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Binding and purification of plasmid DNA using multi-layered carbon nanotubes

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ABSTRACT

We propose a new method for the separation of nucleic acids using multi-layered carbon nanotubes (CNTs) as an adsorbent. According to agarose gel electrophoresis, oxidized water-stable CNTs adsorb certain forms of nucleic acids, such as high molecular weight RNA, chromosomal DNA, linear and denatured forms of plasmid DNA. However, CNTs do not adsorb supercoiled form of plasmid DNA. Nucleic acids bound to CNTs can be readily removed by centrifugation whereas supercoiled plasmid DNA remains in solution. Upon the addition of divalent metal ions supercoiled plasmid DNA forms relatively stable complexes with CNTs due to chelation. Thus, new details about association of nucleic acids with CNTs were revealed and stoichiometry of the complexes was estimated. Our results can be used for fine purification of supercoiled plasmid DNA for gene therapy applications as well as manipulation of nucleic acids for biosensor design.

1. Introduction

Development of materials and devices for manipulation of biological objects is one of the main problems of biotechnology. The use of nanomaterials for such purpose is reasonable as their dimensions are comparable to that of biomolecules in addition to broad range of physicochemical and biological characteristics exhibited by nanomaterials (Kane and Stroock, 2007). In this respect carbon nanotubes (CNTs) with organized graphene-like structure are one of the most promising nanomaterials which are capable to controllably affect biological systems at different levels (Yang et al., 2008).

CNTs are a candidate for developing materials for tissue engineering applications. In particular, CNTs and their composites with proteins are used for fabricating cell culture substrates. These substrates were shown to be non-toxic and biocompatible towards mammalian cells including stem cells in vitro (Harrison and Atala, 2007; Mooney et al., 2008). CNTs can provide a good support for the adhesion and proliferation of mammalian cells as well as for modulation of their differentiation (Abarrategi et al., 2008).

The mechanisms by which CNTs affect cell behaviour may involve the adsorption of cellular factors and extracellular matrix proteins (Abarrategi et al., 2008; Kam and Dai, 2005), direct effect on mechanical properties (Wang et al., 2005) and electrical conductivity (Malarkey et al., 2009) of matrixes. Doping of polymeric coating with CNTs allowed direct electrical stimulation of osteoblasts to assist their proliferation and production of bone components (Supronowicz et al., 2002).

Owing to high surface area and capability for chemical modification CNTs are a promising carrier for biomacromolecules. Proteins can be readily attached to the surface of water dispersed CNTs through physical adsorption (Munge et al., 2005) and chemical linking (Prashanth et al., 2006). CNT–enzyme conjugates exhibit high catalytic activity and stability (Prashanth et al., 2006) and can be used as ultra-sensitive labels for DNA detection (Munge et al., 2005).

While proteins are non-specifically adsorbed on CNTs by means of hydrophobic interactions (Kam and Dai, 2005), the binding of nucleic acids to CNTs seems to occur in a more complicated manner depending on molecular weight and conformation of nucleic acids. Single-stranded oligonucleotides were shown to wrap around CNTs due to adsorption of nitrogen bases onto nanotube wall (Zheng et al., 2003). It was shown that single-layered CNTs bind to major groove of DNA in preference to GC sequence resulting in destabilization of DNA duplex (Li et al., 2006).

One of the promising applications of interactions between CNTs and nucleic acids is the separation of nucleic acids based on their conformations. This biotechnological problem particularly covers large-scale isolation of plasmid DNA for gene therapy and vaccination (Ferreira, 2005). Plasmid DNA produced by bacteria and some

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eukaryotes has compact supercoiled structure which considerably differs from that of other forms of nucleic acids, e.g. chromosomal DNA and RNAs. These latter forms are immunogenic and therefore their removal is a prerequisite step in preparing pharmacologically pure plasmid DNA for clinical applications (Ferreira, 2005; Stadler et al., 2004).

