# Ultraviolet Circular Dichroism of Wheat Embryo Ribosomal Ribonucleates*

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ABSTRACT: The ultraviolet circular dichroism spectra of wheat embryo ribosomal ribonucleic acid has been examined from 300 to 185 mμ in the Cary Model 6001 circular dichroism attachment to the Cary Model 60 recording spectropolarimeter. These investigations have revealed the presence of an ellipticity band in the neighborhood of 187 mμ, which is unique because of its relatively large magnitude and extreme sensitivity to changes in conformation of the ribosomal ribonucleic acid in solution. In the present investigation, the effects of pH, ionic strength, formylation, and organic solvents (formamide, urea, and ethylene glycol) on the circular dichroism spectra of ribosomal ribonucleic acid have been examined. The results indicate that conformational changes in the ribonucleic acid are reflected by a large change in the circular dichroism band at 187 mμ, as well as by alterations of lesser magnitude in other regions of the spectrum. It is concluded that the extreme sensitivity of this ellipticity band to conformation will be of great importance, from both an empirical and a theoretical standpoint, in future circular dichrosim studies on ribonucleic aicd.

Brahms and Mommaerts (1964) have shown by comparative circular dichroism studies on DNA and RNA that the features of the circular dichroism spectra of nucleic acids reflect principally the secondary structure. Examination of the circular dichroism of various RNAs between 300 and 225 mμ in a Jouan dicrograph showed that all had similar spectra, and upon heating or addition of alcohol the intensity of the positive ellipticity band at 265 mμ was reversibly reduced. The gradual disappearance of the band was interpreted as indicative of the conversion of an ordered structure into a random coil arrangement. Beychok (1966) has reported that poly C at pH 4.6, where it exists in a double-stranded conformation, exhibits a circular dichroism spectrum similar to that for DNA, while at pH 7, where it is represented by an ordered single-stranded structure, poly C portrays a typical RNA-like circular dichroism spectrum. These observations indicate that circular dichroism is a sensitive probe of the solution conformation of RNA.

The optical rotatory dispersion of nucleic acids has been examined in detail, both empirically (Samejima and Yang, 1964, 1965; Lamborg and Zamecnik, 1965; Sarkar and Yang, 1965) and theoretically (Cantor *et al.*, 1966; Cantor and Tinoco, 1965; Vournakis and Scheraga, 1966; Bush and Scheraga, 1967). Wolfe *et al.* (1968), employing solvent conditions designed to favor either single-stranded stacked conformations or double-helical base-paired ones, have examined the optical rotatory dispersion of high molecular weight RNA from wheat embryo ribosomes. Their results support the hypothesis that both hydrogen bonding and base stacking play significant roles in the stabilization of the structure of the RNA. Since circular dichroism possesses the inherent advantage over optical rotatory dispersion in presenting discrete bands whose widths are comparable with the associated spectral bands, and recent technical advances have made available instrumentation capable of detecting and recording circular dichroism in the ultraviolet region down to 185 mμ, this study was undertaken to examine the effects of conformational changes on the ultraviolet circular dichroism of high molecular weight RNA from wheat embryo, over the range 300–185 mμ.

## Materials and Methods

*Preparation of RNA and Its Formylation.* Wheat embryo rRNA was isolated from whole embryo of the Thatcher variety of wheat *via* the phenol extraction procedure, described by Singh and Lane (1964). The isolated RNA was reprecipitated three times from 1 M NaCl, washed each time in alcohol and ether, air dried, and stored at −20°. The RNA sedimented as a two-peak system in the analytical ultracentrifuge, with $s^0_{20,w}$ values of 18 and 26 S, respectively.

The extinction coefficient, $E^{1\%}_{1\,cm}$ 260 mμ, for wheat embryo rRNA in 0.1 M phosphate at pH 7.0, was determined from dry weight–absorbance measurements as 200 OD units. In all experiments, the RNA was freshly dissolved in distilled, deionized water and Millipored. Dilutions from these stock solutions were made into 0.1 M phosphate at pH 7.0 and concentrations were measured by absorbance.

