Low-frequency, Raman-active vibrational modes of poly(dA)·poly(dT)

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The Raman activity of low-frequency (20–300 cm^{-1}) vibrational modes of dehydrated, oriented fibers of the sodium salts of poly(dA)·poly(dT) and random sequenced DNA have been measured. Distinct bands near 60, 75–100, and 125–140 cm^{-1} are resolved in poly(dA)·poly(dT). The Raman activity of the two lowest bands correlate with the previously observed infrared activity of poly(dA)·poly(dT). The apparent reduction in spectral line broadening for poly(dA)·poly(dT), as demonstrated by this and previous measurements of a number of different polynucleotides, is considered as possible evidence for inhomogeneous line broadening.

In 1987 Genzel and co-workers[1] published results of a far-infrared (40–500 cm^{-1}) investigation of long-wavelength vibrational models in polynucleotides. Measurements of vacuum-dried, free-standing, unoriented films of poly(dA)·poly(dT), poly(dA-dT)·poly(dA-dT), poly(dG)·poly(dC), and poly(rA)·poly(rU) under various temperature and salting conditions were reported. The spectra of poly(dA)·poly(dT) were unique in that, for the appropriate salting conditions, three sharp bands at 62, 80, and 95 cm^{-1} were resolved in room-temperature spectra and, in addition, a detailed spectrum of "outer" modes from 125 to 250 cm^{-1} were observed. These experimental results were described in terms of a lattice-dynamical model that predicts Davydov pairs of vibrational modes that are split by a few wave numbers. One mode of the pair will be principally infrared active and the other principally Raman active. The purpose of this Brief Report is to present the results of low-frequency (20–300 cm^{-1}) Raman measurements of poly(dA)·poly(dT) and random sequenced DNA. Before describing these results, however, a brief summary of related research is presented.

Prior to the far-infrared measurements, Urabe and Tominaga published low-frequency Raman measurements[2] of nucleic acids including the observation of a broad feature near 85 cm^{-1}. Together with co-workers they also observed modes at frequencies below 50 cm^{-1},[3] as have Lindsay and co-workers.[4] Since the appearance of the far-infrared measurements several complementary investigations have been reported. Prohofsky and co-workers[5] have presented a lattice-dynamical calculation that addresses the temperature dependence of these modes. In addition, during the preparation of this manuscript Weidlich and Lindsay[6] reported measurements of polynucleotide gels (70–150 g/l) which revealed features in difference spectra where a water background has been subtracted from the gel spectra.

For this investigation the nucleic acid samples were prepared as follows. Single fibers of the sodium salts of poly(dA)·poly(dT) (Sigma Chemical Company, lots 127F03421 and 127F03431) and of calf thymus DNA (Sigma Chemical Company, lot 17F-9610) were pulled yielding DNA molecules that are essentially oriented along the fiber directon.[7] The fibers were then dialyzed in a 73% (by volume) ethanol and 27% (by volume) distilled water solution to remove excess salt and then dried in a desiccated environment as done previously.[1] The spectra were obtained using a Spex Model 1403 double monochromator and a Coherent Inova 90 argon-ion laser. The laser was operated at 488 nm with a power at the fiber of 10 mW. Spectra were obtained in a 90° geometry and polarization occurred along the fiber axis. Scattered light was detected by an RCA C3103M GaAs photomultiplier tube. Liquid-nitrogen-temperature spectra were taken under vacuum with a cold-finger bath cryostat. For some spectra the plasma emission lines have been partially filtered. Experimental spectra present raw data; no averaging was used and there has not been subtraction of any type of background. Collection times of many hours were required to achieve acceptable signal-to-noise ratios. The performance of the spectrometer has been confirmed with more conventional solid-state samples and multiple fibers were investigated to ensure that the features are reproducible.

Figure 1(a) presents a room-temperature spectrum of poly(dA)·poly(dT) showing a distinct band in the 75 to 100 cm^{-1} region. Figure 1(b) presents a lower resolution, room-temperature spectrum of poly(dA)·poly(dT); for reduced photon counts additional bands near 60 cm^{-1} and 125–140 cm^{-1} are observed. For comparison, in Fig. 1(c) the room-temperature spectrum from a fiber of calf thymus DNA is presented demonstrating a broad feature near 85 cm^{-1}; changes of scale do not reveal any additional structure. For these purposes calf thymus DNA may be viewed as having a random sequence.