In contrast to labor and time consuming chromatographic techniques, particulate adsorbents are very promising alternative for rapid isolation of plasmid DNA from bacteria (Paril et al., 2009). To date, cationic micro- and nanoparticle based adsorbents with high binding capacity towards plasmid DNA have been developed (Chiang et al., 2005; Paril et al., 2009). However, such cationic adsorbents are intrinsically low-selective and readily adsorb other forms of nucleic acids along with plasmid DNA. In current study we propose an effective method of purifying supercoiled plasmid DNA from contaminating nucleic acids using water suspension of nanosized negatively charged multi-layered CNTs. The method is suitable for fine purification of supercoiled plasmid DNA preparations after chromatographic or particle-based isolation.

2. Materials and methods

2.1. Preparation of carbon nanotubes

Multi-layered carbon nanotubes (Sigma–Aldrich) were produced by CVD (purity > 90%, inner diameter 1–3 nm, outer diameter 3–10 nm, length 0.1–10 μm, specific surface area 300–400 m^2/g). Chromosomal calf thymus DNA was purchased from Sigma–Aldrich. All solutions were prepared with the use of deionized water and salts of analytical grade.

1 g of CNTs was added to 100 ml of a mixture of concentrated nitric and sulfuric acids (1:3, *v:v*). The mixture was sonicated for 3 h at room temperature using sonicator UZDN-A (Selmi, Ukraine) at frequency of 22 kHz and power of 70 W. Next, CNTs were precipitated and washed 3 times by repeated centrifugation at 5000 × *g* in distilled water. The precipitate was resuspended in 10 ml of deionized water or a buffer solution resulting in stable black-colored suspension of CNTs. Optical absorption spectra of CNT suspensions were measured by UV–vis spectrophotometer Lambda-35 (PerkinElmer). One absorbance unit of the suspension at 260 nm in 1 cm cuvette was found to correspond to ~10 μg/ml of CNTs. We used this ratio to estimate mass concentration of CNTs in different samples.

2.2. Preparation of nucleic acids

We used *E. coli* strain JM-109 transformed with green fluorescent protein (GFP) coding plasmid pEGFP-N2 (Clontech). Bacteria were grown overnight at 37 °C in LB medium supplemented with 30 μg/ml kanamycin and collected by centrifugation. Plasmid DNA was isolated from bacterial lysate and purified using a PureYield™ Plasmid Midiprep System (Promega). In order to prepare linear form of plasmid DNA, supercoiled plasmid DNA was digested by restriction enzyme EcoRI (Promega) followed by the purification of DNA using EZ10 Spin column PCR purification kit (Bio Basic).

Chromosomal DNA was used without additional purification. For denaturation, supercoiled plasmid DNA and chromosomal DNA were subjected to heating at 100 °C for 5 min and 15 min, respectively followed by rapid cooling with ice. All DNA samples were characterized by optical absorption ratio 260/280 nm > 1.8 indicating high level of DNA purity. Total cellular RNA was isolated from harvested HeLa cells using SV Total RNA Isolation System (Promega).

2.3. Binding of nucleic acids to carbon nanotubes

An aliquot of nucleic acid solution (10–100 μg/ml) in 15 mM phosphate buffer (pH 7.0) or water was mixed with CNTs (25–300 μg/ml). The mixture was kept at room temperature for 60 min to allow nucleic acids adsorb on CNTs. Binding of DNA to CNTs in the presence of divalent metal ions was performed similarly in 15 mM Tris–HCl buffer (pH 7.0) with 2–5 mM Mg^{2+}, Ca^{2+}, Mn^{2+} or Ba^{2+}. No CNT precipitate was observed during the reactions. Samples of nucleic acids and CNTs were analyzed before and after removal of CNTs with adsorbed components from solution by centrifugation at 5000 × *g*.

2.4. Characterization of carbon nanotubes and their complexes with nucleic acids

Hydrodynamic diameter and electrokinetic potential of CNTs in water suspension were measured by Zetasizer NanoZS instrument (Malvern Instruments). Dynamic light scattering (DLS) values were an average of three measurements which were performed at 25 °C using six sub-runs for each scan.

