roche Pharmaceuticals were used without further purification.

Stock solutions of 500 μg mL^{-1} dsDNA, 200 μg ml^{-1} poly[G] and poly[A], and 1 mg mL^{-1} BEVA were prepared in deionised water and kept at 4 °C. All solutions were diluted to the desired concentration in 0.1 M acetate buffer pH = 4.5 or 0.1 M phosphate buffer pH = 6.9 supporting electrolytes, prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity ≤ 0.1 μS cm^{-1}).

Microvolumes were measured using P20, P200 and P1000 μL pipettes (Gilson S. A., Villiers-le-Bel, France). The pH measurements were performed with a Crison microph 2001 pH-meter with an Ingold combined glass electrode.

All experiments were carried out at room temperature (25 ± 1) °C.

2.2. Voltammetric parameters and electrochemical cells

Voltammetric experiments were carried out using a μAutolab running with GPES 4.9 software, Metrohm/Autolab, Utrecht, The Netherlands. Measurements were performed using a glassy carbon working electrode (GCE) (d = 1.5 mm), a Pt wire counter electrode, and an Ag/AgCl (3 M KCl) as reference electrode, in a 500 μL one-compartment electrochemical cell.

The experimental conditions for differential pulse (DP) voltammetry were: pulse amplitude 50 mV, pulse width 70 ms, interval time 0.4 s, and scan rate 5 mV s^{-1}.

The GCE was polished using diamond spray (particle size 1 μm, Kement, Kent, UK) before each electrochemical experiment. After polishing, the electrode was rinsed thoroughly with Milli-Q water for 30 s. After this mechanical treatment, the GCE was placed in buffer supporting electrolyte and DP voltammograms were recorded until a steady state baseline voltammogram was obtained. This procedure ensured very reproducible experimental results.

2.3. Acquisition and presentation of voltammetric data

All the DP voltammograms presented were baseline corrected using the automatic function included in the software. The mathematical treatment of the original voltammograms was used in the presentation of all experimental data for a better and clearer identification of the peaks. The values for peak currents were determined from the original untreated voltammograms after subtraction of the baseline.

2.4. BEVA denaturation

The immobilization of BEVA at the GCE surface was carried out by covering successively the GCE surface with 3 drops each of 5 μL from a 10 mg mL^{-1} BEVA stock solution. After placing each drop the electrode surface was dried under a constant flux of N_2.

The GCE with immobilized BEVA was incubated in 10 mM DTT during 30 min. Then, the electrode was removed from the solution, washed with deionised water to remove the excess of DTT and placed in the electrochemical cell containing only the supporting electrolyte where DP voltammograms were recorded.

2.5. Gel electrophoresis procedure

Nondenaturing agarose (1%, ultrapure DNA grade from Sigma) gel was prepared in TAE buffer (10 mM Tris base, 4.4 mM acetic acid and 0.5 mM EDTA, pH 8.0). The solutions of 25 μl of 100 μg mL^{-1} dsDNA control, 25 μl of 500 μg mL^{-1} BEVA control, and 25 μl of 500 μg mL^{-1} BEVA incubated with 100 μg mL^{-1} dsDNA during different time periods (with 0.25% bromophenol blue in water) were loaded into wells. The electrophoresis was carried out in TAE buffer for 4.5 h at ~100 V. After 0.5% ethidium bromide (EtBr) stained DNA was visualized and photographed under UV, λ = 312 nm, transillumination to visualize DNA mobility.

2.6. Procedures

Procedure 1 – BEVA-dsDNA in incubated solutions – BEVA-dsDNA solutions were prepared incubating 100 μg mL^{-1} dsDNA with 10, 100 or 500 μg mL^{-1} BEVA, in 0.1 M phosphate buffer pH = 6.9,