MICROWAVE-FIELD-DRIVEN ACOUSTIC MODES IN DNA

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ABSTRACT The direct coupling of a microwave field to selected DNA molecules is demonstrated using standard dielectrometry. The absorption is resonant with a typical lifetime of 300 ps. Such a long lifetime is unexpected for DNA in aqueous solution at room temperature. Resonant absorption at fundamental and harmonic frequencies for both supercoiled circular and linear DNA agrees with an acoustic mode model. Our associated acoustic velocities for linear DNA are very close to the acoustic velocity of the longitudinal acoustic mode independently observed on DNA fibers using Brillouin spectroscopy. The difference in acoustic velocities for supercoiled circular and linear DNA is discussed in terms of solvent shielding of the nonbonded potentials in DNA.

INTRODUCTION

We are investigating the absorption of microwave energy by aqueous solutions of DNA at room temperature. Aqueous solutions, because of the polar nature of water, absorb significantly at microwave frequencies. This absorption is well described by Debye relaxation theory (1, 2). Our earlier studies have indicated that in some cases solutions of DNA isolated from *Escherichia coli* (*E. coli*) absorb more microwave energy than the solvent (3, 4). The enhanced absorption of DNA solutions relative to solvent, observed in our earlier studies, is broadband and shows little frequency-specific structure. Indeed, Debye theory does not predict frequency-specific coupling of the microwave field to DNA molecules. In the past, broadband absorptions have been attributed to hydration layers and described by mixture relations consistent with Debye relaxation theory (5).

An alternative model for describing the enhanced absorption has been developed by Prohofsky, Van Zandt, and co-workers (6–17). Computer calculations using normal mode analysis and lattice dynamics to account for the acoustic velocity observed with oriented DNA fibers (18, 19) predict vibrational modes that could directly couple to the microwave field. This early model did not, however, address the damping of the normal modes by solvent molecules. Solvent damping was introduced with hydrodynamic calculations (15, 16) that describe extensively damped normal modes: the observation of the longitudinal acoustic mode with oriented DNA fibers is attributed to reduced solvent damping resulting from the structure and orientation of water and DNA molecules within the fiber. This hydrodynamic model predicts the solvent will overdamp the acoustic modes for aqueous solutions of DNA.

To further understand the absorption of microwave energy by aqueous DNA solutions, we have characterized the nature of *E. coli* DNA solutions that result in enhanced absorption. In addition, we have investigated the absorption mechanism using cloned DNA of uniform length and known sequence. We demonstrate experimentally that DNA solutions can exhibit microwave resonances that have a definite frequency-length dependence. These resonances are unexpectedly sharp and are in remarkable agreement with an acoustic mode model. Here we expand on this model and the observations preliminarily described previously (20, 21). In addition, we suggest a plausible explanation for the observed difference in acoustic velocities for supercoiled circular and linear DNA.

MEASUREMENT METHODS

The amount of microwave energy absorbed by sample solutions is measured by two different methods using a semi-automated network analyzer system, shown schematically in Fig. 1. Knowing the sample depth, the complex permittivity of the sample solution is determined from the change in phase and amplitude of the radiation reflected from the sample relative to the incident radiation. The absorption coefficient α is related to the complex permittivity ϵ^* by

$$\alpha = \sqrt{2}\,(\omega/c_o)\,\sqrt{\epsilon'}\,\sqrt{\sqrt{1+\tan^2\delta}-1},$$

where

$$\epsilon^* = \epsilon_o\,(\epsilon' - i\epsilon'')$$

$$\tan\delta = \epsilon''/\epsilon'.$$

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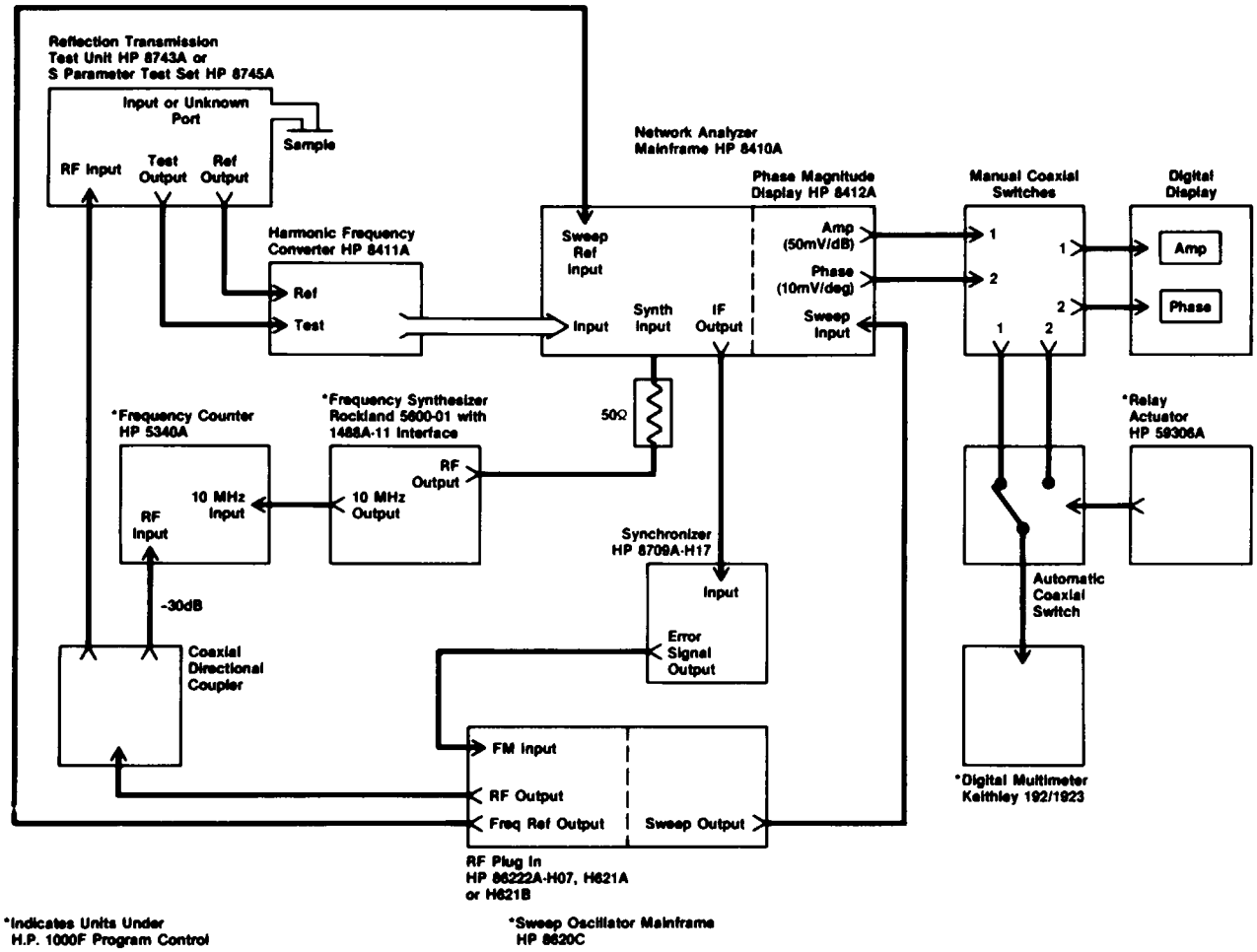

