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Millimeter Wave-induced Vibrational Modes in DNA as a Possible Alternative to Animal Tests to Probe for Carcinogenic Mutations

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Developing methods for alternative testing is increasingly important due to dwindling funding resources and increasing costs associated with animal testing and legislation. We propose to test the feasibility of a new and novel method for detecting DNA mutagenesis using millimeter wave spectroscopy. Although millimeter wave spectroscopy has been known since the 1950s, the cost was prohibitive and studies did not extend to large biological proteins such as DNA. Recent advances have made this technology feasible for developing laboratory and field equipment. We present preliminary findings for lesion-induced vibrational modes in DNA observed from 80 to 1000 gigahertz (GHz). These findings suggest that there are vibrational modes that can be used as identification resonances. These modes are associated with localized defects of the DNA polymers. They are unique for each defect/lesion, and should be easy to detect. We described a field-detecting detector based on the local modes. © 1997 John Wiley & Sons, Ltd. *J. Appl. Toxicol.*, Vol. 17(4) 243–246 (1997)

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INTRODUCTION

It has long been the goal of scientists to develop rapid *in vitro* scans for carcinogenic substances that are both accurate and relatively inexpensive[1–5] and also do not involve the direct use of animals.[6–8] Cancer is thought to arise in most cases from damage to DNA, either directly or indirectly. Humans are constantly exposed to chemicals in the environment that can potentially damage DNA.[1] Standard methods for detecting DNA damage are indirect and relatively coumbersome.[9–12]

It is the goal of this paper to examine the feasibility of using spectroscopic techniques[13–17] as a probe for possible detection of damage to DNA. In particular microwave and millimeter wave technology will be examined in detail.[18–21] The two strands of DNA are held together with relatively weak hydrogen bonds between the base pairs of the DNA. Phonon modes or sound waves can propagate up the DNA helix, causing a slight displacement of the hydrogen bond distance between the base pairs as the sound wave moves by the base pairs. It is probable that resonances caused by these sound waves could change dramatically as the DNA is damaged. It has been predicted that the phonon modes in DNA can be excited by electromagnetic radiation in the 80–1000 GHz region of the spectra.[18–21] However, these phonon modes have never been measured experimentally until now. This paper represents the first direct measurement of phonon modes in DNA.

Recent advances in understanding the interaction between microwave/millimeter wave (mm wave) radiation and living matters[18–22] have opened new avenues in the detection and identification of microorganisms. In particular, deoxyribonucleic acid (DNA) is thought to interact with electromagnetic radiation in the mm wave region of the spectra due to the presence of phonon modes and plasmon modes of base pairs along the double helix of the DNA chain. Solitons may also be present in the DNA chain, but in nature these are non-linear waves and would require significant excitation energies.[18] Phonon modes are primarily mechanical in nature and travel along the DNA chain at a speed of about 2 km s^{-1}. The plasmon modes are essentially charge density waves and are thought to travel along the DNA chain at a speed of about 36 km s^{-1}.[19,20]

There is some evidence that the plasmon modes may be overdamped and thus are extremely difficult to observe.[19] On the other hand, the phonon modes are well-defined resonances over a wide range of the frequency spectrum (from a few GHz to several THz), and should be readily accessible to experimental observation.[21]

RESULTS AND DISCUSSION

Traditionally, the 80–1000 GHz region of the electromagnetic spectrum has been difficult for spectral obser-

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vations. Not until very recently have techniques become available for such spectroscopic studies. The purpose of this work is to use some of these techniques to observe and identify some of the phonon modes of the DNA polymers. In the following, we report on our preliminary findings.

In the frequency range of interest here (i.e. from a few GHz to several THz) the spectral features predicted by theoretical studies mainly arise from localized motions, spread over one or more entire base-pair units. Detailed descriptions will depend on the strength and range of the interactions, such as van der Waals interaction, electronic exchange interactions, coulombic interactions, and hydrogen bonding. Based on available physical parameter values and reasonable assumptions, a series of resonances are predicted in this spectral region. These are in frequency at 88, 89, 110, 172, 232, 300, 382, 418, 503, 561, 638, 784, 891, 920, and 1019 GHz. These previous predictions may only serve as a guide insofar as some of the parameters are not known exactly and, more importantly, the theories are model dependent and may not be completely accurate. For example, shifted frequencies and change intensities are to be expected for DNA polymers in different environment, e.g. in water solutions or other solutions, as compared with dry samples. However, it is reasonable to assume that at or near some of the resonant frequencies predicted some spectral features should be detectable.

