THz-Spectroscopy of Biological Molecules

T.R. GLOBUS¹, D.L. WOOLARD², T. KHROMOVA¹, T.W. CROWE¹, M. BYKHOVSKAIA³, B.L. GELMONT¹, J. HESLER¹ and A.C. SAMUELS⁴

¹*University of Virginia, Charlottesville, VA*

²*U.S. Army Research Laboratory, RTP, NC*

³*Dept. of Biological Sciences, Lehigh University, Bethlehem, PA*

⁴*U.S. Army SCBRD, Edgewood, MD*

Abstract. The terahertz frequency absorption spectra of DNA molecules reflect low-frequency internal helical vibrations involving rigidly bound subgroups that are connected by the weakest bonds, including the hydrogen bonds of the DNA base pairs, and/or non-bonded interactions. Although numerous difficulties make the direct identification of terahertz phonon modes in biological materials very challenging, our research has shown that such measurements are both possible and fruitful. Spectra of different DNA samples reveal a large number of modes and a reasonable level of sequence-specific uniqueness. In an attempt to show that the long wavelength absorption features are intrinsic properties of biological materials determined by phonon modes, a normal mode analysis has been used to predict the absorption spectra of polynucleotide RNA Poly[G]-Poly[C]. Direct comparison demonstrated a correlation between calculated and experimentally observed spectra of the RNA polymers, thus confirming that the fundamental physical nature of the observed resonance structure is caused by the internal vibration modes in the macromolecules.

In this work we demonstrate results from Fourier-Transform Infrared (FTIR) spectroscopy of DNA macromolecules and related biological materials in the terahertz frequency range. Careful attention was paid to the possibility of interference or etalon effects in the samples, and phenomena were clearly differentiated from the actual phonon modes. In addition, we studied the dependence of transmission spectra of aligned DNA and polynucleotide film samples on molecule orientation relative to the electromagnetic field, showing the expected change in mode strength as a function of sample orientation. Further, the absorption characteristics were extracted from the transmission data using the interference spectroscopy technique, and a strong anisotropy of terahertz characteristics was demonstrated.

Key words: Absorption, anisotropy, biological molecules, terahertz, transmission spectroscopy, vibration modes

1. Introduction

There exists considerable interest in both the experimental and theoretical investigation of the low-frequency internal vibrations associated with Deoxyribonucleic acid (DNA) polymers. The study of molecular dynamics, achieved via scattering or absorption spectroscopy, is a viable and proven approach that has been applied widely for the general characterization of molecular structure [1]. Since the terahertz frequency regime (i.e., ~ 0.1 – 10 THz) is predicted to be fairly rich with

spectral features that are dependent on DNA internal vibrations spread over large portions of the complex molecule [2], it is reasonable to expect results that are dependent on the primary sequence of the molecule. The focus of this paper is to demonstrate the use of terahertz spectroscopy for the characterization of DNA polymers and for the future interpretation of phonon modes.

Most spectral investigations of DNA have been performed at frequencies above 10 THz. Below this range, a very limited number of spectroscopic studies have been reported until recently because of the many challenges related to the terahertz frequency band which spans from 100 GHz through 10 THz. [see for example, 3–8]. Although numerous difficulties make the direct identification of terahertz phonon modes in biological materials very challenging, our recent research on the experimental and theoretical aspects of terahertz transmission spectroscopy of biological macromolecules has shown that such measurements are both possible and fruitful. Detailed experimental spectra of DNA macromolecules and complete cellular biological samples over the spectral range 10 cm^{-1} to 500 cm^{-1} have been obtained [9–12]. The results demonstrated evidence of high level numerical multiple resonances due to low frequency vibrational phonon modes in qualitative agreement with the theoretical prediction [13]. Computational methods used to predict the low frequency absorption spectra of short artificial DNA and RNA have also been demonstrated [14, 15]. In addition, the preliminary application of Neural Network Analysis for the raw data generalization of very far FTIR spectra of genetic materials from fish indicated a high degree confidence in the recognition of DNA samples [10]. Absorption spectra of oligonucleotides with known base-pair sequences (a 12 base pair RNA fragment Poly[G]-Poly[C]) (G-Guanine, C-Cytosine) have been measured and calculated in the sub-millimeter-wave range [15]. The results demonstrate a correlation between the calculated and experimentally observed spectra of RNA homopolymers confirming the fundamental physical nature of the observed resonance structures that are caused by the internal vibration modes in macromolecules.

