Observation of Low-Lying Raman Bands in DNA by Tandem Interferometry

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A tandem Fabry-Perot interferometer has been used to locate Raman bands at 12 cm^{-1} (*B*-DNA) and 15 and possibly 12 cm^{-1} (*A*-DNA). The Raman shift of these bands is dispersionless and the scattering process appears to require quasimomentum conservation. We assign these bands to the lowest-lying Raman-active optic phonons.

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It is important to locate the lowest-lying zone-center optic phonon of the DNA double helix because of the proposal that the molecule changes its conformation through softening of this mode.[1] Recent experiments appear to show resonant microwave absorption by DNA[2] adding to the need for a systematic spectroscopic search for excitations in the inverse centimeter to gigahertz region. The frequencies and polarization characteristics of the lowest bands reported to date[3,4] are not consistent with the predictions of a lattice-dynamics calculation.[5] This is almost certainly a consequence of the limitations of grating monochromators when used below a few tens of inverse centimeters with samples of poor optical quality (such as DNA fibers and films). Sophisticated methods for operating Fabry-Perot interferometers in tandem have been developed recently[6] and we have applied the technique to find what we believe are the lowest-lying Raman bands due to optic phonons in the two common conformations of DNA (the so-caled *A* and *B* conformations).

A tandem Fabry-Perot can achieve better resolution and stray-light rejection than a multiple-grating monochromator, albeit at the cost of reduced luminosity and increased complexity. The reduced transmission (at a given angular acceptance) is largely a consequence of a relatively small aperture, though the many reflecting surfaces of a practical system[6] contribute further losses, so that signal aquisition times are much longer (typically one thousand times longer) than with the usual Raman monochromators. Practical restrictions on the alignment of a tandem Fabry-Perot limit its scanning range[6] while the ultimate contrast is set by spontaneous emission from the laser. To our knowledge tandem interferometry has not been applied to Raman spectroscopy in the way we outline here, and so we have tested our method in a number of ways (illustrated in Fig. 1). For the spectra we show, the interferometer plate gap was set to 200 μm (determined interferometrically[7] to ± 5 μm) resulting in instrumental ghosts at +25 cm^{-1} (which have been removed for clarity). The overall bandpass is controlled by a 10-nm interference filter (a grating is impractical at this scanning range). 10 μW of the 514.5-nm laser line fed into the interferometer at full aperture gives the response shown in Fig. 1(a). There is a line at 13-cm^{-1} Stokes shift (due to a strong plasma emis-

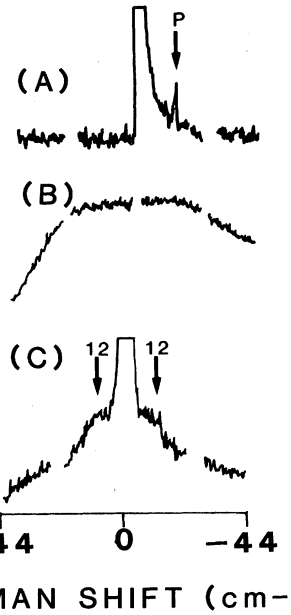

FIG. 1. Performance of the tandem interferometer as a Raman spectrometer. The three spectra are (a) 10 μW of the 514.5-nm laser line fed into the spectrometer, (b) white light, and (c) a 12-cm^{-1} LA mode in high-density polyethylene (the band center is marked with arrows). The 13-cm^{-1} plasma line is labeled *P* on this and subsequent figures.

sion) and a tail caused by phototube dynode heating, but the background is otherwise flat (and about twice the dark count of 0.1 count/s). Departure from the requirement that the plate gaps be simultaneously zero[6] limits the uniformity of the transmission, and we show a typical response to white-light illumination in Fig. 1(b) (minimum transmission is about 50% of the maximum). This profile varies a little from day to day (because of thermal drift in the plate gaps) and so we record such a transmission profile before and after each spectrum by focusing a spot of white light onto the sample. The response below 25 cm^{-1} is quite uniform. Figure 1(c) is a spectrum obtained from a sample of high-density polyethylene showing an LA mode at 12 cm^{-1} in excellent agreement with the results obtained with a triple monochromator.[8] The instrumental full width (for the spectra shown here) was 0.5 cm^{-1}.

The $\theta = 0$[9] optic modes are of most interest[2] but they are difficult to observe because of the requirement that the incident and scattered electric fields lie along the helical axis (z).[10] Taken together with quasimomentum conservation, these conditions are approximately satisfied in the geometry shown in Fig. 2(a) (with $\vec{E}_i$ and $\vec{E}_s$ in the scattering plane). A mode of related character may be probed in the experimentally easier backscattering geometry shown in Fig. 2(b) with the fiber tilted so that q is inclined at about 45° to z. In these geometries, modes at $\theta = \Psi$ (the helix pitch angle) scatter with $\vec{E}_s$ perpendicular to the scattering plane.[10]

