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13. ABSTRACT (Maximum 200 words)

Low frequency phonon modes of a helical DNA olygomers were calculated and compared. The customized program packages JUMNA and LIGAND (developed in Dr. Lavery's group, IBPC, Paris) were was adopted for IBM PC and employed to minimize potential energy and to calculate vibrational frequencies of a molecule. The initial geometry for the B-helix of oligonucleotides d(TATATATATATA)d(ATATATATATAT) and d(AAAAAAAAAAAA)d(TTTTTTTTTTTT) was created by program JUMNA. The energy of interatomic interactions was calculated using a semiempiric force field FLEX. The conformational energy was minimized in the space of internal coordinates of a molecule (i.e. dihedral and valence angles for all the covalent bonds). Vibrational frequencies were calculated using second derivative of the conformational energy with the program LIGAND. 360 normal modes with vibrational frequencies ranging from 0 to 900 cm-1 were found. A new method for the analysis of optical activity for each normal mode was developed. The essence of the method is to calculate oscillator strengths from vibrations of the dipole moment of a molecule. All the modes were analysed for their ability for the infrared absorbtion. For this pupose we calculated the motions of the DNA molecule along each mode and analysed the deviations of dipole moments. The described calculations clearly demonstrated that 1) DNA double helix has at least hundreds of vibrational modes in far infrared region; and 2) theses normal modes may differ many folds in their ability for the radiation absorption. The dependend of absorption coefficient on the wave length was calculated and compared for the two molecules under the study. We have demontstated that the differences in nucleotide sequence produce significant differences in the absorption spectra. The calculated positions of the resonance peaks were found to be in an excelent agreement with those known from the experiment.

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THEORETICAL ANALYSIS OF DNA LOW-FREQUENCY PHONON MODES

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Objectives:

To calculate far IR absorption spectra of DNA double helixes with known nucleotide sequences and to analize the affect of nucleotide sequences on IR absorption.

Approach:

DNA potential energy is calculated and minimized using a semi-empiric potential for interatomic interactions (molecular mechanics method). Normal modes are calculated as eigenvectors and eigenvalues of the matrix of second derivatives of molecular energy. The ability of each normal mode to add to the DNA absorption spectra is evaluated by analyzing of charge motions produced by the corresponding vibration. The dependence of the absorption coefficient on the wavelength is calculated from normal vibrations of dipole moments of the molecules.

Research focus and impact

Far-IR spectroscopy has a potential to become a detection tool for biological war-fare agents. Nucleic acids are bearers of genetic information, therefore their detection is important. The long-term of the project is to create a theoretical basis for detection of certain patterns of DNA structure and topology, i.e. to detect a signature of particular DNA structures in its far-IR absorption spectrum. The present project is intended to create a classification of DNA low-frequency vibrations in terms of their correlation with nucleotide composition, three-dimensional structure, and ability for radiation absorption. Such a classification is fundamental for the understanding of DNA biological function, because the function of DNA crucially depends on its dynamics. In addition, detection of DNA anomalies is important for the understanding of genetic diseases. As the first step for this long-term goal, we calculated and analyzed low-frequency vibrations of a DNA with know nucleotide composition and far-IR absorption spectra.

Technology transfer and research collaborations

The software for molecular modeling and normal mode analysis (program packages JUMNA and LIGAND) was obtained from the Lab of Dr. Lavery, Institute of Biochemistry and Physical Chemistry, Paris, France. The procedure for DNA normal mode analysis was originally developed by Drs. K. Zacrzewska and Tap Ha Duong (Dr. Lavery's Lab), who consulted us about the usage of this software.

The method for evaluation of radiation absorption by different resonance frequencies was proposed by Dr. B.Gelmont, Dept. of Electrical Engineering, University of Virginia.

The farther investigation of DNA spectroscopy is planned in collaboration with Dr. T.Globus, Dept. of Electrical Engineering, University of Virginia.

Concepts and main results

We calculated vibration frequencies of double helical DNA fragment $(TA)_6$ and and $(A)_{12}$ and evaluated optical properties of all thevibrations. The above DNA sequences were chosen because its far IR absorption spectrum have already been measured (range cm^{-1}, Powell et al., 1987).