The discovery of terahertz vibrational spectra of biological molecules is important for a number of reasons. The far-infrared region of the DNA absorption spectra, $3\text{--}300\text{ cm}^{-1}$ (or $0.1\text{--}10\text{ THz}$), reflects low-frequency molecular internal motions. In this range, the spectral features from bio-polymer molecules arise out of poorly localized low-frequency molecular internal vibrations such as twisting, bending and stretching of the double helix, sugar pseudorotational vibrations and fluctuations of weakest bonds or non-bonded interactions (van der Waals forces, dispersion forces, and hydrogen bonding) [see for example, 16–20]. The internal motions, dependent on the weak hydrogen bonds of the double-helix base-pairs [21], are extremely sensitive to DNA composition and topology and have an impact on the main processes related to the transfer of genetic information, such as replication, transcription and viral infection [22]. Hence, phonon modes that arise in this range should reflect features specific to the DNA code. Furthermore, theoretical studies have predicted the occurrence of DNA phonon frequencies throughout this region

[19, 23–25]. Therefore, the investigation of the far-infrared region of the DNA absorption spectra and the identification of DNA low-frequency internal motions are direct ways to obtain information about the peculiarities of DNA topology and internal motion. Terahertz spectroscopy coupled with theoretical prediction can become a powerful tool for the investigation of the DNA structure and possible biological function.

The identified physical mechanisms of interactions between radiation and the biological material in the terahertz region are also important to bio-detection since this initial work demonstrated that a large number of species-specific phonon modes might be detected relatively easily. Thus, phonon modes that arise in the very far-infrared region can serve as a signature of specific DNA-molecules.

This paper presents results of spectroscopic studies of biological molecules in submillimeter range. Transmission spectra of aligned DNA and polynucleotide film samples were measured depending on molecule orientation relative to the electromagnetic field of radiation. Absorption characteristics are extracted from transmission data using the interference spectroscopy technique, and a strong anisotropy of optical characteristics is demonstrated. Furthermore, these experimental measurements confirm our earlier conclusion that the measured resonance modes are not the result of any sort of interference effects in the samples.

2. Experimental Technique

Fourier Transform Infrared (FTIR) spectroscopic investigations of DNA films and other biological materials were performed over the spectral range from $\sim 10\text{ cm}^{-1}$ to 25 cm^{-1} [9–12]. The spectral studies in transmission mode utilized a Bruker IFS-66 spectrometer equipped with a mercury-lamp source and a liquid-helium-cooled Si-bolometer ($T = 1.7^\circ\text{ K}$) for signal detection. All the measurements were made with samples in a vacuum to eliminate any influence of atmospheric water-absorption lines. Both the material absorption characteristics and the refractive indexes have been investigated. Test measurements with a wire polarizer ($25\text{ }\mu\text{m}$ wire diameter and $75\text{ }\mu\text{m}$ spacing between wires) were performed indicating 80% polarization of radiation in the FTIR spectrometer in the spectral range of interest and the electric field vector E oriented preferably in the vertical direction.

Film samples were prepared from a water or buffer solution gel using the technique described earlier [10–12]. Film thickness varied in the range $3\text{--}200\text{ }\mu\text{m}$. The ratio of liquid to dry material content in the initial gel was in the range from 5:1 to 25:1. Free-standing films were used for measurements in most cases, although polycarbonate membranes with thickness $8\text{ }\mu\text{m}$ and about 98% transmission were used as supporting substrates for the thinnest samples. To receive good resolution, samples of at least $1/2''$ diameter were fabricated. Herring, salmon and calf thymus DNA sodium salts, as well as single stranded RNA – potassium salt, poly[A], and double stranded RNA – sodium salts: Poly[C]-Poly[G] and Poly[A]-Poly[U] from Sigma Chemicals were used for this investigation.