We have used wet spun films of calf thymus DNA prepared by one of us (A.R.) as described elsewhere.[11] Most (> 80%) of the chains have a molecular weight in excess of 10^6 daltons (1,500 base pairs). The films are both highly and uniformly birefringent,with the helix axis lying along the draw direction (z in Fig. 2). Samples salted at 1% excess NaCl and held at 66% relative humidity (r.h.) are in the A conformation while samples with 4% excess LiCl are in the crystalline B conformation at 66% r.h. In the forward scattering geometry of Fig. 2(a) the B-DNA yields the spectrum shown in Fig. 3(a). A shoulder is evident at about 12 cm^{-1}. The backscattering geometry [Fig. 2(b)] leads to less elastic scattering and a distinct band is observed at 12 cm^{-1} [Fig. 3(b)]. In A-DNA fairly sharp bands are seen at 15 cm^{-1} [Fig. 3(c)—22-cm^{-1} peaks are hidden by the ghosts]. Spectra obtained at higher resolution show features which we attribute to acoustic[12] or local[13] modes. We have not, however, found any lower-lying bands that we

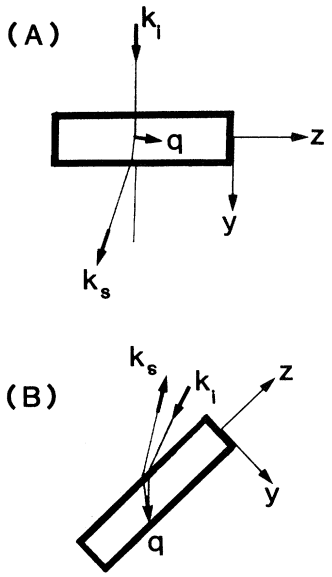

FIG. 2. Scattering geometries used. (a) Near forward scattering with the scattering vector (q) nearly along the fiber axis (z). (b) Near backscattering with the film tilted so that q lies about 45° from z.

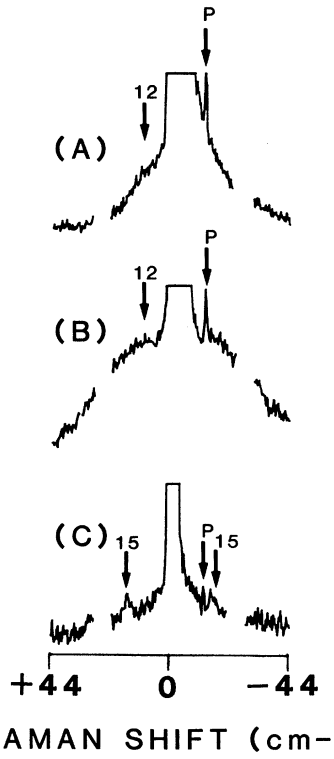

FIG. 3. Spectra of DNA films. (a),(b) B-DNA (LiCl, 66% r.h.) in the forward and backscattering geometries, respectively. (c) A-DNA (NaCl, 66% r.h.). The arrows mark the band center frequencies referred to in the text.

can assign to optic phonons.

These spectra were all obtained with 15 mW of laser power focused to a 100-μm spot, signal averaging for typically 4 d for each spectrum. This is above the power density at which disruption of the Brillouin spectra occurs,[12] but the low-lying Raman bands appear far less susceptible to these effects[5] although, even at these low power levels, visible damage occurs after about 5 d of laser exposure, a phenomenon we do not understand.

The films are optically active and polarization analysis is difficult—in the spectra shown both x and y polarizations are collected. However, by using the backscattering geometry and focusing tightly onto the surface (so that the sample is probed to a depth of only a few microns) we appear to be able to obtain consistent results, albeit at very poor signal-to-noise levels as the losses introduced by the polarization analyzer cannot be compensated for by extending the duration of the experiment. The 12-cm^{-1} band in B-DNA appears to be fully (zz) polarized while the 15-cm^{-1} band appears depolarized (zx). In approximate zz polarization the central feature in the A-DNA spectrum is resolved into very feeble 12-cm^{-1} bands.[5]

These features do not change position with scattering angle and appear to weaken as q is moved away from alignment with z. They are therefore most probably due to optic phonons. We cannot rule out the possibility that there are lower-lying optic phonons which are not Raman active, or are viscously or inhomogeneously broadened so as to be unobservable as distinct bands; however, the frequencies and polarization of the bands we report here are in remarkable agreement with the predictions of a lattice-dynamics model in which the nonbonded potentials have been fitted to the observed acoustic velocities.[5] We do indeed find an association between softening of low-lying Raman bands and conformation changes of the DNA double helix although we also find that inter-double-helical interactions play a major role in stabilizing the A or crystalline B conformations.[14] These factors may be important in leading to dynamical and structural differences between chromosomal or condensed prokaryotic DNA and the molecule in solution. In consequence, however, of these complications, there are significant differences between the crystalline form of B-DNA and the semicrystalline or "wet" form of B-DNA. A fuller report of this work will present evidence to substantiate the view that these are really two distinct forms of DNA, and so we emphasize the point that the results presented here for the crystalline B form do not apply to, for example, sodium salted DNA at high humidity, also, nominally, B-DNA.

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