Vacuum ultraviolet circular dichroism as an indicator of helical handedness in nucleic acids

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ABSTRACT

Calculated circular dichroism spectra are presented for double-stranded polynucleotides of regular sequences in A-RNA, A-DNA, B-DNA, and Z-DNA conformations. Quantum mechanical matrix method calculations were carried out in the near and vacuum ultraviolet regions. In the near UV, the calculated spectra agreed qualitatively with the measured spectra. However in the far and vacuum UV, the calculated CD compared nearly quantitatively with the experimental spectra. The calculations show that the sign of the CD in the vacuum UV, in contrast to that in the near UV, can be correlated with the handedness of the helix.

INTRODUCTION

In the last few years, circular dichroism (CD) spectra have been measured in the vacuum ultraviolet region for natural DNAs and for synthetic polynucleotides of regular sequence (1-5). The large difference in the observed vacuum UV CD of polynucleotides in right- and left-handed conformations has led to the suggestion that the vacuum UV CD can be used to identify the sense of helix winding.

The first vacuum UV CD measurements of polynucleotides were made by Sprecher et al. (1) on the right-handed A and B forms of natural DNA. A positive band at about 187 nm and a negative band at about 170 nm were found to be characteristic of these forms. Further evidence that these bands are characteristic of right-handed helical forms was presented by Brahms et al. (3). They found the vacuum UV CD spectra of poly (rG-dC):(rG-dC) and of a natural RNA to be similar to the A-form spectra measured by Sprecher et al. (1).

Sutherland et al. (2) provided the first evidence that the vacuum UV may be sensitive to helical handedness. They measured the vacuum UV CD of poly d(G-C):d(G-C) under conditions which produce the Z-form (left-handed) conformation [70% trifluoroethanol (TFE)]. The long-wavelength CD is characterized by a negative band at 290 nm and a positive band at 263 nm while

the vacuum UV has a large negative band at 194 nm and an equally large positive band below 180 nm. The existence of a different spectral Z-form was shown by Brahms et al. (3) for the same deoxy polymer in 80% TFE, 0.4 mM KF, 1 mM phosphate buffer. The vacuum UV CD was nearly identical to that measured by Sutherland et al. (2), however the near-UV CD appeared to reflect the A-form conformation with a positive band at about 280 nm and no near-UV negative CD at 290 nm.

The reliability of the vacuum UV CD as an indicator of helical handedness was further demonstrated by Sutherland and Griffin (4). The long-wavelength CD of poly d(I-C):d(I-C) is similar to that observed for the Z-form of poly d(G-C):d(G-C) in 70% TFE. However, Sutherland and Griffin (4) showed that the vacuum UV CD bands are characteristic of the B-form. Subsequent proton NMR measurements have also indicated that this polymer is in the B-form (6).

Recently, poly r(G-C):r(G-C) has been shown to undergo a transition from A-form to Z-form (7). Riazance et al. (5) measured the vacuum UV CD of poly d(G-C):d(G-C) in the A-, B-, and Z-form conformations and poly r(G-C):r(G-C) in the A and Z conformations. While the long-wavelength CD spectra that characterize the B- to Z-DNA transition are very different from the spectra that characterize the A- to Z-RNA transition, the vacuum UV CD are quite similar. All the right-handed forms have a positive CD band near 185 nm, and all the left-handed forms have negative CD between 185 to 200 nm followed by positive CD below 180 nm. Furthermore, the CD of the A-form RNA was found to be remarkably similar to the DNA in the A-form. In addition, the Z-form DNA measured by Brahms et al. (3) [80% TFE, 1 mM phosphate buffer, 0.4 mM KF] is similar to the Z-form RNA measured by Riazance et al. (5).

Only a few polynucleotides of regular sequence in right- and left-handed conformations have been measured in the vacuum UV. Inversions in the long-wavelength CD for poly d(A-C):d(G-T) and poly d(A-T):d(A-T) characteristic of the B-Z transition of poly d(G-C):d(G-C) have been reported (8-10). In this paper, we present theoretical calculations of the vacuum UV CD for several polynucleotides of regular sequence. The results demonstrate that the characteristic peaks of right- and left-handed conformations in the vacuum UV are independent of sequence, and that the vacuum UV CD can be useful in establishing the helical sense of nucleic acids.

