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Voltammetric study of interaction between polymers (PEI and TMO) and pDNA on a hanging mercury drop electrode

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Abstract

Electrochemical DNA biosensors can become a powerful tool for the investigation of potent changes on the plasmid DNA structure caused by polymers used as non-viral vectors in gene delivery. Trimethylated chitosan oligomer (TMO) and polyethylenimine (PEI), offering biocompatibility, low immunogenicity and minimal cytotoxicity, are being studied as model non-viral carriers. The information obtained is intended to serve as a basis for developing a new analytical system for the study of the effect of any physically or chemically synthesized polymer on DNA structure.

Keywords: Polyethylenimine (PEI); Chitosan oligomers; Vectors; Gene delivery; DNA intercalators; HMDE

1. Introduction

Gene therapy has become an attractive concept for a broad variety of biomedical applications. Although the majority of gene delivery approaches so far have involved adenoviral or retroviral vectors, more recently non-viral vectors are receiving increasing attention as gene delivery vehicles because of several advantages, such as ease of manipulation, stability, low cost, safety, and high flexibility regarding the size of the transgene delivered [1].

Non-viral synthetic vectors are based essentially on the condensation of negatively charged DNA into compact particles by electrostatic interactions with condensing compounds, protecting the DNA from degradation. For efficient transfection, a multistep process has to be mediated by the delivery vector, which includes DNA condensation, uptake into the cell, endosomal release, migration through the cytoplasm, and uptake into the nucleus, and finally decondensation of the DNA into a transcribable form [1].

The process for gene delivery in vivo is complicated enough due to problems coming from the anatomical size constraints and protection from non-specific interactions with biological fluids, extracellular matrix and non-target cells [1].

Cationic polymers have been used to condense and deliver DNA both in vitro and in vivo. Several cationic polymers have been investigated that lead to increased transfection efficiencies. They form polyelectrolyte complexes with plasmid DNA, in which the DNA becomes better protected against degradation by nucleases. They show structural variability and versatility including the possibility of surface modification with specific targeting ligands for gene expression mediated through specific receptors. Although polycations of natural origin are not abundant, they can become an alternative to viral or lipid mediated gene transfection offering bio-

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compatibility, low immunogenicity and minimal cytotoxicity [2].

Chitosans are natural polysaccharides obtained in various molecular weights and represent a novel class of cationic carriers for gene delivery that are potentially safe, efficient and cost-effective. Chitosan derivatives, such as lactosylated chitosan vectors, galactosylated chitosan-graft-dextran vectors and trimethylated chitosan polymers, were studied for their transfection efficiency. In addition, trimethylated chitosan polymers with different degrees of quaternization were investigated as potential absorption enhancers. In addition, the polycation polyethylenimine has recently been used for the design of DNA delivery vehicles and proved to present target specificity and improved biocompatibility for in vivo application. The PEI transfection mechanism is known as 'the proton sponge' mechanism and is completely different compared to the mechanism of viral vectors [3–5].

The interaction of the above mentioned polymers with DNA have been studied so far with gel electrophoresis. This technique provides information concerning the conformational changes of DNA caused by its interaction with the polymer, but it is time-consuming and demands high concentrations of polymers. In order to overcome these two disadvantages, new analytical methods should be developed and applied in conjunction to gel electrophoresis. Electrochemical methods offering simplicity and low-cost could provide information more easily acting in a complementary way to gel electrophoresis. They are based on the electrochemical properties of DNA using different electrode materials. Differential pulse voltammetric measurements on carbon paste electrodes (CPE) are realized due to the oxidation of guanine and/or adenine residues, while alternating current measurements on hanging mercury drop electrodes (HMDE) are realized due to the reduction of the absorbed biomolecule via phosphodiesteric bonds or via bases.

Electrochemical measurements of DNA at mercury electrodes present more sensitivity to small changes in DNA structure compared to solid electrodes like CPE. Nucleic acid electrochemistry is focused on the effect of planar organic molecules containing several aromatic condensed rings after their interaction with DNA. A number of these compounds belong to known carcinogens (polycyclic aromatic hydrocarbons, aromatic amines) or antitumor antibiotics (daunomycin, doxorubicin, echinomycin, bleomycins, etc.). Binding of an intercalator to DNA leads to a significant change in the DNA conformation [6,7].

