
NONLINEAR LIGHT SCATTERING

Raman Scattering in Dried DNA and Crystalline Amino Acids

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Received February 10, 2016

Abstract—Raman spectrum characteristics of dried deoxyribonucleic acid (DNA) and two types of crystalline amino acids (L-lysine, D-asparagine) are compared in a wide range of frequencies, including the regions of lattice (7 to 200 cm⁻¹) and intramolecular (200 to 4000 cm⁻¹) vibrations. It is found that the spectral position of the low-frequency band in the Raman spectrum of DNA with a peak near 26 cm⁻¹ correlates with the Raman spectrum of high-Q low-frequency modes that manifest themselves in the crystalline amino acids under investigation. The low-frequency band of DNA refers to a twist-like vibrational mode of nucleobases. The intensities of this DNA mode and the high-Q lattice modes of the crystalline amino acids L-lysine and D-asparagine are several times as high as those of the Raman lines corresponding to the intramolecular modes. Resonant coupling of low-frequency modes of DNA and amino acid molecular chains is analyzed.

DOI: 10.3103/S1541308X1604004X

1. INTRODUCTION

Molecules of deoxyribonucleic acids (DNAs) and amino acids are important components of biological structures. As has been established by now [1–4], the DNA molecule stores genetic information on organisms and plays a crucial part in metabolic processes that maintain life and reproduction of cells of biological objects. The structure of the DNA molecule is a double helix that can exist in various conformations [4]. The DNA molecule consists of two chains, sugar-phosphate strands, bound together by complementary nucleobase pairs. The nucleobases are joined to one another in long polynucleotide chains. In most cases (except for some viruses that have a single-stranded DNA genome), these chains are bound pairwise to form the secondary structure of the double helix. The double helix of DNA is stabilized by hydrogen bonds that bind the complementary nucleobases A, G, C, and T. This spiral is most commonly right-handed. The width of the double helix ranges from

2.2 to 2.4 nm, its period is 3.4 nm, and the distance between the neighboring base pairs is 0.34 nm [4].

The information stored in DNA is transmitted via codes of sequences of nucleobases specifying the type of amino acid molecules of the corresponding proteins that perform various vital functions and exist in the form of particularly oriented chains of various amino acid molecules [1]. Thus, protein molecules are formed in compliance with the program stored in DNA via interaction of DNA components with chains of amino acid molecules. The microstructure of the DNA molecules and the structure of the amino acid molecular chains are directly related to secondary radiation spectra, in particular to photoluminescence and Raman spectra [5–11]. Parameters of vibrational spectra of DNA molecules and amino acid crystals change from one microstructure to another, and the data on them can be obtained by the Raman scattering method. Raman spectra of DNA in different structural states were investigated in a lot of works. First, Raman spectra were investigated [4–8] for intramolecular modes of aqueous solutions of DNA in the range of 600 to 1800 cm⁻¹, and frequencies of intramolecular modes of nucleobases were established. In [9] Raman spectra of A-DNA and B-DNA in the form of aqueous gel were recorded in the region of 50 to 300 cm⁻¹, where cluster modes of water and

