A Computer Probe of the Circular Dichroic Bands of Nucleic Acids in the Ultraviolet Region. II. Double-Stranded Ribonucleic Acid and Deoxyribonucleic Acid†

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ABSTRACT: The circular dichroism (CD) and optical rotatory dispersion (ORD) between 181 and 300 nm were measured for *Micrococcus lysodeikticus* and calf thymus DNA and the double-stranded RNAs from rice dwarf virus and cytoplasmic polyhedrosis virus. The CD spectra can be resolved into six Gaussian bands for DNA and five for RNA, plus an extrapolated negative band near 165 nm. The positive ellipticity between 260 and 300 nm for DNA can best be fitted with two bands; DNA also has a small positive band near 221–222 nm, but a negative one near 310 nm is too small to be included in the curve resolution. The ORD computed from the CD spectrum by the Kronig–Kramers transform gives a good fit with the experimental ORD. Both the positive band around 180 nm and the negative one near 165 nm are conformation dependent. For RNA the 210-nm band is much stronger than the one around 240 nm, but the reverse is true for DNA. These two bands are closely related to the geometry of the base pairs relative to the helical axis, *i.e.* the A and B forms.

The major conformational aspects of nucleic acids are the number of strands involved and the orientation of the bases with respect to the sugar–phosphate backbone. Under different experimental conditions DNA can exist in any of three forms, A, B, or C. Double-stranded RNA is in a conformation similar to the A form of DNA, in which the base pairs are tilted from the perpendicular to the helical axis (Arnott *et al.*, 1967). The CD[1] spectra of DNA and RNA indicate that the band positions are nearly the same for the A and B forms but their absolute values, in particular, the relative magnitudes of certain bands, are quite different. Thus, the CD profiles can be used to detect various conformations of nucleic acids (see, for example, Yang and Samejima (1969)). Recently, Ivanov *et al.* (1973) have thoroughly measured the CD spectra of DNA in solution for which the base tilt of the DNA molecules is altered by changing the solvent composition and salt concentration. Maestre (1970) has reported the CD patterns of DNA films which can exist in various forms under different relative humidities.

Until recently, all the CD spectra have been confined to wavelengths above 200 nm. We now extend measurements to about 181 nm and observe a CD band around 181–188 nm, which dominates the other bands. Independently, D. G. Lewis and W. C. Johnson, Jr. (private communication) have now reported an intense positive band at about 188 nm and a negative one at about 168 nm for DNAs (see Results). In this work we study both double-stranded RNA and DNA, which exemplify the A and B forms. We use the method of analysis described in the preceding paper (Wells and Yang, 1974) to probe the ultraviolet region below 180 nm and again predict a strong negative band near 165 nm. These two bands between 140 and 200 nm are conformation dependent and therefore can be used to study the secondary structure of nucleic acids.

Experimental Section

The double-stranded RDV-RNA and CPV-RNA were gifts from Professor K. Miura. The CT-DNA (lot No. 14) and ML-DNA (lot No. 9) were purchased from Miles Laboratory. The samples were dissolved in 0.15 M KF (the pH of the solutions had been adjusted with KOH to 7.3) and exhaustively dialyzed against the same solvent. No buffer was employed so that the spectra could be measured to as low a wavelength as possible. The polymer concentrations were determined by phosphorus analysis (Chen *et al.*, 1956); the mean residue weights are 321 for both RNAs which have 43% (G + C), and 309 for CT-DNA (42% (G + C)) and ML-DNA (72% (G + C)).

The CD was measured on a Durrum-Jasco J-5, SS 10 modified circular dichrometer and the ORD on a Cary 60 spectropolarimeter. All CD spectra were resolved into Gaussian bands on a Du Pont curve resolver. The Kronig–Kramers transform from CD to ORD was done on a CDC 6400 (Thiéry, 1969). Experimental details and method of analysis have been described in the preceding paper (Wells and Yang, 1974).