METHODS

Calculations were done with a quantum mechanical matrix method. One obtains eigenvalues and eigenfunctions of the Hamiltonian matrix using monomer

functions as a basis set. This method and the type of monomer parameters that are required for its use have been discussed in detail (11,12). The location of each polymer CD band directly depends on the specific frequency of each monomer band from which it is derived.

Given in Table I are the wavelengths at the absorption maxima, and the directions and magnitudes of the monomer electric transition dipoles used in the calculations. The values of the absorption maxima and the dipole moment magnitudes refer to solution spectra. The dipole moment directions for guanine and cytosine were obtained from crystal polarized reflection spectra for the bases (13,14). Transition II and III of adenine are those of transitions I and II of the crystal spectra (15,16), respectively. The longest wavelength transition (I) of adenine has been located at 270 nm based on CD difference spectra (17) and its direction was arbitrarily chosen as

TABLE I

Base	Transition	Wavelength (nm)	θ_{DT}^* (degrees)	/u/ (Debye)
Adenine	I	270	-87	1.07
	II	263	-3	3.96
	III	235	-87	1.00
	IV	216	-87	3.71
	V	195	-3	3.70
	VI	187	-87	4.17
Guanine	I	272	-4	2.76
	II	248	-75	3.18
	III	204	-75	3.92
	IV	187	-9	3.92
	V	159	-4	2.76
	VI	154	-75	1.84
	VII	145	-4	2.30
Cytosine	I	272	6	2.86
	II	231	-46	2.07
	III	214	76	2.44
	IV	196	-27	4.43
	V	163	0	2.17
	VI	151	60	2.44
Thymine or Uracil	I	265	-19	3.27
	II	215	71	2.14
	III	195	-36	2.04
	IV	177	-20	3.88

* DeVoe-Tinoco angle (See ref. 22)

being perpendicular to transition II. Transition IV of adenine was chosen to be perpendicular to the 260 nm transition (II) based on polarized fluorescence measurements (18). The remaining two transitions (V and VI) were made perpendicular and parallel to transition IV, respectively. The dipole moment directions for transitions I and II of thymine/uracil were taken from Eaton and Lewis (19) who gave -19° for transition I and suggested that the second transition was perpendicular to the first. The choice of the remaining thymine/uracil directions (transitions III and IV) were based on crystal data for 1-methyl thymine (20) and directions derived from a semi-empirical procedure matching calculated and observed CD data (25). If there was ambiguity in the directions of the transition dipole moments from the crystal data, we used the set which gave the best agreement between the calculated and measured spectra.

The Hamiltonian matrix is obtained from the absorption maxima and the charge distributions of the bases. The charge distributions for the longest-wavelength transitions were recalculated from CNDO/MO calculations (22) using Lagrangian multipliers (23), so that they generate correct directions and magnitudes of the electric transition dipole moments. This was done for three transitions in adenine, four in guanine, five in cytosine and two in thymine/uracil. The remaining charge distributions were calculated using Lagrangian multipliers starting from an initial set of transition monopoles equal to zero.

In all the calculations, the dielectric constant was set to two.

RESULTS AND DISCUSSION

In this section, we report the results of calculations performed using fiber A-form and B-form geometries (24,25) and crystallographic Z-form geometries (26). These results are compared with the experimental data of double-stranded polynucleotides believed to be in the various conformations.

The calculated spectra of A, U, and T polynucleotides are compared with measured spectra in Figure 1. The calculated spectra for all four polymers show remarkably good agreement in terms of sign, shape, and magnitude with the experimental spectra, the only exception being the near-UV region of poly dA:dT (Fig. 1c). In this region of the poly dA:dT spectrum, the calculated spectrum has a single positive maximum at 265 nm, whereas the measured spectrum has two weaker positive CD bands at 280 and 255 nm. Experimental spectra for the deoxy polymers [poly dA:dT and poly d(A-T):d(A-T), Fig. 1c and 1d] have been measured into the vacuum UV region (27). As can be seen in Fig.

