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# Optical Rotatory Dispersion Studies of Yeast Alanine and Tyrosine Transfer Ribonucleic Acids. Evidence for Intramolecular Hydrogen Bonding and Discussion of Conformational Aspects*

John N. Vournakis and Harold A. Scheraga

ABSTRACT: Optical rotatory dispersion measurements were made on solutions of yeast alanine and tyrosine transfer ribonucleic acids (t-RNA's) at neutral pH, in the presence and absence of $Mg^{2+}$ ion, and over the temperature range 5–90°. The data were interpreted in terms of neighbor–neighbor base stacking and intramolecular hydrogen bonding. Comparison of t-RNA data with those for the poly-(G-C) and poly-(A-U) double helices shows that alanine and tyrosine t-RNA's have a significant amount of double-stranded structure (primarily G-C hydrogen bonding) at low temperatures. Upon heating, the hydrogen bonds and most of the stacking interactions are disrupted. The addition of $Mg^{2+}$ ion stabilizes the low-temperature form, presumably by binding along the phosphate backbone, thus reducing electrostatic repulsions in the t-RNA molecules, so that the transition temperature is increased. Calculations of ORD curves are performed, assuming several conformations (*i.e.*, single-strand with no hydrogen bonding, a hydrogen-bonded conformation, a hypothetically hydrolyzed sample, etc.) including ones previously proposed by R. W. Holley and J. T. Madison for alanine and tyrosine t-RNA, respectively. These calculated curves are compared to experimental data; they agree quite well with the observed curves.

The use of optical rotatory dispersion as an experimental tool for the investigation of the conformation of polyribonucleotides has been well demonstrated (Fasman *et al.*, 1964; Holcomb and Tinoco, 1965; Lamborg *et al.*, 1965; Brahms *et al.*, 1966; Poland *et al.*, 1966; Vournakis *et al.*, 1966). It has been shown that neighbor–neighbor base stacking (without hydrogen bonding) exists in single-strand oligomers of adenylic acid, and that the state of stacking of a given base pair is essentially independent of the state of stacking of the rest of the chain (Brahms *et al.*, 1966; Poland *et al.*, 1966). An ORD study of the 16 dinucleoside phosphates corresponding to the 16 possible pairings of A, U, C, and G[1] with one another

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[1] Abbreviations used: G, guanosine; C, cytidine; U, uridine; A, adenosine.