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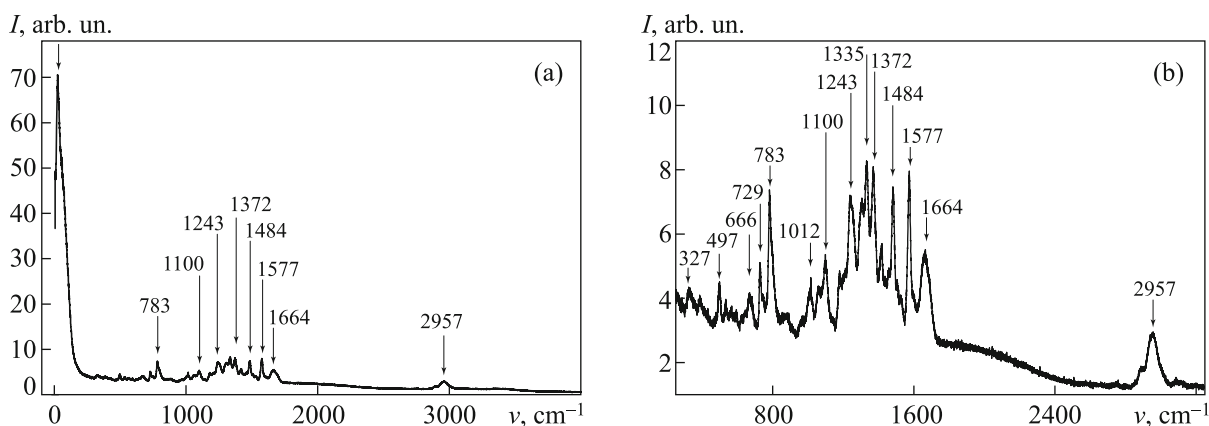


Fig. 1. Raman spectrum of the dried calf DNA in a wide spectral region (a) and in the region of intramolecular modes (b) at room temperature.

low-frequency vibrations of DNA molecular components interacting with polar modes of water manifest themselves. Later, the dependence of the Raman spectra of DNA on the relative ambient humidity in the region of 10 to 300 cm^{-1} was studied [10–13] and interactions of DNA molecules with amino acids were investigated [14, 15]. In [16] experimental data on characteristics of Raman spectra of solid (dried) DNA were obtained.

Amino acid crystals have low point symmetry and are constructed from chiral zwitterions with a structural formula $(\text{NH}_3)^+-(\text{HCR})-(\text{COO})^-$. Raman spectra of crystalline amino acids were investigated in [17–26]. By now the Raman spectra of the intramolecular modes of amino acids corresponding to the middle- and high-frequency regions have been mainly studied [27–32].

The goal of this work was to compare Raman spectrum characteristics of dried calf DNA and two crystalline amino acids (L-lysine, D-asparagine) in a wide spectral interval including both the region of low-frequency modes responsible for the secondary structure of DNA and the region of intramolecular modes corresponding to vibrations of atoms of nucleobases, sugar-phosphate groups, and hydrogen bonds. It was intended to investigate Raman spectrum parameters for solid DNA in a wide temperature interval, with both heating and cooling the sample under investigation.

2. EXPERIMENTAL PROCEDURE

Samples of dried calf DNA fibers and the polycrystalline L-lysine and D-asparagine amino acids were used for the investigation. The argon laser beam with a wavelength of 514.5 nm was directed by a semitransparent mirror to a quartz substrate with a small amount ($\sim 10\text{ }\mu\text{g}$) of dried DNA strands or L-lysine or D-asparagine polycrystals. The laser beam

was focused on the sample with a short-focus lens to a spot a few micrometers in diameter. The laser power was about 1 to 10 mW, which insignificantly heated the sample (by 2 to 4°). The temperature of the sample was controlled by analyzing the relative intensities of the Stokes and anti-Stokes components of the Raman scattering. The area for the analysis of the Raman spectra was chosen using a microscope. The Raman scattering radiation was sent to the entrance slit of the Horiba Jobin Yvon T64000 triple monochromator, which allowed Raman spectra to be recorded with a resolution of 1 cm^{-1} both in the immediate vicinity of the spectral position of the exciting radiation line (514.5 nm) beginning with 5 to 7 cm^{-1} and in a wide spectral region (up to 4000 cm^{-1}). In the latter case, different spectral regions linked at their boundaries were recorded. The exposure needed for recording a low-frequency Raman spectrum was a few minutes long. It took about an hour to obtain the complete Raman spectrum. The dried DNA was heated and cooled using an optical cryostat that allowed Raman spectra to be recorded in a range of -193 to 80°C .