Visualization of CNTs was performed by an atomic force microscope NTEGRA Prima (NT-MDT) with scanner 50 μm and silicon cantilevers (curvature 10 nm, spring constant 0.4–2.7 N/m). An aliquot of CNT suspension was evenly spread out on the surface of freshly cleaved mica and dried. The samples were analyzed on air using tapping mode with a resonance frequency 80 kHz, scan rate 1 Hz and resolution 256 × 256 pixels.

Electrophoresis of complexes of nucleic acids with CNTs was performed in 1% agarose gel at a voltage of 10 V/cm. Nucleic acids were stained in the gel with ethidium bromide; CNTs were observed directly without staining. GeneRuler DNA ladders (Fermentas) were used to estimate molecular weight of nucleic acids.

Electrochemical measurements were performed in a three-electrode cell composed of glassy carbon electrode (working electrode), silver–silver chloride reference electrode and nickel plate as a counter electrode. Polished electrode 1.5 mm in diameter was covered with oxidized CNTs by casting 2 μl of CNTs suspension onto working surface followed by solvent evaporation.

An aliquot of DNA solution (0.5 mg/ml) was placed on CNT-covered electrode and incubated 10 min to allow DNA adsorb on CNTs. Adsorbed DNA was detected by Autolab PGSTAT12 potentiostat (EcoChemie) using linear sweep voltammetry in the supporting electrolyte (0.01 M sodium acetate in 0.1 M NaCl, pH 5.0).

3. Results

3.1. Characterization of carbon nanotubes

In order to prepare water-stable suspension of CNTs, pristine multi-layered CNTs were oxidized in the mixture of concentrated nitric and sulfuric acids under sonication as originally described in Liu et al. (1998). Such a treatment results in cutting nanotubes and introduces oxygen-containing groups to graphene-like walls of CNTs. It also allows removal of general impurities such as amorphous carbon and metal catalysts which are more readily oxidized than CNTs (Datsyuk et al., 2008).

Under experimental conditions, optimal time for the oxidation of CNTs was about 3 h. Shorter treatment resulted in less stable CNTs which were prone to precipitation in the presence of nucleic acids and salts whereas after more extended oxidation CNTs exhibited low adsorption capacity towards nucleic acids. Typical atomic force microscopy image of resulting CNTs is presented in Supplementary material (Fig. 1S).

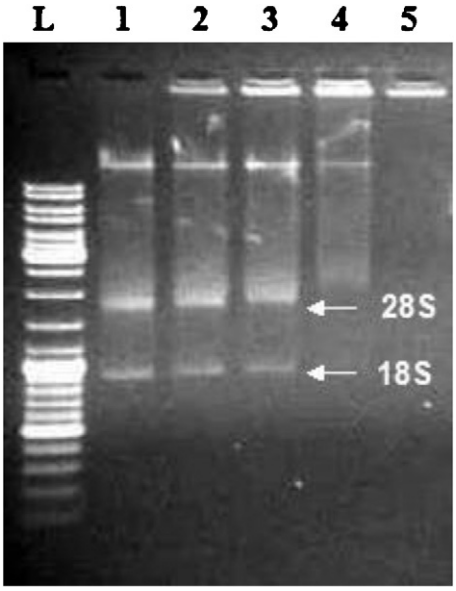

Fig. 1. Agarose gel electrophoresis of total cellular RNA (100 μg/ml) treated with carbon nanotubes in 15 mM phosphate buffer (pH 7.0). L, DNA ladder; 1, RNA (control); 2–5, RNA with carbon nanotubes (38, 75, 150, 300 μg/ml, sequentially).

