A REINTERPRETATION OF THE INFRARED LINEAR DICHROISM OF ORIENTED NUCLEIC ACID FILMS AND A CALCULATION OF SOME EFFECTIVE PARTIAL CHANGES ON THE RIBOSE PHOSPHATE BACKBONE

C. P. BEETZ, JR. AND G. ASCARELLI, *Department of Physics*
S. ARNOTT, *Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907 U.S.A.*

ABSTRACT In this paper we show, based on symmetry considerations, that structural information cannot be obtained from the linear infrared dichroism of the dioxy vibrations of the phosphate group of nucleic acids. Consequently, the discrepancies between the results of x-ray structure measurements and linear dichroism measurements are not meaningful. The linear dichroism measurements are instead important for a calculation of transition dipole moments that involve both the vibrations of all the atoms of the nucleotide and their charges. Independent information on either the atomic displacements contributing to a given vibration or the atomic charges permits a refinement of the unknown quantities. Based on the molecular dynamics calculations of Prohofsky et al., atomic charges of DNA are calculated to reproduce the observed linear dichroism results. Some of the resulting charges are unexpected and may reflect the inadequacy of the molecular dynamic calculation.

INTRODUCTION

The orientation of the phosphate group in the nucleic acid backbone has been determined for several DNA conformations primarily by two experimental means: x-ray diffraction measurements (1–4) and infrared linear dichroic absorption measurements. (5–9). The results do not agree with each other. It is the purpose of this paper to investigate the possible sources of this discrepancy.

In general, the quality of the x-ray diffraction obtained from nucleic acid fibers is poor compared with that of a well-annealed single crystal: even in the case of homopolymers, it is neither possible to obtain single crystals of nucleic acids nor even to orient perfectly all the molecules in the fibrous sample. The resulting limit on the resolution (0.3 nm) does not permit the direct determination of the atomic coordinates.

To obtain the coordinates of the atoms in a repeating unit of the nucleic acid backbone, the low resolution x-ray data is supplemented with the results of a structural analysis of either single-crystal nucleosides or single-crystal nucleotides (1). The central assumption used is that the bond lengths and bond angles that exist in the single crystals are maintained in the polynucleotides. The validity of this assumption has been confirmed by the observation of the relatively invariant nature of the purine, pyrimidine, and deoxyribose rings in several different compounds. (10, 11). The problem of the determination of DNA structure is then reduced to

the definition of a set of conformation angles (2, 4) (Fig. 1) that locate the various subgroups relative to one another. These angles are determined in such a way so as to optimize agreement between the calculated and observed diffraction patterns (4). The orientation of the phosphate group in the backbone is automatically determined in the process of finding the conformation angles.

The assumptions that bond lengths and bond angles can be transferred from simpler compounds to the nucleic acids may introduce a symmetry in the "x-ray determined" coordinates that does not necessarily exist in the polynucleotide. For example, a phosphate group is modeled as having C_{2v} symmetry despite the fact that counter ions and water of hydration are likely to decrease its symmetry. The x-ray structure determinations are not sensitive enough to distinguish configurations in which either two atomic positions vary in bond length by about 0.1 Å or bond angles vary by about 5°.

The IR linear dichroism measurements, in contrast, can directly determine the orientation of the transition dipole moment associated with a given molecular vibration (12).

As an example, the IR absorption spectra of partially oriented nucleic acid films show two absorption bands that have been assigned to the molecular vibrations of the PO_2^- group. The dioxy stretching vibrations occur at ~1,230 and ~1,090 cm^{-1} and have been respectively assigned (13) to the so-called "antisymmetric" and "symmetric" motions of the dioxy oxygen atoms.

The dioxy absorption bands in oriented nucleic acid films are dichroic and allow a determination of the orientation of the transition dipole moment associated with these two vibrations with respect to the helix axis. The direction of the transition dipole moments of these two vibrations with respect to the phosphate group has been assumed to be such that the 1,230 cm^{-1} antisymmetric mode dipole is along the O2—O3 line (Fig. 2) and the 1,090 cm^{-1}

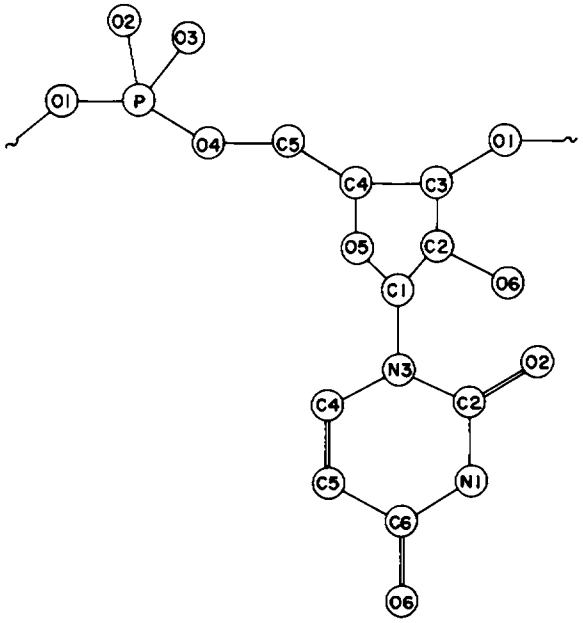

FIGURE 1 Conformation angles.