In a preliminary set of experiments we attempted to detect some of the resonances using microwave absorption spectroscopy. For example, Fig. 1 shows spectral data taken for two different DNA samples using an HP 8510 W-band (i.e., 85–110 GHz) tester. Here, the dried DNA salts were mechanically loaded into a 100-mil long shorted section of waveguide and S_{11} data was taken. For the Herring DNA data in Fig. 1(A), a broad absorption peak (i.e., S_{11} valley) is indeed clearly visible centered about 90 GHz. This may actually correspond to multiple frequency resonances separated by several GHz, which is beyond the resolution of our experiment. Figure 1(B) shows the same spectral region but for absorption of salmon DNA. Both the center of the major peak and the relative intensity are changed compared with Fig. 1(A), reflecting the subtle differences between relatively similar DNA species.

Figure 2 shows the microwave absorption spectrum of the same Herring DNA sample in the frequency region of 180–220 GHz. Here, a Millitech frequency-domain up-conversion unit was used to perform transmission measurements through a 75-mil section of Herring DNA contained within a Teflon sample holder. As shown, the solid DNA yields a broad absorption region with a maximum starting at approximately 212 GHz and extending outside the testing window.

Measurements of the Herring DNA sample were also taken over a much broader frequency range utilizing a Bell Labs T-Ray source.[22,23] Here, femtosecond laser pulses are down-converted using an Austin switch to arrive at electromagnetic pulses with time-domain variations of fractions of picoseconds. Standard Fourier transformation techniques were then applied to determine the frequency-domain transmission characteristics through a very thin layer (i.e., 1–4 mm) of the same Herring DNA sample. Figure 3 shows power-absorption results, for transmission through four different locations of the sample layer, in the frequency region of 100–400 GHz. Here, relatively well-defined peaks were resolved at 160, 180, 230, 260, 290, 330 and 390 GHz. It should be noted that reflection effects had to be removed from this data in the time-to-frequency domain transformation step. Hence, additional measurements will need to be performed to confirm the resonances observed in Fig. 3.

In all the above experiments the DNA samples used were obtained from Sigma Chemical Co. and were