Figure 1. Image (40X) of the Salmon DNA sample (free standing film with thickness about 10 μm) in polarizing Microscope (OLYMPUS, model BX60). Gel concentration 1:10.

DNA, as other typical rod-like polymers, spontaneously forms ordered liquid crystalline phases in aqueous solution above some critical concentration (exceeding 100 milligrams per milliliter) [26] with the long rod axis preferentially aligned in one direction. In drying process, DNA film samples are characterized by their microscopic textures with periodic variations in refractive index and fringe patterns observed with polarizing microscope (Figure 1). Also, the gel-samples can be mechanically aligned to provide preferable orientation of long rod axes in one direction. However, the film texture depends on the concentration of molecules in solution and on drying conditions as well.

Figure 2 demonstrates the sensitivity of the measurements. It shows the typical intensity change in the fine resonance structure of biomolecules measured with a resolution 0.25 cm^{-1} . The transmission of a polycarbonate membrane substrate (PC) and the 100% transmission line (free path measurements) are also shown. Although the observed amplitude of the resonant modes is not greater than several percent, the signal-to-noise ratio of the instrument is good enough to detect these features in spectra of free standing films, as well as of films on polycarbonate substrates. The fine structure observed here requires spectral resolution better than 0.5 cm^{-1} , which is difficult to achieve because of the very low source energy in the terahertz range and because of easy to generate standing waves in a measurement system. This requirement may partially explain why earlier FTIR investigations were not able to detect phonon resonances.

Far IR spectra show a great sensitivity to factors that are affected by differences in sample preparation, and it is not immediately clear to what extent resonant

Figure 2. Transmission spectra of calf thymus (CT) DNA. Reproducibility of background spectrum (100% line) and transmission of polycarbonate membrane substrate (PC) are also shown. The structure around 18.5 cm^{-1} is due to traces of water absorption.

features in Figure 2 are reproducible and represent features intrinsic to the DNA molecules. Films of various thicknesses were studied to confirm that the observed resonances were directly attributable to the absorption characteristics of the biological material. The thinnest samples (less than ten micron thickness) had reasonably good planarity, although they sometimes had an imperfection density (i.e., voids) of about 5–10%. In this study we present results on samples with aligned DNA chains.

3. Experimental Results and Discussion

An extensive series of measurements in the terahertz frequency region with a resolution of 0.25 cm^{-1} revealed resonance features in the spectra that can be more or less pronounced depending on the quality of material and sample preparation and measurement conditions. Peaks appear with a density of approximately one per cm^{-1} in the interval between 10 and 200 cm^{-1} . This general density of modes had been predicted earlier in theoretical studies [20, 14] if oscillator decay is smaller than 1 cm^{-1} . The intensity of absorption lines gradually drops towards the upper end of the band. However, at the lower end of the band the measurements were limited by the power of the source.

Figure 3. Two independent measurement results of Poly [A] RNA film transmission spectra taken with the same orientation. Resonance features are observed at the background of large scale fringes caused by the multiple reflection at the film surfaces.

As was found in our previous works [11], the terahertz characteristics are sensitive to sample preparation and measurement conditions. Most importantly, in order to successfully resolve phonon resonances in biological materials one must consider several important effects that are dependent on the sample and measurement geometry. For example, the mode coupling strength can be affected by the polarization of the electromagnetic field in relation to the dipole moment of the DNA oscillators. Thus sample rotation and the polarization level of the source can affect the structure of the spectra. Even more importantly, films of uniform thickness are well known to exhibit interference fringes in their reflection and transmission spectra. These interference phenomena can obscure fine resonance features or be misinterpreted as modes themselves. Therefore great care must be taken to understand these effects and to differentiate them from the measured mode structure.

