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Raman and CARS spectroscopy of interactions of nanodiamonds with DNA strands in water

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ABSTRACT

This paper presents the results of studying the interactions of DNA chains with nanodiamonds (ND) with NV centers by CARS spectroscopy. As a result of a comparative analysis of the obtained CARS spectra of DNA in water and in aqueous suspension of ND, a sign of the destruction of DNA chains as a result of interaction with the surface of ND was found. It was confirmed that DNA molecules interact with the surface groups of nanodiamonds by means of electrostatic interactions: no new CARS spectral bands were detected in the CARS spectra of DNA in the ND suspensions.

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1. Introduction

Recently, carbon nanoparticles are actively used in biomedical applications. They are non-toxic,[1] have intense stable luminescence[2] and high adsorption properties.[3] Nanodiamonds (ND) and ND-based complexes are promising for biomedical applications, since they can be used as multifunctional theranostic agents that combine the properties of luminescent marker and means for targeted drug delivery.[4–6] It is very important that nanodiamonds have a chemically developed multifunctional surface. Currently, methods have been developed that allow controlling the properties of this surface, in particular, by means of functionalization or modification of the surface.[5–7]

Thus, today there are all possibilities to attach drugs or bioactive substances to the surface of the nanodiamond and deliver them to cells or to diseased organs. However, it is obvious that the main issue that needs to be solved before using biomedical carbon agents is the question of the interaction of nanoparticles with biomolecules – proteins, lipids, amino acids, DNA, etc. It is known that as a result of such interactions, carbon nanoparticles can form bioconjugates for different applications,[8] for example, electronic and sensing applications.[9] These interactions also affect the stability and functional activity of enzymes – it can significantly increase relative to changes of environmental parameters – pH, temperature.[10,11] The adsorption of lysozyme on the surface of carboxylated nanodiamonds has been well studied.[12,13] Many studies have shown that lysozyme adsorbed on nanodiamonds remains catalytically active, but in some cases its activity can significantly decrease.[12,14,15]

There are studies of interactions of nanodiamonds and surfactants and the effect of these interactions on the properties of surrounding molecules.[16,17] As it is known, the main structural elements of biological tissues are micelles: cell membranes consist of micelles-bilayers, the most important functions are performed by bile acid micelles, phospholipid renewal is associated with the processes of restoring the structural micelles of membranes, etc.[18] Therefore, the results of such studies allow us to expand our understanding the processes occurring when using nanoparticles in biology, and ensure the development of safe carbon theranostic agents.

It is very important to study the interaction of nanodiamonds with DNA chains, because all genetic information is contained namely in DNA. To date, the question of safety of ND in relation to DNA chains remains open. Thus, the authors[19] found a partial destruction of DNA chains during interaction with oxidized ND. On the other hand, ND show almost complete absence of cytotoxicity,[20] which opens up prospects for their use in cellular nanomedicine. The available contradictory data stimulate further studies of the interactions of nanodiamonds with DNA molecules. This publication presents the results of such studies for nanodiamonds with NV centers and DNA chains.

Raman spectroscopy is effectively used to study the interactions of DNA molecules with the environment.[21,22] This method allows one to obtain information about structural changes in the studied molecules. Unfortunately, when studying luminescent nanodiamonds with NV centers in the biological medium, the potential of spontaneous Raman spectroscopy is limited by the need to isolate a low-intensity useful Raman signal of DNA molecules against the background of intense luminescence of both nanodiamonds and the medium itself (autoluminescence). An alternative approach in this case is to use the method of coherent anti-Stokes Raman scattering (CARS). This method assumes the

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registration of DNA Raman signal in the anti-Stokes region of the spectrum, where there is no signal of autoluminescence. This allows to eliminate the need to solve the problem of isolating useful Raman signal of biomolecules against the background of nanoparticle luminescence and autoluminescence of the medium.

CARS spectroscopy is quite actively used in the studies of the structural features of biomolecules and cells, for the analysis of protein conformation, for the identification of amino acids.[23–25] CARS microscopes are used for cell imaging and visualization of nanoparticles in cells.[26,27] In Ref.[28] CARS microscopy was used to detect a new COVID-19 virus, the epidemic of which humanity faced in 2020. The authors[29] showed with the help of CARS that when lysozyme is adsorbed on the surface of carboxylated nanodiamonds, the conformation of lysozyme molecules in the first adsorption layer changes, and the conformation of molecules adsorbed in the second layer practically coincides with the conformation of free lysozyme molecules.