The objective of the project is the application of novel analytical methods for the investigation of the effect of non-viral vectors on the structure of DNA due to their interaction. This work is proved that, besides the importance of electrochemical DNA biosensors in anticancer research, they could play a dominant role in the investigation of the non-viral vectors' reaction mechanism in order to improve their effectiveness in humans.

2. Experimental

2.1. Reagents

The RSV-α3 luciferase plasmid used was propagated in *Escherichia coli* and isolated using anion exchange columns (Endo-Free Plasmid Giga kit, QIAGEN, Leusden, The Netherlands). The *N*,*N*,*N*-trimethyl chitosan (44% quaternised, chitosan starting material from RC Biochemical, 3–6 kDa) and PEI (25 kDa) were purchased from Sigma.

For the electrochemical behavior on the HMDE surface the stock solutions of plasmid DNA (150 mg/L) were prepared with 0.1 M NaCl in 10 mM Tris–HCl at pH 7.5. Stock solutions of PEI and TMO were prepared with doubly distilled and sterilized water, while dilute solutions were prepared just before use. The supporting electrolyte of alternating current voltammetric experiments was 0.3 M NaCl in 50 mM sodium phosphate buffer solution (pH 8.5).

All water and pipette tips were sterilized by autoclaving for 20 min. The electrochemical cells were cleaned with diluted nitric acid, rinsed with water and sterilized for 20 min. Ultrapure argon was used to bubble the solutions of dissolved oxygen for 5 min before each experiment.

2.2. Apparatus

Alternating current voltammetric measurements were performed with a Metrohm 647 VA-Stand controlled by a 646 VA-Processor. The working electrode for the alternating current voltammetric measurements was a hanging mercury drop electrode, the reference electrode was a saturated Ag/AgCl/3 M KCl and the counter electrode was a platinum wire electrode.

2.2.1. Hanging mercury drop electrode

Plasmid DNA was absorbed at the electrode surface from 10 μl of solution containing 10 mM Tris–HCl pH 7.5 for 120 s. The DNA modified electrode was washed twice by distilled water and by background electrolyte solution. It was then transferred to deareated blank background solution, which was initially bubbled with argon for 100 s. The initial potential (E_I) was applied at the electrode for 15 s prior to the voltage scan.

3. Procedures

3.1. Treatment of DNA with PEI or TMO in solution and immobilization on the HMDE surface

A 10 μL of the pDNA solution were adsorbed at the surface of the electrode for 120 s and the modified electrode was washed twice with doubly distilled water and then with background electrolyte. The electrode was then immersed into the blank background electrolyte which was previously deareated with argon.

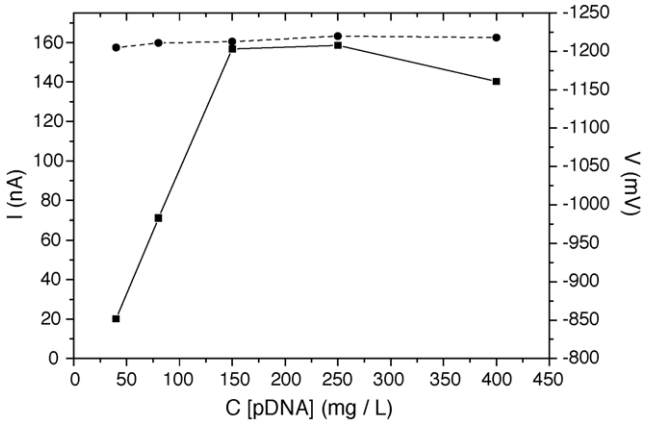

Fig. 1. Dependence of peak current and peak potential of the characteristic peak of pDNA at −1.21 V, in relation with increasing concentrations of immobilized pDNA on the HMDE surface.