TABLE I
COMPARISON OF RESULTS FROM IR DICHROISM MEASUREMENTS AND X-RAY STRUCTURE DETERMINATIONS

	PO_2^- bisector	O3—O2 line	
IR*	45	65	A-DNA
X-ray‡	15	79	
IR*	70	56	B-DNA
X-ray‡	54	75	
IR§	63	57	B'-DNA
X-ray‖	36.5	77	
IR¶	62	48(A+T rich DNA)	C-DNA
	64	51(Calf thymus)	
	67	48(Salmon sperm)	
X-ray**	56	73	
IR‡‡	62	60(Poly(dA)*poly(dT)	D-DNA
	65	65(Crab light DNA)	
X-ray§§	47	73	
IR‖‖	40	70	A-RNA
X-ray	39.9	62.9	

*Table II, p. 396, ref. 5.
‡Coordinates from ref. 1.
§Table I, p. 1871, B* DNA, ref. 6.
‖Table 2, p. 513, ref. 15.
¶Table 1, p. 3353, ref. 7.
**Table II, ref. 16.
‡‡Table I, p. 1871 and Table III, p. 1872, D* DNA, ref. 6.
§§Table 1, p. 525, ref. 17.
‖‖Table II, p. 396, ref. 5.

symmetric mode dipole is along the O2—P—O3 angle bisector (13, 14). With this assumption, the determination of the orientation of the dipole moments in the phosphate group with respect to the helix axis uniquely determines the orientation of the phosphate group in the nucleic acid backbone. Table I shows a comparison of the results of the x-ray and IR data determination of the angles that the O2—P—O3 angle bisector and the O2—O3 line form with the helix axis. The disagreement between the two sets of data is well outside the accepted experimental errors.

To determine whether or not the discrepancy was due to the x-ray diffraction modeling procedure or to inherent errors associated with the IR dichroism assumptions, the phosphate orientation angles, as determined by the IR dichroism measurements, were used as additional contraints on the conformation angles in the x-ray structure determination. The reproduction of the observed x-ray diffraction with the above constraints imposes severe deformations of the generally accepted bond lengths and bond angles of the dioxy ribose ring. Such deformations are well outside of the range of the normally observed variations for these quantities.

Finally, Arnott and collaborators[1] have reviewed their previous results in the light of better

[1]Arnott, S. Unpublished results.

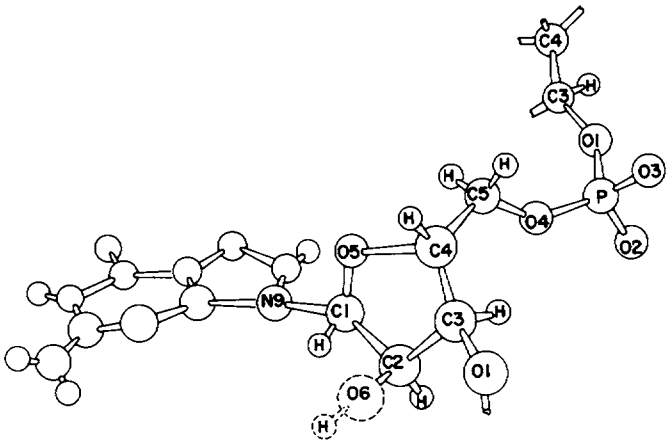

FIGURE 2 Pictorial view of the (deoxy) ribose-phosphate backbone.

data and of refinement procedures available since some of the earlier models were published. They conclude that only trivial changes in the conformation angles are warranted. The conclusion of the next section is that the bulk of the discrepancy between the two sets of phosphate orientation angles appears to arise from a misinterpretation of the IR dichroism data.

ANALYSIS OF THE IR DICHROISM MEASUREMENTS

The extraction of specific molecular group orientation angles from the IR dichroism data is a two-step process (14). The first step involves the measurement of the dichroic ratio and the calculation of the resultant angle the transition dipole makes with respect to the helix axis.