FIGURE 1 Semi-automated network analyzer system used for dielectrometric measurements. The system is controlled through a Hewlett-Packard Interface Bus (Hewlett-Packard Co., Palo Alto, CA). The test frequency is produced by the sweep oscillator (servo-locked to the frequency synthesizer for test frequencies <2 GHz). The test unit divides the signal into test and reference channels. The test channel signal is shifted in phase and amplitude by the sample solution. The network analyzer measures these shifts. The phase information is sampled 100–200 times at the digital multimeter and the mean and variance calculated. If the variance exceeds a predetermined threshold the measurement is rejected. If accepted, the digitized phase and amplitude information, which determines the dielectric properties of the sample, is stored in computer memory.

ω is the frequency of the microwave field, ϵ_0 is the permittivity of free space, and c_0 is the speed of light in vacuum. The network analyzer measures the reflection coefficients and has been described elsewhere (22).

One measurement technique uses the shorted line method, similar to that described by Roberts and Von Hippel (23). The network analyzer is calibrated at the outer plane of a Teflon-filled section of coaxial line (0.4–9 GHz) or waveguide (8–12 GHz); this corrects for any systematic errors and mismatches due to the Teflon plug. The sample, contained in a section of coaxial line or waveguide terminated in an electrical short, is attached at the outer plane of the Teflon plug. To maximize system resolution, the length of the precisely measured section containing sample must approximate a quarter wavelength in the sample solution.

The other technique uses the network analyzer system to measure the reflection coefficients with an open-ended coaxial line, as described by Athey et al. (22). This method takes advantage of the lossy character of aqueous solutions. The open-ended coaxial line (141 line) is submerged in sample solution of sufficient dimensions to minimize or eliminate the coupling of evanescent modes to the surfaces of the sample container. The complex permittivity can be calculated from the measured reflection coefficients.

Measurements using sections of waveguide are maintained at 25°C within 0.2°C through the use of a specially constructed chamber connected to a circulating water bath. Measurements using sections of coaxial line or the open-ended coaxial line are made at room temperature. The temperature is monitored during data acquisition and does not vary by more than 0.5°C.

The shorted line technique offers a wider high-resolution frequency range. It requires, however, frequent handling of the sample solution at the expense of limited recovery of sample; the dismantling of the waveguide sample holder that is necessary after measurement makes recovery of the sample solution difficult. Measurements with the open-ended coaxial line do not require transfer of sample solution. This is an attractive feature considering that several milliliters of sample solution containing approximately a milligram of DNA per milliliter are needed. This experimental convenience is, however, accompanied by loss of resolution at higher frequencies relative to the shorted line method.

SAMPLE PREPARATION

E. coli DNA was isolated and purified by standard phenol extraction and ethanol precipitation techniques and dissolved in 9 g/l NaCl, 50 mM Tris

at pH 8, resulting in a typical A_{260}/A_{280} of 1.9. Plasmids p9.32 gpt-ori (5,880 bp) and pUC8.c2 (2,740 bp) were grown in *E. coli* strains RR 1 and HB 101, respectively. These cloned DNAs were isolated by standard plasmid extraction procedures and dissolved in 10 mM Tris, 10 mM NaCl, and 1 mM EDTA at pH 7.5. DNase I was purchased from Worthington (Diagnostics Division, Millipore Corp. Freehold, NJ); restriction endonucleases from Bethesda Research Laboratories, Inc. (Gaithersburg, MD) were used according to the purveyor's recommendations. ECOR I cleaves once within pUC8.c2 and results in a linear molecule 2,740 bp in length. PVU I cleaves pUC8.c2 twice and gives two fragments, 948 bp and 1,792 bp. Extractions and digestions are routinely verified by agarose gel electrophoresis with standard markers.