Results

DNA. The CD and ORD spectra for ML-DNA are presented in Figure 1; the solid lines are experimental curves and the points are the extrapolated CD band (Gaussian) and the ORD computed by means of Kronig–Kramers transform. The observed CD spectrum can be resolved into six Gaussian bands, excluding band 1 at 310 nm which is too small to appear on the curve resolver (Table I). The positive ellipticity between 260 and 300 nm cannot be fitted by a single Gaussian band and is therefore split into two bands (2 and 2′) with extrema at 265 and 280 nm. The area of band 3 approximates the combined areas of bands 2 and 2′, but the ellipticities are

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[1] Abbreviations used are: CD, circular dichroism; ORD, optical rotatory dispersion; ML-DNA, *Micrococcus lysodeikticus* DNA; CT-DNA, calf thymus DNA; RDV-RNA, rice dwarf virus RNA; CPV-RNA, cytoplasmic polyhedrosis virus RNA.

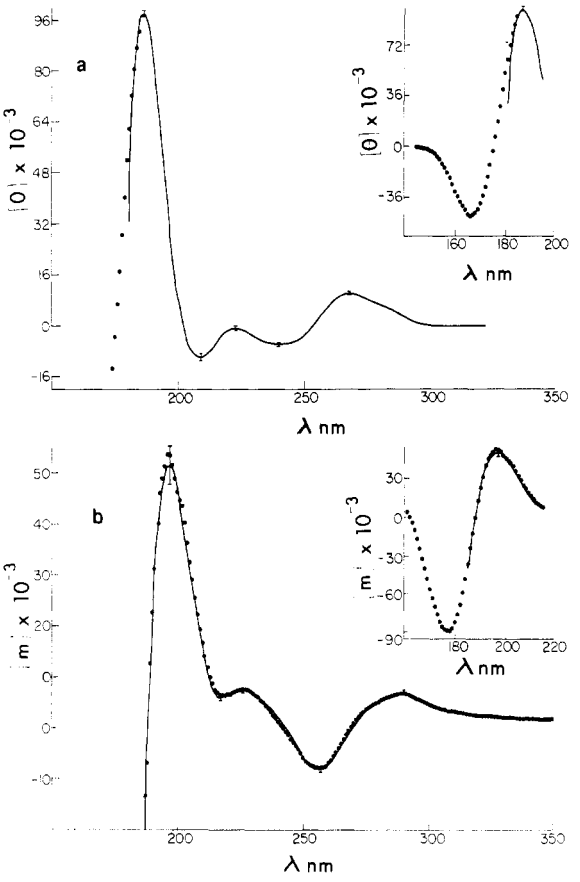

FIGURE 1: (a) The CD spectrum of *Micrococcus lysodeikticus* DNA in 0.15 M KF at 25°. Solid line, experimental; points, extrapolated according to item 3 of Table I. (b) The ORD spectrum. Solid line, experimental; points, computed from (a) including the extrapolated band.

opposite in sign. They arise mainly from the exciton splitting of the π–π^* transition and such a spectrum has been termed "conservative" (Bush and Brahms, 1967). The resolved band 4 is positive, although the actual ellipticity at that wavelength is slightly negative. Band 5 has a rotational strength close to that of band 3. The most striking feature in Figure 1a is the dominance of band 6 at 187 nm. It has a rotational strength of 68.0×10^{-40} erg cm^3 rad, whereas the sum of R_i for the first five bands above 200 nm is only -2.0×10^{-40} (Table I).

Computation of the Kronig–Kramers transform from CD to ORD for ML-DNA indicates that the best ORD fit can only be obtained by including an extrapolated CD band around 165 nm just as in the case of tRNAs (see Wells and Yang (1974)). Table II presents the various extrapolations for this band for ML-DNA and also the other three nucleic acids. A band at 167 nm giving an ORD fit of 0.07 (item 3) is the extrapolation plotted in Figure 1a and used for computing the ORD in Figure 1b (only a few selected computations are listed in Table II for illustration).

