# CIRCULAR DICHROISM MELTING STUDIES ON R17 PHAGE RNA

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Received March 3, 1972

SUMMARY

Ultraviolet circular dichroism measurements were made on solutions of freshly prepared RNA from the bacteriophage R17 at pH 7.0, in both the absence and presence of $Mg^{2+}$ ion, and over a temperature range of 26 - 76°. A small negative band is detected at 298 nm adjacent to the strong positive band at 266 nm with and without $Mg^{2+}$ ion. This band is thermolabile, disappears irreversibly and might suggest the presence of some sulfur-containing bases in R17-RNA. The intense positive band at 266 nm increases upon addition of $Mg^{2+}$ ion, and temperature studies indicate that three distinct melting regions are now apparent, whereas in the absence of $Mg^{2+}$ ion and in solution of low ionic strength only a sigmoidal heating curve is observed.

Some exciting current work on RNA phages is concentrated upon those parts of the chromosome which are not translated into protein, but which serve as signals for initiation or termination of translation. The single-stranded RNA molecule from the R17 phage contains about 3300 nucleotides, and the nucleotide sequence is rapidly becoming known (1-3). It is generally assumed that most nucleotides in a single-stranded and linear RNA molecule interact to form a secondary structure which consists of intramolecular helical regions and unpaired loops. Further folding would define a unique biologically active tertiary structure. However, even for molecules of known sequence it is difficult to determine a secondary and tertiary structure by pure contemplation, by model building or by computer methods. Recently an interesting simple method for estimating the secondary structure of an RNA molecule from knowledge of its sequence and assessments of some thermodynamic parameters was developed (4).

In the present study circular dichroism (CD) is used to follow changes in R17 RNA conformation accompanying thermodenaturation in solution of 0.1X SSC buffer (0.015M NaCl, 0.0015M sodium citrate, pH 7.0) with and without the presence of $4 \times 10^{-3}$ $Mg^{2+}$. It is well established that CD is a sensitive probe to gain insight into the forces stabilizing the RNA structure, and the contribution of base paired regions and base stacking to the R17 RNA structure can be estimated by this method. This is feasible due to results of many investigations on appropriate model systems which have focused upon the intense positive CD band at about 265 nm, and its relation to the secondary structure of RNA (5-8).

## MATERIALS AND METHODS

Preparation of R17-RNA. RNA was prepared according to the procedure of Gesteland et al. (9). To check the absence of hidden breaks a fraction of each preparation was sedimented through a sucrose density gradient containing formaldehyde (10).

CD measurements. Ultraviolet CD measurements were made on a Cary Model 6002 CD attachment to a Cary 60 recording spectropolarimeter, equipped with a thermostatable cylindrical cell holder. Results are reported in terms of mean residue molecular ellipticity, $[\theta]$, which is calculated on the basis of nucleotide residue molar concentration. The units of $[\theta]$ are degree x $cm^2$/decimole. Measurements were made in 10 mm cells and the concentration was about 0.1 mM (nucleotide residues). Constant nitrogen flushing was employed over the wavelength examined. Water from two Haake thermostats, coupled with a water bath cooler, was circulated through a thermostatable cylindrical cellholder. One Haake bath was used in order to keep a temperature of about $26^0$ in the cell compartment, whereas with the second bath the cell was heated to the desired temperature. The temperature of the bath was increased at a rate of 2-3 degrees at a time, and 15-20 minutes were allowed for equilibration, before a CD spectrum was recorded between 310 and 250 nm. Tempera-

ture measurements were made with a sensitive thermistor which was inserted through a teflon stopper directly into the top of the cell. The temperature was determined during each scanning at 310, 266 and 250 nm, and it was found that the temperature at the three wavelengths was within $0.5^0$ the same. The reported temperature was recorded at 266 nm. No correction for thermal expansion of the solutions was made.