3. RESULTS OF EXPERIMENTAL INVESTIGATIONS

Figure 1 shows the Raman spectrum of the dried calf DNA recorded in a wide spectral region. As is evident from Fig. 1(b), in the region of 3000 cm^{-1} there is a band corresponding to the C—H bond and in the range of 700 to 2000 cm^{-1} there are a lot of combination peaks corresponding to the intramolecular modes of the nucleobases and sugar-phosphate groups. In the low-frequency region (Fig. 1(a)) there is a wide intense Raman scattering band with a peak at a frequency of 26 cm^{-1} . The intensity of this band is almost an order of magnitude higher than the intensity of the combination satellite lines corresponding

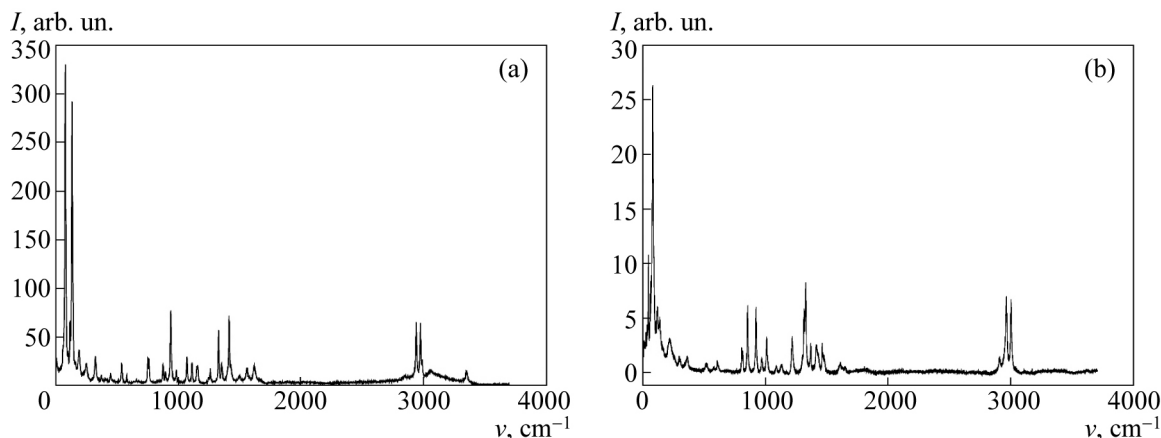


Fig. 2. Raman spectra of the polycrystalline amino acids D-asparagine (a) and L-lysine (b) in a wide spectral region at room temperature.

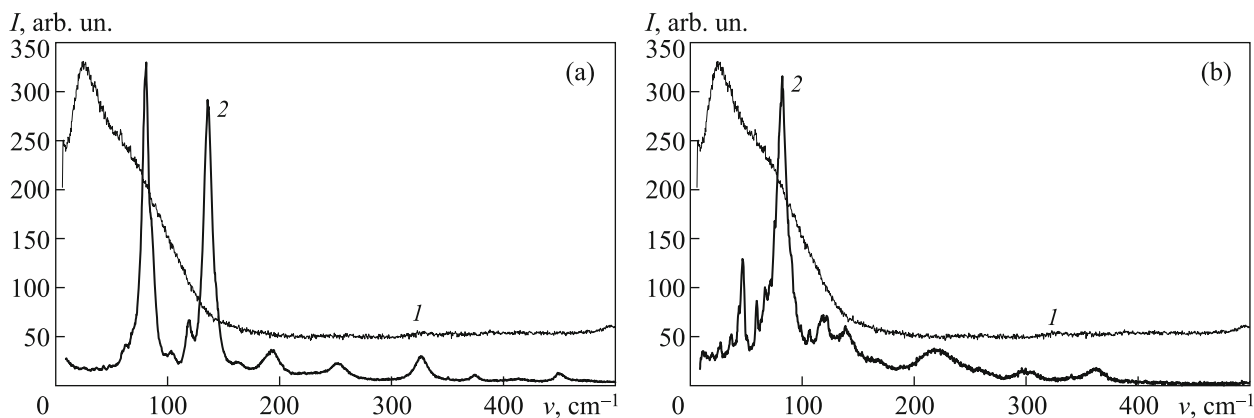


Fig. 3. (a) Comparison of the low-frequency Raman spectra of the dried DNA (1) and polycrystalline D-asparagine (2); (b) comparison of the low-frequency Raman spectra of the dried DNA (1) and polycrystalline L-lysine (2).

to the intramolecular modes in the middle- and high-frequency spectral regions.