The CD and ORD of CT-DNA were treated in the same manner as those of ML-DNA. The extrapolated band in Figure 2 is based on item 6 in Table II. The CD spectrum between 200 and 300 nm has been reported by Allen *et al.* (1972). The profiles in Figures 1a and 2 are very similar; the band positions and bandwidths of the corresponding Gaussian bands (Table I) are also very close. The absolute values of most of the bands for CT-DNA are smaller than those for ML-DNA probably because of the difference in (G + C)

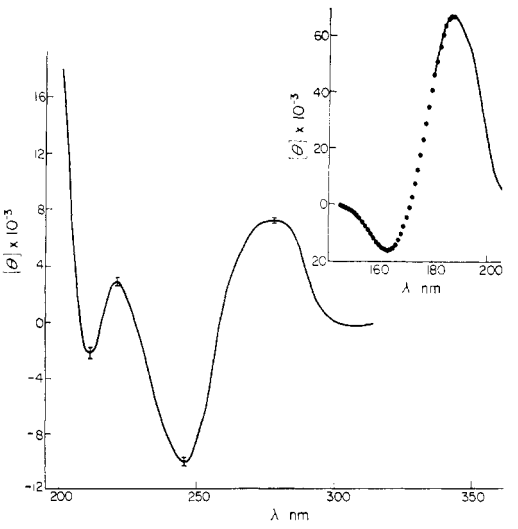

FIGURE 2: The CD spectrum of calf thymus DNA in 0.15 M KF at 25°. Solid line, experimental; points, extrapolated (see item 6 of Table I).

content. One significant exception is band 3 at 248 nm of CT-DNA, which is twice as large as that of ML-DNA at 242 nm. Band 3 in Figure 2 is much stronger than band 5 at 211 nm, whereas the reverse is true for ML-DNA. Band 6 at 188 nm is again the strongest among all the observed bands; for CT-DNA it has a rotational strength of 52×10^{-40} erg cm^3 rad as compared with -1.3×10^{-40} for the sum of R_i of the five bands between 200 and 300 nm. Table III summarizes the experimental and computed values of the ORD extrema for CT-DNA and the other nucleic acids. The agreement is good for both DNAs. One unexpected observation is that the rotational strength of the extrapolated band at 165 nm for CT-DNA is only about one-third that of ML-DNA. It is the smallest among all the DNAs and RNAs we have studied, including the tRNAs in the preceding paper (Wells and Yang, 1974).

Upon completion of the dissertation (by B. D. Wells), we learned of the work of D. G. Lewis and W. C. Johnson, Jr. (private communication, July 1973) on the CD in the vacuum ultraviolet of five DNAs in 10^{-3} M NaF. These authors extended their measurements to about 164 nm and detected a strong negative band at about 168 nm. They also observed a strong positive band at about 188 nm. These two bands are conformation dependent and their magnitudes are markedly reduced upon heat denaturation. Thus, the experimental approach and computer probe of the CD bands of DNAs between 160 and 180 nm reach essentially the same conclusion. Direct measurement of vacuum ultraviolet CD is definitely an advantage, but not without technical problems. There is always the danger of artifacts arising from orientation of the macromolecules or other factors because exceedingly short light paths (a few microns) must be used to minimize solvent absorption. The concentrations of the solutions must then be increased to ensure detectable CD signals. To avoid linear dichroism Lewis and Johnson found it necessary to reduce the viscosity of the DNA solutions (about 1% by weight) by shearing them through a syringe needle. Therefore, our method of analysis can serve to complement the vacuum ultraviolet CD measurements and help us to determine the accuracy of such results. On the other hand, we can even probe and predict any CD bands below 160 nm if accurate results of both CD and ORD in the vacuum ultraviolet region become available.