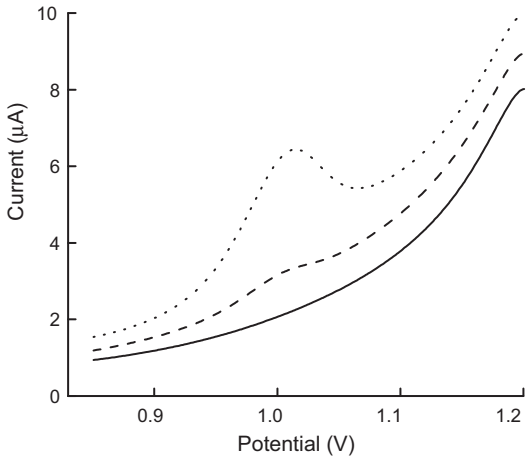

Fig. 2. Linear sweep voltammograms of 0.5 mg/ml chromosomal DNA pre-adsorbed on surface of carbon nanotube-covered electrode. (– – –), Native DNA; (·····), heat-denatured DNA; (—), background curve. Scan rate 0.1 V s^{-1}.

Dynamic light scattering (DLS) showed that oxidized CNTs were almost monodispersed with hydrodynamic diameter of 137 nm and polydispersity index of 0.279 (see also Supplementary material, Fig. 2S and Table 1S). CNTs were also characterized by well-defined zeta potential peak at −56.2 mV (pH 7.0) as if nanotubes formed stable negatively charged colloidal system.

CNTs did not precipitate in diluted inorganic or organic buffers at pH ∼ 7 during prolonged incubation for at least 1 month at room temperature. However, the decrease of pH as well as the increase of ionic strength of the solution substantially promoted precipitation of CNTs. This indicates that colloidal stability of CNTs is determined by negatively charged groups, such as carboxylic ones, formed upon extensive oxidation of CNTs.

UV–vis spectroscopy showed that CNTs absorbed light in UV region with a maximum at 260 nm (Supplementary material, Fig. 3S). Similar spectra are observed for polycyclic aromatic hydrocarbons (Stern and Timmons, 1970) which exhibit structural similarity to graphene walls of CNTs. Optical absorption of diluted suspensions of CNTs at 260 nm was linearly proportional to the dilution factor. By measuring absorption spectra of CNTs we estimated their mass concentration in suspensions as described in Materials and methods.

3.2. Adsorption of RNA and chromosomal DNA

Initially, we studied the adsorption of total cellular RNA and chromosomal DNA (ch-DNA) onto oxidized CNTs using agarose gel electrophoresis. Nucleic acids were mixed with CNTs and incubated in phosphate buffer at different concentrations of the adsorbent. We found that after the incubation 28S and 18S RNAs were bound by CNTs and retarded in agarose gel proportionally to the amount of nanotubes in the mixture (Fig. 1). At the concentration of ∼300 μg/ml CNTs totally bind 100 μg/ml of RNA under experimental conditions.

Similarly to RNA, native ch-DNA was also adsorbed and retarded by CNTs in agarose gel (Supplementary material, Fig. 4S). Gel retardation of RNA and ch-DNA in the presence of CNTs was little dependent on buffer composition and pH. However, electrophoretic mobility of nucleic acids treated with CNTs was restored after addition of 0.1% Tween-20, non-ionic surfactant which reduces hydrophobic interactions (data not shown). Such an effect is apparently due to surfactant molecules are being adsorbed onto hydrophobic walls of CNTs and displace nucleic acids bound to CNTs by means of hydrophobic interactions. The effect is not expected to occur when nucleic acids are adsorbed onto CNTs by means of electrostatic interactions.

Interaction of CNTs with heat-denatured ch-DNA, in contrast to native nucleic acids, did not notably affect DNA gel mobility probably due to its relatively flexible structure. However, denatured ch-DNA was found to impart mobility to CNTs as evident by a dark band of CNTs in agarose gel (Supplementary material, Fig. 4S) indicating that denatured ch-DNA is also adsorbed by CNTs.

In order to compare the adsorption of nucleic acids by CNTs we also applied voltammetric method which is based on the detection of purine nucleotides upon their electrochemical oxidation (Abdullin et al., 2009b). As we demonstrated earlier, purine bases and nucleotides are intensively adsorbed on electrode covered with CNTs presumably due to hydrophobic interactions with nanotubes (Abdullin et al., 2008). We found that the oxidation current of denatured ch-DNA freely adsorbed on CNT-covered electrode was almost 4 times higher than that of native ch-DNA at the same concentration (Fig. 2). As the current is proportional to the amount of surface-attached molecules, this indicates that denatured DNA is more efficiently adsorbed on modified electrode. The difference in electrochemical behaviour of DNAs can be explained by that denatured DNA possesses relatively flexible structure with more single-stranded regions available for interaction with CNTs compared with native DNA.