The dichroic ratio for a partially oriented polymer in which only a fraction f of the molecules have their axis along a given direction z is given by (12)

$$R\left(\frac{\parallel}{\perp}\right) = \frac{O'D_{\parallel}}{O'D_{\perp}} = \frac{f\cos^2\theta + 1/3(1-f)}{f/2\sin^2\theta + 1/3(1-f)}, \tag{1}$$

where $R(\parallel/\perp)$ is the ratio of the optical densities for light polarized parallel and perpendicular to the helix axis, and θ is the angle between the transition dipole direction and the helix axis. The use of this equation has been extensively discussed in the literature (5–9, 12). Its limitations and its use will not be repeated here. Suffice it to say that it is not at the origin of the discrepancy. The second step must relate the direction of the measured transition dipole moment with respect to the orientation of the molecular group that is associated with the specific optical absorption.

In the case of the phosphate dioxy stretching vibrations in the nucleic acid backbone, the simple classification of the modes as symmetric and antisymmetric have been made by comparison with model compounds involving the PO_2^- structure (13). The symmetry of the model compounds is C_{2v} for the hypophosphite anion $H_2PO_2^-$ and C_2 for the dimethyl phosphate anion $(CH_3O)_2PO_2^-$. However, inspection of the phosphate group in its environ-

ment in the nucleic acid backbone clearly reveals the symmetry to be C_s since the C3 and C5 carbons attached to the O1 and O4 oxygens are not equivalent.

The symmetric and antisymmetric vibrations refer to the motion of atoms in the reflection plane containing the two hydrogen atoms and the phosphorus atom of the $H_2PO_2^-$ ion. Such a reflection plane disappears when the symmetry is reduced from C_{2v} to C_s. As an example, in the normal mode calculations performed by Shimanouchi et al. (13) on $H_2PO_2^-$, $D_2PO_2^-$, it is clear that when the symmetry of the two former molecules is lowered from C_{2v} to C_s (by substituting one H with a D) the normal mode described as symmetric PO_2^- changes its characteristics. Furthermore, modes such as the symmetric and antisymmetric motions of the oxygen atoms are not limited to the motion of these individual atoms but involve other atoms in the molecule as well, in particular the phosphorus atom and all the other atoms attached to it.

As an example, consider the motions corresponding to the 1,160 cm^{-1} (1,042 cm^{-1}) and 1,180 cm^{-1} (1,153 cm^{-1}) bands of $H_2PO_2^-$ ($D_2PO_2^-$). These bands are accurately described as symmetric and antisymmetric PO_2^- stretching modes of $H_2PO_2^-$ ($D_2PO_2^-$) that have C_{2v} symmetry. By this we mean that the corresponding motions belong to the A_1 and B_2 representations of C_{2v} (i.e., they transform as z and $|x|$ or y, respectively). When the symmetry is lowered to C_s, the motion that is usually improperly labeled antisymmetric PO_2 stretching (it transforms like the B representation of C_s; its basis function can be taken as y) is described by a linear combination of motions corresponding to the several motions belonging to different A_2 and B_2 (the A_2 and B_2 representations of C_{2v} transform, respectively, as $|z|$ or xy and $|x|$ or y) representations of C_{2v}. This mixing of the irreducible representations of C_{2v} to form the irreducible representations of C_s is due to the fact that C_s is a subgroup of C_{2v} and is evident from inspection of the group compatibility table (Table II). These arguments are supported by detailed calculation of the normal modes (13) of $HDPO_2^-$, which has, indeed, the C_s symmetry.

According to the normal mode calculations of Shimunouchi et al. (13), the 1,071 cm^{-1} mode of $HDPO_2^-$ derives 57% of its potential energy distribution (PED) from a symmetry coordinate describing the symmetric PO_2^- stretching and 37% from a symmetry coordinate describing an HPD bending motion. The remaining 6% is derived from other motions. In the case of the 1,160-cm^{-1} band of $HDPO_2^-$ it derives 90% of its PED from a symmetry coordinate describing a symmetric PO_2^- stretch, and 5% from a representation describing an HPD bending, and the remaining 5% of the PED from other motions of the atoms in the group.

The motions of the different atoms in a molecular group having C_s symmetry should be

TABLE II

C_{2v} – C_s COMPATIBILITY TABLE

C_{2v}	C_s (σ_{xz})
A_1	A
A_2	B
B_1	A
B_2	B

viewed as linear combinations of motions that belong to different representations of C_{2v}. The relative importance of the contributions belonging to different symmetries cannot be obtained from group theoretical arguments. The admixtures of different representations may be significant despite the fact that the vibrational frequencies are not appreciably changed. The perturbed energies can be calculated by using the characteristics of the more symmetric Hamiltonian; however, the displacements are in general not appropriate to the perturbed Hamiltonian. This is a paraphrase of the well-known result of perturbation theory (18), i.e., the correction to the energy to ith order requires a knowledge of the eigenfunction only to the (i-1)th order.

