Difference Raman spectroscopy of DNA molecules

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Abstract. In this paper the micro-Raman spectra of calf DNA for different points of DNA sample have been recorded. The Raman spectra were made with help of difference Raman spectroscopy technique. Raman spectra were recorded with high spatial resolution from different points of the wet and dry samples in different spectral range (100÷4000 cm\textsuperscript{-1}) using two lasers: argon (514.5 nm) and helium -neon (632.8 nm). The significant differences in the Raman spectra for dry and wet DNA and for different points of DNA molecules were observed. The obtained data on difference Raman scattering spectra of DNA molecules may be used for identification of DNA types and for analysis of genetic information associated with the molecular structure of this molecule.

1. Introduction and background

Important components of biological objects are nucleotides, which enter the composition of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The fundamental function of these molecules is to keep and transfer the information that is necessary for existence and reproduction of biological objects. Therefore, any modification of the nucleotide structure of an object affects strongly its vital functions. A structural modification of DNA and genes changes also the genetic information. The microstructure of biological objects can be modified by different chemical and physical impacts, in particular, under electromagnetic irradiation. Methods of molecular spectroscopy, including Raman scattering and photoluminescence, are widely used to study the structural modification of polynucleotides.

A large number of studies are devoted to DNA Raman spectra recording. Raman spectra of an aqueous solution (pH=7) of calf thymus DNA in a frequency range of 600 to 1800 cm\textsuperscript{-1} and at temperatures of 25, 80, and 98\textdegree C, in the presence of 0.01 mol/l sodium cacodylate and 0.001 mol l\textsuperscript{-1} ethylenediaminetetraacetic acid were reported in [1, 2]. The total concentration of Na\textsuperscript{+} ions was 0.0075 mol l\textsuperscript{-1}. It was found that the intensities of individual lines due to internal vibrations of all DNA bases increased when approaching the melting temperature (80\textdegree C). The Raman spectra of both an aqueous solution of calf thymus DNA and dry DNA were investigated in the frequency range of 600 to 1800 cm\textsuperscript{-1} at temperatures of -100, 20, and 130\textdegree C [3]. The frequencies corresponding to adenine and guanine were found to redshift upon cooling, while the frequencies corresponding to thymine and cytosine remained invariable.
The conformational changes in DNA aqueous solutions with Na$^+$, Cs$^+$, Mn$^{2+}$, and Mg$^{2+}$ counter ions introduced into different sites in the macromolecule were studied in [4–6]. These counterions neutralize phosphate groups in the double helical backbone of the DNA molecule. As a result, low frequency Raman spectra in the range of 60 to 140 cm$^{-1}$ were also recorded.

In [1] and [7], the data on changes in the Raman spectra at conformational transitions of DNA molecules in aqueous solutions by the addition of NaCl salt at a concentration of 3-4% and a relative humidity of 75 and 92% in the frequency range 600-1800 cm$^{-1}$ were presented. It was found that when the relative humidity exceeds 92% of the DNA transferred from A- to B-form.

In [8-10] the influence of aspirin, Adriamycin and ethidium bromide on DNA Raman spectrum has been investigated. It was studied the changes DNA structure changing as a result of double helix interaction with these compounds. Accomplished studies are important in terms of influence rhenium aspirin microstructure DNA, as well as in view of the fact that Adriamycin used for cancer treatment, and ethidium bromide is a potent mutagen. It became clear from these studies, that in the frequency range of 600-1800 cm$^{-1}$ substantial change ha ve undergone, which indicates on significant interaction of DNA molecules with these compounds.

Despite a large number of studies devoted to DNA Raman spectra, the low-frequency (0 to 600 cm$^{-1}$) and high-frequency (to 4000 cm$^{-1}$) spectral ranges have been poorly studied. Completed to present days studies of the Raman spectra of DNA were performed in macroscopic volumes of test samples. The purpose of our paper was to analyzing of DNA Raman spectra in a wide frequency range (100 to 4000 cm$^{-1}$) with high spectral resolution (1 cm$^{-1}$) for different points of the sample. In this work we used calf DNA samples and have studied the effect of water influence on the Raman spectrum.