## RESULTS AND DISCUSSION

The ultraviolet CD of R17-RNA is shown in Figure 1 at low ionic strength with and without $Mg^{2+}$. In the absence of $Mg^{2+}$ the RNA exhibits maxima at 266 and 223 nm, as well as troughs at 298, 235, and 210 nm. If $4 \times 10^{-3}$ M $Mg^{2+}$ is added to the solution, a small increase (~10%) is observed in the 266 nm peak, as well as an increase of the trough at 210 nm. In addition, the weak band at 223 nm decreases in magnitude and undergoes a bathochromic shift of ~ 3nm,

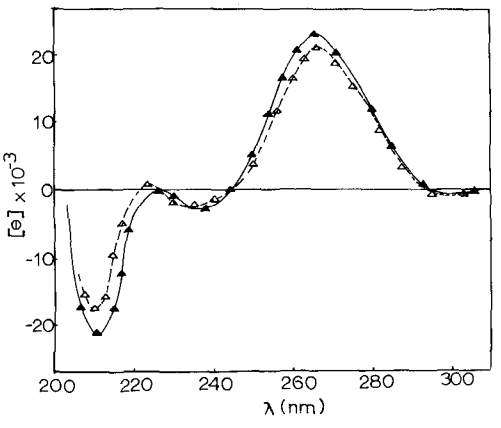


FIGURE 1. Circular dichroism spectra of R17-RNA at $27^0$. Δ-Δ, RNA in 0.1 X SSC; ▲-▲, RNA in 0.1 X SSC and in the presence of $4 \times 10^{-3}$ M $Mg^{2+}$.

whereas the troughs at 235 and 298 nm remain virtually unchanged. The observed bathochromic shift is most probably due to an increase of the negative band at 210 nm. On the other hand, an increase in intensity of the 266 nm band would be consistent with more stacking interactions. This long wavelength, intense positive band has been discussed at several occasions with

respect to its relation to the secondary structure of RNA (5-8). The presence of the low-intensity band at 298 nm is of great interest, although its origin is not known with certainty. It has also been observed in some ribosomal RNA (11-13), and it was suggested (12) that sulfur-containing bases may be responsible for this high-wavelength negative band, if they are in an asymmetric environment. Melting studies indicate that this band is thermolabile and disappears irreversibly. Such an observation would favor in R17-RNA the presence of some sulfur-containing bases which can be hydrolyzed easily.

For the thermal denaturation studies of R17-RNA the CD peak of 266 nm was chosen for scrutiny. It is assumed that the separation of paired bases causes a red shift in the position of the Cotton effect of this band with with a minor change in its magnitude. A decrease in magnitude can on the other hand be attributed to the disruption of stacking interactions (5-8). In Figure 2 a typical set of CD melting curves are shown which are observed when R17-RNA is subjected to heating in 0.1 X SSC in the presence of

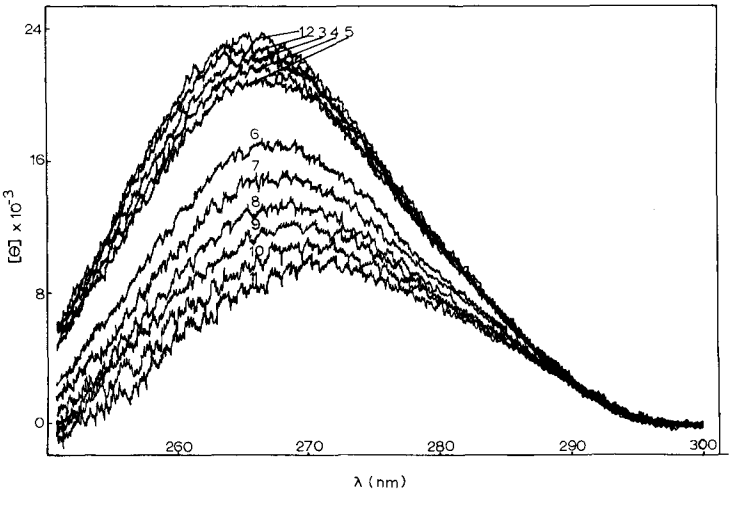


FIGURE 2. Circular dichroism spectra of R17-RNA in 0.1 X SSC and $4 \times 10^{-3}$ M $Mg^{2+}$ at various temperatures. (1) 28°; (2) 34.9°; (3) 39.5°; (4) 44.0°; (5) 49.3°; (6) 60.5°; (7) 64.8°; (8) 67.1°; (9) 69.5°; (10) 72.2°; (11) 75°.

4 x $10^{-3}$ M $Mg^{2+}$. Only those CD spectra are shown, which are significantly different from one another. In figure 3 all the measured $[\theta]$ at 266 nm are reported on the left ordinate as a function of temperature in the absence of $Mg^{2+}$ and in the presence of 4 x $10^{-3}$ M $Mg^{2+}$ in 0.1X SSC. In addition, the shift of the peak ellipticity is plotted for each melting experiment on the right ordinate.