To know the transition dipole moment direction for a given vibrational mode, it is necessary to calculate

$$\mu_{mo} = \sum_j q_j \mathbf{r}_{jom}, \tag{2}$$

where μ_{mo} is the electric transition dipole between states o and m, q_j is the charge on atom j, and $\mathbf{r}_{jom}$ is the oscillating position vector describing the (om) vibration of the jth atom. This description is classical. For its quantum mechanical analogue $\mathbf{r}_{jom} = <\psi_m|\mathbf{r}_j|\psi_o>$, where $\mathbf{r}_j$ is the position vector of the jth atom, and ψ_o and ψ_m are the wave functions describing two different vibrational states of the whole molecule. These vibrational wave functions must transform as basis functions of the group describing the actual symmetry of the molecule.

In the case of polynucleotides, the fact that nonequivalent carbons (C3 and C5) are attached to the O1 and O4 oxygens decreases the symmetry of the potential necessary to describe the PO_4^{--} group from T_d to C_s. In C_s there are no motions that can be described as symmetric and antisymmetric with respect to the two reflection planes present in either T_d or C_{2v}. As mentioned before, the symmetric and antisymmetric motion of the O2 and O3 oxygens is not limited to these atoms but is shared by the whole PO_4^{--} group. This motion is in turn coupled to the motion of the remaining atoms in the deoxyribose ring through the displacements of the O1—C3 and O4—C5 bonds. The relative amplitude of the motion of the atoms in the deoxyribose ring compared to the motion of the O2 and O3 oxygens is a result that can be obtained from a complete calculation of the normal modes of the polynucleotides.

Recently Forrest and Lord (19) in a study of cyclic nucleotides introduced experimental evidence focusing on the importance of substitutions in the ribose ring and their effect on the frequency of the diester and dioxy modes of the phosphate group of nucleotides. This can only take place if there is an appreciable delocalization of the PED. This delocalization is particularly important for the symmetric phosphate modes.

The results of two recent normal mode calculations (20–22) for the A-DNA, B-DNA, and A-RNA structures are shown in Table III. This table shows that the PED's for the 1,094 cm^{-1} and 1,209 cm^{-1} are not due to pure PO_2^- dioxy stretching modes. There is considerable admixture of other modes from the backbone, and as a result the transition dipole moment is given by

$$\mu_{mo} = <\psi_m|\sum_i q_i \mathbf{r}_i|\psi_o>, \tag{3}$$

where the sum involves not only the dioxy oxygens but also the other atoms contributing to the PED. Note that the PED given by Brown and Peticolas is the sum of the PED associated with the P—O2 and P—O3 motion. They do not contribute equally to the PED.

TABLE III

NORMAL MODE CALCULATION RESULTS AND POTENTIAL ENERGY DISTRIBUTIONS

	(Lu, Prohofsky, and Van Zandt)						(Brown and Peticolas)			
	A-RNA		A-DNA		B-DNA		A-DNA		B-DNA	
	1,107 cm^{-1}	1,211 cm^{-1}	1,105 cm^{-1}	1,209 cm^{-1}	1,094 cm^{-1}	1,209 cm^{-1}	1,098 cm^{-1}	1,209 cm^{-1}	1,093 cm^{-1}	1,210 cm^{-1}
C3—01	—	—	—	—	3.5	4.1	12.0	—	25.0	—
C3—C4	—	—	—	1.7	—	—	—	—	—	—
C4—C5	—	—	—	—	—	—	—	—	—	—
C5—04	18.0	—	8.3	—	15.5	—	—	—	—	—
P—04	7.2	—	5.8	—	—	—	—	—	—	—
P—02	32.1	41.7	35.0	45.6	21.3	42.3	62.0	84.0	56.0	83.0
P—03	27.5	42.7	31.6	43.8	17.5	43.0				
P—01	2.3	—	2.1	—	—	—	—	—	—	—
C4—05	—	—	—	—	1.6	1.0	—	—	—	—
05—C1	—	—	1.5	13.7	0.6	—	—	—	—	—
C1—C2	—	—	—	1.6	—	—	—	—	—	—
C2—C3	—	2.8	2.8	—	4.2	0.7	—	—	—	—
C2—06	—	3.6	—	—	—	—	—	—	—	—

Further, since Eq. 3 involves a vectorial addition, small contributions at right angles to the O2—P and O3—P stretching directions can produce a considerable rotation of the transition dipole moment from a direction along either the O2—P—O3 angle bisector or the O2—O3 line.