The formylation of 0.5% RNA solutions was conducted according to Boedtker (1967) in 1 M HCHO solutions at 63° for 10 min. The reaction mixture was cooled

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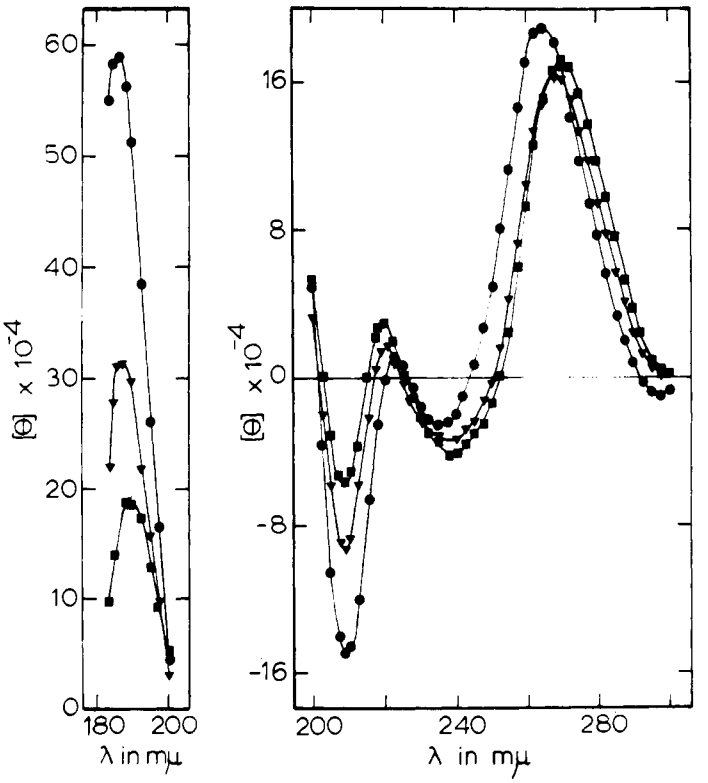


FIGURE 1: Molar ellipticities per residue of wheat embryo RNA at neutral pH as a function of ionic strength. Experimental results in the presence of 0.1 M phosphate (●–●–●), 0.001 M phosphate (▼–▼–▼), and $H_2O$ (■–■–■).

to room temperature, diluted into experimental solvents, and examined immediately in the circular dichroism apparatus.

*Circular Dichroism Measurements.* Ultraviolet circular dichroism measurements were made on a Cary Model 6001 circular dichroism attachment to a Cary 60 recording spectropolarimeter, equipped with a water-cooled lamp housing maintained at 27°. The instrument was calibrated with an aqueous solution of *d*-10-camphorsulfonic acid (Eastman Organic Chemicals, recrystallized) with a difference in molecular extinction coefficient ($\epsilon_L - \epsilon_R$) of 2.16. Constant nitrogen flushing was employed over the wavelength range examined (185–300 mμ). Measurements were made in 0.5- and 0.1-mm cells at RNA concentration ranges of 0.05–0.07 and 0.10–0.14%, respectively.

The results are reported in terms of mean residue molecular ellipticity, $[\theta]$, given by the relation, $[\theta] = \theta M/100lc$, where $M$ is the mean residue weight (317 atomic mass units; Lane, 1965), $\theta$ is the observed ellipticity in degrees, $l$ is the path length in decimeters, and $c$ is the concentration of RNA in g/ml. The units of $[\theta]$ are deg cm² per dmole.

## Results and Discussion

*The Effect of Ionic Strength.* The ultraviolet circular dichroism of wheat embryo rRNA as a function of ionic strength is shown in Figure 1. In 0.1 M phosphate buffer at pH 7.0 the RNA exhibits maxima at 265, 223, and 187 mμ, as well as troughs at 235 and 209 mμ. These data agree well with that reported by Brahms and Mommaerts (1964) for that portion of the spectrum which they examined, from 300 to 225 mμ. The low-intensity negative band at 295 mμ, described by Beychok (1966) has also been confirmed.

Upon reduction of the ionic strength to 0.001 M, a small reduction (~8%) is observed in the 265-mμ maximum, as well as a red shift of the peak and crossover of 3 and 7 mμ, respectively. The weak band at 223 mμ increases in magnitude and undergoes a blue shift of ~3 mμ. Most probably, this peak is not sensitive to ionic strength variations, and exhibits changes merely due to the reduction of the influence of the negative band at 209 mμ. The most dramatic effect observed is on the positive dichroic band at 187 mμ.[1] This peak decreases in magnitude by 28,000° upon reduction of the ionic strength from 0.1 to 0.001 M phosphate at pH 7.0. Further reduction of ionic strength, to essentially salt-free conditions, brings about a further reduction of 10,000° in this 187-mμ dichroic band, while changes in the rest of the spectrum are relatively minor. This circular dichroism band, then, is certainly highly sensitive to minor conformational alterations, as evidenced by the dramatic changes in its amplitude.