EXPERIMENTAL RESULTS

Demonstration of a Length Dependence

Our earliest studies of the absorption properties of aqueous solutions of *E. coli* DNA used the shorted line technique with the sample contained in a section of waveguide. Measurements repeatedly demonstrated enhanced broadband absorption (Fig. 2); we only accepted that data showed enhanced absorption when the enhancement was well above systematic error (this error is 2–3% for the waveguide shorted line method). Although the enhancement was consistently reproducible for a given extraction, there was significant variability between extractions. Biochemical analysis showed that these samples contained RNA and protein impurities and the DNA had been extensively sheared by improper handling. In addition, carefully prepared samples of high molecular weight *E. coli* DNA (>200,000 bp) free of protein and RNA failed to exhibit enhanced absorption. In an effort to redemonstrate enhanced absorption, this high molecular weight extraction procedure was systematically altered to produce sample solutions approximating selected characteristics of our original poor-quality sample solutions. Combinations of variable protein and RNA content and RNase and DNase I activity recovered differing degrees of enhanced absorption (Fig. 3). In addition, mechanically sheared

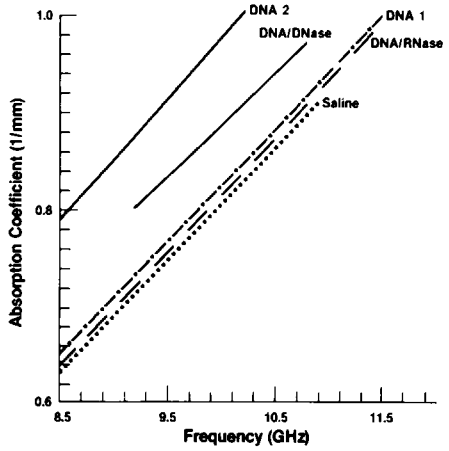

FIGURE 3 Absorption properties of *E. coli* DNA solution relative to 0.9% saline solvent for four different preparation protocols. DNA 2 (3.7 g/l) is sheared DNA with RNA and protein impurities. DNA 1 (5.5 g/l) is high molecular weight, purified DNA. DNA/DNase (7.0 g/l) is initially high molecular weight DNA that has been partially digested before measurement. DNA/RNase (3.25 g/l) is high molecular weight DNA and digested RNA.

DNA absorbs more than nonsheared DNA. Crudely classifying the treatment of the DNA as either rough or gentle and normalizing the concentration reveals that roughly treated DNA samples exhibit enhanced absorption one order of magnitude greater than gently treated DNA samples. This suggests the previously observed variability was due to the state of the DNA molecule.

To investigate the possibility of a length dependence, high-quality solutions of *E. coli* DNA of high molecular weight, which initially fail to exhibit enhanced absorption, are measured during DNase I activity. A 9 mg/ml DNA solution is adjusted to 10 mg/ml $MgCl_2$ and treated with 0.1 mg/ml DNase I at 37°C for 30 min (time = 0 to 30 min). The sample is returned to room temperature in 5 min (time = 30–35 min) and measurements are made at 10-

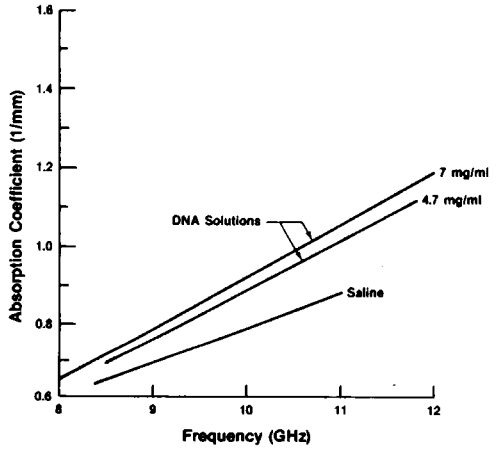

FIGURE 2 Early demonstration of enhanced absorption of *E. coli* DNA solutions relative to 0.9% saline solvent. See text for discussion of shearing and impurities.

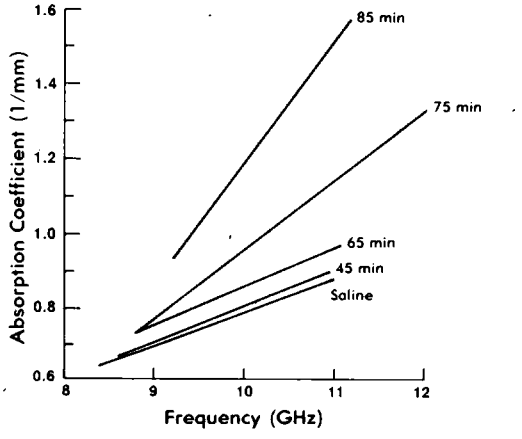

FIGURE 4 Variation of the absorption coefficient of *E. coli* DNA exposed to DNase I for 85 min. 1.75 g/l *E. coli* DNA (>50,000 bp before DNase activity) dissolved in 9 g/l NaCl, 0.1 g/l DNase I, and 10 g/l $MgCl_2$ solution.

min intervals (time = 35, 45, etc. min) (Fig. 4). Enzymatic activity can be halted by addition of EDTA, resulting in a static enhanced absorption. A plausible explanation for the observed dramatic increase in absorption is that the enzymatic nicking produces a dynamic length distribution whose mean length decreases with time. Enhanced absorption occurs as the sample length distribution moves through the region giving significant absorption in the experimental frequency range.