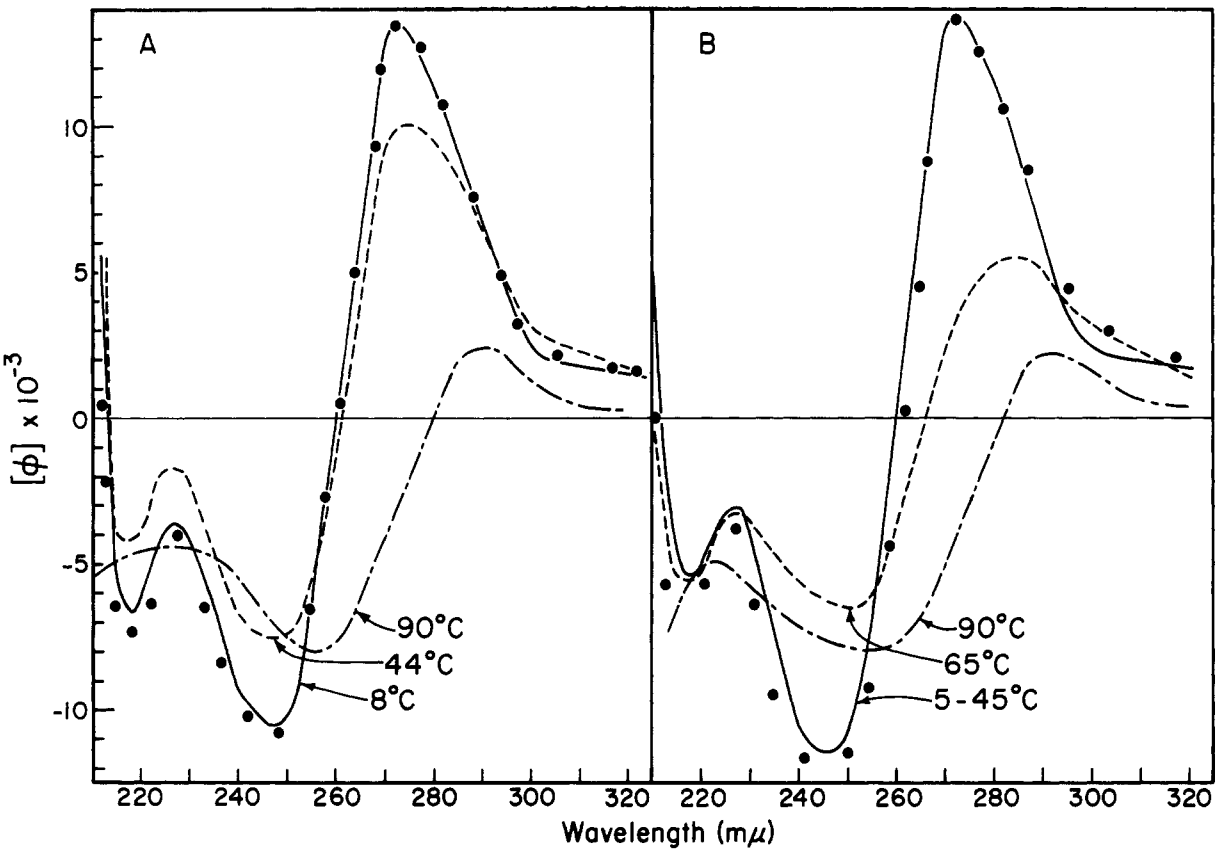


FIGURE 1: Optical rotatory dispersion curves of alanine t-RNA (0.10 M phosphate, 0.15 M KCl) at three temperatures. (A) At pH 6.80 in the absence of $Mg^{2+}$ ion. Solid circles are data obtained by cooling to 8.0° after solution had been heated to 90° for 1 hr. (B) At pH 6.75 in the presence of 0.003 M $Mg^{2+}$ ion. Solid circles are data obtained by cooling to 10° after solution had been heated to 90° for 1 hr.

has provided values for the mean residue rotation for each base pair over the 320–210-m$\mu$ wavelength region at 25° (Warshaw and Tinoco, 1965; Warshaw, 1966). Further, ORD data are available for double-stranded poly-(G-C) and poly-(A-U) complexes in which interchain hydrogen bonding is present (Sarkar and Yang, 1965a,b); these data are also available in the 320–210-m$\mu$ wavelength region. Given this information, it now becomes possible to begin to interpret, within limitations, the ORD behavior of a polyribonucleotide of a known base sequence, in terms of its conformation in solution. With the availability of yeast alanine and tyrosine transfer ribonucleic acids (t-RNA's), we have carried out ORD studies on solutions (at neutral pH and 0.15 M KCl) of the two t-RNA's, the primary sequence of these polyribonucleotides having been recently determined (Holley *et al.*, 1965; Madison *et al.*, 1966). These measurements were made over a range of temperature, in both the absence and presence of $Mg^{2+}$ ion, which stabilizes the conformation of RNA in solution (Boedtker, 1960; Fuwa *et al.*, 1960); it is also a necessary factor in protein synthesis along with t-RNA (Nirenberg and Leder, 1964). Using data on model compounds, and having the primary sequence data at hand, theoretical ORD curves were calculated for alanine and tyrosine t-RNA's. Comparison of these calculated curves with experimental data lends support to previously proposed conformations for these molecules in solution (Holley *et al.*, 1965; Madison *et al.*,1966).

## Experimental Section

*A. Materials.* Yeast alanine t-RNA was prepared according to the procedure of Apgar *et al.* (1962), and given to us by R. W. Holley. Yeast tyrosine t-RNA was prepared according to Holley *et al.* (1963), and given to us by J. T. Madison. The t-RNA's were stored as partially hydrated (~15% $H_2O$) amorphous solids at about 2° before use in spectrophotometric and spectropolarimetric measurements. Both transfer RNA's consisted of better than 85% of one component (*i.e.*, one base sequence), the impurity being primarily t-RNA's of different base sequences.