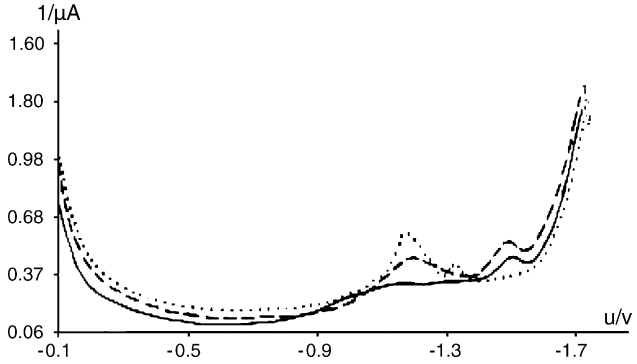

Fig. 2. Alternating Current Voltammograms: (1) Immobilized mixture [pDNA (150 mg/L) + PEI (15 mg/L)], peak 1 at −1.21 V and peak at −1.5 V due to the complex formation; (2) Immobilized mixture [pDNA (150 mg/L) + PEI (30 mg/L)], decreased peak 1 at −1.21 V and increased peak at −1.5 V; (3) Immobilized pDNA (150 mg/L), peak 1 at −1.21 V.

The mercury electrode should be fully covered by DNA and the optimal concentration for the full coverage of the HMDE surface is 150 mg/L for the pDNA. The results are shown in Fig. 1. The interaction between the plasmid DNA and increasing concentrations of PEI or TMO was studied by immobilizing each time the mixture on the HMDE surface. The transduction was carried out in blank background electrolyte with an initial potential of −0.1 V, a scan rate of 20 mV/s, a frequency of 230 Hz and a peak to peak amplitude of 10 mV.

4. Results

4.1. Alternating current voltammetric responses of plasmid DNA (pDNA) at full electrode coverage

The mercury electrode was immersed into a 10 μL drop of the DNA solution for an accumulation time of t_A = 120 s. The electrode was washed and transferred into the electrolytic cell containing a blank background electrolyte and the alternating current voltammetric measurement was performed.

Plasmid DNA yielded two reduction peaks one at −1.21 V (peak 1) due to the reorientation of the helical regions adsorbed mainly via the phosphodiesteric bonds, which increases by increasing the concentration, and another at −1.35 V probably due to a minor amount of open circular DNA coming from the isolation procedure of plasmid DNA, which is very weak and does not change with increased concentrations.

The behavior of the two polymers was also examined. The mercury electrode was immersed into a 10 μL drop of the PEI or TMO solution for different accumulation periods (including the accumulation time of t_A = 120 s). The electrode was washed and transferred into the electrolytic cell containing a blank background electrolyte and the alternating current voltammetric measurement was performed each time. No signal was obtained leading to the conclusion that neither of these polymers is adsorbed on the HMDE surface. Experiments were also performed by adding different amounts of PEI or TMO into the electrolytic cell containing blank background electrolyte solution using the mercury electrode. Alternating current voltammetric measurements were performed and no signal was obtained leading to the conclusion that neither of these two polymers are electrochemically active.

4.2. Interaction between PEI or TMO and plasmid DNA (pDNA) on the HMDE surface

Plasmid DNA and PEI were incubated for different time intervals at room temperature by changing the concentration of the polymer and keeping constant the concentration of pDNA. PEI concentrations ranged from 0 to 30 mg/L. The incubation time (45 min) was selected according to the appearance of the peak at −1.5 V, which declares the formation of a complex according to previous published work [13]. A 10 μL of this solution was immobilized on the HMDE surface and alternating current voltammetry was performed in blank supporting electrolyte. By increasing the concentration of PEI, peak 1 decreases, while the peak at −1.5 V appears when the PEI concentration equals 15 mg/L. Fig. 2 presents the differentiations into the configuration of pDNA after interaction with increasing concentrations of PEI.

The interaction between pDNA and TMO (ranging concentration 0–20 mg/L) after incubation for 15 min and immobilization on the HMDE surface was also studied with the same way. The incubation time was selected according to the appearance of peak 3 at −1.43 V, which declares the introduction of ‘free ends’ according to previous published work [8,9]. The results are shown in Fig. 3. Fig. 4 presents the differentiations in the pDNA form after incubation with increasing concentrations of TMO into the incubated solution.