1c and 1d, the agreement between calculated and experimental spectra in the vacuum UV is very good in terms of both shape and magnitude.

In Fig. 2 the calculated spectra of double-stranded polynucleotides of G and C are compared with experimental spectra. The overall shape and magnitude of the measured spectra are reproduced in the calculated spectra, with the exception of the near-UV region of the Z-form calculation (Fig. 2f). All of the calculations based on right-handed geometries (Figs. 2a-e) appear to be

Figure 1. Measured (—) and calculated (---) CD spectra for: (a) poly rA:rU, measured (ref. 29), calculated using A-RNA geometry (ref. 24); (b) poly r(A-U):r(A-U), measured (ref. 29), calculated A-RNA geometry (ref. 24); (c) poly dA:dT, measured (ref. 27), calculated B-DNA geometry (ref. 25); and (d) poly d(A-T):poly d(A-T), measured (ref. 27) and calculated B-DNA geometry (ref. 25).

red-shifted when compared to their respective experimental spectra. And, none of the calculated spectra agree with the near-UV (280-300 nm) region of the measured spectra. The calculated near-UV (260-300 nm) CD of the Z-form spectrum of poly d(G-C):d(G-C) is particularly bad; the calculated CD being nearly zero in this region (Fig. 2f). We have found this region of the spectrum to be extremely sensitive to both geometry and to monomer transition dipole moment data. There could also be a contribution to the CD from an enhanced n- π^* transition found in this region in the spectrum of Z-DNA single

Figure 2. Measured (—) and calculated (---) CD spectra for: (a) poly rG:rC, measured (ref. 29), calculated using A-RNA geometry (ref. 24); (b) poly r(G-C):r(G-C), measured (ref. 5), calculated A-RNA geometry (ref. 24); (c) poly dG:dC, measured (ref. 27) and calculated B-DNA (ref. 25); (d) poly d(G-C):d(G-C), measured (ref. 5) and calculated B-DNA (ref. 25); (e) poly d(G-C):d(G-C), measured (ref. 5) and calculated A-DNA (ref. 25); (f) poly d(G-C):d(G-C), measured (ref. 5) and calculated Z-DNA (ref. 26).

crystals (Prof. A. Rich, personal communication). However, the agreement between the calculated and measured spectra in the vacuum UV region is nearly quantitative, although the calculated spectra are red-shifted slightly with respect to the measured curves (Figs. 2a-f).

The measured and calculated CD spectra of polynucleotides containing 50% (A-U or T) and 50% (G-C) base pairs are shown in Figure 3. In Fig. 3a and b the calculated spectra of the ribo and deoxy alternating A-C polymers are compared to their experimental spectra. The calculated results, although

Figure 3. Measured (—) and calculated (---) CD spectra for: (a) poly r(A-G):r(C-U), measured (ref. 29) and calculated using A-RNA geometry (ref. 24); (b) poly d(A-G):d(C-T), measured (ref. 30) and calculated B-DNA (ref. 25); (c) poly r(A-C):r(G-U), measured (ref. 29) and calculated A-RNA (ref. 29); (d) poly d(A-C):d(G-T), measured (ref. 30) and calculated B-DNA (ref. 25).

generally red-shifted, show good agreement in both shape and magnitude with their experimental spectra. However, the near-UV negative CD band at 290 nm of the measured spectrum of poly r(A-C):r(G-U) is not reproduced in the calculated spectrum (Fig. 3a). Similar results were obtained for poly r(A-G):r(C-U) and poly d(A-G):d(C-T) [Figs. 3c and d], in which the positive 270 nm positive CD band of the calculated spectrum of poly d(A-G):d(C-T) is actually located 10 nm to the blue of the measured spectrum (Fig. 3d). Unfortunately, no vacuum UV spectra for these polymers have appeared in the literature, so no comparisons between measured and calculated curves in this region can be made at this time.