TABLE I: Resolved CD Bands of Nucleic Acids at 25°.[a]

Band[b]	ML-DNA	CT-DNA	RDV-RNA	CPV-RNA
(1) λ_1 (nm)	(310)[c]	(307)[c]	296	294
$[\theta_1^0]$	(−74)[c]	(−170)[c]	−4280	−3000
Δ_1 (nm)			12	7
$R_1 \times 10^{40}$			−2.1	−0.9
(2) λ_2 (nm)	280	285		
$[\theta_2^0]$	6360	2860		
Δ_2 (nm)	11	6		
$R_2 \times 10^{40}$	2.9	0.7		
(2′) $\lambda_{2'}$ (nm)	265	269	261	261
$[\theta_{2'}^0]$	11,000	8,690	24,900	25,700
$\Delta_{2'}$ (nm)	11	18	15	16
$R_{2'} \times 10^{40}$	5.6	7.2	18.0	19.0
(3) λ_3 (nm)	242	248	238	237
$[\theta_3^0]$	−6,470	−12,700	−4710	3,640
Δ_3 (nm)	18	15	8	8
$R_3 \times 10^{40}$	−5.9	−9.5	−2.0	−1.5
(4) λ_4 (nm)	221	222		
$[\theta_4^0]$	2,120	3400		
Δ_4 (nm)	8	8		
$R_4 \times 10^{40}$	0.9	1.5		
(5) λ_5 (nm)	209	211	210	209
$[\theta_5^0]$	−10,400	−4,190	−27,600	−28,200
Δ_5 (nm)	9	5	8	9
$R_5 \times 10^{40}$	−5.5	−1.2	−13.0	−15.0
(6) λ_6 (nm)	187	188	181	182
$[\theta_6^0]$	103,000	71,900	98,800	84,100
Δ_6 (nm)	10	11	12	12
$R_6 \times 10^{40}$	68.0	52.0	81.0	68.0
Total $R \times 10^{40}$	66.0	50.7	81.9	69.6

[a] Solvent, 0.15 M KF (pH 7.3). [b] $R_i = 1.233 \times 10^{-42} [\theta_i^0] \Delta_i / \lambda_i$ erg cm^3 rad; $[\theta_i]$ in deg cm^2 dmol^{-1}. [c] The numbers in parentheses are experimental values.

RNA. The CD spectra of RDV-RNA and CPV-RNA between 200 and 300 nm have been reported by Samejima *et al.* (1968) and Miura *et al.* (1968), but a new maximum is now found at 181–182 nm (Figures 3a and 4). The whole spectrum can be resolved into five Gaussian bands (Table I). Unlike DNA, RNA has only a single Gaussian band between 240 and 290 nm and it lacks the small positive band near 220 nm. The band positions, bandwidths, and rotational strengths of various CD bands do not vary much between the two RNAs, noting that their (G + C) content is about the same (43%). On the other hand, band 6 at 181–182 nm and the extrapolated band at 160–163 nm (Table II, items 9 and 14) are smaller for CPV-RNA than for RDV-RNA. Like DNA, the CD bands between 200 and 300 nm of the two RNAs have a net rotational strength of 0.9×10^{-40} erg cm^3 rad for RDV-RNA and 1.6×10^{-40} for CPV-RNA but the band at 181–182 nm has a rotational strength of 81×10^{-40} and 68×10^{-40}, respectively, which is the same order of magnitude as the corresponding value for band 6 of DNAs.

The ORD spectrum of RDV-RNA is shown in Figure 3b; that of CPV-RNA is very similar. The agreement between the observed and computed ORD for the two RNAs is not as good as that found in DNAs probably because of the limited supply of samples which restricted the number of replicate experiments performed. In particular, we observed slight shift in the positions of the extrema between the experimental and computed ORD (Table III). Our data were somewhat better for RDV-RNA than for CPV-RNA as exemplified by the ORD fits in Table II.