To ensure that these observations were not artificially induced by the salt itself, the studies were repeated in the presence of $5 \times 10^{-3}$ M $Mg^{2+}$, which has been shown to stabilize the solution conformation of RNA. Addition of $5 \times 10^{-3}$ M $Mg^{2+}$ to RNA dissolved in either 0.1 M phosphate, 0.001 M phosphate, or essentially salt-

[1] The experimental uncertainty in the absolute values of the ellipticity bands will vary with wavelength, in view of the higher noise level of the instrument at lower wavelengths. In our case, the maximum errors for the 185-, 190-, and 209-mμ regions are ±4000, ±2000, and ±1000°, respectively. At wavelengths above 209 mμ the error is estimated to be no greater than ±500°.

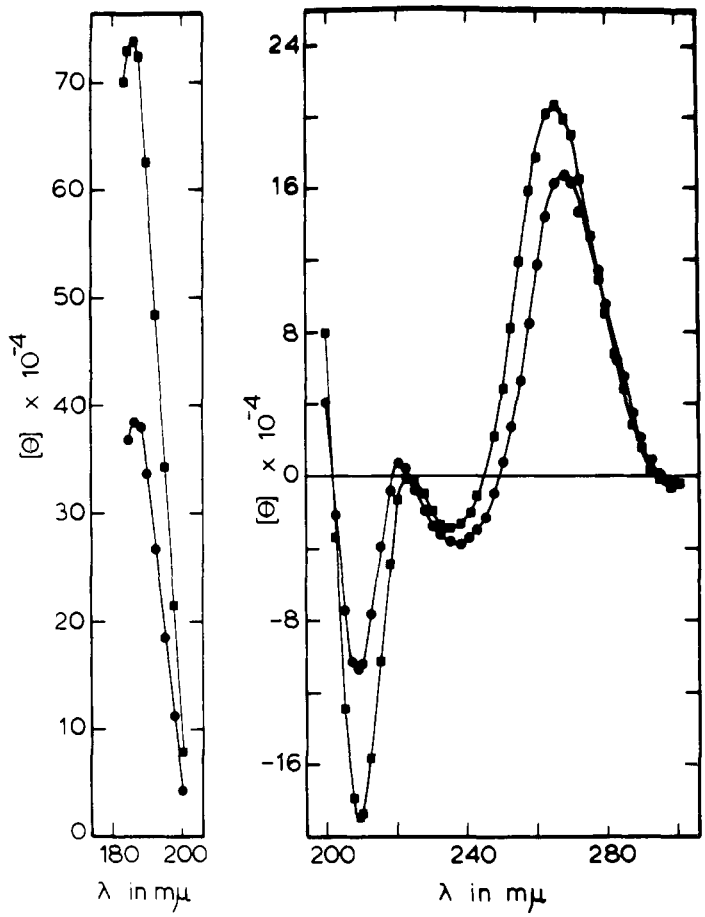


FIGURE 2: Molar ellipticities per residue of RNA at neutral pH and in the presence of $5 \times 10^{-3}$ M $Mg^{2+}$ (with or without added phosphate; see text for details) (■–■–■); in the presence of 0.001 M EDTA (●–●–●).

free solutions resulted in identical circular dichroism behavior (Figure 2). In fact, the amplitude of the 187-mμ peak was increased by about 18% over the 0.1 M phosphate case alone, perhaps reflecting a greater compacting of the RNA structure. On the other hand, RNA in 0.001 M EDTA produced a circular dichroism spectrum comparable with that in 0.001 M phosphate, definitely confirming the role of $Mg^{2+}$ as a stabilizing agent.