Demonstration of Resonant Microwave Absorption

We have demonstrated that solutions of *E. coli* DNA can be prepared to exhibit dramatically enhanced microwave absorption. The dynamic DNase I digestion of DNA demonstrates a frequency dependence on the degree of enhancement (Fig. 4). The absorption mechanism, however, remains unclear. Consider two possibilities involving a resonant mechanism. A heavily damped absorption mechanism could exist which has a fixed resonant frequency above our experimental frequency range; the results shown in Fig. 4 would be due to the low frequency wing of a broad, increasing resonant peak. Alternatively, a not so heavily damped resonant mechanism could exist; the increasing absorption shown in Fig. 4 could be due to a distribution of relatively sharp peaks summing to yield the observed results.

Standard cloning and restriction endonuclease digestion techniques provide extremely well-characterized DNA solutions for physical measurements. These biochemical techniques yield DNA samples of uniform length and known sequence. To conserve sample, measurements of cloned DNA are made using the open-ended coaxial line method. This method limits the resolution at higher frequencies relative to the previous results.

Plasmids were selected for length and restriction sites. The plasmid p9.32 gpt-ori (5,880 bp) showed no enhanced absorption in the supercoiled circular state or when linearized. Treatment with DNase I, however, gave dynamic enhanced absorption qualitatively similar to previous DNase I treatments (data not shown). This provided an upper bound for the absorbing lengths in the experimental frequency range.

Similarly, a lower bound could be determined. Digesting plasmid with restriction endonucleases provides a number of DNA fragments of known length. Solutions of various restriction endonuclease digestions were measured in an attempt to demonstrate enhanced absorption by a known DNA fragment. When enhanced absorption did not occur, the sample solution was exposed to DNase I. Eventually a solution containing several DNA fragments of known length failed to give dynamic enhanced absorption when exposed to DNase I (data not shown). This provided a lower bound for the absorbing lengths in the experimental frequency range.

Recognition of the length window and the measured acoustic velocity (18, 19) suggested an appropriate length distribution for study. Based on our intuition about the mechanism of microwave absorption and using the acoustic velocity data of Hakim et al. (19), we were guided towards measurement on DNA samples whose lengths were in a range up to a few wavelengths of sound waves on the double helix. Our expectations of what length of DNA molecules would exhibit enhanced absorption were borne out experimentally. A solution of supercoiled circular DNA molecules of 2,740 bp has resonant frequencies near 2.55, 4.00, 6.60, and 8.75 GHz (Fig. 5). A solution of 2,740 bp linearized DNA has resonant frequencies near 2.75, 4.15, and 5.60 GHz (Fig. 6). A solution of equimolar 948 and 1,792 bp linear DNA fragments has resonant frequencies near 2.65 GHz (Fig. 7) and 4.10 GHz (Fig. 8).

It is surprising to observe such sharp microwave resonances in dense solutions at room temperature. It has generally been believed that such resonances should be overdamped. If not overdamped, the resonances were expected to be heavily damped, and thus very broad.

DISCUSSION

Acoustic Mode Model

Prohofsky, Van Zandt, and co-workers (6–17) have performed theoretical calculations based on lattice dynamics and normal-mode analysis that describe acoustic modes on

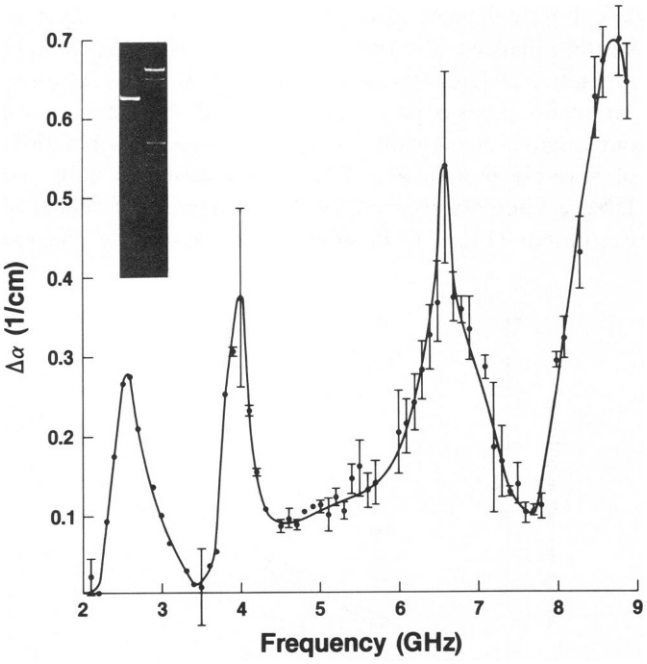

FIGURE 5 Relative absorption coefficient of the fundamental and first three harmonics of 0.53 g/l 2,740 bp supercoiled circular DNA. DNA sample verified by agarose gel electrophoresis with standard markers (λ digested with Hind III and ΦX174 digested with Hae III). Relative measurements have background absorption subtracted. Error bars represent total spread in the data. DNA is dissolved in storage buffer (10 mM TRIS, 10 mM NaCl, 1 mM EDTA, pH 7.5).