Our results show that water dispersed oxidized CNTs bind both RNA and ch-DNA. The binding seems to be based on reversible adsorption of nitrogen bases of single-stranded (denatured) regions presented in the nucleic acids in different extent.

3.3. Adsorption of plasmid DNA

Plasmid DNA (p-DNA) is a useful model for studying interactions of nucleic acids with CNTs owing to homogeneity of p-DNA and availability of its different structural forms. Using agarose gel electrophoresis we studied the adsorption of p-DNA pEGFP-N2 in different conformations onto oxidized CNTs.

As shown in Fig. 3, co-incubation of CNTs with supercoiled p-DNA did not result in its gel retardation even at relatively high concentrations of the adsorbent. Under the same conditions, linear form of p-DNA, a result of EcoRI restriction enzyme digestion, bound to CNTs proportionally to the amount of the adsorbent which

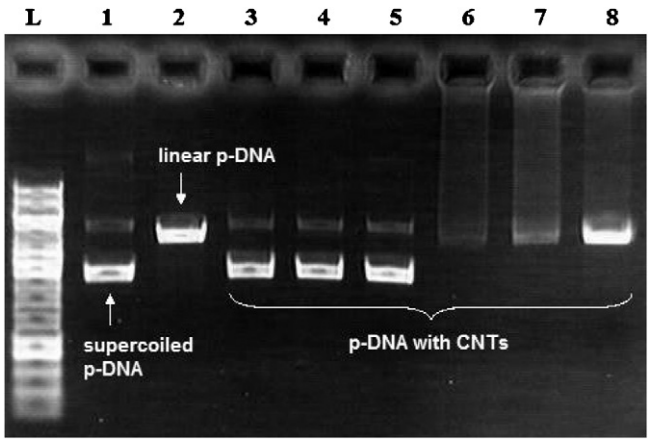

Fig. 3. Agarose gel electrophoresis of plasmid DNAs (10 μg/ml) after co-incubation with carbon nanotubes in 15 mM phosphate buffer (pH 7.0). L, DNA ladder; 1, supercoiled p-DNA (control); 2, linear form of p-DNA (control); 3–5, supercoiled p-DNA with carbon nanotubes (200, 100, 50 μg/ml); 6–8, linear form of p-DNA with carbon nanotubes (200, 100, 50 μg/ml).

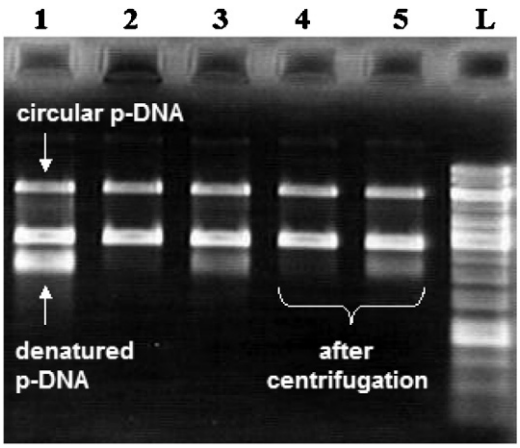

Fig. 4. Agarose gel electrophoresis of heated plasmid DNA (10 μg/ml) after co-incubation with carbon nanotubes in 15 mM phosphate buffer (pH 7.0). L, DNA ladder; 1, heated p-DNA with circular, supercoiled and denatured forms; 2 and 3, heated p-DNA with carbon nanotubes (100 and 50 μg/ml); 4 and 5, heated p-DNA with carbon nanotubes (100 and 50 μg/ml) after centrifugation.

remained in the gel well (Fig. 3). This shows that CNTs predominantly bind linear form of p-DNA and slightly interact (if at all) with supercoiled p-DNA which is characterized by tightly packed structure with limited spatial availability of nitrogen bases.