*The Effect of Protonation.* In Figure 3, the circular dichroism scans of RNA in 0.1 M phosphate are presented at varying acid pH values. Again, the most dramatic changes are observed in the 187-mμ positive band, as it progressively decreases in amplitude with increasing acidity. The effects of the presence of excess protons on RNA structure have been discussed elsewhere (Fasman *et al.*, 1964; Sarkar and Yang, 1965) and are thought to arise from the protonation of cytosine residues and the resulting disruption of hydrogen bonding between guanine–cytosine base pairs. This effect will most certainly disrupt the double helical segments of the molecules of RNA, and this disruption is definitely reflected by the decrease in magnitude of the 187-mμ dichroic band. At pH 3.1, where all possible protonation has occurred, this band has been reduced to only about 6% of its magnitude at pH 7.0. Other changes in the spectra include a marked reduction in the magnitude of the negative band at 209 mμ, a substantial decrease in magnitude of the positive band at 265 mμ, as well as a red shift of the peak of this band, and in the extreme case at pH 3.1, a conversion of this band into one of bimodal character. These results are not straightforward in interpretation, but it is clear that the circular dichroism spectral intensities decrease markedly as the forces which stabilize the solution conformation of the RNA are perturbed, and a more random state is achieved.

*The Effects of Organic Solvents.* The effects of organic solvent systems on the optical rotatory dispersion of wheat embryo rRNA have been described in detail by Wolfe *et al.* (1968). In order to examine these effects in terms of circular dichroism spectra, experimental conditions identical with those used in the optical rotatory dispersion studies were utilized, with formamide, urea, and ethylene glycol the principal solvents studied. Application of circular dichroism measurements to these solvent systems is complicated by the high absorbance of formamide and urea below 250 and 205 mμ, respectively. The ethylene glycol systems (59 and 80 vol %) were amenable to analysis down to 185 mμ. The results of these studies are shown in Figure 4. The amplitude of the 265-mμ peak has been reduced in all solvent systems, and most markedly in the 80 vol % ethylene glycol system, where the peak is ~25% of its value in 0.1 M phosphate at pH 7.0. Again, the greatest alterations are seen at the lowest wavelengths. The amplitidue of the 187-mμ dichroic band has been reduced to 16 and 3% of the reference value for RNA in 0.1 M phosphate (pH 7.0) in the 50 and 80 vol % ethylene glycol

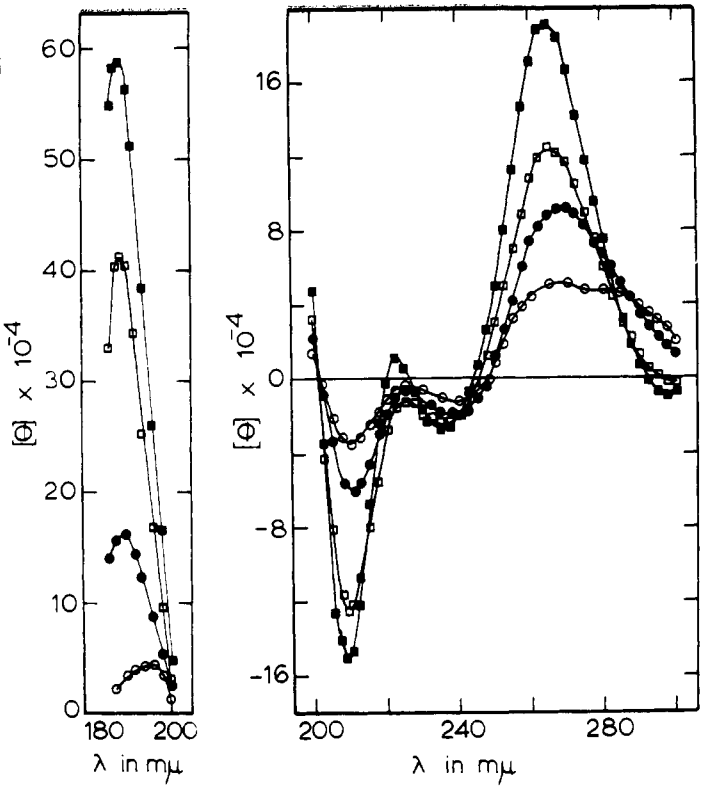


FIGURE 3: Molar ellipticities per residue of RNA at constant ionic strength (0.1 M phosphate) as a function of pH. Experimental results are reported at: pH 7.0 (■-■-■), pH 4.5 (□-□-□), pH 4.0 (●-●-●), and pH 3.1 (○-○-○).