*B. Preparation of Solutions.* Solutions of t-RNA were prepared by dissolving the solid in 0.04 M $NaH_2PO_4$, 0.06 M $Na_2HPO_4$, 0.15 M KCl, the final pH being 6.80 ± 0.02. Concentrations were determined from the ultraviolet absorbtion at 260 m$\mu$, knowing that, for both t-RNA's, a concentration of 1 mg/ml gives

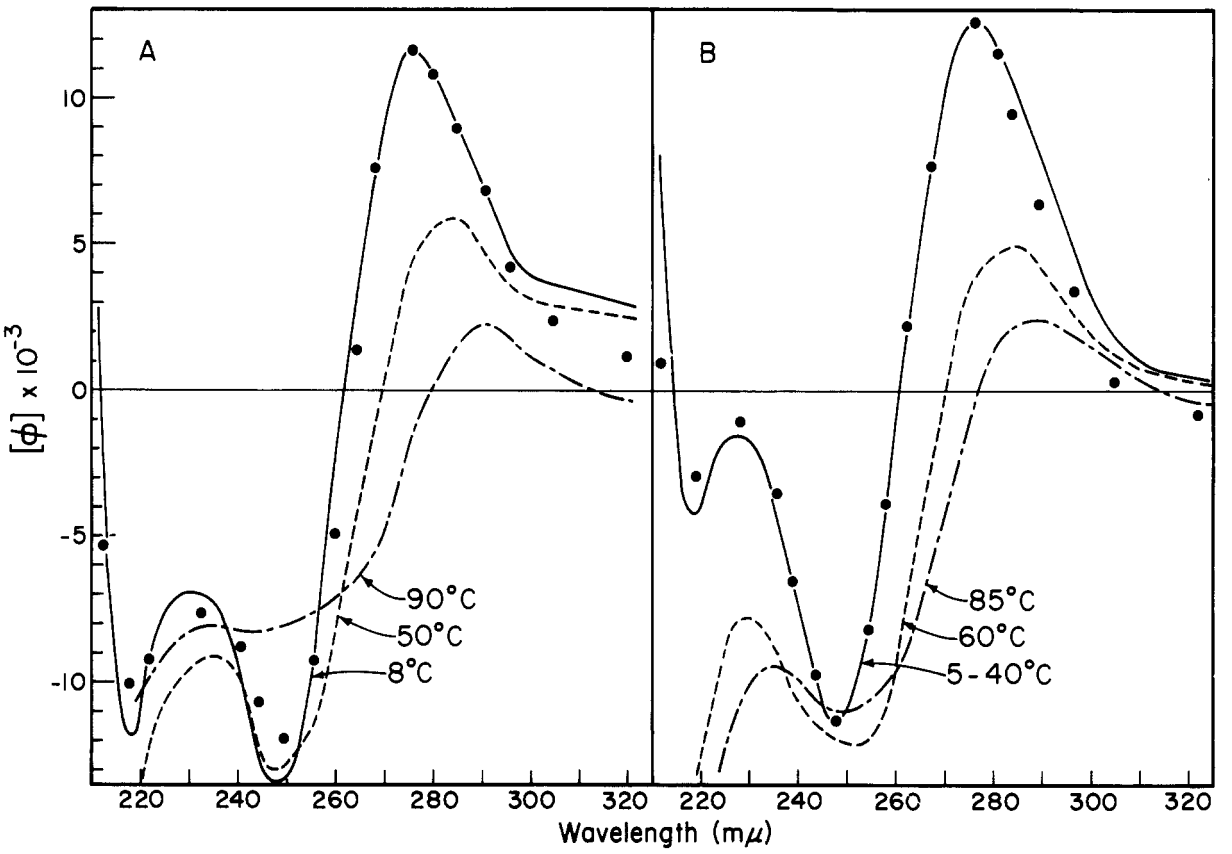


FIGURE 2: Optical rotatory dispersion curves of tyrosine t-RNA (0.10 M phosphate, 0.15 M KCl) at three temperatures. (A) At pH 6.80 in the absence of $Mg^{2+}$ ion. Solid circles are data obtained by cooling to 8° after solution had been heated to 90° for 1 hr. (B) At pH 6.75 in the presence of 0.003 M $Mg^{2+}$ ion. Solid circles represent data obtained by cooling to 10° after solution had been heated to 90° for 1 hr.