In the absence of $Mg^{2+}$ a sigmoidal heating curve for $[\theta]$ peak versus temperature is observed which has a midpoint (Tm) at about 46°. A more accurate Tm value cannot be given at the present time due to the somewhat arbitrary procedure of limiting the high and low temperature values. The plot for the shift of the wavelength maximum of the main band gives also a sigmoidal curve. If the criterion used for Tm is the position of the peak ellipticity of the intense positive band, the Tm is also about 46°. Slow cooling of the solution after melting restores with the exception of the 298 nm band the original CD spectrum. Thus, the temperature-dependent shift of the Cotton effect maximum at about 266 nm and the decrease in rotational strength appear to be completely reversible. In the presence of 4 x $10^{-3}$ M $Mg^{2+}$ it is seen that the two curves in Figure 3 are quite different from those ob-

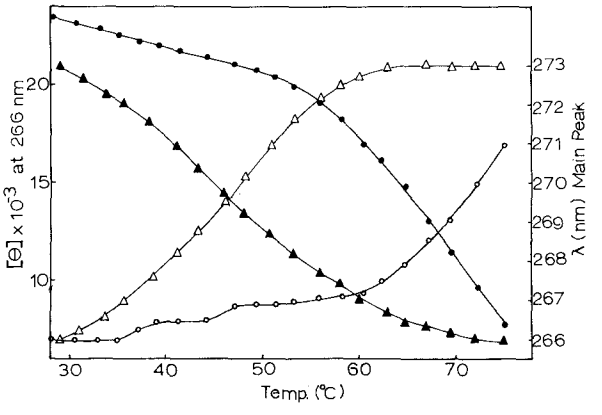


FIGURE 3. Circular dichroism melting curves of R17-RNA in 0.1 X SSC with and without 4 x $10^{-3}$ M $Mg^{2+}$. Two parameters are plotted: on the left ordinate the peak residue ellipticity at 266 nm, ▲-▲ without $Mg^{2+}$, ●-● with $Mg^{2+}$; on the right ordinate the red shift of the peak of the intense positive band, Δ-Δ without $Mg^{2+}$, o-o with $Mg^{2+}$.

tained without the presence of $Mg^{2+}$. If one considers the variation of $[\theta]$ two distinct melting regions are apparent (one below $\sim 50^{0}$ having less of a slope than the one above $\sim 50^{0}$). In addition, the transition curves are sharper and the temperature transition occurs at higher temperature in the presence of $Mg^{2+}$. Also, the transition below $\sim 50^{0}$ occurs with little change in $[\theta]$. It has to be pointed out that an apparent decrease in $[\theta]$ at 266 nm is not necessarily due to loss of stacking, but will also occur with the bathochromic shift of the intense positive band. Therefore we have examined carefully the effect of temperature on the position of the peak of this band. As can be seen in Figure 3, the shift in wavelength of the band maximum contains discontinuities. At lower temperature 2 small cooperative shifts of the band maximum with transition midpoints of about $37^{0}$ and $46^{0}$ can be distinguished. These two shifts are followed by a large one having a transition midpoint greater than $65^{0}$. As stated before a red shift in the position of the Cotton effect of the band under investigation is assumed to result from separation of paired bases (5-8). Thus, the existence of cooperative shifts in wavelength of the band maximum would indicate the presence of 3 distinct melting regions.

The CD results just presented can be summarized by stating that the total denaturation of R17-RNA is not cooperative in $4 \times 10^{-3}$ M $Mg^{2+}$ ion and 0.1 X SSC. The presence of three phases suggests the existence of at least 3 cooperatively melting regions. The break between the phases is sharp, and it seems likely that the melting of the more stable region does not start until the melting of the less stable region is essentially complete. At the present time we shall not favor either one of the two following possible explanations for this phenomenon. It is feasible that each melting phase represents the cooperative melting of a separate helical region containing different proportions of A-U and G-C pairs. On the other hand the data could also be interpreted as resulting from changes in tertiary structure occurring at the lower two Tm values, whereas the high Tm would correspond to the melting of the secondary structure consisting of intramolecular helical regions and unpaired loops.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Bruce M. Alberts (Princeton University) and Dr. Raymond F. Gesteland (Cold Spring Harbor Laboratory) for their gifts of E. coli $K_{12}$Hfr RNase$^-$ $D_{10}$ and phage R17, respectively.

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