It must be also noted that these (20, 22) calculated PED are obtained assuming the polynucleotide structure is "determined" by x-rays where the PO_4^{-2} group is assumed to have C_{2v} symmetry. The force constants for the P—O2, P—O3 bonds are assumed equal. This gives rise to a contribution of the PO—2, PO—3 bonds to the dipole moment that is close to parallel (or perpendicular) to the O2—P—O3 bisector. The direction of the total dipole moment (Eq. 3) may, however, differ appreciably from the O2—P—O3 bisector and is very sensitive to minor changes in the force constants that produce negligible effects ($<10\ cm^{-1}$) on the calculated energies. For example, when an asymmetry of $\pm 5\%$ in the P—O2, P—O3 force constants of B-DNA is introduced in the normal mode calculation (22) for the 1,092-cm^{-1} vibration, the angle between μ and the O2—P—O3 bisector changes from 3.68° to 14.9°. Refinements (20, 22) of the force field in the normal mode calculations that do not significantly change the frequency of the vibrations we are considering can easily alter the orientation of μ for a particular vibrational band. In nature there is no a priori reason to expect the terms in Eq. 3 to add up to give a vector along the O2—P—O3 angle bisector or the O2—O3 line. Therefore it is not valid to compare the x-ray data for the direction of the O2—O3 angle bisector or the O2—O3 line direction with the angles determined by the IR dichroism measurements. Information about the orientation of a molecular group can only be obtained from the IR dichroism data by using a detailed normal coordinate analysis and by a knowledge of the appropriate partial charge distribution. At present neither is known with sufficient accuracy.

CALCULATION OF THE EFFECTIVE PARTIAL CHARGE DISTRIBUTION

One important quantitative use of the experimental IR dichroism data coupled with a calculated normal mode analysis is either to provide a more realistic effective partial charge distribution for the atoms in the nucleic acid backbone or, given a charge distribution, to refine the quality of a calculated set of eigenvectors describing the atomic motions. We chose the former because it is simpler.

We have attempted to construct the summation of Eq. 3 by choosing the partial charges q_j in such a way that the angle that the transition dipole moment makes with the helix axis is the same as that obtained from IR linear dichroism measurements. The amplitudes of vibrations, the displacements of Eq. 3, are taken from a normal mode calculation (20, 23) which in turn is rather insensitive to the magnitude of the partial charges on the different atoms.

Our calculation is carried out by calculating

$$\cos \theta_\nu = \frac{\mathbf{k} \cdot \mu_\nu}{|\mu_\nu|} \tag{4}$$

where **k** is the unit vector along the helix axis and the subscript υ indicates the frequency of the absorption band and has replaced the subscripts (mo) in the previous expression for the transition dipole moment, Eq. (3). The only unknowns in Eq. 4 are the partial charges q_j that appear in Eq. 3 defining $\mu\nu$.

There are n unknown charges q_j that can in principle be determined if we have experimental information on n dichroic bands. In the case of the polynucleotide backbone this requires knowledge of the linear dichroism of 11 bands. Experimentally, instead, there are only five well-characterized bands in A-DNA, five in B-DNA, and two in RNA for which the IR linear dichroism data is available. The five A-DNA bands are at ~1,230 cm^{-1} and 1,090 cm^{-1} assigned (13) to the O2—P—O3 stretching modes, 1,060 cm^{-1} assigned to C5-O4 stretch (20, 24), 970 cm^{-1} assigned to the C4-C5 stretch (23, 24), and 900 cm^{-1} assigned to a ribose-phosphate vibration (20, 24). The B-DNA bands are at 1,230 cm^{-1} and 1,090 cm^{-1} assigned (14) to the O2—P—O3 stretching modes, 1,060 cm^{-1} assigned to C5-O4 stretch (20, 24), 970 cm^{-1} assigned to the C4-C5 stretch (23, 24), and 782 cm^{-1} assigned (23, 24) to the O1—P—O4 diester symmetric stretching mode. The RNA bands are at 1,178 cm^{-1} and 1,080 cm^{-1} assigned (23) to the O2—P—O3 stretching modes. These data are summarized in Table IV. In several cases the dichroic ratios were not previously calculated in the papers in which the experimental data were published. In these instances we calculated the dichroic ratios and the corresponding angles from the available data. These cases are noted in Table IV.

To overcome this obvious underdetermination of the system of equations, we make use of the fact that not all atoms contribute equally to θ_ν. Therefore we can neglect the contributions of some of them, thus decreasing the number of unknowns.

We have chosen two procedures to calculate the partial charge distribution: in one case, the partial charges for A- and B-DNA were calculated independently; in the other case, the partial charges for A- and B-DNA were constrained to be equal. Thereafter both calculations were carried out in the same way.