Figures 2(a) and 2(b) show Raman spectra of the polycrystalline amino acids D-asparagine and L-lysine, respectively. As is seen, Raman spectra of polycrystalline amino acids exhibit a lot of sharp strong lines. The doublet band in the region of 3000 cm^{-1} correlates well with the CH bond band observed in the Raman spectrum of the dried DNA (see Fig. 1). In addition, the D-asparagine spectrum shows a sharp peak at the frequency of 3400 cm^{-1} corresponding to the OH bond vibration.

In the Raman spectrum of the crystalline amino acids the lines corresponding to intramolecular vibrations are rather narrow (see Fig. 2). The most strong Raman lines are observed in the low-frequency region of the spectrum that falls within the range of the low-frequency Raman band of the dried DNA (see Fig. 1(a)). The intensity of the low-frequency (lattice) modes of the crystalline amino acids under discussion is several times higher than the Raman

intensity of the corresponding intramolecular vibrations (see Fig. 2). The low-frequency Raman spectra of the dried DNA and crystalline amino acids under discussion are illustrated in more detail in Fig. 3.

As is evident from Fig. 3, the low-frequency band in the DNA Raman spectrum (curve 1) consists of two overlapping components in the region of 0 to 150 cm^{-1} . A lower-frequency component is characterized by an intensity peak at the frequency of 26 cm^{-1} . The low-frequency spectra of the crystalline D-asparagine and L-lysine amino acids (curves 2 in Fig. 3) consist of several Raman lines, the narrowest and most strong of which falls within the region of the second component of the DNA low-frequency band.

Figure 4 shows how the low-frequency Raman spectra of the dried calf DNA vary with temperature as the samples were heated and cooled. When the DNA is heated, the spectral position of the low-frequency Raman band with a peak near 30 cm^{-1} at -193°C is seen to shift to 25 cm^{-1} at 80°C . In addition, when the sample is heated, the

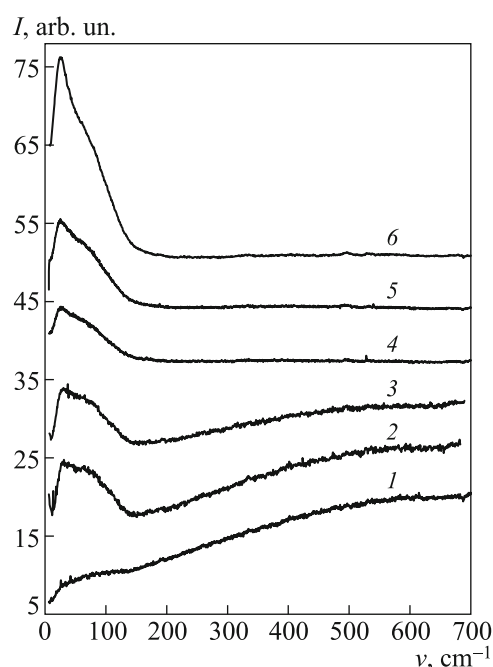


Fig. 4. Temperature dependence of the Raman spectra of the dried calf DNA; the low-frequency Raman spectra are recorded at the temperature of -193°C (1), -100°C (2), -50°C (3), 23° (4), 40° (5), and 80° (6).

integral intensity of the low-frequency Raman band under discussion appreciably increases and the shape of this band changes. At 80°C a quite sharp peak is formed (see Fig. 4, curve 6), which corresponds to the low-frequency component of this band in the Raman spectrum.

