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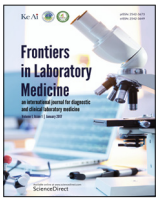

Pegylated doxorubicin gold complex: From nanovector to potential intercalant agent for biosensor applications

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ABSTRACT

We report an original approach to synthesize hybrid gold nanostructures in which doxorubicin (DOX), mixed to Poliethylenglycole diacid (PEG-COOH) led to original hybrid gold nanovector (DOX IN PEG AuNPs). In this work, we investigate the ability of DOX IN PEG-AuNPs to detect the amplification of the hybridization process by a sensitive Quartz crystal Microbalance with dissipation (QCM-D) by intercalation process. The sensing layer was carried out by self-assembled monolayer of β mercaptoethylamine (cysteamine) on gold-coated quartz crystal sensor composed by a rigid homobifunctional cross-linker 1,4 phenilenediisothiocyanate (PDITC) linked covalently with amino-probe oligonucleotides. By QCM characterization in the range from 8 μM to 20 nM, we demonstrate high specificity of DOX IN PEG-AuNPs-DNA with a limit of detection (LOD) of 9 nM. This result is very promising for development of sensitive and effective nanoparticle-based biosensor for quantifying small biomolecules concentration in physiological liquids. These results open a possibility to realize a new class of nanovector which will be tailored for different biomedical application, such as imaging, targeting and drugs delivery.

Introduction

Recently, great advances have been made in the use of gold nanoparticles (AuNPs), for biomedical applications, owing to their stability, chemical reactivity, non toxic nature, and scattering properties.[1–3] High biocompatibility, tunable surface chemistry and unique optical properties make nanogold a desirable platform for many biomedical and diagnostic applications.[4–6] For instance biomolecule- and/or biopolymer-conjugated AuNPs are largely used as biomarkers or biodelivery vehicles, as well as for cosmetics, as anti-aging components for skin protection.[7–9] In the last few years, many research works were devoted to the understanding of the effects of antitumor drugs on biological cells.[2,10] Other researchers have focused attention on small drugs that intercalate directly into the double helix of DNA as chemotherapeutic agents.[11] Doxorubicin is an anthracycline antibiotic. It is photosensitive and it works by intercalating DNA, while the most serious adverse effect is life-threatening heart damage.[12] A bulky sugar groups and a planar aglycon chromophore allows the insertion to the base pairs. The binding process hence induces large conformational deformations to the DNA helix, which in turn means that binding kinetics are slow.[13,14] The doxorubicin (DOX) has been conjugated to AuNPs (DOX-AuNPs) in order to improve the DOX therapeutic efficiency and the targeting of tumour cells reducing side effect but also to improve the imaging contrast or the photothermal cancer therapy.[15,16] Recently authors have demonstrated the ability of doxorubicin to be grafted on gold nanoparticles by carbodiimide chemistry. The so-called DOX-ON-PEG-AuNPs[17–19] were characterised by extinction spectroscopy (observation of the LSPR shift) and by Raman spectroscopy (observation of the band shift and of new Raman bands) to demonstrate the hybridization of the DOX to the AuNPs surface.[17,19]Successively H. Moustaouiet al. have designed a new nano-therapeutic agent based on a gold-DOX complex called DOX-IN-PEG-AuNPs.[20] Chemical-physical characterizations and biological "*in vitro*" studies, have fully elucidating that, the change of DOX conformation during the formation of gold-nanostructure by complexation have a large influence its therapeutic activity. The purpose of this study is to demonstrate the interaction of doxorubicin before (DOX *free*)

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and after complexation of gold nanoparticles (DOX IN PEG-AuNPs) with duplex sequences oligonucleotides, expecting a better amplification of hybridization process during the intercalative process. The main advantages of QCM in sensing fields include high sensitivity, high stability, fast response and low cost. Under ideal conditions, the QCM technique, can detect mass changes of 1 ng/cm.[21] On the basis of the work of Sauerbrey[22], at 25 MHz, each shift of 1 Hz in the resonant frequency at the crystal corresponds to a mass change of 3.5 ng/cm.[2]

This remarkable mass sensitivity, has been exploited to study a variety of DNA based interactions including the detection of single base-pair mismatches during peptide nucleic acid-DNA hybridization[23,24], doxorubicin-DNA binding[25] and immobilization of DNA via a self-assembled monolayer of intercalative molecules.[26,27]To achieve detection of DOX free and DOX IN-PEG AuNPs to DNA (28mer) through a mass change, doxorubicin interact with DNA conjugated to a cross-linker cysteamine monolayer. A number of methods have been employed to bind DNA to surfaces for use in sensing devices such as localized surface plasmon resonance (LSPR)[19,28,29]and Raman spectroscopy.[17] The key to the application of the QCM technique to the study of biomolecular interactions is the formation of suitably immobilized biomolecular films. When considering the choice of immobilization method, the final desired coverage, molecular orientation, and point of DNA attachment are important factors.[30,31] Direct physical adsorption, complexation, cross-linking[32] and direct covalent attachment have all been employed.[17,20] The use of short alkanethiols linkages has the added benefit of placing the DNA strands close to the Quartz Crystal Microbalance sensor surface. Here we have chosen to immobilize first β mercaptoethylamine (cysteamine) on the gold electrodes of the crystal sensor. In a second step, the amino-groups on surface has been activated by 1,4phenilenediisothiocyanate (PDITC). Finally, amino-probe oligonucleotides has been linked covalently in order to monitored intercalation process after hybridization with doxorubicin-gold-nanoparticles (DOX IN PEG-AuNPs). For instance, force spectroscopy studies using the atomic force microscope (AFM-Tapping mode) were carried out to characterize the variation of gold surface morphology during intercalation experiments.