This paper presents the results of the study of the interactions of nanodiamonds with NV centers with DNA molecules in aqueous suspensions obtained by the method of CARS.

2. Materials and methods

2.1. Raman spectroscopy

In this work, three experimental systems were used to obtain Raman spectra. For registration the signals of spontaneous Raman scattering of samples in the powdery state Horiba Jobin Yvon LabRAM HR-800 confocal laser spectrometer (wavelength 473 nm, the practical spectral resolution 1 cm^{-1}) was used. For registration spontaneous Raman spectra of aqueous mixtures-suspensions of nanodiamonds and DNA molecules, a Raman spectrometer was used, in which an argon laser was used as a source of exciting radiation (λ_{exc}=514.5 nm, power in the volume of the sample – 130 mW). The system of registration of spectra consisted of monochromator Acton 2500i and PMT Hamamatsu H-8259-01. This spectrometer is described in details in Ref.[30] For obtaining CARS spectra, degenerate CARS (ω_P=ω_{Pr}) was implemented: the second harmonic (532 nm, pulse duration – 10 ns, pulse repetition rate – 100 Hz) of pulsed Nd:YAG laser (model LQ629-100, Solar Laser Systems, Belarus) was used as pump and probe sources. The third harmonic (355 nm) of the same Nd:YAG laser was used to pump optical parametric oscillator LP603 (Solar Laser Systems, Belarus), the radiation of which was used as Stokes wave ω_{Stokes} in the CARS.

In the experiment on the interaction of ND with DNA, the wavelength of the Stokes component varied in the range from 560 to 580 nm. The radiation power of the pump and the Stokes component were adjusted by turning the Glan-Taylor GT10A prisms (Thorlabs). The beams of ω_P, ω_{Pr}, ω_{Stokes} were focused in the sample volume using the lens with focal length of 55 mm. CARS signal was obtained in the forward-detection scheme (FCARS). A set of filters (FES0550, NF533-17) was used to separate the signal only in the anti-Stokes region. The system of registration of CARS spectrum consisted of the monochromator with focal length of 500 mm, assembled according to the Czerny-Turner scheme, equipped with 600 lines/mm grating and CCD camera (Horiba-Jobin Yvon, Synapse BIUV). The practical spectral resolution of the experimental setup was 4 cm^{-1}.

2.2. Materials

In this study nanodiamonds with NV centers (SKU NDNVN90nmMd10ml, Adámas Nanotechnologies (Raleigh, USA)) with carboxyl and hydroxyl groups on the surface were used. Deoxyribonucleic acid sodium salt from calf thymus (Sigma-Aldrich, CAS Number: 73049-39-5) was used as a DNA sample.

Aqueous suspension of nanodiamonds with concentration of 0.5 mg/ml and aqueous solution of DNA with concentration of 4 mg/ml were used in all experiments. In order to prepare aqueous mixture of ND suspension and of DNA solution the equal volumes of ND suspension with the concentration 1 mg/ml and of DNA solution with the concentration 8 mg/ml were mixed together. The mixture was incubated for 4 hours in order to provide interactions between ND particles and DNA chains. The sizes of nanoparticles in the initial suspension were measured by dynamic light scattering. They were found to be 102.0 ± 3.2 nm. The zeta potential of the prepared ND suspension was −28 ± 0.2 mV.

3. Results and discussion

As it was mentioned above, Raman spectroscopy is a powerful tool for studying various physical and chemical processes, including biomolecular ones. Figure 1 shows the Raman spectra of DNA samples in powdered state and in aqueous solution with concentration of 4 mg/ml. As it can be seen from Figure 1, in the region from 900 to 1800 cm^{-1} one can observe large number of spectral bands corresponding to various vibrations of bonds in DNA chains. In the

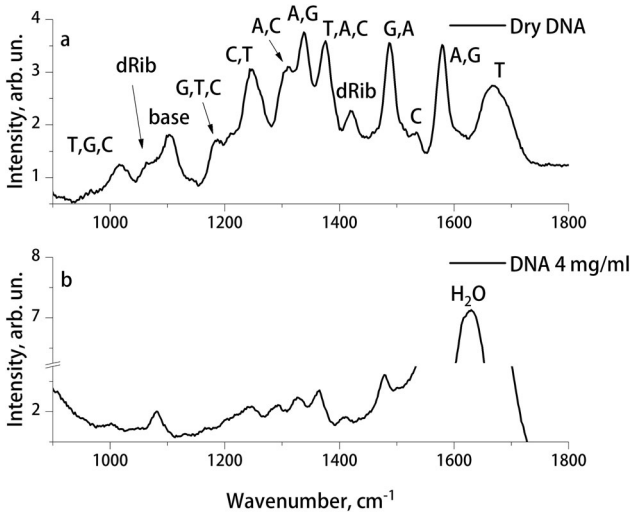

Figure 1. Raman spectra of powdered DNA (a) and aqueous solution of DNA with concentration 4 mg/ml (b).