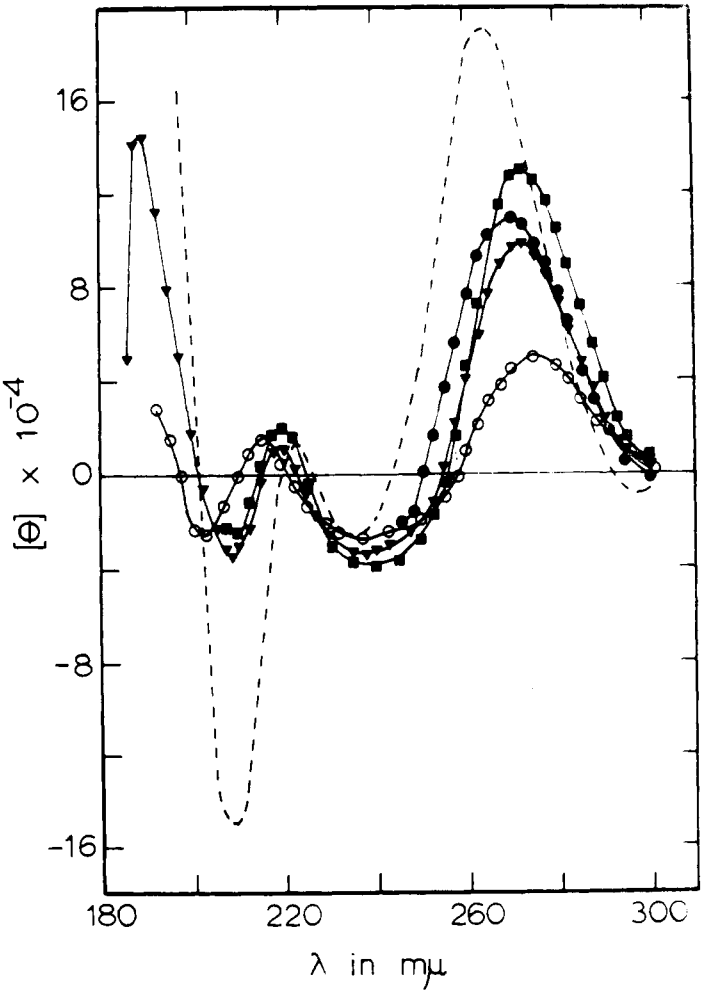


FIGURE 4: Molar ellipticities per residue of RNA in various organic solvents: 90 vol % formamide (●-●-●), 8 M urea (■-■-■), 50 vol % ethylene glycol (▼-▼-▼), and 80 vol % ethylene glycol (○-○-○). (The dotted line represents RNA in 0.1 M phosphate at pH 7.0, included for comparison purposes.)

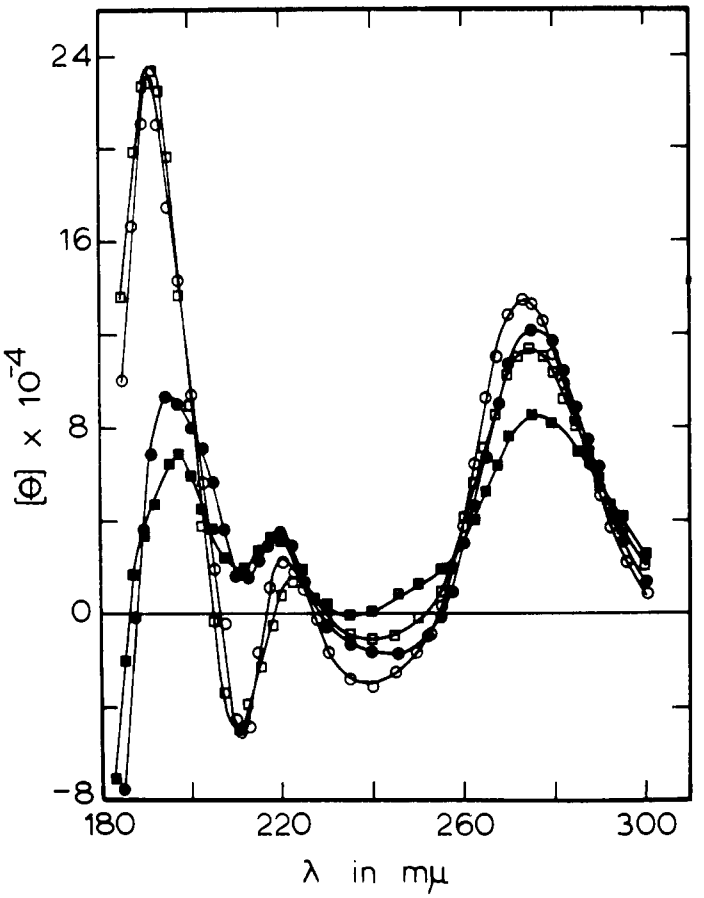


FIGURE 5: Molar rotation per residue of RNA heated in the presence of 1 M HCHO to 63° for 10 min and then fast cooled to 20°. Spectra were obtained in 0.1 M phosphate (pH 7.0) (O–O–O), 0.001 M phosphate (pH 7.0) (●–●–●), $H_2O$ (pH 7.0) (■–■–■), and $5 \times 10^{-3}$ M $Mg^{2+}$ (□–□–□). (All solvents also contained 1 M HCHO.)