Experimental section

Materials

All chemicals were reagent grade or higher and were used as received unless otherwise specified. Tetrachloroauric acid ($HAuCl_4$), sodium borohydride ($NaBH_4$), Pyridine (98%), Dimethylformammide anhydrous (98%) ethanol (99%), 1,4phenylenediisothiocyanate (PDITC), sodium hydroxide (NaOH), phosphate buffered saline (PBS, 0.1 M, pH 7.4), dicarboxylic PolyEthylene Glycol (PEG)-600 (PEG), and doxorubicin hydrochloride (98%), were purchased from Sigma Aldrich. All solvents were used without any further purification. Experiments were carried out at room temperature if not specified otherwise.

Synthesis

Synthesis of pegylated- gold nanoparticles (PEG-Au NPs)

Synthetic procedures were carried out as previously described[18,19,34].

Synthesis of doxorubicin-gold nanoparticles (***DOX IN PEG-Au NPs***)

Synthetic procedures were carried out as recently described with some modifications.[20]

Briefly 20 ml $HAuCl_4$ aqueous solution ($2.5 * 10^{-4}$ M) was added to DOX (5 ml, $1.72 * 10^{-4}$ M in water) and aged for 10 min. After 10 min, 500 μl of dicarboxylic PEG was added and mixed by magnetic stirring for 10 min at room temperature. Finally, 20 ml of aqueous 0.01 M $NaBH_4$was added at -once. The as-prepared **DOX IN PEG-Au NPs** solution was centrifugated at 6.000 rpm for 20 min for three times and then the supernatant was discarded and the residue was redispersed in an equivalent amount of Buffer solution (PBS pH: 7). This was repeated twice principally to remove excess of doxorubicin and PEG diacid. Stock solutions were stored at 27–29 °C and characterized using UV–Vis spectroscopy and transmission electron microscopy (TEM).

Intercalation of doxorubicin pegylated-Au nanoparticles to DNA in solution (DNA-DOX-IN-PEG-Au NPs)

The intercalation process between doxorubicin-pegylated-gold nanoparticles and DNA oligonucleotides was conducted at room temperature under ionic conditions. 200 μL of **DOX IN PEG-Au NPs** solution (20 nM in 0.1 M PBS) was treated with 30 μL of 10% NaCl. After this process, 40 μL of 100 nM H_2N-DNA (probe1) and the complementary strands (target1), were added onto the **DOX IN PEG-Au NPs** solution for 4 h at room temperature in PBS buffer (1 M NaCl, 100 mM phosphate buffer, pH 7). The resultant colloidal solution was stirred for 1 h at room temperature and characterized by UV–Visible absorption and TEM.

DNA hybridization

For end-point measurement, the surface was exposed to the complementary targets (labelled and non-labelled) at 21 °C during 1 h in a hybridization chamber at pH 7. The denaturation of hybridized DNA was performed using NaOH (1 mM) during 1 min followed with rinsing with PBS.

Intercalation of DOX and DOXIN-PEG-AuNPs with DNA stabilized PEG-AuNPs

50 μl of an aqueous solution of doxorubicin, was added into 5 mL of DNA stabilized PEG-AuNPs for 2 h. This experiment was repeated with DOX IN-PEGAuNPs and the intercalation processes were recorded at a precise short interval time by UV VIS absorption spectra and then confirmed by QCM-D measurements.

QCM substrate preparation

The schematic diagram of the chemical immobilization method is depicted in Scheme 1. The chemical procedures for the formation of a cysteamine SAM on the planar gold surface, and the binding of the PDITC linker in absolute ethanol have been described previously[32].

The 28^{mer} oligonucleotides were purchased from Eurogentec and have the following sequences:

- DNA oligonucleotides on QCM surface (probe 1): 5′-H_2N-TTT-TGG-GAT-GGT-TGA-GGG-TGC–CTC-TGG-C-3′.
- Complementary DNA in solution (target 1): 5′-GCC-AGA-GGC-ACC–CTC-AAC-ACT-CCC-A3′.

Similar solutions of non complementary oligonucleotides and "free" pegylated gold nanoparticles (PEG-AuNPs), were prepared as control for all experiments. For drug binding study, doxorubicin (DOX) solution (4 and 8 μg/ml) was prepared in PBS buffer (1 M NaCl, 100 mM phosphate buffer, pH 7). All solutions were filtered and sterilized prior to use.

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