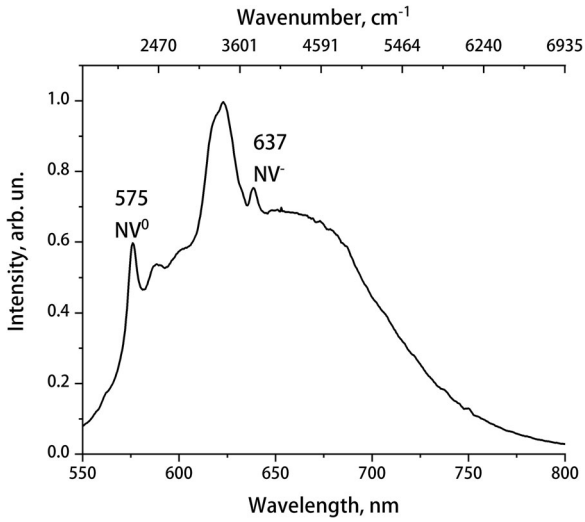

Figure 2. Raman and fluorescence spectrum of aqueous mixture-suspension of ND and DNA solution.

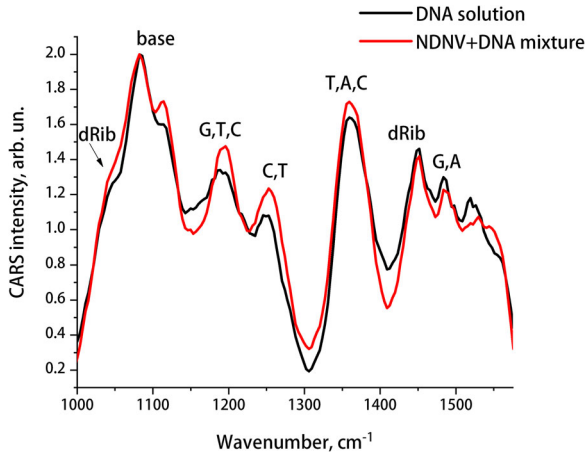

Figure 3. CARS spectra of aqueous DNA solution and a mixture–aqueous suspension of ND and aqueous DNA solution. The spectra are normalized to the maximum intensity at the frequency of 1088 cm^{-1} for the convenience of visual comparison.

Raman spectrum of powdered DNA, bands caused by vibrations of individual nitrogenous bases (adenine – A, guanine – G, cytosine – C, thymine - T), deoxyribose (dRib) and phosphate backbone (base) were identified.[22,31]

From the comparison of the presented spectra, it can be concluded that the Raman spectra of aqueous DNA solutions, even with relatively high concentration (4 mg/ml), are significantly less contrasting and less informative than the spectra of DNA in the powdered state. However, in order to conduct a correct analysis in the study of DNA, it is necessary to take into account the influence of the water environment, since it can have a significant impact on the object of study.[32,33]

Figure 2 shows the Raman and fluorescence spectrum of an aqueous mixture-suspension of ND and DNA solution when excited by argon laser with wavelength of 514.5 nm.

As it can be seen from Figure 2, the Raman and fluorescence spectrum of aqueous mixture-suspension of ND and DNA solution has bands in the region of 575 nm (corresponds to the zero phonon line of NV^0-centers), 623 nm (corresponds to the valence vibrations of OH groups of water molecules), 637 nm (corresponds to the zero phonon line of NV^- centers). Due to the intense fluorescence of ND in the low-frequency region of the spectrum, it is impossible to distinguish the Raman spectral bands caused by vibrations of DNA molecules (Figure 1). This, in turn, does not allow to perform precision analysis of changes of the vibrational bands in the spectrum of spontaneous Raman scattering of DNA molecules as a result of their interaction with nanodiamonds.