The differences in the CD spectra between DNA and RNA can be briefly summarized as follows. Band 1 of RNA at 294–296 nm is located about 15 nm lower than that of DNA at 307–310 nm; it is also much stonger for RNA than for DNA. Band 2′ at 261 nm of RNA is not as broad as the combined bands 2 and 2′ of DNA; it is much stronger in the case of RNA. The absolute value of the rotational strength of band 2′ for RNA is an order of magnitude higher than that of band 3; such a spectrum has been called "nonconservative." Band 4 near 220 nm is only found in DNA. For RNA, band 5 around 210 nm is much stronger than band 3. In both RNA and DNA, band 6 between 180 and 190 nm and the extrapolated band around 165 nm overshadow the other observed CD bands.

Discussion

Just as in the case of tRNAs (see Wells and Yang (1974)) the sum of the rotational strengths of various Gaussian CD bands including the extrapolated band at 165 nm does not become zero for double-stranded RNAs and DNAs. This suggests the existence of other CD bands outside the wavelength range studied (140–320 nm). But the net ORD contribution of these missing bands happens to be insignificant in the wavelength range of interest. Thus, we are able to ob-

TABLE II: An Extrapolated CD Band of Nucleic Acids.

Extrapolated Band[a]			Total[b]	ORD[c]
λ_i (nm)	$[\theta_i^0]$	$R_i \times 10^{40}$	$R \times 10^{40}$	Fit
		ML-DNA		
1. 165	−58,900	−44.0	22.0	0.09
2. 165	−50,100	−37.4	28.6	0.08
3. 167	−50,100	−37.0	29.0	0.07
4. 165	−37,900	−28.3	37.7	0.12
		CT-DNA		
5. 163	−25,500	−19.3	31.4	0.11
6. 165	−17,100	−12.8	37.9	0.08
7. 167	−26,100	−19.3	31.4	0.12
8. 165	−25,800	−19.3	31.4	0.11
		RDV-RNA		
9. 163	−63,200	−47.8	34.1	0.11
10. 163	−60,500	−45.8	36.1	0.15
11. 165	−50,700	−37.9	44.0	0.13
12. 165	−68,000	−50.8	31.1	0.15
		CPV-RNA		
13. 165	−41,200	−30.8	38.8	0.26
14. 160	−43,400	−35.0	34.6	0.21
15. 163	−46,300	−35.0	34.6	0.23
16. 160	−49,300	−39.0	30.6	0.23

[a] See footnote *b* in Table I; Δ_i is preset at 10 nm. [b] Sum of total R in Table I and R_i of the extrapolated band. [c] Fit = $\{\Sigma(E_j - C_j)^2/\Sigma E_j^2\}^{1/2}$, where E_j = experimental $[m]_j$ and C_j = calculated $[m]_j$ at wavelength j.

tain a good ORD fit merely by the introduction of an extra CD band around 165 nm.

The CD band at 181–188 nm and the extrapolated band around 165 nm are conformation dependent (see Wells and Yang (1974)), a conclusion which should be valid for all nucleic acids. In double-stranded RNAs the bases are fully paired and stacked, as contrasted with partial base pairing and stacking in tRNAs. Accordingly, the rotational strengths of the two bands for unfractionated yeast tRNA and *E. coli* $tRNA^{Val}$ (Table II of the preceding paper) are smaller than those of double-stranded RNAs. If the R_6 at 181 nm for RDV-RNA is assumed to represent 100% double-stranded helix, the corresponding R_6 of *E. coli* $tRNA^{Val}$ and yeast $tRNA^{Phe}$ at 185 nm would have about 60% and 45% double-stranded helices, respectively. These estimates compare favorably with the predicted 55% of the cloverleaf model (see, for example, Cramer, 1971; Blum *et al.*, 1972). Similarly, we could estimate the per cent of double helix for $tRNA^{Val}$ from the rotational strength of the extrapolated band at 165 nm, which turned out to be only about 30% (the lack of this 165-nm band for yeast $tRNA^{Phe}$ has been mentioned in Wells and Yang (1974)). Any agreement between these estimates and the predicted value of the cloverleaf is probably fortuitous. Nevertheless, the qualitative statement that the 181-nm band and possibly the extrapolated band at 165 nm are related to the secondary structure of nucleic acids remains valid.