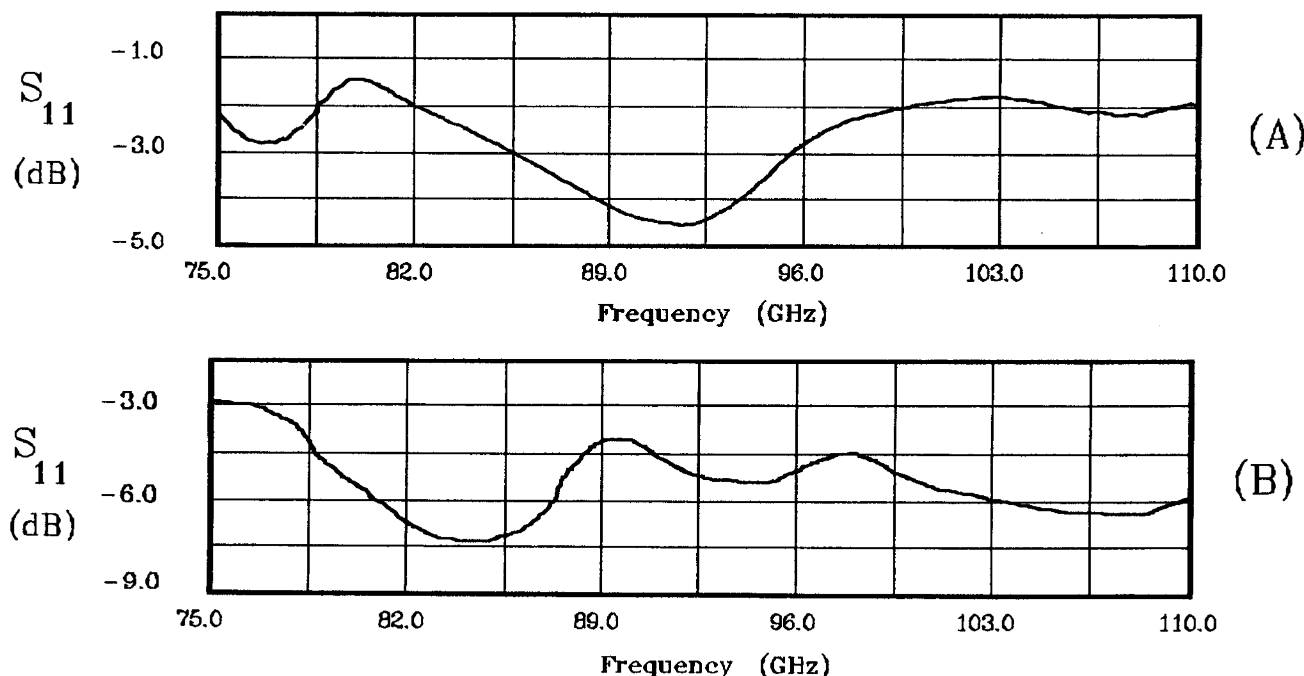

Figure 1. S_{11} data taken from a cavity filled with (A) herring DNA sample and (B) salmon DNA sample.

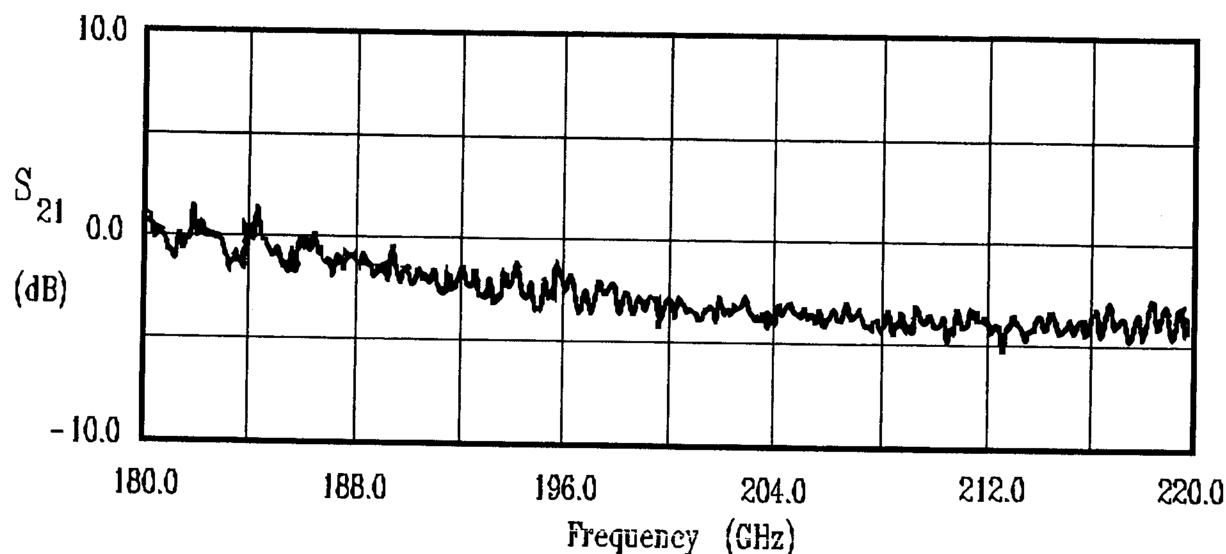

Figure 2. S_{21} data taken from transmission through a 75-ml thick section of herring DNA sample.