Figures 3 and 4 demonstrate the importance of interference effects and sample orientation in the electromagnetic field of radiation. Two transmission spectra of Poly [A] RNA with the same orientation (Figure 3) show the high sensitivity and reasonably good reproducibility of the measurements. For this relatively thick sample, the fine mode structure is resolved on the envelope of the wide interference pattern caused by the multiple reflections at the film surfaces. The position of trans-

Figure 4. Transmission spectra of partially aligned Poly[A] sample measured at two different orientation of a long rod axes Z relative to the electric field of radiation E.

mission maxima and minima of the interference pattern can be used to estimate the refractive index of the biomaterial, n , from the equation

$$nd = \frac{m\lambda_{extr}}{4}, \quad (1)$$

where λ_{extr} is the wavelength of transmission maximum or minimum and m is an integer number corresponding to the order of extremum, including maxima and minima ($m = 1$ for the first transmission minimum, and $m = 2$ for the following transmission maximum) [27]. The order of extremum m are found from the wavelengths of two adjacent extrema in the spectral range where n does not change appreciably when m is increased by one. The first transmission maximum corresponding to $m = 0$ occurs at zero frequency. The position of fringes is very sensitive to the thickness of the film, d , and the fringe with the same number shifts to lower frequencies with reducing d . The estimates of n are not very accurate due to distortion of the interference pattern by resonance features and the imperfect uniformity of the sample thickness.

Figure 4 shows significant difference in transmission spectra of a partially aligned Poly[A] sample measured at two different orientations of a long rod axes Z relative to the electric field of radiation E. The thickness of this sample is only $16 \mu\text{m}$ and the first transmission minimum occurs at much higher frequencies than 25 cm^{-1} . Thus, for such a thin sample the interference pattern is virtually eliminated from the data. Also, the transmission is strongly effected by the sample rotation, which

Figure 5. Transmission spectra of herring DNA film measured at two orientations.

would certainly not be the case if the remaining modes were caused by a ‘etalon effect.’ Rather, the much lower transmission measured with the electric field of radiation E perpendicular to the long axes of molecules, indicates a higher mode absorption for this orientation. The fact that the resonance structure is much more pronounced at this orientation correlates with the higher oscillator strengths, as was predicted by the modeling results [15]. Higher absorption in the sample can suppress the interference pattern, as is demonstrated on Figure 5 for herring DNA films. From comparison of two curves presented in Figure 5 it is clear that the refractive index is 5–10% higher in the case E perpendicular to Z .

Measured transmission spectra were used to extract the absorption coefficient spectra and refractive index of biological material by applying an interference spectroscopy technique (IST) for proper modeling of the multiple reflection behavior. [28, 29] The estimated values of refractive index varied between 1.7 and 2.3. These results are in qualitative agreement with a refractive index of natural DNA at relative humidity 1% near 6 cm^{-1} measured to be 1.9 using a polarizing millimeter wave interferometer [30].

Absorption spectra of a Poly[A] film as derived at two orientations are demonstrated in Figure 6. Strong anisotropy of terahertz characteristics of biological molecules is obvious. This result is also confirmed by the modeling results for absorption intensities of longitude and transverse modes as described in [15] and by the direct comparison of measured absorption spectra of 12 base pair RNA fragment Poly[G]-Poly[C] derived from transmission measurements with the theoretical prediction. This comparison, shown on Figure 7, demonstrates a correl-

Figure 6. Absorption spectra of partially aligned Poly[A] sample measured at two different orientation of a long rod axes Z relative to the electric field of radiation E (obtained from transmission data in Figure 4).

Figure 7. Absorption spectrum of Poly[C]-Poly[G] from measurements and modeling results for longitude and transverse absorption modes.

ation between calculated and experimentally observed spectra of RNA polymer, thus confirming that the fundamental physical nature of the observed resonance structures is caused by the internal vibration modes in macromolecules.