4. DISCUSSION OF THE EXPERIMENTAL RESULTS

The table presents frequencies and intensities of the lines and bands observed in the Raman spectrum of the dried DNA. The column "Assignment" shows the assumed types of vibrations corresponding to the external and intramolecular vibrations of the constituent molecular groups of the DNA. The assignment is based on the comparison of the present data with the results obtained in previous Raman studies of DNA molecules [33–38]. The Raman spectra corresponding to the low-frequency vibration region of the DNA molecule were earlier analyzed within various models [33–40]. In particular, the strong band with a peak near 26 cm^{-1} was assigned [14] to the twist-like mode, libratory antiphased nucleobase vibrations.

The DNA Raman spectra were approximated in the low-frequency region (7 to 170 cm^{-1}) by two Gaussians. An example of this approximation for the spectrum at temperature -50°C is presented in Fig. 5(a). The peaks of two components of this band at this temperature are near 33 and 68 cm^{-1} . Fig-

Parameters and assignment of lines and bands in the Raman spectrum of the dried DNA

No.	Frequency, cm^{-1}	I , rel. un.	Assignment
1	26	670	twist-like mode
2	327	10	ext. vibr.
3	386	8	A
4	497	12	d
5	534	7	G, T
6	666	10	A, C
7	729	20	G
8	783	43	T, d
9	1012	13	d
10	1100	23	d
11	1243	44	T
12	1308	43	A
13	1335	55	A
14	1372	53	A, T, G
15	1484	50	A, G
16	1577	53	A, G
17	1663	28	T, G
18	2957	17	d

Note. A, G, C, and T are the nucleobases adenine, guanine, cytosine, and thymine, respectively; d is deoxyribose; twist-like mode denotes libratory antiphased vibrations of nucleobases; ext. vibr. denotes external vibrations of DNA molecular groups.

ure 5(b) illustrates temperature dependences of the frequency positions ν [cm^{-1}] for the intensity peaks of the components obtained by the approximation of the shape of the low-frequency band in the Raman spectrum of the DNA.

As is evident from the figure, a change in the temperature entails a resonant coupling between two modes corresponding to the components of the Raman low-frequency band. With heating from low temperatures to 0°C , this coupling causes softening of the first component of the low-frequency band in the Raman spectrum of the DNA. With further heating, the frequency of this mode is stabilized, but the Raman peak intensity of the first component considerably increases (see curve 6 in Fig. 4). The mode (1) frequency stabilization during the heating can probably be due to an additional low-frequency high-Q mode in the vibrational spectrum, which interacts with the libration-type low-frequency antiphased vibrations of the DNA nucleobases. A lot of theoretical studies have been carried out to investigate into the vibration dynamics of atoms in DNA strands [41–45]. In particular, possible man-