The exciton splitting of the 260-nm absorption band in double-stranded nucleic acids predicts a conservative CD spectrum in which the sum of the positive and negative rotational strengths of the CD bands around 260 nm equals zero (Johnson and Tinoco, 1969; Tinoco, 1968). Neither of the CD spectra between 230 and 300 nm (bands 2, 2′, and 3) for the two DNAs (Figure 1a and 2) is quite conservative in the literal sense. The CD spectrum for the two RNAs is highly nonconservative (Figures 3a and 4); this has been attributed to the base tilting with respect to the helical axis (Johnson and Tinoco, 1969; Yang and Samejima, 1969). Most recently, Ivanov *et al.* (1973) have suggested that there might exist families of A and B forms in DNAs so that the B form spectra are more nearly conservative about the 260-nm absorption band than the A form. Our CD spectra of RNAs also fit with the scheme proposed by Ivanov *et al.* for the A form with a large maximum above 250 nm and a small minimum below.

The CD region between 200 and 230 nm appears to be sensitive to the base composition at least in the case of DNA as reported by Allen *et al.* (1972). The 210-nm band is greatly intensified in ML-DNA (72% (G + C)) as compared with CT-DNA (42% (G + C)). RDV-RNA and CPV-RNA have almost identical base composition and therefore their 210-nm band in relation to the (G + C) content cannot be assessed. In the preceding paper (Wells and Yang, 1974), we note that the 210-nm band is stronger for $tRNA^{Val}$ (61% (G + C)) than for $tRNA^{Phe}$ (53% (G + C)). However, a definite correlation between the rotational strength at 210 nm and base

TABLE III: ORD of Nucleic Acids.[a]

	ML-DNA		CT-DNA		RDV-RNA		CPV-RNA	
Extrema	E	C	E	C	E	C	E	C
λ_1 (nm)	291	290	290	291	279	275	279	282
$[m_1]$	7,380	7,250	8370	7,490	15,400	16,200	15,200	15,400
λ_2 (nm)	256	256	256	257	250	250	250	250
$[m_2]$	−8,510	−8,330	−6,730	−7,010	−15,400	−15,900	−14,400	−16,200
λ_3 (nm)	223	226	227	228	230	228	230	230
$[m_3]$	7970	8,120	15,900	15,200	−3,980	−4,870	−2,180	−4,770
λ_4 (nm)	217	219	217	218	219	220	220	219
$[m_4]$	6340	6,750	13,300	12,100	−7,320	−8,360	−5,850	−10,000
λ_5 (nm)	197	196	199	200	193	195	196	200
$[m_5]$	54,500	56,800	45,500	42,900	55,100	51,000	63,600	53,100
λ_6 (nm)		178		177		171		170
$[m_6]$		−94,300		−56,000		−102,000		−82,300

[a] Dimension of mean residue rotation, deg cm^2 dmol^{-1}; E, experimental; C, computed from the CD spectra.