DNA is a biopolymer comprised of nucleotides as monomers [11 - 13]. Each nucleotide consists of a phosphoric acid residue attached to a deoxyribose sugar to which is attached through a glycosidic bond one of the four nitrogenous bases adenine (A), guanine (G), cytosine (C) and thymine (T). DNA polymer has a fairly complex structure [14-17]. The skeleton of each of the DNA strands is composed of alternating sugar and phosphate [16].

2. Results

Raman spectra were excited and recorded with using of typical experimental micro-Raman setup. Laser beam was directed by a transparent mirror onto the substrate with the material studied. The secondary radiation (Raman signal) was directed (using a semitransparent mirror) to the input slit of a spectrograph Renishaw InVia Reflex. Spectrograph equipped with a microscope Leica DMLM, having a resolution of ~1 μm and lens 5x, 20x, 50x and 100x. An argon and helium-neon lasers with wavelength of 514.5 and 632.8 nm generations were used as an excitation source.

Figure 1 shows the Raman spectrum obtained from three different points of dry DNA when excited by an argon laser. From this figure we can see that in the Raman spectra present background photoluminescence. This pattern is different for different points of the target DNA. We can see the essential Raman spectra difference from each other at high frequencies. Near the edge of the low frequency (≈ 100 cm$^{-1}$) there is the peak, which was investigated at room and liquid nitrogen temperatures before [18, 19].

Figure 2 shows Raman spectra of wet DNA obtained from three different points with excitation by helium-neon laser. As we can see the Raman spectra is on photoluminescence background. The interaction of DNA with water results in the some changes of several bands frequencies, corresponding to $PO_2^-$, C-O and C-C vibrations, as well as all components of DNA nucleic bases [3, 20].
Figure 1. Raman spectra of dry DNA excited by an argon laser: 1, 2, and 3 corresponds to first, second, and third points of DNA molecule, respectively.

Figure 2. Raman spectra of wet DNA excited by a helium-neon laser: 1, 2, and 3 corresponds to first, second, and third points of DNA molecule, respectively.
To establish quantitative differences in the Raman spectra obtained from different points of the DNA dry and wet samples we were constructed difference spectra [11, 21-24] using the ratio of

$$K_X^B (\nu) = 1 - |I_X (\nu) - I_B (\nu)|.$$  \hspace{1cm} (1)

Here $I_X (\nu)$ and $I_B (\nu)$ - the normalized Raman spectra of the current point (X) and the base point (B). For the base point adopted the first point. On the basis of the difference spectra were calculated integral coefficients of compliance with the following formula

$$K = \frac{\sum_{j=1}^{N} (1 - |I_X (\nu) - I_B (\nu)|)}{N}.$$ \hspace{1cm} (2)

Figure 3 shows the difference Raman spectra excited by argon laser between the second and first and third and first points of the dry DNA. Figure 4 illustrate the difference Raman spectra of wet DNA obtained for the two points (the second and first and third and first). In this case the excitation of Raman spectra was with using of helium-neon laser. The frequency range is indicated by arrows.

![Figure 3. Difference Raman spectra of dry DNA when excited by an argon laser: 1 corresponds to second and first points, with a coefficient of compliance of $K = 0.91$; 2 corresponds to third and first points, with a coefficient of compliance of $K = 0.95$.](image-url)
Figure 4. Difference spectra of wet DNA when excited by a helium-neon laser: 1 corresponds to first and second points, with a coefficients of compliance of $K = 0.96$; 2 corresponds to third and first points; with a coefficients of compliance of $K = 0.89$.

As can be seen from figure 4, Raman spectra of wet DNA at different points differ considerably, indicating that the DNA strongly interacts with water.

3. Conclusions
Thus, in this paper Raman spectra of dry and wet calf DNA in a wide frequency range with high spatial resolution (~10 μm) have been examined. By using difference spectroscopy technique we have revealed the significant difference in these spectra, recorded from different points of dry and wet DNA. Obtained results on difference Raman spectroscopy of DNA molecules is interesting for DNA characterizing and also for the study of DNA structural changing as a result of different type perturbations.

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