The dependence of permittivity ε on radiation frequency ω can be described by the Kramers – Heisenberg dielectric function:

$$\varepsilon(\omega)=\varepsilon_\infty + 4\pi N \sum_k S_k/3(\omega_k^2-\omega^2 - i\,\gamma_k\,\omega/2\pi) , \qquad (1)$$

where index k denotes normal modes, ω_k are vibrational frequencies, S_k are oscillator strengths, γ_k are oscillators dissipations, and N is the number of molecules.

Absorption coefficient (α) is proportional to the imaginary part of permittivity:

$$\alpha= (\omega/nc)\,\mathrm{Im}(\varepsilon), \qquad (2)$$

hence its dependence on frequency ν ($\nu=\omega/2\pi$) is determined by:

$$\alpha(\nu) \sim \nu^2 \sum_k S_k \gamma_k / ((\nu_k^2-\nu^2)^2 + (\gamma_k \nu)^2) \qquad (3)$$

Normal mode analysis enables calculations of normal frequencies ν_k and oscillator strengths S_k, while oscillator dissipations γ_k remain to be determined from the comparison of theory and experiment. In the first approximation, however, the decay can be considered frequency independent. In this simplified form the absorption will depend on the frequency as:

$$\alpha(\nu) \sim \nu^2 \gamma \sum_k S_k / ((\nu_k^2-\nu^2)^2 + (\gamma\nu)^2) \qquad (4)$$

The realistic estimation of γ obtained from the measurements of (poly dA)(poly dT) absorption spectra is from 2 to 7 cm^{-1} [10].

Standard B-helical conformations were created by the program package JUMNA [15-17]. Conformational energy was calculated as a sum of an energy of van der Waals and electrostatic interactions, torsion rotation potentials, harmonic potentials of bond angle deformations, and the energy of hydrogen bonds deformations using FLEX force filed [18]. The energy was minimized in the space of internal coordinates of a molecule (torsion and bond angles) using software the package LIGAND [19].

Normal modes (eigenfrequencies **W** and eigenvectors **A**) were calculated in the space of internal variables as solutions of the equation:

$$\mathbf{HAW = FA} \qquad (5)$$

where W is a diagonal matrix with elements ω_k^2 (vibrational frequencies); **F** is a matrix of second derivatives of potential energy and **H** is a matrix of second derivatives of kinetic energy. The normal modes (matrixes **A** and **W**) were calculated as described in [7] using the program LIGAND.

Each normal vibration produces fluctuations of the dipole moment of a molecule. Oscillator strength S_k corresponding to k^{th} normal mode can be expressed as a square of normalized amplitude (**p**) of the dipole moment deviation [20]:

$$S_k = (\mathbf{p}^k)^2 \qquad (6)$$

The normalized dipole moment **p** can be expressed as:

$$\mathbf{p} = \sum_i e_i \mathbf{a}_i / \sqrt{m_i} \qquad (7)$$

where index i denotes atoms, e_i are partial atomic charges, m_i are atomic masses, and $\mathbf{a}_i$ are eigenvectors (atomic displacements) normalized in such a way that:

$$\sum_i (\mathbf{a}_i)^2 = 1 \qquad (8)$$

The equation (7) was obtained [20] under the classical mechanical approximation ($h\nu << k_B T$). However, we show in the Appendix that (6) is also applicable in ultraquantum approximation ($h\nu >> k_B T$).

The procedure consists of :
1) Initial optimization of the B-helix by the program JUMNA;
2) Energy minimization and calculation of normal modes (eigenfrequencies ω_k and eigenvectors $\mathbf{A_k}$) by the program LIGAND.
3) Calculation of three dimensional structures of a molecule along normal vibrations [7].
4) Calculation of oscillator strengths. The variable dipole moment of a molecule **(P)** is calculated as:

$$\mathbf{P} = \sum e_i \mathbf{r}_i \qquad (9)$$

Where the index i denotes atoms and the vector $\mathbf{r}_i$ is a displacement of i^{th} atom from equilibrium. Oscillator strengths S_k are calculated as:

$$S_k = (\mathbf{P^k})^2_{max} / \sum m_i (\mathbf{r}_i)^2 \qquad (10)$$

where $\mathbf{P^k}$ is produced by k^{th} vibration.
5) Calculation of absorption spectra $\alpha(\nu)$ (Eq. 4).

