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19 ABSTRACT (Continue on reverse if necessary and identify by block number)
We have used Raman and Brillouin scattering to study the role of water, ions base sequence, and crystal morphology on the structure and dynamics of DNA. We find that the interactions between DNA double helices (as measured by the frequency of Raman bands in the solid state), elastic properties, internal geometry (conformation) and optical properties are all dominated by the specific counter ion, changing in a systematic manner with counter ion hydrated radius. The primary hydration shell relaxes at about 2.5 GHz and consists of about one monolayer of water molecules attached to the DNA by one hydrogen bond, on average. The hyperpolarizability of DNA films is about 1% of that of quartz (A10)

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ANNUAL REPORT ON CONTRACT N00014-87-K-0478

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PRINCIPAL INVESTIGATOR: S.M. LINDSAY

CONTRACT TITLE: OPTICAL NONLINEARITY IN DNA FILMS

START DATE: JUNE 1, 1987

RESEARCH OBJECTIVE: To study the linear and nonlinear optical properties of DNA films.

1. PROGRESS: YEAR 2

1.A. The role of Ions, Water and Base-sequence in the Structure and Dynamics of DNA

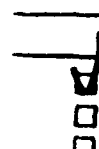
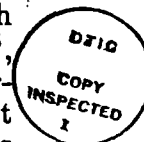
We have used Raman and Brillouin spectroscopy to study the role of water, ions, base sequence and crystal morphology on the structure and dynamics of DNA. The role of the hydration layer is significant, and we have extended our work to simple electrolytes (LiCl) and, in recent (unpublished) work to supercooled water. We summarize some of this work as follows:

(1.A.i) Many of the interactions of DNA (including interactions that control conformation) are counter-ion-mediated. The effects appear to be controlled by counter-ion size (as opposed to mass) and counter-ion charge. DNA (and, presumably, other similar polyelectrolytes) belong to a class of materials whose microscopic and macroscopic properties are controlled by counter-ion doping¹ (see Appendix D for further results).

(1.A.ii) The base sequence (in the sense of the number of bridging hydrogen bonds) has *relatively* little influence on the structure, and almost no influence on the low frequency dynamics. Although recent work shows a correlation between sequence and the conformation of DNA in crystals², we have shown that external factors, such as crystal packing³, can dominate. It is possible that the influence of sequence is indirect, perhaps through the propensity of certain sequences to form ordered water spines (see 1.A.v). Soumpasis has demonstrated the role of ion-packing effects⁴ in the diffuse ion cloud. Experiments with Poly(dA)·Poly(dT), modified so as to have an NH_2 group at the C2 position of adenine⁵, have indicated the role that water ordering may play. We have only recently begun to clarify the role of sequence (using homopolymers). Earlier work with calf thymus DNA not only obscured this aspect of structure, but is complicated by the fact that the differences in stacking energy between the A and B conformations is a minimum at the G/C ratio of calf thymus DNA.⁶

(1.A.iii) With the exception of the lowest mode, the low frequency Raman bands reflect the conformation of the backbone; results for some A and B sequences have been published,^{7,8}.

(1.A.iv) Our analysis of the Raman lineshapes (including unpublished measurements made at low temperatures) indicate that fundamental (unresolved) problems arise in modeling the temperature dependence of the lineshapes of broad (anharmonic) low frequency spectra. Specifically, the normal fluctuation-dissipation theorem approach to calculating



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lineshapes may be invalid⁹ (though we have little choice but to use it at present). Monte-Carlo simulations¹⁰ show that quite anomalous temperature dependences might occur; for example, band intensities might *increase* as temperature is lowered.

(1.A.v) The lineshapes of the various intrahelical modes are very broad, yet don't appear to depend on temperature, crystallinity or sequence! The only hint as to their origin lies with the noticeable sharpening in the spectra of Poly(dA)·Poly(dT) (where an ordered water spine exists in a modified minor groove^{11,12}). Thus, if these lines are inhomogeneously broadened, *their width reflects a distribution of water structures even in a homopolymer or oligonucleotide crystal.*

Finally, we note that Urabe and Tominaga^{13,14} have studied the temperature dependence of the lowest frequency (interhelical) mode in *solutions* at low temperatures. The ion dependence of the mode is somewhat different than in the solid state¹⁴ but the fact that the mode exists at all in solutions implies that it is *not* just an 'inter-double-helix' mode, but it involves the motions of the counterions explicitly! If this is the case, then even the rather sophisticated potential-of-mean-force (pmf) description of Soumpasis⁴ is too simple. This is because the motions of the counter-ion cloud are treated statistically (i.e., averaged over to produce a net effective pmf). There are probably several ways in which ions modulate DNA structures, and this may have many consequences in biology.¹⁵

1B. Experimental Progress in measuring OHG in DNA Films

The measurements we reported in our original proposal were completely qualitative (and possibly corrupted by OHG from our make-shift beam splitter). We now have a functioning OHG apparatus and some signals from samples in controlled conditions. OHG in these films is weak; we will have to modify our arrangement somewhat in order to make systematic measurements. However, the pseudo inversion symmetry of the double helix *should prohibit any OHG!* (The inversion symmetry is exact for a homopolymer or 'palindromic' sequence). We discuss the possible implications of OHG in DNA films in the next section. Here we outline our experiments to date.