The calculation of the partial charges was carried out as follows. A set of $n + 1$ equations was solved for $n + 1$ effective partial charges q_1, where n is the number of available

TABLE IV

A-DNA, B-DNA, AND A-RNA TRANSITION DIPOLE MOMENT DIRECTIONS FROM IR LINEAR DICHROISM DATA

Frequency	Assignment	Dipole moment direction with respect to helix axis	
A-DNA cm^{-1}			
~1,230	02—03 antisymmetric stretch	65° ± 5°‡	65° ± 5°*
~1,090	02—03 symmetric stretch	50° ± 2°‡	45° ± 5°*
1,060	C5—04 stretch	—	61° ± 5°*‖
970	C4—C5 stretch	87°‡	—
900	ribose-phosphate	—	51° ± 5°*‖
B-DNA			
~1,230	02—03 antisymmetric stretch	64° – 90°‡	56° ± 5°*
1,090	02—03 symmetric stretch	75° ± 4°‡	70° ± 5°*
1,060	C5—04 stretch	—	63° ± 5°*‖
970	C4—C5 stretch	60°‡	—
782	01—04 diester oxygen symmetric stretch	—	48° ± 5°*‖
A-RNA			
1,178	02—03 antisymmetric stretch	70° ± 4°§	
1,080	02—03 symmetric stretch	40° ± 4°§	

*References 5–8.
‡Reference 9.
§Reference 5.
‖Values calculated from the data of reference 8.

experimental angles θ_ν. An extra equation arises from the condition that the net charge on the backbone be one electron charge. This allows the determination of $n + 1$ effective partial charges. Of the $n + 1$ equations, n are nonlinear in the partial charges having the form (4). The entire system of equations is solved on the computer by an interative scheme known as the secant method for solution of nonlinear equations (25).

The set of n equations having the form of Eq. 4 are rearranged for the calculation to be of the form $F(q) = 0$, where

$$F_\nu(q) = \sum_{i=1}^{\nu} \sum_{j=1}^{\nu} q_i q_j A_{ij}^{\nu}$$

and the coefficient

$$A_{ij}^{\nu} = {}^{\nu}r_{ix}\,{}^{\nu}r_{jx} + {}^{\nu}r_{iy}\,{}^{\nu}r_{jy} + \left(1 - \frac{1}{\cos^2\theta_\nu}\right){}^{\nu}r_{iz}\,{}^{\nu}r_{jz}.$$

The superscript n indicates the particular IR absorption band for which the angle θ has been calculated from the dichroism data, the r's are the x, y, and z components of the cartesian

eigenvectors of the normal mode calculation for the νth IR band under consideration. The $n + 1$th equation has the form

$$\left(\sum_{i=1}^{n+1} q_i\right) + 1 = 0.$$

A set of partial charges from a linear combination of atomic orbitals (LCAO) molecular orbital calculation (26) were used as the initial solution. These charges were varied by the computer program until the best possible fit to the $n + 1$ equations was determined.

In the case of the individual A- or B-DNA refinements only five dichroic bands are available, allowing six of the 11 charges to be calculated. In these instances the six charges with the largest eigenvectors that contribute to the five bands were allowed to vary in the calculation. In the case of the combined A- and B-DNA calculation the system of equations was completely determined and all 11 backbone charges could be calculated. In the case of A-RNA the calculations were not carried out since IR dichroism data is available for only two bands. However, we wish to point out that the agreement of the results of the RNA x-ray data and the IR dichroism data for the angles that the O2—P—O3 angle bisector and the O2—O3 line form with the helix axis is fortuitous for the reasons discussed earlier.

The results of the calculations are shown in Table V. The calculated partial charges shown represent the best solutions we were able to attain by varying within the experimental errors the values of the various angles, θ_ν, of the transition dipole moments. In the case of B-DNA, the eigenvectors for the C3 and C4 atoms have comparable magnitudes for the vibrational modes considered. Since there is not enough experimental data available to simultaneously include both C3 and C4 in the calculation, two separate calculations were made, one with C3, the other with C4. The charges obtained are very different. When the observed values of θ_ν are varied within their experimental errors, we only obtain a set of reasonable charges in the case of B-DNA in which the motion of C4 is included. The other calculations all give unreasonable charges for the phosphorus atom, and the O2 and the O3 oxygen atoms. Note that, as expected from symmetry considerations, the charges obtained for O_2 and O_3 are different.