In an attempt to resolve the 75 to 100 cm^{-1} band in poly(dA)·poly(dT) we have paralleled the far-infrared experiments by making low-temperature measurements as
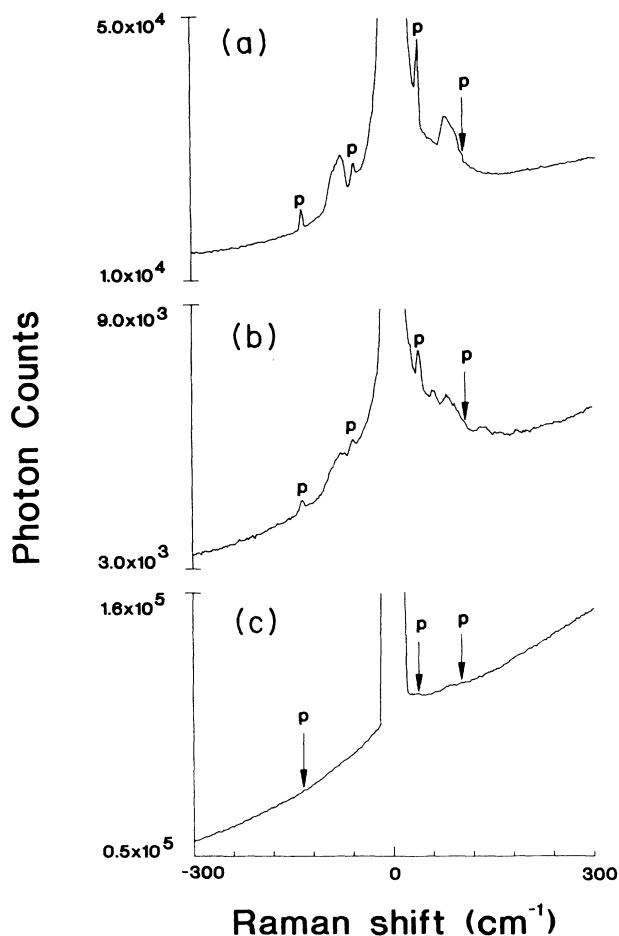
FIG. 1. Low-frequency Raman spectra of DNA at room temperature: (a) poly(dA)·poly(dT), approximately 10^4 counts; (b) poly(dA)·poly(dT), approximately 10^3 counts; (c) random sequenced DNA. Plasma emission lines of the argon-ion laser are indicated by p.

presented in Fig. 2. Unfortunately complications associated with the use of the cryostat led to a decrease in the signal-to-noise ratio. In addition, the laser line causes heating of the fiber. It should be noted that our low-temperature spectra show indications of a band near 25 cm^{-1} as previously observed.[8]

To determine eigenfrequencies the spectra have been modeled as a sum of oscillators.[9] A spectrum was first symmetrized to remove the fluorescence background and plasma lines. The symmetrized spectrum was then modeled as a sum of Lorentzian curves; the spirit of this process is to fit a spectrum with as few oscillators as is reasonable. As an example, the multioscillator fit to the spectrum shown in Fig. 1(a) is presented in Fig. 3. Multioscillator fits of the data in Figs. 1(a) and 1(b) clearly assign modes at 78 and 92 cm^{-1} and 58, 78, 94, and 134 cm^{-1}, respectively; although additional oscillators were required to achieve reasonable fits, these modes are not obvious in the original spectra. These "weak" modes include 52, 60, and 84 cm^{-1} modes in Fig. 1(a). In addition, both spectra required a mode near 35 to 40 cm^{-1}; the intensity of this mode was particularly sensitive to the fitting of the central feature. The spectral features of poly(dA)·poly(dT) are summarized in Table I and are compared with previous observations of infrared- and Raman-active modes. It may be interesting to point out that far-infrared measurements of polyethylene and other polyethylenelike polymers demonstrate a mode at 73 cm^{-1} for room temperature and a weak 108 cm^{-1} mode for low temperature.[10]

There are several aspects of these data to be discussed. First, Raman-active modes lie at 58–60, 78, and 92–94 cm^{-1} in definite correlation to the infrared-active modes observed at 62, 80, and 95 cm^{-1} by previous room-temperature measurements.[1] Although Landau splitting predicts the Raman-active modes to lie at higher frequency, the splittings are small and, given the resolution of the respective measurements and limitations in comparing Fourier transform infrared (FTIR) and Raman spectra, these differences are not significant.
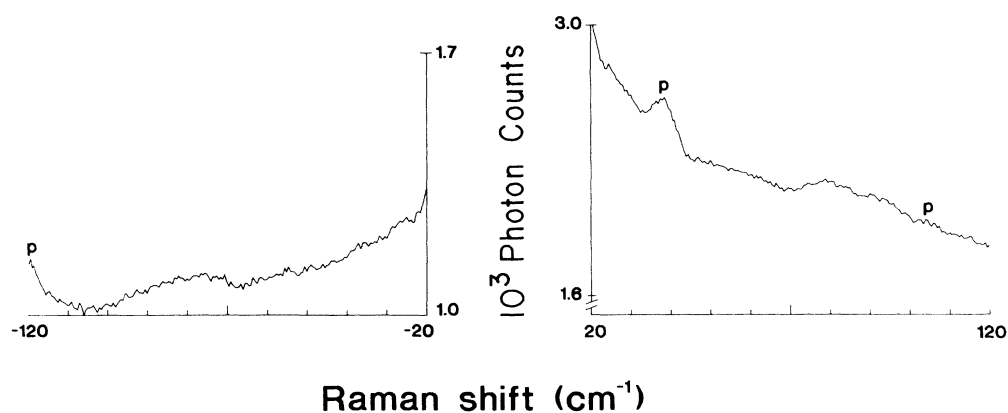