The most difficult problem is the optical quality of the films. We originally squeezed the film between a pair of optical flats, but this arrangement made humidification difficult. We now use a cell in which one of the windows is a long focal length biconvex lens. Not only does this permit more controlled flattening of the center of the film, but it also leaves space for humidified air to be flushed through. A piece of DNA film is swollen slightly by being left at 100% r.h. for ten minutes or so. It is then placed on the center of the lens, and gentle pressure is applied as moist air is flushed through the cell. The center usually flattens into a completely clear region of some 5mm diameter. Birefringence measurements show that the material maintains its alignment. The water in the gas humidification system is replaced by the appropriate saturated salt solution and the sample is equilibrated at the desired r.h. (its water content can be measured accurately by Brillouin scattering¹⁶). We have exposed samples made in this way to mode-locked, Q-switched IR pulses of up to 160 kW in a 3mm dia. beam (well over 2 MW/cm² with no *visible* sign of damage (but see below). The optical quality is such that the Rayleigh scattering from the film is only a little more than the scattering from the surface of the optical components. It is difficult to quantify our initial results, because derivation of the appropriate elements of the hyperpolarizability tensor depends critically on the geometry both of the film, and of the arrangement of the microcrystallites (or whatever is responsible for the OHG) within the film.¹⁷ However, we now measure our signal relative to a standard. Using a 1mm thick piece of z-cut quartz, with the Nd:YAG beam incident normally, and at the same power

density as at the DNA film, a 20 μm Na-DNA film (1% excess NaCl), equilibrated at 75% r.h., produces a signal (on first exposure) that is no more than about 1% of the quartz reference signal. The second harmonic signal appears to decay with time, *even though no visible damage occurs*. We have only just begun to investigate the first sample, and further improvements and experiments will be carried out during a one year, no-cost extension.

2 WORK PLAN (No cost extension, year 3)

In addition to studies of OHG as a function of humidity, we plan to attempt to measure OHG as a function of scattering angle. Our original proposal pointed out that symmetry considerations should prohibit OHG in DNA (briefly, the second harmonic polarization varies as the square of the incident field, so information about inversion of the sample can only be conveyed by inversion of the hyperpolarizability; an impossibility in materials with an inversion symmetry). However, we also pointed out that the double helix geometry is incompatible with three dimensional packing, and so interhelical contacts must break the crystal symmetry.³ Studies of piezoelectric effects in DNA films may also indicate a permanent polarization (inconsistent with an inversion symmetry),¹⁸⁻²¹ although interpretation of electrical measurements in terms of ferroelectric behavior is disputed.^{21,22} Nonetheless, the primary requirement for piezoelectricity is the absence of inversion symmetry: DNA should not be piezoelectric!

Piezoelectricity has been investigated widely in many other biopolymers, in addition to many tissues.²³⁻²⁸ If regions of spontaneous polarization are indeed present, they may be significant in biological processes. For example, electric fields can induce conformation changes in DNA.^{29,30} On a more general level, electric fields in polyelectrolyte systems will modify ion transport and chemical (reaction) oscillations.³¹ Electrical measurements are notoriously difficult to make on biological tissue, particularly in its hydrated state. The best data (of which we are aware) appears to have been obtained on collagen fibers. The pyroelectric effect³² (appearance of a polarization with changes in temperature) *implies a permanent polarization*, while the large value for certain piezoelectric coefficients³³ is another clear indication of macroscopic polarization. These data are subject to the usual concerns about the quality of electrical measurements on biological samples. Rat-tail tendon, like DNA films, should not have a macroscopically polar structure. Although the individual collagen molecules are polarized (unlike DNA), there is good structural evidence that the number of 'up' fibrils equals the number of 'down' fibrils, and that the fibril distribution is homogeneous.³⁴ These conflicting results were brilliantly resolved (from an experimental viewpoint) in a series of optical measurements by Isaac Freund's group at Bar-Ilan University. They used OHG as a probe of polarization in hydrated samples, finding strong evidence for spontaneous polarization.³⁵ The conflict with structural data was resolved (in a sense!) by their use of *Optical Second Harmonic Microscopy*.³⁶ By probing the OHG from rat tail at 50 μm resolution, they found that the polarization occurred in macroscopic patches whose distribution was not associated with the known distribution of collagen fibrils in the tendon. Although these experiments have replaced one mystery with another, they do not challenge the well established structural models, and explain a considerable body of electrical data. At a microscopic level, the occurrence of spontaneous polarization might be understood through the formation of ordered water spines.

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5 TRAINING ACTIVITIES

The following students graduated:

PhD

Nonjain Tao, *Structure and Dynamics of the DNA Hydration Shells*—(1988).

Thomas Weidlich, *Raman Spectroscopy from the low frequency vibrations of DNA in Highly Crystalline Films, Oligonucleotide Crystals and Polynucleotide Solutions*—(1989).

MS

Qi Rui, *Low Frequency Raman Spectra of RNA Homopolymers*—(1988).

One is a women, all three are non-citizens.