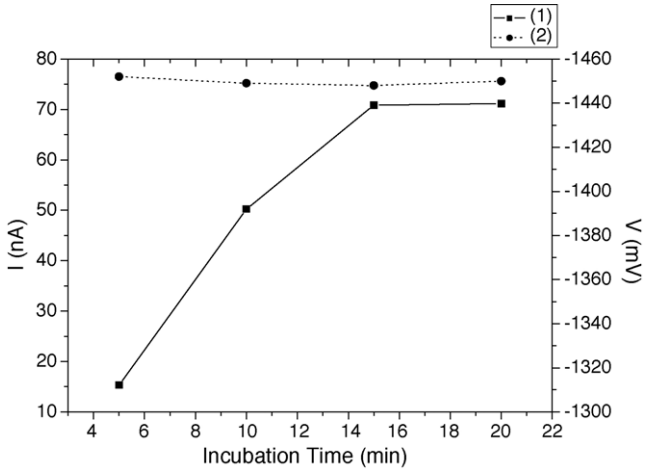

Fig. 3. The effect of incubation time on peak 3 (−1.43 V) between pDNA (150 mg/L) and a constant concentration of TMO (0.5 mg/L).

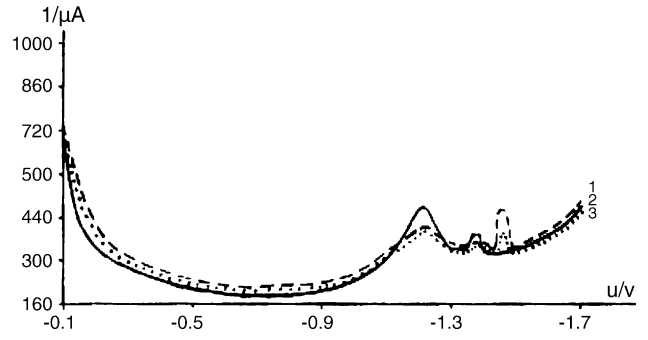

Fig. 4. Alternating Current Voltammograms: (1) Immobilized mixture [pDNA (150 mg/L) + TMO (15 mg/L)], decreased peak 1 at −1.21 V and increased peak at −1.43 V (peak 3); (2) Immobilized pDNA (150 mg/L), peak 1 at −1.21 V; (3) Immobilized mixture [pDNA (150 mg/L) + TMO (10 mg/L)], peak 1 at −1.21 V and peak at −1.43 V showing introduction of 'free ends' to the structure of DNA.

The interaction between pDNA and TMO presents the same results (appearance of peak 3) as in the case of the interaction between plasmid DNA and ethidium bromide (EB) [8,9]. Peak 3, which appears due to the desorption of the DNA segments firmly adsorbed via bases, is a very important indicator of the introduction of 'free ends' to the structure of DNA at the electrode surface [8–10]. The interaction between pDNA and PEI results in the appearance of a new peak at −1.5 V, declaring the formation of a complex, like in the case of plasmid DNA–actinomycin D (ACTD) interaction [13]. The appearance of these two peaks (peak 3 and peak at −1.5 V) proves the different way of interaction between each polymer and DNA.

In addition, the amount of PEI interacting with pDNA is higher than the amount of TMO interacting with the same concentration of pDNA due to the different mechanism of action and the structure of each polymer.

5. Conclusions

In our previous studies [11–13], we have shown that adsorptive transfer stripping voltammetry with alternating current as stripping mode offered great advantages to the study of interactions between DNA and known intercalators by presenting their different mechanism of action.

So far, electrochemical DNA biosensors with either carbon paste electrodes [14–17] or hanging mercury drop ones have been used to study interactions between biomolecules, such as RNA and DNA, and compounds presenting toxicity. Furthermore, hybridization electrochemical biosensors have been developed for the detection of sequences responsible for inherited diseases [18,19].

The present work extends the application of electrochemical DNA biosensors at mercury electrodes to the study of the reaction mechanism of polymers used in gene delivery and their effect in DNA structure during condensation. Compared to the already established electrophoretic methods, electrochemical biosensors take advantage of very low concentrations of the analyte [20], offer simplicity, low cost and reliability, thus proving them to be an ideal analytical tool.

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