The above linear form of p-DNA is probably adsorbed onto CNTs through its bases in single stranded regions at the "sticky" ends. We also obtained other linear forms of p-DNA including blunt-ended forms which were also capable of binding to CNTs in a similar manner (data not shown). Lack of difference in the adsorption of sticky-ended p-DNA and blunt-ended p-DNA (lacking single-stranded sites) can be explained by partial unwinding of DNA duplexes in the presence of CNTs. As demonstrated earlier (Li et al., 2006), when interacting with DNA single-layered CNTs induce destabilization of the duplex. DNA destabilization and subsequent unwinding by CNTs presumably involves DNA terminal areas which are characterized by thermodynamically less stable association than internal polynucleotide areas.

High quality purified p-DNA predominantly consists of supercoiled form of p-DNA. Other forms of p-DNA, such as linear, relaxed circular and denatured forms, can also be found in purified p-DNA samples. Supercoiled p-DNA was subjected to brief heating and cooling to generate circular and denatured forms. We found that partially denatured form of p-DNA moving in front of supercoiled p-DNA was adsorbed by CNTs proportionally to their concentration (Fig. 4) similarly to linear form of p-DNA (Fig. 3).

The adsorption of partially denatured p-DNA apparently occurs due to its single-stranded sites generated after heating of supercoiled form of p-DNA. In contrast to denatured form of p-DNA, CNT-treated circular p-DNA was observed in agarose gel indicating no significant interaction of circular p-DNA with CNTs similarly to supercoiled form of p-DNA (Fig. 4). The fact that circular form of p-DNA is not adsorbed on CNTs unlike linear form of p-DNA can be explained by better stability of circular p-DNA upon destabilization of the duplex by nanotubes.

It should be noted that CNTs removal from samples by centrifugation prior to electrophoresis did not alter distribution pattern of p-DNAs in the gel (Fig. 4). This indicates that only adsorbed DNA (here, denatured form of p-DNA) was removed from the solution along with the adsorbent. Similarly, different linear forms of p-DNA adsorbed on CNTs in solution were also removed by centrifugation (data not shown). Altogether these results demonstrate that CNTs can be used for the adsorption of damaged p-DNA forms (linear and denatured p-DNA) and subsequent removal of adsorbed DNA from the solution by centrifugation.

3.4. Divalent metal ions mediated interaction of DNA to carbon nanotubes

Above results were obtained when DNA was incubated with CNTs in phosphate or organic buffers. We found that the interaction of p-DNA to CNTs considerably altered in the presence of divalent metal ions (DMIs): Mg^{2+}, Mn^{2+}, Ca^{2+}, Ba^{2+}. Fig. 5 shows electrophoregram of supercoiled form of p-DNA after co-incubation with CNTs in Tris–HCl buffer supplemented with 2 mM Mg^{2+} or Mn^{2+}.

In those solutions supercoiled form of p-DNA was efficiently bound to CNTs and lost its mobility in agarose gel (Fig. 5) unlike to phosphate buffer without DMI where supercoiled p-DNA did not interact with CNTs (Fig. 3). No precipitate was observed in the reaction after 1-h incubation at room temperature whereas in the absence of DNA the DMI induced aggregation and precipitation of CNTs.

This shows that DMIs mediate binding of supercoiled p-DNA to CNTs apparently due to formation of chelate bonds between metal ions, carboxyl groups of oxidized nanotubes and phosphate groups of DNA. Similar interaction was observed when linear form of p-DNA was used (data not shown). The binding of DNA by CNTs in the presence of DMI occurred at the adsorbent concentration notably