Figure 4. Calculated CD spectra in A-DNA (ref. 25) [---], B-DNA (ref. 25) [...], and Z-DNA geometry (ref. 26) [—] for: (a) poly d(A-T):d(A-T), (b) poly d(G-C):d(G-C), (c) poly d(A-C):d(G-T).

Shown in Fig. 4 are the calculated CD spectra of the various alternating purine-pyrimidine sequence double-stranded polymers in A-, B-, and Z-form geometries. In general, the sign of the measured long wavelength CD bands of polynucleotides has not proven to be conclusive of helical handedness. For example, the Z-form of poly r(G-C):r(G-C) has positive CD in the near UV (7), whereas the CD of Z-form poly d(G-C):d(G-C) is negative (27). While the sign of the calculated CD above 200 nm presented here (Fig. 4), appears ambiguous with regard to helical handedness, the sign of the CD below 200 nm is not. A large difference between right- and left-handed structures is evident. The calculated results (Fig. 4) show that, for all cases, a transition from a right-handed to a left-handed structure involves a change in sign of CD from generally positive above 180 nm (right-handed) to negative (left-handed) and a change in sign of the CD below 180 nm from generally negative (right-handed) to positive (left-handed). These results not only mimic what has been observed for both the A-Z transition of poly r(G-C):r(G-C) (7) and the B-Z transition of poly d(G-C):d(G-C) (2), but predict what should be observed if the alternating purine-pyrimidine A-U or A-T and A-C double-stranded polymers undergo an A-Z or B-Z transition.

CONCLUSION

The discoveries of left-handed Z-forms of DNA (26) and RNA (5) have shown that double-stranded polynucleotides are conformationally flexible and that their conformation is dependent on the environment. The calculations presented in this paper have shown that the sign of the CD in the vacuum UV region can be used to identify helical handedness and to follow conformational changes from one form to another. The validity of these conclusions are based on the overall excellent agreement we obtained between measured and calculated spectra in the vacuum UV CD (Figs. 1 and 2).

Thus far, the conformational flexibility of alternating G-C sequence polynucleotides is best understood. Transitions from A-Z forms have been observed for poly r(G-C):r(G-C) (5,7) and B-Z and A-Z forms for poly d(G-C):d(G-C) (4,28-29). The calculated results of the vacuum UV CD in these forms are in excellent agreement with the measured spectra. In this regard it can be noted that the monomer input properties are experimentally most certain for G and C. Other alternating purine-pyrimidine sequence polynucleotides have been postulated to undergo B-Z and A-Z transitions based on changes of their near-UV CD (8-10). Unfortunately, vacuum UV CD for these polynucleotides have not yet been measured. However, we have presented

calculated results which indicate what changes in the vacuum UV CD should be expected for double-stranded alternating A-C and A-U or A-T sequence polynucleotides undergoing such transitions (Fig. 4).

In general, left-handed Z-form spectra of alternating purine-pyrimidine double-stranded polynucleotides are characterized by a large negative maximum ($\Delta\epsilon < -20$) below 200 nm that immediately precedes a large positive maximum ($\Delta\epsilon > 30$) below 180 nm. The spectra of right-handed conformations, regardless of sequence, are characterized by positive CD bands between 180 and 200 nm followed by a negative CD band below 180 nm. Thus, these calculations add further evidence that the vacuum UV CD, in contrast to the near UV CD, is a good indicator of the helical-handedness of double-stranded nucleic acids.

Lastly, the theoretical circular dichroism calculations presented here require a knowledge of the orientations of the transition moments within the nucleic acid bases. This data is experimentally best understood for G and C (derived from crystal polarized reflection spectra) and our calculated results are excellent for the G C polymers. Since transition moment data for A, U, and T have not yet been derived for transitions in the vacuum UV, the results presented for polymers containing those bases are more speculative. However, given the good agreement we obtained between the measured and calculated vacuum CD of A and T polymers (when available); we can conclude that the relative orientations of the transition moments used in our calculations (with respect to each other) are correct.

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