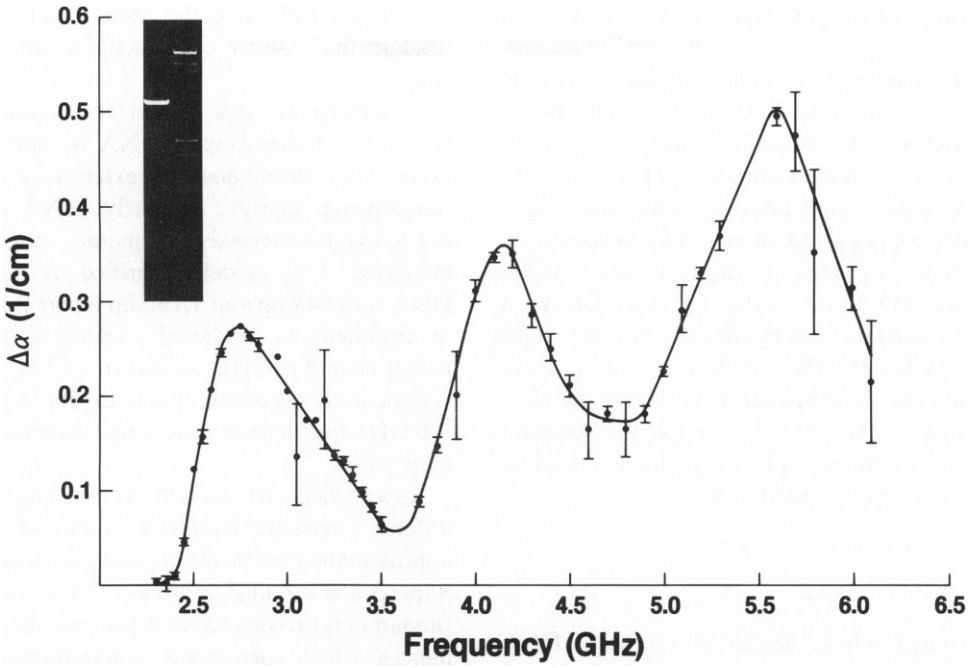

FIGURE 6 Relative absorption coefficient of the first three harmonics of 0.50 g/l 2,740 bp linear DNA dissolved in storage buffer. The corresponding fundamental expected near 900 MHz is not observed.

linear DNA double helices. An acoustic velocity associated with a thermally populated longitudinal acoustic mode has been measured on oriented DNA fibers by Brillouin spectroscopy (18, 19). These fibers likely contain ordered, linearized DNA fragments and yield an acoustic velocity of 1.69 km/s for the fully hydrated fiber (19). The early theory (7), which considered only chemical bonds in modeling intermolecular interactions within a given DNA molecule, predicted an acoustic velocity for the longitudinal mode of approximately 60 m/s and that these acoustic modes could couple to the electromagnetic field. To account for the necessary stiffening of the polymer, Prohofsky and Van Zandt recognized the ionic distribution in double helical DNA. The addition of electrostatic and extensive van der Waal's interactions in the double helix accounted theoretically for the observed acoustic velocity. In addition, the revised theory predicted low-lying acoustic

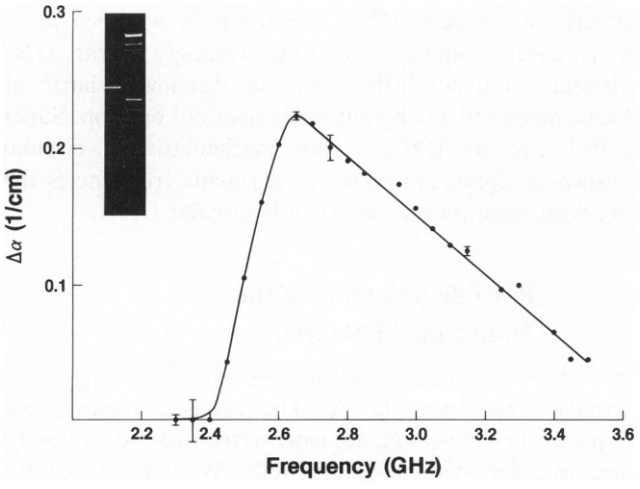

FIGURE 7 Relative absorption coefficient assigned to the fundamental of 0.22 g/l 948 bp fragment dissolved in storage buffer. Resonance assigned to 1,792 bp fragment shown in the next figure.

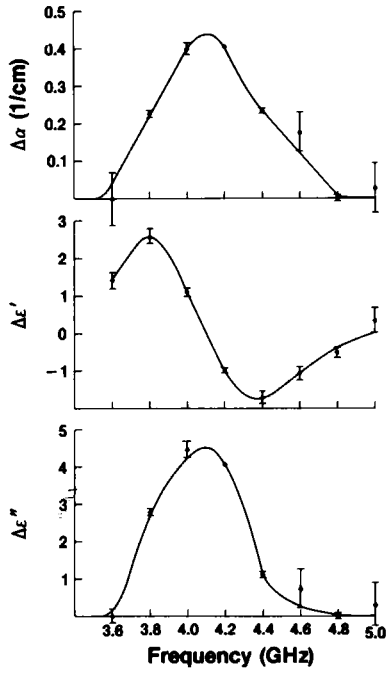

FIGURE 8 Relative absorption coefficient ($\Delta\alpha$), and real ($\Delta\epsilon'$) and imaginary ($\Delta\epsilon''$) parts of the dielectric constant assigned to the first harmonic of 0.22 g/l 1,792 bp fragment dissolved in storage buffer. These curves exhibit a classical absorption resonance. The corresponding fundamental expected near 1.4 GHz is not observed.

modes that could directly couple to a microwave field (13).