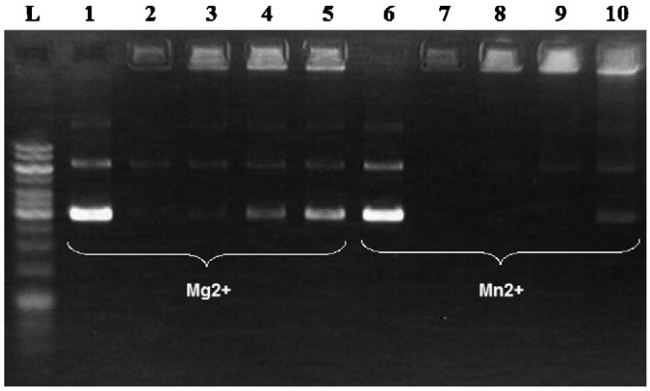

Fig. 5. Agarose gel electrophoresis of supercoiled plasmid DNA (10 μg/ml) after co-incubation with carbon nanotubes in the presence of 2 mM divalent metal ions (in 15 mM Tris–HCl, pH 7.0). L, DNA ladder; 1 and 6, p-DNA (control); 2–5, p-DNA with Mg^{2+} and carbon nanotubes (200, 100, 50, 25 μg/ml, sequentially); 7–10, p-DNA with Mn^{2+} and carbon nanotubes (200, 100, 50, 25 μg/ml, sequentially).

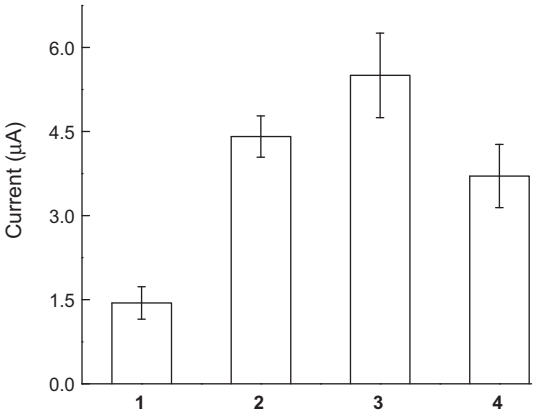

Fig. 6. Effect of divalent metal ions (5 mM) on oxidation current of chromosomal DNA pre-adsorbed on surface of carbon nanotube-covered electrode. 1, DNA; 2, DNA + Mg^{2+}; 3, DNA + Mn^{2+}; 4, DNA + Ca^{2+}.

lower (~10 times) than required for the adsorption of nucleic acids without DMI.

DLS data showed that hydrodynamic diameter of the colloidal system increased from 137 nm for CNTs alone to 258 nm for their chelate complexes with supercoiled p-DNA and Mg^{2+}. No increase in CNT size was observed after the addition of either supercoiled p-DNA or linear form of p-DNA in the absence of DMI (Supplementary material, Table 1S).

Our results demonstrate that DMIs induce the formation of relatively stable and compact complexes between p-DNA and CNTs apparently through the association of DNA molecules on the surface of oxidized nanotubes. This interaction does not exhibit notable specificity to DNA conformation in contrast to simple adsorption in the absence of DMI. It allows efficient binding of supercoiled form of p-DNA to CNTs.

The effect of DMI was somewhat unequal for different ions studied. According to electrophoresis data, Mn^{2+} induced more efficient binding of supercoiled p-DNA to CNTs than Mg^{2+} resulting in 2–4 times lower concentration of the adsorbent required for total DNA binding (Fig. 5). The effect of Ca^{2+} and Ba^{2+} was similar to that of Mg^{2+} and Mn^{2+}, respectively (data not shown).

We also found that DMIs increase the oxidation current of DNA on CNT-covered electrode (Fig. 6) to different extent (3–4 times) as if the loading of DNA adsorbed on CNTs was increased in the presence of the ions. In particular, the signal was highest for Mn^{2+} and was similarly lower for both Mg^{2+} and Ca^{2+}. These results can be explained by the effect of DMI size on binding of DNA to nanotubes where the larger the ion (e.g. Mn^{2+} and Ba^{2+}) the more efficient interaction it induces.