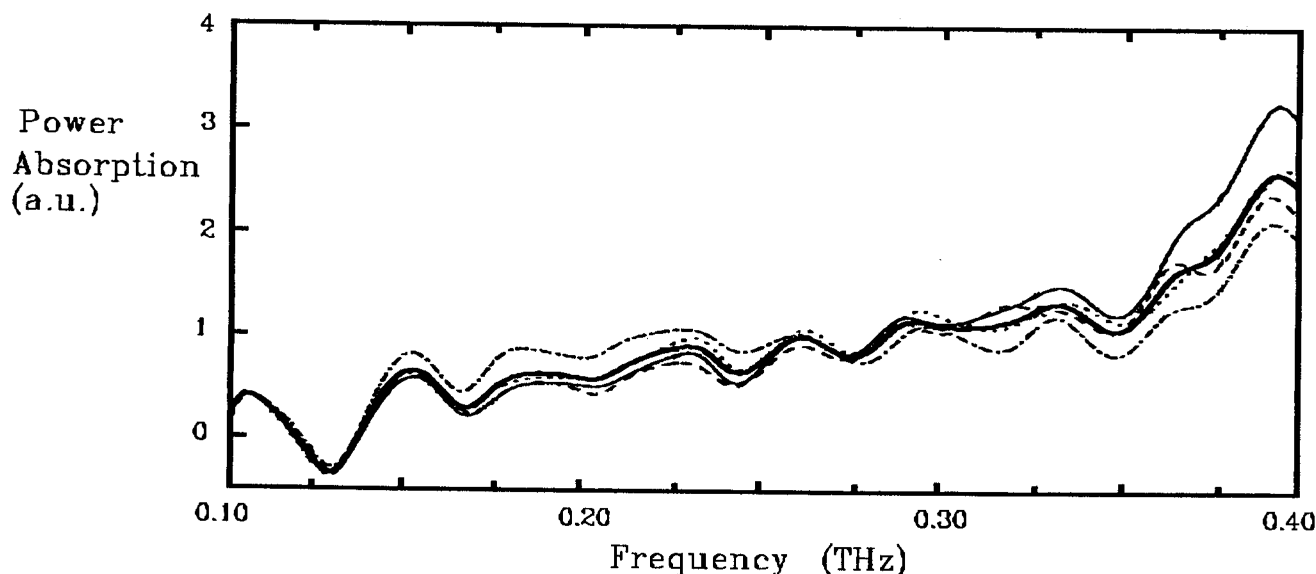

Figure 3. Power absorbtion spectrum for herring DNA. Thick solid line is average over four locations.

tested in their original dry sodium (DNA-Na) forms. Traditionally, Spectra studies similar to those reported here utilize samples that are prepared as dry oriented films[24] or ones that are dissolved in aqueous solutions.[25] It is appropriate to mention here that DNA has a very complex structure which is strongly dependent on sample preparation, humidity, temperature, etc. Hence, the microwave spectra obtained will be significantly influenced by the previous factors. The decision to utilize unoriented dry films for these initial studies was primarily a result of the foreseen potential application as a detector of viral agents. Since viruses are the simplest microorganisms (i.e., are almost exclusively DNA material) and generally exist in a dehydrated state in nature, it seems appropriate to investigate the inhomogeneous effects that arise in randomly oriented DNA samples.

We believe the distinct features in the previous far-infrared spectra may be the signature of lesion-induced vibrational modes. When the translational symmetry of the long DNA chain is broken, by defects such as broken bond, missing atoms or groups, additional dimers, substitutional impurities, etc., vibrations of atoms in the vicinity of the defect leads to distinctive modes which are essentially localized spatially about the defects. This contrasts with the more common sound-like modes of vibration of the long-chain DNA polymers in which every atom of the chain partakes in the motion. For each different defect a spectroscopically distinctive characteristic local-mode frequency exist. Hence the local modes bear direct information on the nature of the defect and can thus serve as a diagnostic signature of the polymer chain lesion. It is expected that the lines corresponding to local modes will be sharper, and should be easier to detect.[26] The allowed vibration frequencies associated with the localized defect, and the corresponding relative motion of the nearby atoms, are of great potential interest for

their diagnostic possibilities. Spectroscopic observation of these should provide characteristics of particular lesions and be related to the irregularities of the DNA polymer structure. The relative intensities of spectral features characteristic of each lesion will be a direct measure of the degree of damage to the DNA polymer. Thus an analysis of the absorption spectra of known defects/lesions in DNA samples will be a powerful tool for obtaining information about the particular inter-atomic interaction and can be used to identify the species in question.

One could envision a working field detector as being composed of three components. The first component is a data bank with all spectra of defect-related DNA local modes for a class of substances; this may be comprised through systematic experiments and supplemented with numerical computations. A second component required is a spectrometer composed of a broadband mm wave signal source and detector. A third component performs the comparison of the spectra obtained with those in the data bank. Our work along this line has just begun, and we hope to report on it further in the near future.

SUMMARY

In summary, we propose a scheme for detecting DNA-based microorganisms using microwave absorption spectroscopic techniques. Preliminary experiments performed by us show that it is promising. We discuss the feasibility and advantages of using a particular set of modes of the DNA polymers as identification resonances. These modes are associated with localized defects of the DNA polymers. They are unique for each defect/lesion, and should be easy to detect. We described a field-detecting detector based on the local modes.

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