Oligonucleotides (Poly dA)$_{12}$(Poly dT)$_{12}$ and (Poly dAdT)$_6$ (Poly dTdA)$_6$ have 360 degrees of freedom in the space of internal coordinates of a molecule (torsion and bond angles). Respectively, 360 normal modes were found for each sequence. Their frequencies lie below 900 cm-1 (Fig. 1), so there is almost no overlap with vibrations of covalent bonds which have frequencies above 750 cm^{-1} [21]. The two oligonucleotides have similar mode densities in the region above 250 cm^{-1} , which essentially involve vibrations of valence angles [7]. It could be expected that the modes that reflect vibrations of torsion angles would be more sensitive to the conformation and flexibility of the helix. Indeed, the calculated density spectra of the two oligos differ markedly in the region below 250 cm^{-1}. Specifically, (Poly dAdT)$_6$ (Poly dTdA)$_6$ has very

distinct peaks around 15 and 175 cm^{-1}, while homoplymer (Poly dA)(Poly dT) with unique conformational properties [14] has a broad spectrum with no pronounced peaks (Fig. 1). The earlier normal mode analysis of $(Poly\ dAdT)_6$ $(Poly\ dTdA)_6$ performed with rigid valence angles [7] revealed only one peak at 15 cm^{-1}, which indicates that the second peak at 175 cm^{-1} essentially involves vibrations of valence angles.

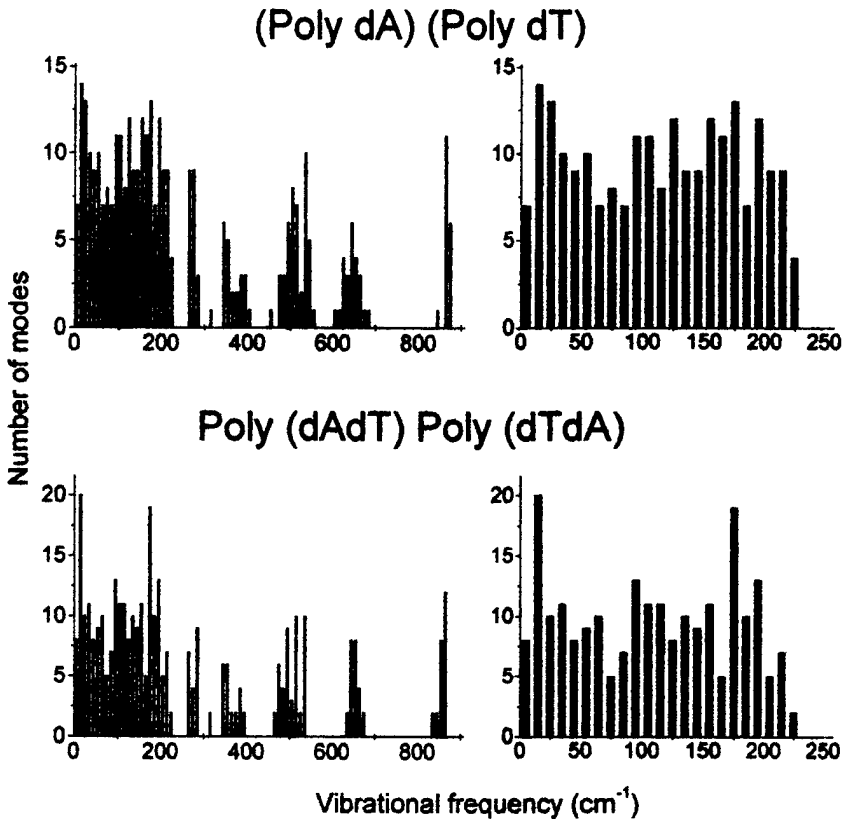

Fig. 1. Density of normal modes. Histograms on the left represent all the calculated modes. The scale is expanded (right panels) to show the lowest frequency modes.

Both Poly(dA)Poly(dT) and Poly(dAdT)Poly(dTd A) have a resonance absorption at the frequency 60 cm^{-1} [10], therefore we examined deviations of a dipole moment $\mathbf{P^k}$ (Eq. 7) produced by the strongest mode with the frequency near 60 cm^{-1} (Fig. 2). This mode was previously assigned to vibrations along the helix axis (Z), however, Fig. 2 demonstrates that the traverse component P_x has comparable amplitude.

