Complexes of carbon nanotubes with oligonucleotides in thin Langmuir–Blodgett films to detect electrochemically hybridization

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Abstract. Self-assembled complexes consisting of thin multi-walled carbon nanotubes (MWCNTs) and DNA oligonucleotides which are able to a cooperative binding to complementary oligonucleotides have been investigated. It was established a high-performance charge transport in nanostructured Langmuir–Blodgett complexes thin MWCNTs/DNA. A method to electrochemically detect DNA hybridization on the self-organized structures has been proposed.

1. Introduction

An unique property of nucleotide bases is to be arranged by stacking interactions on a surface of carbon nanotubes (CNTs). CNT can be complexified with DNA due to the property. Carbon nanostructures such as graphene and CNTs are characterized by very weak scattering of charge carriers. Langmuir–Blodgett (LB) technique is a nanotechnology which allows to fabricate highly ordered two-dimensional (2D) clusters of functionalized CNTs. These clusters can be spontaneously polarized by virtue localization of the carriers ballistically moving in "zig-zag"-CNT [1]. In [2] we have proposed a method to synthesize new DNA-oligonucleotide complexes of few-walled (thin MWCNTs with diameter $d \sim 2–5$ nm) and multi-walled carbon nanotubes (thick MWCNTs with diameter of 10 nm) under conditions of self-organization.

One needs bioactive ligand-binding DNA-containing model structures with carboxylated carbon nanotubes to carry out an effective nanobiodetection of DNA hybridization. It is possible to develop a new generation of biosensors – bionanodetectors when fabricating of synthetic carbon nanotubes with low dispersion. Bionanodetecting of DNA – genotyping of SNP (Single Nucleotide Polymorphism) should combine mutually exclusive properties. On the one hand, the oligonucleotides must interact with detector high sensitivity ("reporter") sites more strongly than with nonspecific molecular agents, but, on the other hand – form a specific complex with DNA targets through weak hydrogen bonds. As
known [3, 4], methods of covalent binding "reporter" groups distort significantly the conformation of the DNA, and, respectively, the hybridization process is not sufficiently specific (ambiguous). It was shown [5] that pyrenyl conjugates of oligonucleotides can "coagulate" on the single-walled nanotubes surface into self-organized electron-dense nanostructures. In this case, the self-assembly was a side effect, which prevents the molecular recognition. However, we will represent self-assembled complexes MWCNTs/DNA, which are able to a cooperative binding to complementary oligonucleotides.

A goal of the paper is to establish a high-performance charge transport in nanostructured LB-complexes thin MWCNTs/DNA and show the ability of such heterostructure to efficient DNA hybridization.

2. Materials and methods
Oligonucleotide ON1 (structure sequence: 5’-GCCATATACTCTCCTTGGTGACA-3’), oligonucleotides ON2 and ON4, complementary and non-complementary to the ON1, as well as oligonucleotides ON5 and ON3 with one and three non-complementary bases, respectively, were synthesized at «Praymteh" (Minsk, Belarus). We will utilize MWCNTs obtained by chemical vapor deposition method with subsequent covalent modification of their surface by carboxyl groups.

A complexification is performed by two methods. First method is ultrasound treatment of a mixture from ON1 with thin MWCNTs, suspended in TE buffer at various concentrations. Second method is to form MWCNT/oligonucleotide complexes by LB-technique with additional non-covalent functionalization of MWCNTs by stearic acid molecules [6].

Electron microscopy images are obtained with help of a transmission electron microscope (TEM) JEM-100CX TEM (JEOL, Japan) at an accelerating voltage of 100 kV.

Electrochemical detection was carried out by the method [7]. A sensor has an interdigital structure from the \( N \) pairs of twisted aluminum electrodes, which are arranged on the pyroceramics substrate and have octagonal shape. One utilizes the electrodes system with \( N = 10, 15 \). Each pair of electrodes is an capacity of “open”-type. A nanocomposite dielectric coating of electrodes is a metal-containing LB film of thiophene-pyrrole series oligomer on sublayer of nanoporous anodic oxide [8].