There are two considerations for field-induced acoustic modes: the acoustic wavelength must satisfy specific boundary conditions and the microwave field must be capable of coupling power into the mode (matching). The boundary conditions limit linear molecules to full- and half-wavelength acoustic modes and the circular molecules to full-wavelength acoustic modes, i.e., the acoustic vibrations must reflect (linear DNA) or overlap (circular DNA) in phase. Matching considerations eliminate full-wavelength acoustic modes on linear DNA molecules (for a more detailed discussion see the Appendix). Equating the electromagnetic energy to the acoustic energy, the resonant frequency ν for DNA molecules of length ℓ (determined by the number of base pairs) are related by

$$(n + 1)\, \nu_{\text{circular}} = v\, \ell$$

$$(n + \tfrac{1}{2})\, \nu_{\text{linear}} = v\, \ell,$$

where v is the acoustic velocity for circular or linear DNA molecules; n indicates the nth harmonic, the fundamental being described by n equal to zero. Dispersive acoustic velocities are not constant but depend on the acoustic wavelength (i.e., molecular length ℓ).

Resonance Assignment

Linearized 2,740 bp DNA molecules exhibit three resonant frequencies approximately related as 3:5:7. An acoustic mode model with a constant acoustic velocity predicts a 1:3:5:7 resonant frequency spacing in the experimental frequency range; deviations in this spacing will arise for wavelength-dependent acoustic velocities. The observed spacing corresponds to acoustic velocities of 1.67, 1.52, and 1.46 km/s, respectively. The associated fundamental resonance expected near 0.9 GHz is conspicuously absent.

A plausible explanation for the missing fundamental is suggested from considerations of the observed relaxation times and the development of acoustic modes. The observed resonant microwave absorption is a length-dependent event. In some sense the vibrational excitation must know the extent of the DNA molecule to develop into a coherent mode. The electromagnetic field exerts forces on charges along the DNA molecule. Field-induced motion is propagated through a DNA molecule by intramolecular potentials. A coherent acoustic mode develops when excitations reflected from the ends of linear DNA molecules constructively interfere to yield a standing wave.

The lifetime of the coherent mode can be described by the relaxation time τ, which is determined from the line shape: $\tau = (2\pi\, \Delta\nu)^{-1}$; where $\Delta\nu$ is the full frequency width at half-maximum–enhanced absorption. The observed relaxation times (~300 ps) and acoustic velocities suggest an excitation can travel on the order of 1,500 bp on linear DNA before dispersing. Thus 2,740 bp linear DNA is too long to allow development of a coherent mode. The presence of the nth harmonic results from $2n + 1$ times the fundamental energy being coupled into the DNA molecule.

According to this model a fundamental resonance should be observed when DNA is short enough to allow propagation throughout the extent of the molecule at the fundamental energy. Similarly, DNA molecules should fail to exhibit successive harmonics as molecular length is increased. This model is verified by digesting 2,740 bp DNA into 948 bp and 1,792 bp linear fragments. The 948 bp fragment, as predicted, exhibits a fundamental resonance near 2.6 GHz, as shown in Fig. 7. The 1,792 bp fragment, as expected, fails to exhibit a fundamental near 1.4 GHz but does exhibit a first harmonic near 4.2 GHz (Fig. 8).

Supercoiled circular DNA molecules are subject to a different acoustic boundary condition that predicts an approximate 1:2:3:4, etc., spacing of resonant frequencies. Supercoiled circular 2,740 bp DNA molecules exhibit a fundamental resonance and the first three harmonic resonances, which correspond to significantly faster acoustic velocities (2.33, 1.83, 2.01, and 2.00 km/s, respectively). For the fundamental acoustic velocity an excitation can travel on the order of 2,100 bp. In addition, for circular DNA molecules an excitation need travel only half the molecular extent before constructive interference can develop.

The calculations presented in the Appendix show that some harmonics allowed by acoustic considerations are disallowed by matching considerations. This occurs when power absorbed over one half-wavelength is completely out of phase with that absorbed over another half-wavelength. Only a fundamental resonance is predicted for mathematically circular DNA. Deviations from the mathematically linear or circular configuration result in different power deposition for offsetting DNA segments: this allows net power to be coupled into the molecule. In solution, linear DNA closely approximates a bent rod; however, in solution supercoiled circular DNA is extensively wrapped into a noncircular configuration. Approximately linear DNA absorbs relatively little power at disallowed harmonic frequencies and a coherent mode does not develop. Supercoiled circular DNA is not mathematically circular, absorbing significant power at harmonic frequencies that are disallowed for mathematically circular DNA.

Possible Shielding of the Nonbonded Potentials

We observe different acoustic velocities for supercoiled circular and linear DNA. The acoustic mode theory depends, however, on nonbonded (electrostatic and extensive van der Waal's) interactions. Whereas in solution linear DNA molecules approximate randomly bent rods, supercoiled circular DNA molecules are extensively wrapped into a noncircular configuration. DNA in solution

is a polyanionic macromolecule surrounded by a counterion cloud. The influence of these counterions on the nonbonded interactions is an open question.

When DNA molecules condense, electrostatic repulsion is opposed by hydrated counterions bridging the anionic DNA molecules (24), i.e., there is sharing of the counterion cloud between DNA molecules. We propose that the supercoiling of circular DNA excludes solvent and is characterized by counterion bridging between segments of a given DNA molecule. This sharing of counterions results in less solvent shielding of the nonbonded interactions, an effective increase in the interaction potential. Eyster and Prohofsky (8, 9) have theorized that dehydration decreases shielding, increasing the nonbonded interactions on B DNA. In addition, the theory describes the onset of the B to A conformational transition as the nonbonded potentials are increased. Our differing acoustic velocities for supercoiled circular DNA and linear DNA may reflect a reduction in counterion shielding of the interaction potential in the supercoiled configuration. Alternatively, it has been suggested that for certain localized disturbances propagating on a DNA molecule there may be time delays associated with reflections at the end of the molecule, effectively decreasing the apparent acoustic velocity for linear molecules, relative to circular molecules (Scott, A. C., personal communication).