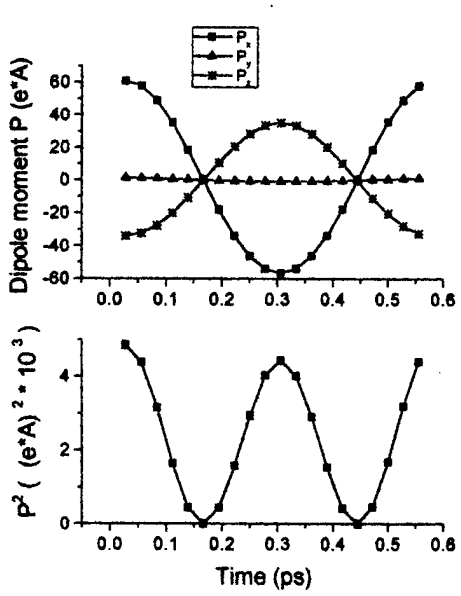

Fig. 2. Fluctuations of the dipole moment P^k of the molecule $(Poly\ dAdT)_6$ $(Poly\ dTdA)_6$ corresponding to a normal vibration with a frequency 60.3 cm^{-1} at the room temperature. The double helix is oriented along the axe Z. The amplitude of $(P^k)^2$ fluctuation is used in Eq. 10.

The amplitude of $\mathbf{P^2}$ fluctuations determines the strength of each vibration (Eq. 9). Normal modes of the two examined oligos demonstrated very different distributions of oscillator strengths. As would be expected, for the molecule with homogeneous strands Poly(dA)Poly(dT) the oscillator strengths appeared to be very uniform, while heterogenity in the strands composition introduces many-fold variance (Fig. 3). The two lowest-frequency modes (2.1 and 2.6 cm^{-1}) have strengths 3-4 times greater than the average

strength for the other modes. These two lowest-frequency vibrations are have strong P components perpendicular to the helical axe, and they apparently reflect helical bending [7, 22]. The strongest modes of the heteropolymer (Fig. 3 A) also lie in the lowest frequency range (mostly below 50 cm^{-1}). This result is not surprising, for the strongest modes reflect the relative motions of the strands, which in turn involve low-frequency torsion rotations. Correspondingly, the normal modes spectrum weighted by oscillator strengths is essentially shifted towards low-frequencies (Fig. 4A compared to Fig 2A) for the heteropolymer, while for the homopolymer heterogenity in oscillator strengths has a minor effect compared to the normal mode density itself.

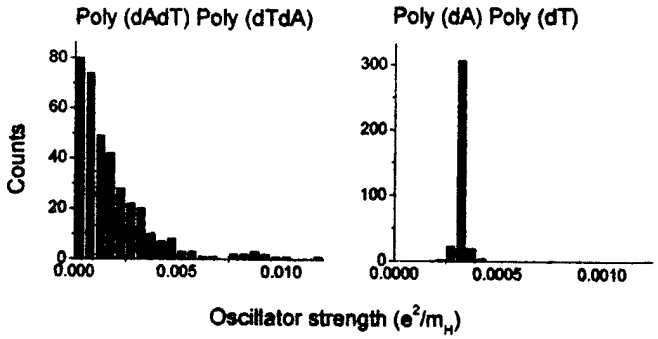

Fig.3. Distributions of oscillator strengths for normal modes.

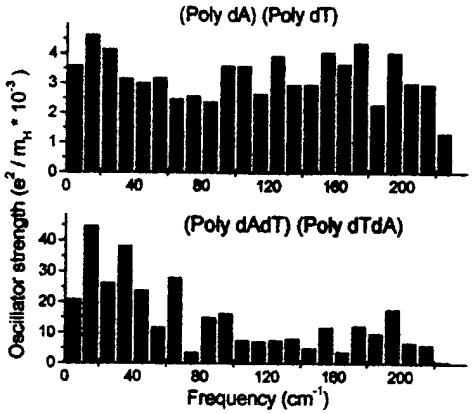

Fig. 4. Density of normal modes weighted with their oscillator strengths. Data are normalized to represent the oscillator strength per normal mode

We calculated absorption spectra of the two molecules (Fig.5) according to Eq. 4 using two reasonable approximations for the band width γ obtained experimentally (2 and 7 cm^{-1}, [10]). The positions of resonance peaks do not depend on the band width (Fig. 5), and in the longer wave range (below 220 cm^{-1}) they are remarkably similar to those observed experimentally (Table 1).