Impedance analysis has been performed in deionized (resistivity>18.0 M \( \Omega \) cm at 25 °C).

3. Results and discussion

3.1. Structural analysis
MWCNTs have open ends (see figure 1a). Figure 1b demonstrates that flexible ultrathin CNTs are helically twisted with a pitch of helix ~ \( 1/d \).

![Figure 1. TEM-images of MWCNTs.](image-url)
A distribution of ss-DNA-oligonucleotide / MWCNT (ss-DNA/MWCNT) in TE buffer after the ultrasonic treatment is shown in figure 2. ss-DNA/MWCNT complexes hold three-dimensional (3D) structure of cable type (figure 2), a core of which is the conductive MWCNT, and a shell (“casing”) – 3D self-organized oligonucleotide layer dark-colored in comparison with bright white CNT-image with sharply delineated dark edge. The dark color indicates a high density of the nucleotide self-organized on the surface of CNT, and, hence, the complexes under investigation are электронно-плотные наноструктуры. The stacking interactions decrease a polarization of the CNT in direction transverse to CNT surface (spontaneous polarization) and stiffen CNT walls, whereby the pitch and the radius of helix becomes very large (figure 2b).

Figure 2. TEM-images of complexes ss-DNA/MWCNT in TE buffer.

3.2. Electro-chemical properties
A electrochemical detecting is performed for two frequency ranges: low-frequency (~ 100 kHz) and high frequency (~ 400 – 800 kHz) ones. A Maxwell polarization of conducting inclusions gives main contribution to polarization in the low-frequency region. A dipole polarization vector of molecules or molecular groups relaxes in the high-frequency region. 2D LB-CNT-clusters effectively shield the electrodes due to charge ballistic transport in MWCNTs. Therefore, ionization of water molecules in an electric field of the electrodes is suppressed, and, respectively, the capacitance $C_{CNT}$ of the sensor with 2D LB-CNT-clusters is smaller than the capacitance of the pure sensor. A decrease of $C_{CNT}$ in the high frequency region is $\Delta C_{CNT} \approx -1 \div -1.5 \text{ pF}$ and practically does not depend on the geometry of the sensor. The dielectric permittivity of the oligonucleotide shell in ss-DNA/MWCNTs LB-complexes is high, and a shielding degree is considerably lowered because the charge transport happens mainly transversely to helix axes. Therefore, an increase of capacitance $C_{ss-DNA/CNT}$ by $\Delta C_{ss-DNA/CNT} > 0$ takes place. $\Delta C_{ss-DNA/CNT}$ in the high frequency region is practically independent on the sensor geometry also, but has a larger range of values: from +2 to +6 pF.

The sensors with ss-DNA/MWCNT LB-complexes were incubated about 10 minutes in solutions of different nucleotides. Then, free oligonucleotide is washed off 10 min. from the sensor surface in water at a temperature of 39 °C.

Recordings in the low frequency region allow to estimate an increase of the conductive 2D LB-CNT-clusters portion when releasing ON1 from ss-DNA/MWCNTs to form duplexes ds-DNA. One can observe in figure 3 that the recognized oligonucleotides forming duplexes ds-DNA, partially liberate CNTs. This leads to a capacitance decrease towards to capacitance values for the sensor with conductive 2D LB-CNT-clusters only. Figure 3 demonstrates $S$-shaped dependence of low-frequency module $|\Delta C|$, $\Delta C < 0$ of electrical capacitance displacement due to the cooperative binding of ON2 with ON1. The value of the capacitance of the sensor with the homoduplexes ON1/ON2 is closest to $C_{ss-DNA/CNT}$ owing to the effect of phase separation in the phase transition from ds-DNA/MWCNTs complexes to double-stranded DNA. This effect is absent when forming of heteroduplexes ON1/ ON$i$, $i = 3, 4, 5$.  

3
4. Conclusion

Thus, a highly effective transport of electric charge holds in such nanoheterostructures as LB-complexes thin MWCNTs/DNA-oligonucleotides. A method to electrochemically detect DNA hybridization on the self-organized structures has been proposed.

5. References

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