The extensive wrapping of supercoiled circular DNA prevents us from assuming that this observed acoustic mode is also a longitudinal acoustic mode. The differing observed acoustic velocities for linear and supercoiled circular DNA may reflect different acoustic modes being driven by the microwave field; however, our intuition is that they are both longitudinal modes and the different acoustic velocities are due to geometrical and excluded solvent effects which decrease counterion shielding of the interaction potential. We are currently studying solutions of varying DNA concentration and configuration to address questions of molecular interactions.

Related Research

As previously alluded to, Prohofsky, Van Zandt, and co-workers (6–17) have extensively modeled double helical DNA. To describe the acoustic velocity observed on oriented DNA fibers, the intermolecular potential includes electrostatic interactions over an extensive number (thirty) of neighboring base pairs (12). Such a long range interaction is surprising: however, we must concede that conventional intuition about biopolymers did not admit the possibility of resonant microwave absorption by DNA molecules.

Prohofsky and Van Zandt's theory, adjusted for the acoustic velocity, predicts longitudinal acoustic modes that might couple to microwave fields (13). In addition, the model describes defect resonances (11) just outside our experimental frequency range. Hydrodynamic calculations (15, 16) predict the longitudinal acoustic mode will only be observed for conditions of diminished solvent damping (i.e., an ordered fiber of DNA molecules). Aqueous solutions of DNA at room temperature are expected to be overdamped.

Modeling DNA solutions is a complicated task. Models of solvent damping demand simplification to make the problem mathematically tractable. Hydrodynamic theory models the solvent as a featureless continuum fluid that drags vibrating DNA molecules. In this case the solvent is characterized by a constant macroscopic coefficient of viscosity and stick boundary conditions are used at the surface of the DNA molecule. Recently it has been suggested that alternative boundary conditions may apply to polymer systems with the dimensions of DNA (26). Water and counterion structure in the molecular grooves could significantly decrease the efficiency of momentum transfer between nonstructured solvent and vibrating DNA molecules. The effective change in damping introduced by alternative boundary conditions will likely be relatively small; microscopic considerations of solvent structure can yield viscosities dramatically less than those of continuum theory. Our experimental results demonstrate that a fundamental mode can develop only for DNA molecules shorter than a critical length. Asymmetric resonances suggest a frequency dependent viscosity (26). These observations directly depend on the nature of the solvent-molecule interaction. A revised hydrodynamic model that takes some of these considerations into account is currently being developed (Van Zandt, L. L., personal communication).

Hakim et al. (19) have measured the velocity of sound of a longitudinal acoustic mode on oriented DNA fibers of varying humidity using Brillouin spectroscopy. Comparing the fundamental resonance of linear DNA (1.67 km/s for 948 bp) with fully hydrated fibers (1.69 km/s), the measured acoustic velocities are in favorable agreement.

All the length dependent resonances that we have observed are consistently described by an acoustic mode model. This model requires a weak wavelength dependence to the acoustic velocity and significantly different acoustic velocities for linear and circular DNA. We also observe a length-independent absorption. Our experimental frequency range has a lower limit of 400 MHz at present. We observe an enhanced absorption near 400 MHz for solutions of linear DNA. This absorption is independent of length of linear DNA molecules and is not present for solutions of supercoiled circular DNA. Lindsay et al. (27) have reported a resonance at 600 MHz that could correspond to the defect resonance predicted by Putnam et al. (11). This defect resonance is associated with the premelting of the ends of DNA molecules and would be observed only in solutions containing linear DNA molecules.

CONCLUDING REMARKS

We have experimentally demonstrated that aqueous solutions of selected DNA molecules at room temperature

resonantly absorb microwave energy. Our experimental results are well described as microwave-induced acoustic modes and are in good agreement with Brillouin spectroscopic measurements. The resonant absorption is, however, in conflict with a recent hydrodynamic model of solvent damping in DNA solutions. We suggest that microscopic considerations may lead to an alternative solvent model that describes noncritically damped acoustic modes.

Our previous studies using the optical heterodyne technique (3, 4) measured the thermal expansion of DNA solutions that resulted from relaxation of excited polymer modes. We intend to further investigate whether these excited states can only relax into thermal energy or if other energetic pathways are available. The acoustic mode is distributed over the entire macromolecule: it is interesting to speculate about the possibility of this or higher excited states evolving into other, possibly localized, energetic forms.

Although there is no demonstrated biochemical effect associated with this phenomenon, these observations indicate that a mechanism exists for frequency-specific deposition of microwave energy in DNA. It will be interesting to see if this mechanism can be used to alter biochemical processes, if similar phenomena exist in other periodic macromolecules, and if acoustic modes possibly provide a mechanism for transporting coherent energy over large biochemical distances.

APPENDIX

Microwave-field–induced acoustic modes can be modeled in a simple manner. Such a model can only give a qualitative description of the absorption mechanism; however, this theory describes distinctive features that are in compelling agreement with experiment. A more sophisticated theoretical approach is reflected in the model of Prohofsky, Van Zandt, and co-workers (6–17).