4. Discussion and conclusion

Our results demonstrate the possibility of using oxidized multilayered CNTs for the adsorption and separation of different forms of nucleic acids. The mechanism of binding of nucleic acids by oxidized CNTs differs from that of single-layered CNTs functionalized with primary amino groups by means of cycloaddition reaction (Pantarotto et al., 2004). The latter cationic CNTs bind negatively charged DNA by means of electrostatic interactions which are generally unspecific towards nucleic acid type and conformation. Unlike these CNTs, the oxidized CNTs used in our study form negatively charged colloidal system and exhibit lack of interaction with supercoiled and circular p-DNA. Notable adsorption of linear forms of DNA and RNA onto oxidized CNTs is apparently associated with their hydrophobic components namely nitrogen bases which are better available in these nucleic acids.

Above results, along with our earlier studies (Abdullin et al., 2008, 2009b) indicate that the adsorption involves hydrophobic interactions between heterocyclic rings of bases and aromatic wall of CNTs. This leads to preferred binding of nucleic acids with spatially available bases (normally at single-stranded sites) to the surface of CNTs. Such a mechanism allows CNTs adsorb nucleic acids but not DNAs with stable ds-structure, here supercoiled and circular p-DNA. We believe that observed adsorption of linear ds-DNAs (e.g. genomic DNA and p-DNA) may occur at single-stranded defects or/and as a result of destabilization of DNA duplex in the presence of nanotubes.

Preliminary oxidation of CNTs is required for preparation of the adsorbent as it converts pristine CNTs to well dispersed stable suspension in water. The introduction of oxygen-containing groups in oxidized CNTs promotes their interaction with nucleic acids probably by increasing wettability of nanotubes and promoting their dispersion to increase their sorption capacity towards nucleic acids.

As shown in Wang et al. (2009), oxidized polycyclic aromatic compounds are formed upon oxidative destruction of graphene layers and strongly adsorbed on the surface of CNTs. Such compounds, classified as fulvic acids, contribute to surface properties of CNTs (Wang et al., 2009). We found that in strongly alkaline solutions prepared CNTs were relatively unstable and prone to precipitation. This indicates that the CNT preparations, even carefully washed in water, may contain certain aromatic substances, which are known to be potentially carcinogenic. Since such substances are stably attached to CNTs at physiological pH (Wang et al., 2010), they are not expected to restrain the use of CNT-based adsorbent for production of DNA drugs. Though, the content and desorption conditions for any aromatic substances should be carefully studied subsequently for biomedical related applications of CNT-based adsorbent.

One of the most interesting applications of the adsorbent is purification of plasmid DNA for biotechnology and gene therapy. After isolation from bacteria p-DNA may contain several admixtures, e.g. proteins and other nucleic acids: chromosomal DNA, RNA, denatured forms of p-DNA. We demonstrated that CNTs can be used for simple binding of these contaminating nucleic acids in the solution. Adsorbed nucleic acids are removed along with the adsorbent by brief centrifugation whereas the desired molecule, supercoiled p-DNA, which does not interact with CNTs, remains in solution.

The advantage of CNT-based adsorbent is its high sensitivity towards RNA, chromosomal DNA and p-DNA forms with structural defects. These nucleic acids are non-active admixtures in the preparations of supercoiled p-DNA which may induce immune reactions (Ferreira, 2005; Stadler et al., 2004). We did not study the adsorption of other immunogenic bacteria components such as proteins onto CNTs. However, as we demonstrated earlier, CNTs rapidly adsorb proteins on electrode surface (Abdullin et al., 2009a) probably by means of interactions with hydrophobic domains of proteins.

Altogether our results suggest that oxidized CNTs can be considered as promising adsorbent for fine purification of supercoiled p-DNA for clinical applications. The adsorbent can be potentially used for purification of supercoiled form of p-DNA isolated from bacterial lysates after removal of majority of proteins. Further experiments are required to study the possibility of isolation of p-DNA from bacterial lysates using CNTs.

Our findings can also be of interest in DNA based devices and sensors design. Earlier, we applied selective adsorption of damaged DNA on CNTs for detecting DNA-damaging agents with electrochemical sensors (Abdullin et al., 2009b). Revealed details about association of nucleic acids with CNTs can be further used in biosensor applications for reversible binding and detection of different forms of nucleic acids.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jbiotec.2011.01.022.

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