solvents, respectively. It is to be noted that the addition of 0.1 M phosphate (pH 7.0) to RNA in 50 vol % ethylene glycol partially reverses the reductions in amplitude of the 265- and 187-mμ peaks. In the case of 80 vol % ethylene glycol this reversal with phosphate addition is far less evident.

Since protonation and ionic strength reduction disrupt hydrogen-bonded double-helical segments, and ethylene glycol promotes unstacking of bases and stabilization of hydrogen bonding (Fasman *et al.*, 1964, 1965), it becomes apparent in view of the diminution in intensity of the 187-mμ dichroic band under all conditions that variations in this band are not reflecting changes in only one specific RNA structural feature. Rather, these observations are consistent with a role for both hydrogen bonding and base stacking in the stabilization of RNA structure, and indeed suggest that the two forces are not independent of each other.

*The Effect of Formylation.* The reaction of formaldehyde with RNA under the conditions described by Boedtker (1967) primarily formylates the exocyclic amino groups of the RNA chain and blocks their participation in hydrogen bonding. Millar and MacKenzie (1967) and Wolfe *et al.* (1968) have discussed the effect of formylation, as well as the qualitative significance of hydrogen bonding in maintaining RNA stability. Figure 5 depicts the ultraviolet circular dichroism of formylated RNA at varying ionic strengths, and also in the presence of $Mg^{2+}$. It can be seen that the peak at 265 mμ in 0.1 M phosphate (pH 7.0) has undergone a red shift by 10 mμ due to formylation, and the amplitude has decreased by about 40%. The peak at 187 mμ has moved to 191 mμ, and has also decreased in intensity by about 60%. The other features of the circular dichroism spectra have undergone decreases in magnitude and shifts comparable with the other solvent systems examined.

An interesting feature is the effect of reduction of ionic strength in the samples of formylated RNA. Further decreases in the amplitude of the circular dichroism bands are observed, which parallels the effect of ionic strength reduction in the case of native RNA; in addition the decreases can be reversed by the addition of $5 \times 10^{-3}$ M $Mg^{2+}$. These results indicate that a structural identity for the RNA persists even in the case of the formaldehyde-treated material, and this structure may be enhanced by the addition of neutral salts and $Mg^{2+}$ cations. Boedtker (1967) suggests that only about 85% of the exocyclic amino groups are affected by formylation, and perhaps the remaining ones are responsible for part of the residual structure persisting in solution after formylation. As well, base-stacking phenomena should also be possible in formylated RNA, which additionally contributes to the intensity of the observed bands.

## Conclusion

The present investigation has described the ultraviolet circular dichroism characteristics of wheat embryo rRNA. In the region 300–225 mμ, this system ex-

hibits an analogous spectrum to that documented by Brahms and Mommaerts (1964), using a Jouan dicrograph, for RNA from various sources. In view of the greater wavelength accessibility of the Cary instrument, this study has also revealed an additional ellipticity band at 187 mμ, whose significance resides in its relatively large magnitude and marked sensitivity to conformational changes. The most dramatic alterations in this band were produced by changes in ionic strength of RNA solutions, and by the addition of $Mg^{2+}$ as an agent for stabilizing RNA conformation. Attempts to assign qualitative significance to the changes in magnitude of the 187-mμ peak in terms of solvents which preferentially interfere with or enhance hydrogen bonding indicated that this band is appreciably reduced, under all solvent conditions examined. Obviously then, this dichroic band is sensitive to the over-all molecular architecture of the RNA (*i.e.*, both the base-stacked and hydrogen-bonded regions), rather than to one isolated structural feature of the molecule. It is apparent that as more instruments capable of resolution of circular dichroism spectra below 200 mμ become generally available, this ellipticity band will be a most important feature of the circular dichroism spectra of RNA, from both theoretical and empirical standpoints.

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