These results indicate that the eigenvectors used in the calculation do not represent the

TABLE V
COMPARISON OF PARTIAL CHARGES FROM LCAO MO CALCULATION WITH THE EFFECTIVE PARTIAL CHARGES CALCULATED IN THIS PAPER

Atom	(LCAO MO)	A-DNA	B-DNA		Combined A- and B-DNA
C3	+0.072	—	—	0.185	−0.151
C4	+0.041	+0.011	+0.069	—	−0.137
05	−0.164	—	—	—	+0.059
C1	−0.005	—	—	—	+0.232
C2	−0.041	—	—	—	−0.234
01	−0.220	—	—	—	−0.214
C5	−0.011	+0.001	+0.048	−0.359	−0.127
04	−0.219	−0.341	−0.136	−0.415	−0.182
P	+0.801	+0.051	+0.649	−0.394	−0.104
02	−0.832	−0.597	−0.705	−0.158	−0.066
03	−0.832	−0.125	−0.971	+0.142	−0.074

motions of the atoms well enough to be used in determining dipole moment directions. Although the eigenvectors from the normal mode calculations give an acceptable fit to the vibrational frequencies, they are not a unique set of displacements and do not give necessarily the best fit to the actual displacements of the atoms in the DNA backbone. These displacements also have not taken into account several perturbations that may be important: the vibrations of the bases and their coupling with the backbone, the presence of water of hydration, and the existence of a counter ion associated with the phosphate group. The latter two may introduce a relatively large asymmetry in the phosphate oxygen force constants. The asymmetry of the O2—O3 charges that appears as a result of the calculation may just reflect the need for this asymmetry.

Support for a deviation of the C_{2v} symmetry of the PO_4 group is obtained from x-ray crystallographic data on nucleotides (27–30) where the phosphate group symmetry is always C_s due to the presence of counter ions and water of hydration. The C_{2v} symmetry of the phosphate group used in the x-ray analysis of the polynucleotides (1–4) is the result of an arbitrary choice allowed on account of the lack of resolution of the x-ray data. The structural asymmetry between the O2—P and O3—P bonds in crystalline nucleotides implies an asymmetry of the corresponding force constants.

There is significant experimental evidence that the dioxy vibrations are not localized (19). As a consequence, even small contributions to the dipole moment perpendicular to the directions of either the O2—O3 direction or to the bisector of the O2—P—O3 angle can significantly change the direction of the observed dipole moment without significantly altering its magnitude.

Finally, the apparent nonphysical charges obtained as a result of our simple-minded calculation must be viewed as the "dynamic charges" that appear in the transition dipole moments (31, 32) that can differ significantly from the equilibrium charges obtained from a molecular orbital calculation. Our imprecise knowledge of both the atomic displacements and atomic charges does not warrant at this point a more refined calculation based on the available data.

Despite the somewhat nonphysical charges that have been obtained in these calculations and the fact that the transition dipole moment does not convey structural information when the PED is not localized in a single band, the IR dichroism measurements appear as one of those few experimental sets of data that will permit a refinement of the atomic displacements of a normal mode calculation when a good set of charges is known. Alternatively, it will allow a refinement of the charge distribution when a good normal mode calculation is available. In the meantime, it appears essential that IR linear dichroism data be measured on other bands of DNA so as to provide a determined set of equations.

The authors are grateful to Doctors K. C. Lu, E. W. Prohofsky, L. L. Van Zandt, and J. M. Eyster for providing them with detailed results of the eigenvectors of their molecular dynamics calculations.

This project was supported in part by National Science Foundation grant DMR74-14367 and National Institutes of Health grant IR01-GM-24443-01.

Received for publication 7 December 1978 and in revised form 5 March 1979.

REFERENCES

1. ARNOTT, S., and D. W. L. HUKINS. 1972. Optimized parameters for A-DNA and B-DNA. *Biochem. Biophys. Res. Commun.* 47:1504.