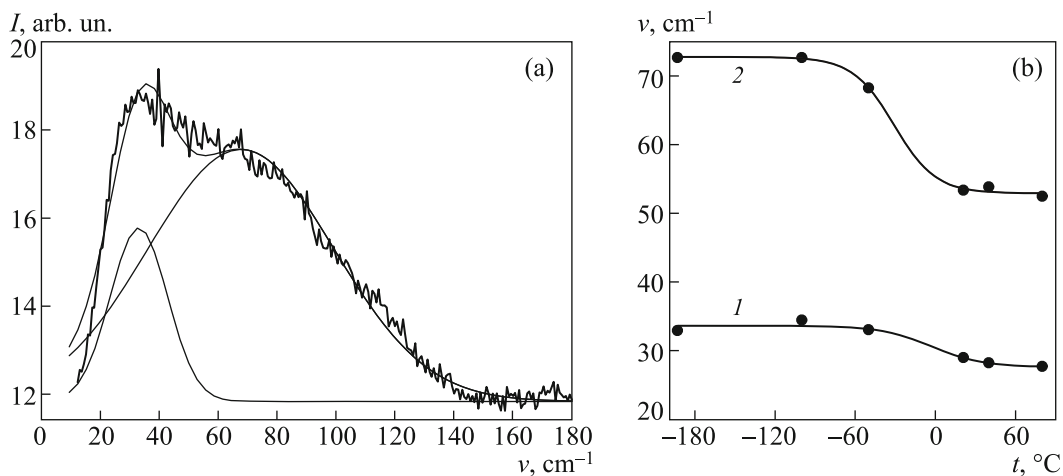


Fig. 5. (a) Approximation of the low-frequency Raman band spectrum at temperature -50°C by two Gaussians; (b) temperature dependences of the frequency positions ν [cm^{-1}] of the intensity peaks of two components of the DNA low-frequency Raman band.

ifestations of soliton mechanisms for transmission of perturbations along the DNA chain are analyzed [46–53]. The experimental results on the dynamics of DNA low-frequency modes obtained in our work appear to be important for comparing theory [46–53] and experiment.

A comparison of the Raman spectra of the dried DNA and the polycrystalline D-asparagine and L-lysine amino acids shows (see Fig. 3) that conditions for the resonant interaction of the amino acid and DNA vibrations arise for both the intramolecular modes and the lattice vibrations. For the lattice modes of the amino acid crystals it is known that the most strong sharp lines in their Raman spectrum correspond to the libratory vibrations of the zwitterions. In our case we observe two such modes (see Fig. 3). The frequency of the most strong line in the Raman spectrum (80 cm^{-1}) of the amino acids under discussion is close to the frequency of the second component of the low-frequency band of the dried DNA. These features of the vibrational spectra of the DNA and the amino acids indicate that resonant interactions are possible between these biological objects.

5. CONCLUSIONS

Raman spectra of the DNA solid phase were recorded in a wide range of frequencies from 7 to 4000 cm^{-1} . It is found that in the low-frequency region there is a two-component band with a peak near 26 cm^{-1} , whose intensity is an order of magnitude higher than the intensity of the Raman lines in the middle- and high-frequency regions of the spectrum. This band corresponds to the antiphased vibrations of the nucleobases in the DNA strands. In the middle-frequency range (600 to 1800 cm^{-1}) there are

a few sharp lines corresponding to the intramolecular modes of the nucleobases. In the high-frequency range a band with a peak near 2957 cm^{-1} is observed. A comparison of the spectra at several points of the sample revealed no statistically significant differences.

The temperature dependence of the DNA low-frequency Raman spectrum measured in the temperature range from -193 to 80°C shows that the change in temperature causes resonant interaction of two components that form the spectrum of the DNA low-frequency Raman band. As the sample is heated, this interaction leads to partial softening of the frequency of the first component and to a sharp increase in its peak intensity in the Raman spectrum. The low-frequency mode of the dried DNA is prevented from complete softening by possible existence of an additional low-frequency mode with the symmetry identical to that of the antiphased libratory mode that manifests itself in the observed Raman spectrum.

Note also that the anomalously high intensity of the libratory modes of the amino acid crystals and the low-frequency Raman band of the DNA indicate a possibility of stimulated Raman scattering by these types of vibration. During the stimulated Raman scattering coherent mechanical waves of the type under discussion are formed, which opens up a possibility of annealing DNA and affecting its microstructure by light. A promising tool for such experiments is a solid-state yttrium aluminum garnet laser generating ultrashort pulses ($\sim 10\text{ ps}$) and characterized by high intensity (10 to 100 GW cm^{-2}) at relatively low pulse energy ($\sim 10\text{ }\mu\text{J}$), which ensures the nondestructive action of its beam on a biological object.

ACKNOWLEDGMENTS

The work was supported in part by the RFBR Projects 14-02-00190 and 16-52-00026 (BRFFR-RFBR F16R-063).

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