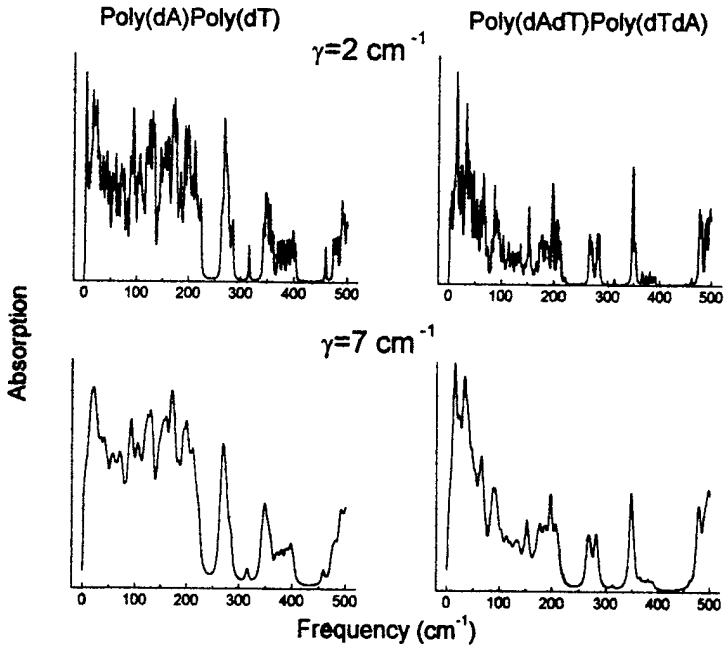

Fig. 5. Absorption spectra (arbitrary units) of $Poly(dA)_{12}Poly(dT)_{12}$ (left) and (Poly dAdT)$_6$ (Poly dTdA)$_6$ (right) calculated at two values (2 and 7 cm^{-1}) of the band width γ.

Table 1. Resonance frequencies for Poly(dA)Poly(dT). All numbers are in units cm^{-1}

Calculations	Experiment [10]
20	unknown
43	unknown
57	62
71	80
94	95
106	106
131	136
160	-
172	170
199	-
211	214
-	238
270	-
348	-
400	-
459	-

Another similarity of the calculated and experimental spectra is the steep fall in absorption intensities as the frequency exceeds 250 cm^{-1} for both sequences. This feature is caused by the decrease in normal mode densities and is not related to their optical properties. Finally, Poly(dAdT)Poly(dTdA) demonstrates higher absorption at lower frequencies (below 100 cm^{-1}) due to the higher oscillator strengths of its lower frequency modes, while Poly(dA)Poly(dT) has a plateau between 20 and 200 cm^{-1} . This difference in the spectra has also been observed experimentally [10].

Our results indicate a strong resonance peak around 20 cm^{-1} for the both sequences. While, no experimental data on IR absorption is available in this frequence range, Raman scattering [24] clearly indicates resonance peaks around 20 cm^{-1} (16.2, 193. 3 and 23.3 cm^{-1})

Publications

T.R.Globus, D.L.Woolard, M. Bykhovskaia, B.Gelmont, J. L. Hesler, T. W. Crowe, A.C. Samuels, "FTIR-Spectroscopic Characterization of DNA Macromolecules" , International Semiconductor Device Research Symposium (ISDRS), Proceedings, Charlottesville, USA, 1999

M. Bykhovskaia, B. Gelmont, T. Globus, D.L. Woolard[3], A. C. Samuels[4], T. Ha-Duong[5], and K. Zakrzewska. Calcuation of DNA low-frequency absorption spectra. (Submitted).

D. L. Woolard, T. R. Globus, B. L. Gelmont, M. Bykhovskaia, A. C. Samuels, D. Cookmeyer, J. L. Hesler, T. W. Crowe, J. O. Jensen, J. L. Jensen and W.R. Loerop. Submilimeter-wave phonon modes in DNA macromolecules. (Submitted).

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