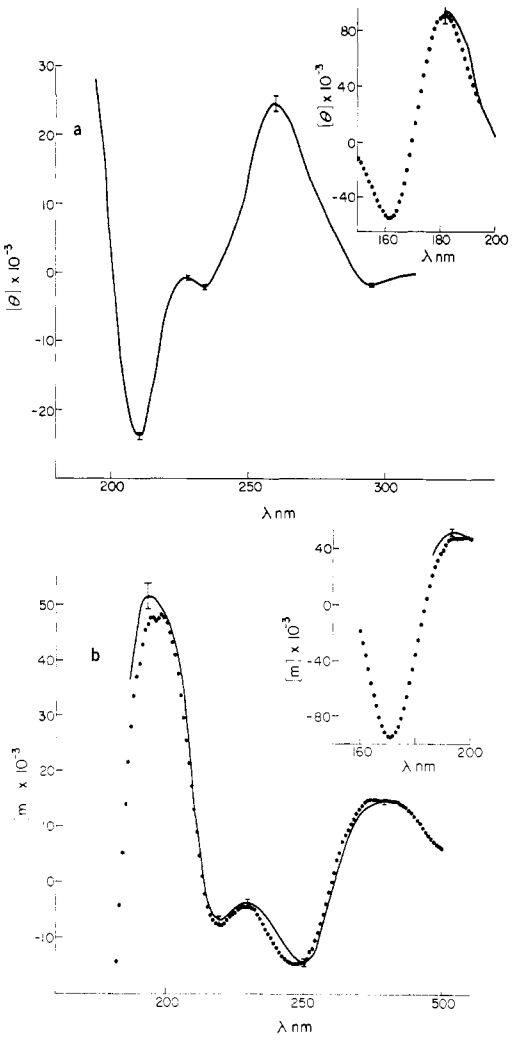

FIGURE 3: (a) The CD spectrum of double-stranded rice dwarf virus RNA in 0.15 M KF at 25°. Solid line, experimental; points, extrapolated (see item 9 of Table I). (b) The ORD spectrum. Solid line, experimental; points, computed from (a).

composition is not possible, since the percent of the (G + C) pairs in the double-stranded region cannot be predicted.

The relative magnitudes of the two negative CD bands at 210 nm and around 240 nm can be used as a spectral discriminator for the A and B forms. The 210-nm band for the A form or the base-tilted form of RNA is considerably stronger than the band near 240 nm; the reverse is true for the B form of DNA except in the case of very high G + C content such as ML-DNA. Studies of *E. coli* DNA films (Maestre, 1970) also show a changing ratio of these two bands that is related to the transformation between the A and B forms. In this respect the positive band around 180 nm and the negative band near 165 nm do not seem to show any obvious trend toward various forms of nucleic acids.

In summary, all the observed CD bands of DNA and RNA are related to their secondary structure. The positive band near 180 nm and the extrapolated band near 165 nm appear to be most sensitive to the conformation of nucleic acids.

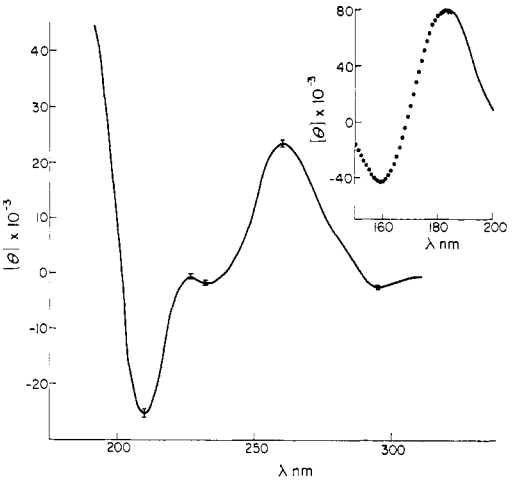

FIGURE 4: The CD spectrum of double-stranded cytoplasmic polyhedrosis virus RNA in 0.15 M KF at 25°. Solid line, experimental; points, extrapolated (see item 14 of Table I).

The 181-nm band in RNA is also dependent on the percentage of its double-stranded helix.

Acknowledgment

We thank Professor K. Miura for the gifts of two double-stranded RNAs, Dr. J. Thiéry for the computer program of the Kronig–Kramers transform, and Professor W. C. Johnson, Jr., for his preprint on the vacuum ultraviolet CD of DNA (Lewis and Johnson, 1974).

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