The overall CARS intensity is not suitable for extracting quantitative information, because of the fact that peak intensities are neither strictly linear nor quadratic in analyte concentration. Vibrational resonances in CARS spectra are shifted by background-dependent frequency and amplitude due to the coherent addition of resonant and nonresonant signal contributions. On the other hand, the imaginary part of the CARS susceptibility, $Im(\chi_R)$, represents the underlying Raman lineshapes and it is possible to use it for quantitative analysis. $Im(\chi_R)$ can be retrieved from the CARS signal once the spectral phase is known, for example, after Hilbert transform[34,35]:

$$\varphi(\omega) = H\big(\chi(\omega'')\big) = -\frac{P}{\pi}\int_{-\infty}^{\infty}\frac{ln|\chi(\omega'')|d\omega''}{\omega''-\omega},$$

where $\chi(\omega) = |\chi(\omega)|\exp(i\varphi(\omega))$, P – the main value of the improper integral.

Figure 3 shows the CARS spectra of aqueous DNA solution (with concentration 4 mg/ml) and mixture-aqueous suspension of nanodiamonds with NV centers (with concentration 0.5 mg/ml) and aqueous DNA solution (with concentration 4 mg/ml) after the Hilbert transformation.

Spectral bands with maximums at 1046 cm^{-1} (dRib), 1088 cm^{-1} (dRib), 1135 cm^{-1} (T), 1195 cm^{-1} (G,T,C), 1250 cm^{-1} (ring mode C,T), 1366 cm^{-1} (ring mode δ_s(C5H3) T,A,C), 1450 cm^{-1} (dRib), 1484 cm^{-1} (G,A), 1520 cm^{-1} (ν(N3C4-C)) were detected and identified in the CARS spectra of aqueous DNA solution.

As it can be seen from Figure 3, the Raman spectra of DNA molecules in the presence and absence of ND differ from each other. Thus, the intensity of the bands in the region of 1135 cm^{-1} (T), 1195 cm^{-1} (G,T,C), 1250 cm^{-1} (ring mode C,T) and 1366 cm^{-1} (ring mode $\delta_s(C5H_3)$ T,A,C) increases when ND is added to the DNA solution, which indicates an active interaction of the surface of nanoparticles and DNA chains.

A comparative analysis of the vibrational bands of DNA molecules in the presence and absence of nanodiamonds revealed a shift of 10 cm^{-1} to the region of high wavenumbers and a broadening of 15 cm^{-1} of the band with a maximum in the region of 1520 cm^{-1} caused valence vibrations of N3C4-C. Similar changes of the specified band of the spectrum of spontaneous Raman scattering were observed in the studies of the authors[31] where DNA chains were irradiated by protons. As it follows from the results of the analysis of the spectral vibrational bands of DNA, when the chains are irradiated by protons, gamma and UV radiation, such changes of the N3C4-C vibrational band indicate damage of the DNA chains.[31,36,37] It should be noted that in

Raman spectra of mixtures of aqueous suspension of ND and aqueous DNA solution no other markers indicating the destruction of DNA chains were found.

No new bands were detected in the obtained CARS spectra of DNA chains in the presence of nanodiamonds with NV-centers. It indicates Coulomb interactions of DNA chains with surface groups of ND (without the formation of new chemical bonds). This conclusion correlates with the previously obtained results of studying the interaction of individual nitrogenous bases (NBs) and their complementary pairs with surface carboxylic groups of detonation nanodiamonds.[38] Basing on molecular modeling using the method of the density functional theory, the interactions of the complementary pairs of DNA NBs and diamond-like nanoparticles were analyzed. The theoretical calculations confirmed the obtained experimental results, namely, the complementary pairs are adsorbed on the ND surface functionalized by carboxylic groups by formation of the hydrogen bonds, i.e., via physical adsorption.[38]

4. Conclusions

In this work the interaction of DNA chains with nanodiamonds with NV-centers was studied by CARS spectroscopy. It was established that the CARS spectra of DNA molecules in the presence and in the absence of ND differ. A comparative analysis of the obtained CARS spectra of DNA in water and in aqueous suspension of ND revealed a sign of the destruction of DNA chains as a result of interaction with the ND surface. It was confirmed that DNA molecules interact with the surface groups of nanodiamonds through electrostatic interactions: no new CARS spectral lines were found in the CARS spectra of DNA in the ND suspensions, which indicates the absence of new chemical bonds between the DNA chains and ND surface.

Disclosure statement

The given paper disclosures no conflict of interests.

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