4. Conclusions

The transmission spectra of partially aligned films of DNA and polynucleotide molecules were carefully measured in the terahertz frequency range. The effect of multiple reflections in films were carefully considered and were easily distinguished from the actual phonon modes. The effect of the orientation of the macromolecules relative to the electromagnetic field of the terahertz radiation was also analyzed. The results were consistent with the theoretical predictions of the dependence of the mode strength on the polarization of the terahertz field with respect to the molecule alignment. Absorption spectra from experimental data were then compared with the modeling results for polynucleotides of known base-pair sequences – the 12 base pair RNA fragment Poly[G]-Poly[C]. A significant correlation between the calculated and experimentally observed spectra of RNA homopolymers was achieved. Taken together these results confirm the fundamental physical nature of the observed resonance structure that is caused by the internal vibrational modes in the DNA macromolecules. Furthermore, these experimental results indicate that theoretical modeling, combined with measured data, may be used to directly assign vibrational modes to specific structural features of the macromolecules

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References

1. Sarkar, M., Sigurdsson, S., Tomac, S., Sen, S., Rozners, E., Sjoberg, B.-M., Stromberg, R. and Graslund, A.: A Synthetic Model for Triple-Helical Domains in Self-Splicing Group I Introns Studied by Ultraviolet and Circular Dichroism Spectroscopy, *Biochemistry* **35** (1996), 4678–4688.
2. Van Zandt, L.L. and Saxena, V.K.: In: R.H. Sarma and M.H. Sarma (eds.), *Structure & Functions*, **Volume 1: Nucleic Acids**, Adenine Press, 1992 and references therein.
3. Beetz, C.P. and Ascarelli, G.: Far Infrared Absorption of dNA and PolyI-PolyC RNA, *Biopolymers* **21** (1982), 1569.
4. Wittlin, A., Genzel, L., Kremer, F., Haseler, S., Poglitsch, A. and Rupprecht, A.: Far-Infrared Spectroscopy on Oriented Films of Dry and Hydrated DNA, *Phys. Rev. A* **34** (1986), 493.
5. Powell, J.W., Edwards, G.S., Genzel, L., Kremer, F., Wittlin, A., Kubasek, W. and Peticolas, W.: Investigation of Far-Infrared Vibrational Modes in Polynucleotides, *Phys. Rev. A* **35** (1987), 3929–3939.
6. Weidlich, T., Powell, J.W., Genzel, L. and Rupprecht, A.: Counterion Effects on the far-IR Vibrational Spectra of Poly (rI):Poly(rC), *Biopolymers* **30** (1990), 477–480.