2. ARNOTT, S. 1970. The geometry of nucleic acid. *Prog. Biophys. Mol. Biol.* **21**:265.
3. ARNOTT, S., and D. W. L. HUKINS. 1973. Refinement of the structure of B-DNA and implications for the analysis of X-ray diffraction data from fibers of biopolymers. *J. Mol. Biol.* **81**:93.
4. ARNOTT, S., S. D. Dover, and A. J. Wonacott. 1969. Least squares refinement of the crystal and molecular structures of DNA and RNA from X-ray data and standard bond lengths and angles. *Acta Crystallogr.* **B25**:2192.
5. PILET, J., and J. BRAHMS. 1973. Investigation of DNA structural changes by infrared spectroscopy. *Biopolymers.* **12**:387.
6. PILET, J., J. BLICHARSKI, and J. BRAHMS. 1975. Conformations and structural transitions in polydeoxynucleotides. *Biochemistry.* **14**:1869.
7. BRAHMS, J., PILET, TRAN-THI PHVONG LAN, and L. R. HILL. 1973. Direct evidence of the C-like form of sodium deoxyribonucleate. *Proc. Natl. Acad. Sci. U.S.A.* **70**:3352.
8. BRAHMS, S., and J. BRAHMS. 1974. Infrared studies on the backbone conformation of nucleic acids. *Isr. J. Chem.* **12**:153.
9. KURSAR, T., and G. HOLZWARTH. 1976. Orientation of the deoxyribose group in A- and B-DNA. *Biochemistry.* **15**:3352.
10. ARNOTT, S., and D. W. L. HUKINS. 1969. Conservation of conformation in mono- and polynucleotides. *Nature (Lond.).* **224**:886.
11. ARNOTT, S., and D. W. L. HUKINS. 1972. The dimensions and shapes of the furanose rings in nucleic acids. *Biochem. J.* **130**:453.
12. FRASER, R. D. B. 1953. The interpretation of infrared dichroism in fibrous protein structures. *J. Chem. Phys.* **21**:1511.
13. SHIMANOUCHI, T., M. TSUBOI, and Y. KYOGOKU. 1964. Infrared spectra of nucleic acids and related compounds. *Adv. Chem. Phys.* **7**:435.
14. TSUBOI, M. 1974. Infrared and Raman spectroscopy. *In* Basic Principles in Nucleic Acid Chemistry. P.O.P. TS'O, editor. **1**:399.
15. ARNOTT S., and E. SELSING. 1974. Structure for poly(dA)·poly(dT) and poly(dT)·poly(dA)·poly(dT). *J. Mol. Biol.* **88**:509.
16. ARNOTT, S., and E. SELSING. 1975. The conformation of C-DNA. *J. Mol. Biol.* **98**:265.
17. ARNOTT, S., R. CHANDRASEKARAN, D. W. HUKINS, P. J. C. SMITH, and LYNDA WATTS. 1974. Structural details of a double-helix observed for DNAs containing alternating purine-pyrimidine sequences. *J. Mol. Biol.* **88**:523.
18. SCHIFF. L. I. 1968. Quantum Mechanics. McGraw Hill Book Company, New York. Third Edition. 246.
19. FORREST, G., and R. C. LORD. 1977. Laser Raman spectroscopy of biomolecules. X-Frequency and intensity of the phosphodiester stretching vibrations of cyclic nucleotides. *J. Raman Spectrosc.* **6**:32.
20. LU, K. C., E. W. PROHOFSKY, and L. L. VAN ZANDT. 1977. Vibrational modes of A-DNA, B-DNA, and A-RNA backbones. An application of a Green function refinement procedure. *Biopolymers.* **16**:2491.
21. BROWN. E. B., and W. L. PETICOLAS. 1975. Conformational geometry and vibrational frequencies of nucleic acid chains. *Biopolymers.* **14**:1259.
22. LU, K. C., E. W. PROHOFSKY, and L. L. VAN ZANDT. 1979. Displacements of backbone vibrational modes of A-DNA and B-DNA. *Biophys. J.* **28**:27.
23. TSUBOI, M. 1969. Application of infrared spectroscopy to structural studies of nucleic acids. *Appl. Spectrosc. Rev.* **3**:45.
24. SMALL, E. W., and W. L. PETICOLAS. 1971. Conformation dependence of the Raman scattering intensities from polynucleotides. III. Order-disorder changes in helical structures. *Biopolymers.* **10**:1377.
25. WOLFE, PHILIP. 1959. The secant method for simultaneous nonlinear equations. *Commun. ACM.* **2**:12.
26. RENUGOPALAKRISHNAN, V., A. V. LAKSHMINARAYANAN, and V. SASISEKHARAN. 1971. Stereochemistry of nucleic acids and polynucleotides III. Electronic charge distribution. *Biopolymers.* **10**:1159.
27. KRAUT, J., and L. H. JENSEN. 1963. Refinement of the crystal structure of adenosine-5'-phosphate. *Acta Crystallogr.* **16**:79.
28. MURAYAMA, W., N. NAGASHINA, and Y. SHIMIZU. 1969. The crystal and molecular structure of guanosine 5' phosphate trihydrate. *Acta Crystallogr.*: **B25**:2236.
29. SHEFTER, E., and K. N. TRUEBLOOD. 1965. The crystal and molecular structure of D(+)-barium uridine-5'-phosphate. *Acta Crystallogr.* **18**:1067.
30. TRUEBLOOD, K. N., P. HORN, and V. LUZZATI. 1961. The crystal structure of calcium thymidylate. *Acta Crystallogr.* **14**:965.
31. DECIUS, J. 1975. An effective charge model for infrared intensities. *J. Mol. Spectrosc.* **57**:348.
32. PRASAD, J. 1978. Infrared intensities: polar tensors and charge flux parameters. *J. Chem. Phys.* **69**:4403.