FIG. 2. Liquid-nitrogen-temperature spectra of poly(dA)·poly(dT). Plasma emission lines of the argon-ion laser are indicated by p.
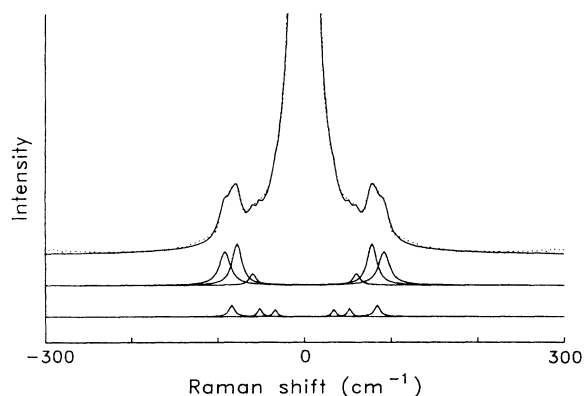
FIG. 3. Multioscillator fit to the spectrum presented in Fig. 1(a). The top curve represents the sum of Lorentzian curves (plus one Lorentzian to model the central feature) and the points represent the symmetrized data (see text). The lower six curves represent the six oscillators used in the model; an offset was introduced for clarity.

It is clear from these results and from the results of previous measurements as summarized above that the low-lying spectral features of poly(dA)·poly(dT), as seen in dehydrated fibers and films and in gels, are sharper than comparable measurements of other nucleic acids. What causes this apparent reduction in spectral line broadening? Of the known line-broadening mechanisms,[11] most relevant to these studies is inhomogeneous

TABLE I. Observed low-frequency modes in room-temperature spectra of Na-poly(dA)·poly(dT). Column one summarizes the results of this investigation; w indicates relatively weak modes (see text). The three modes reported in Ref. 6 are presented in the second column; these modes formed an unresolved band and eigenfrequencies were determined in a method similar to that discussed in the text. In the third column the modes as reported in Ref. 1 are presented. Frequencies are in wave numbers.

| Raman activity | | ir activity |
Figures 1 and 2	Reference 6	Reference 1
22–25w		
35–40w	32	
52w		
58–60		62
	71	
78		80
84w		
92–94	95	95
		106
		122
134		136
		170
		214
		238

broadening due to variations in the local strain. The length of the DNA polymers used for these experiments is on the order of a micron, i.e., thousands of base pairs. These results suggest that poly(dA)·poly(dT), relative to other sequences, may exhibit enhanced base-pair to base-pair structural uniformity. For long-wavelength vibrational modes, lack of structural uniformity along a DNA polymer is equivalent to a distribution of defects in a one-dimensional lattice, i.e., a source of spectral line broadening. This may explain the experimental requirement of dialyzing samples to eliminate randomly distributed salt crystals: measurements of infrared activity in films (1) and Raman activity in fibers (data not shown), in the presence of excess salt, fail to resolve these low-lying features.

It is well known that poly(dA)·poly(dT) has many unique structural and physical properties. Two recent structural determinations[12] of A tracts in DNA dodecamers have reported that tracts of dAdT assume an unusual B-type structure whose characteristics include an extreme propeller twist, maximized base stacking, a system of bifurcated hydrogen bonds, and a narrowing of the minor groove. It has been proposed[13] that a spine of hydration in the minor groove stabilizes this structure. Poly(dA)·poly(dT) does not exhibit the A to B conformational transition as a function of salt and hydration; however, Austin and co-workers[14] have pointed out that for physiological conditions poly(dA)·poly(dT) does exhibit conformational flexibility as a function of temperature. A candidate for a temperature-mediated soft mode associated with this transition is evident in a lattice-dynamical model.[15]

Another aspect of these data is evident from comparison of Figs. 1(a) and 1(b). In the first spectrum the feature near 60 cm^{-1} is not as well resolved as the 75–100 cm^{-1} band. In the second spectrum, however, bands near 60 and 125–140 cm^{-1} are apparent. In our measurements the 60 and 125–140 cm^{-1} bands are resolved in early scans of a fiber but appear to "melt out" as photon counts mount during later scans. One possible explanation is that these modes may be interhelical and the order necessary to support such modes is thermally disrupted. A second possible explanation is that these modes are associated with a temperature-dependent soft mode as discussed in the preceeding paragraph. These issues are of particular interest since two lattice-dynamical models[5,16] differ in their ability to account for the 60-cm^{-1} mode as an intrahelical mode.

In conclusion, we have made measurements of dehydrated, oriented fibers of poly(dA)·poly(dT) with improved resolution of several low lying, Raman-active bands. A multioscillator fit of the spectra assigns modes at 58–60, 78, and 92–94 cm^{-1} that correlate with those observed in far-infrared measurements.[1] These features have a high Q relative to comparable measurements of other DNA sequences and are well described by lattice dynamics. These results suggest that further experiments be done to address whether these modes are indeed inhomogeneously broadened. With regards to the modes near 58–60 and 134 cm^{-1}, their dynamical behavior appears to differ from those modes in the 75 to 100 cm^{-1} band.

ACKNOWLEDGMENTS

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