7. Powell, J.W., Peticolas, W. and Genzel, L.: Observation of the far-IR Spectra of Five Oligonucleotides, *J. Mol. Struct.* **247** (1991), 119.
8. Markeltz, A.G., Roitberg, A. and Heilweil, E.J.: Pulsed Terahertz Spectroscopy of DNA, Bovine Serum Albumin and Collagen between 0.1 and 2.0 THz, *Chem. Phys. Lett.* **320** (2000), 42.
9. Globus, T., Woolard, D.L., Bykhovskaia, M., Gelmont, B., Hesler, J.L., Crowe, T.W. and Samuels, A.C.: *Proc of International Semiconductor Device Research Symposium (ISDRS)*, Charlottesville VA, 1999, p. 485.
10. Globus, T., Dolmatova-Werbos, L., Woolard, D., Samuels, A., Gelmont, B. and Bykhovskaia, M.: Application of Neural Network Analysis to Submillimeter-Wave Vibrational Spectroscopy of DNA Macromolecules, *International Symposium on Spectral Sensing Research Proceedings (ISSSR)*, Canada, Quebec, June 2001, p. 439.
11. Globus, T., Woolard, D.L., Samuels, A.C., Gelmont, B.L., Hesler, J., Crowe, T.W. and Bykhovskaia, M.: Submillimeter-Wave FTIR Spectroscopy of DNA Macromolecules and Related Materials, *J. Appl. Phys.* **91** (May 2002), 6106–6113.
12. Woolard, D.L., Globus, T.R., Gelmont, B.L., Bykhovskaia, M., Samuels, A.C., Cookmeyer, D., Hesler, J.L., Crowe, T.W., Jensen, J.O., Jensen, J.L. and Loerop, W.R.: Submillimeter-Wave Phonon Modes in DNA Macromolecules, *Phys. Rev E* **65** (May 2002), 051903.
13. Van Zandt, L.L. and Saxena, V.K.: Vibrational Local Modes in DNA Polymer, *J. Biomol. Struct. & Dyn.* **11** (1994), 1149–1159.
14. Bykhovskaia, M., Gelmont, B., Globus, T., Woolard, D.L., Samuels, A.C., Ha-Duong, T. and Zakrzewska, K.: Prediction of DNA Far IR Absorption Spectra Basing on Normal Mode Analysis, *Theor. Chem. Acc.* **106** (2001), 22–27.
15. Globus, T., Bykhovskaia, M., Gelmont, B. and Woolard, D.L.: Far-infrared Phonon Modes of Selected RNA Molecules, In: J.O. Jensen and R.L. Spellicy (eds.), *Instrumentation for Air Pollution and Global Atmospheric Monitoring*, Proceedings of SPIE, **4574** 2002, pp. 119–128.
16. Mei, W.N., Kohli, M., Prohofsky, E.W. and Van Zandt, L.L.: Acoustic Modes and Nonbonded Interactions of the Double Helix, *Biopolymers* **20** (1981), 833–852.
17. Young, L., Prabhu, V.V. and Prohofsky, E.W.: Calculation of Far-Infrared Absorption in Polymer DNA, *Phys. Rev. A* **39** (1989), 3173.
18. Feng, Y. and Prohofski, E.M.: Vibrational Fluctuations of Hydrogen Bonds in a DNA Double Helix with Nonuniform Base Pairs, *Biophys. J.* **57** (1990), 547–553.
19. Lin, D., Matsumoto, A. and Go, N.: Normal Mode Analysis of a Double-Stranded DNA Dodecamer d(CGCGAATTCGCG), *J. Chem. Phys.* **107**(9) (1997), 3684–3690.
20. Duong, T.H. and Zakrzewska, K.: Calculation and Analysis of Low Frequency Normal Modes for DNA, *J. Comp. Chem.* **18**(6) (1997), 796–811.
21. Weidlich, T., Lindsay, S.M., Rui, Q., Rupprecht, A., Peticolas, W.L. and Thomas, G.A.: A Raman Study of Low Frequency Intrahelical Modes in A-, B-, and C-DNA, *J. Biomol. Struct. Dyn.* **8** (1990), 139.
22. Sinden, R.R.: *DNA Structure and Function*, Academic Press, San Diego, 1994.
23. Prohofsky, E.W.: *Statistical Mechanics and Stability of Macromolecules*, Cambridge University Press, 1995.
24. McCammon, J.A. and Harvey, S.C.: *Dynamics of Proteins and Nucleic Acids*, Cambridge University Press, 1986.
25. Lafontain, I. and Lavery, R.: Collective Variable Modeling of Nucleic Acids, *Curr. Op. Struct. Biol.* **9** (1999), 170–176.
26. Davidson, M.W., Rill, R.L. and Van Winkle, D.H.:
<http://micro.magnet.fsu.edu/dna/cholesteric.html>
27. Moss, T.S., Burrell, G.J. and Ellis, B.: *Semiconductor Opto-electronics*, Butterworth & Co. (Publishers) Ltd., 1973.

28. Globus, T., Ganguly, G. and Roca i Cabarrocas, P.: Optical Characterization of Hydrogenated Silicon Films using Interference Technique, *J. Appl. Phys.* **88** (2000), 1907.
29. Woolard, D., Globus, T., Brown, E., Werbos, L., Gelmont, B. and Samuels, A.: Sensitivity Limits & Discrimination Capability of THz Transmission Spectroscopy as a Technique for Biological Agent Detection, *Proc. of 5th Joint Conference on Standoff Detection for Chemical and Biological Defence (5JCSD)*, Williamsburg, VA, Sept. 2001.
30. Genzel, L., Poglitsch, A. and Haeseler, S.: Dispersive Polarizing mm-wave Interferometer, *Int. J. Infrared Millimeter Waves* **6** (1985), 741–750.