DNA molecules of several thousand base pairs are exposed to a time varying, essentially uniform field at microwave frequencies. How is it possible to couple electromagnetic energy into a DNA molecule under these conditions? Each base pair has two net negative charges q that experience a force from the field **E**. The resulting mechanical displacement **ds** is coupled into a neighboring base pair by bonded potentials. In addition, it appears there are nonbonded potentials that extend between many base pairs (12). The acoustic velocity depends on these potentials.

Field-induced displacements develop into a coherent mode when the frequency of excitation $\omega/2\pi$ corresponds to an acoustic resonance, i.e., when a standing acoustic wave develops on a finite DNA molecule. Mechanical excitations can draw large amounts of power from the electromagnetic field only when the acoustic resonance condition is satisfied. This is the source of the frequency-length dependence.

The power, P, coupled from the microwave field to a coherent mode on a DNA molecule is

$$P = q \int_{\text{length}} \mathbf{E}(\mathbf{r}, t) \cdot \frac{\mathbf{ds}}{dt}\, dx.$$

For a standing acoustic wave

$$\mathbf{ds} \propto \sin(\omega t) \sin[k(x + x_0)]\, \hat{\mathbf{s}},$$

where $k = 2\pi/\lambda$, and λ is the acoustic wavelength. The phase x_0 is determined by a boundary condition and will be discussed later. Thus

$$P \propto \int_{\text{length}} [\mathbf{E}(\mathbf{r}) \cdot \hat{\mathbf{s}}] \sin[k(x + x_0)]\, dx, \qquad \text{(A1)}$$

which applies for linear or circular DNA and longitudinal or transverse acoustic modes.

Linear DNA

In this model the ends of a linear molecule are equivalent. Excitations reflect from the ends and constructive interference develops when

$$\ell = [(n + 1)/2]\lambda, \qquad n = 0, 1, 2, \ldots$$

or

$$k = [(n + 1)/2](2\pi/\lambda).$$

For the longitudinal acoustic mode $\mathbf{E} \cdot \hat{\mathbf{s}}$ is constant and Eq. A1 reduces to

$$P \propto \int_0^{\ell} \sin[k(x + x_0)]\, dx$$

$$= \begin{cases} 0 & n \text{ odd or } x_0 = \ell/2 \\ \dfrac{2\ell}{(n+1)\pi} \cos\left[\dfrac{(n+1)}{\ell}\pi x_0\right] & \text{elsewhere.} \end{cases}$$

Thus longitudinal acoustic modes on linear DNA may be driven by the microwave field when

$$\ell = (n + 1/2)\lambda, \qquad n = 0, 1, 2, \ldots.$$

The failure of full wavelength excitations to develop into a coherent mode on linear DNA is intuitively explained by recognizing that the power coupled to one half-wavelength is completely out of phase with the neighboring half-wavelength. Upon propagation the excitations destructively interfere. An unbalanced half-wavelength is necessary for the development of a standing wave on linear DNA molecules.

The phase x_0 is determined by a boundary condition on the ends of the DNA molecule. The coupling of the field eliminates the possibility of antinodes occurring at the ends ($x_0 = \pm\ell/2$). This is intuitively pleasing as an antinode maximally punches the solvent and likely results in maximum damping. For the nodal boundary condition ($x_0 = 0, \pm\ell$) the molecule not only maximally couples to the field but also intuitively appears to be minimally damped.

Circular DNA

For circular molecules constructive interference develops when an excitation overlaps itself in phase

$$\ell = (n + 1)\lambda \qquad n = 0, 1, 2, \ldots.$$

Consider a cylindrical coordinate system such that $\hat{\mathbf{z}}$ is normal to the molecular plane and let the component of the field in the molecular plane $E_{\rho\phi}$ define $\theta = 0$. For a longitudinal acoustic mode

$$\mathbf{ds} \propto \sin(\omega t) \sin[k(\theta + \theta_0)]\hat{\theta},$$

where

$$k = n + 1.$$

As before, θ_o allows for indeterminance in locating the nodes. Integrating around the circle (1) becomes

$$P \propto \int_0^{2\pi} [(E_z, E_{\rho\theta} \cos\theta, E_{\rho\theta} \sin\theta) \cdot \hat{\theta}] \sin[k(\theta + \theta_o)]\, d\theta$$

$$\propto \int_0^{2\pi} \sin\theta \sin[k(\theta + \theta_o)]\, d\theta = \begin{cases} 0 & n \neq 0 \text{ or } \theta = \pm\pi/2 \\ \pi \cos\theta_o & n = 0 \end{cases}$$

For mathematically circular DNA a microwave-field induced longitudinal acoustic mode may only be driven at the fundamental frequency.

Transverse and Torsional Modes

Brillouin spectroscopic data indicates that the longitudinal mode is observed on fibers of linear molecules (18). We have also done calculations to see if the transverse mode can couple to the field. The transverse mode gives nearly identical results to the longitudinal mode, except the phase requirement for vanishing absorption on circular DNA is $\theta_o = 0, \pm\pi$ instead of $\pm\pi/2$. The transverse mode seems, however, unlikely as it displaces large amounts of solvent.

This simple model of field-induced mechanical excitations developing into standing acoustic waves does not describe a microwave field driven torsional acoustic mode. Consider the DNA double helix as a linear collection of unit cells. Each cell contains two symmetrical components corresponding to the two helices. Each component has a net negative charge. The essentially uniform field exerts identical forces on each symmetric component; such a system cannot produce the unit cell rotation necessary for the torsional normal mode of DNA.

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