an OD of 20 at this wavelength (R. W. Holley and J. T. Madison, private communication). In some cases, 0.003 M $MgCl_2 \cdot 6H_2O$ was added to the above buffer. The $MgCl_2 \cdot 6H_2O$-containing buffer was tested for precipitate formation as a function of temperature; no precipitation was observed, even up to 90°.

*C. Methods.* Optical rotatory dispersion and ultraviolet absorbtion measurements were carried out as described previously (Vournakis *et al.*, 1966); in addition, the buffer in the presence of 0.003 M $Mg^{2+}$ was tested over the wavelength range for variations in light absorbance and optical rotation as a function of temperature. It was found that neither ultraviolet absorbance nor optical rotation varied from 5 to 90°, thus indicating that, in addition to visible observation of the solutions, no precipitate formed. The light source in the spectropolarimeter was a high-pressure Xenon arc lamp (Osram type XBO-450 W/P). Base lines were obtained with buffer preceding each ultraviolet absorbance and optical rotatory dispersion measurement. Quartz cells (opticell 1 cm) were used in all measurements.

## Results

Examples of the optical rotatory dispersion of alanine t-RNA, both in the absence and in the presence of $Mg^{2+}$ ion, at three temperatures, are shown in Figure 1. Mean residue rotation [$\phi$] (Poland *et al.*, 1966) is plotted against wavelength. Cotton effects are observed, with positive and negative maxima at 273 and 247 m$\mu$, respectively, at low temperature (8° in the absence of $Mg^{2+}$ ion; 5–45° in the presence of $Mg^{2+}$ ion). These maxima are shifted to 290 and 258 m$\mu$, respectively, at high temperature (90° for both cases). The solid circles in Figure 1 are data obtained by slow cooling (~1.3°/min) of solutions, which had remained at 90° for about 1 hr, to low temperature (less than 10° in both the absence and presence of $Mg^{2+}$ ion). The temperature-dependent shift of the Cotton effect maxima and the decrease in magnitude of rotation appear to be completely reversible in both cases.

Figure 2 shows similar data for tyrosine t-RNA, Figure 2A being ORD data at three temperatures

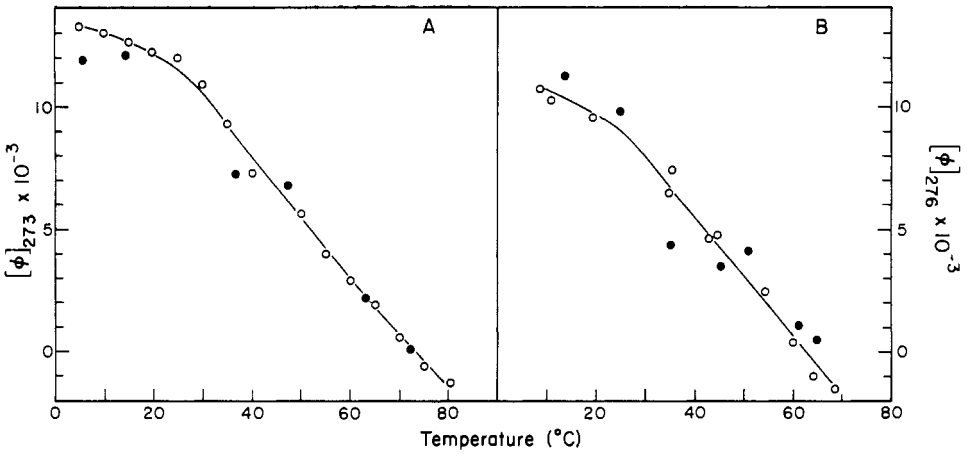


FIGURE 3: Curve of mean residue rotation *vs.* temperature (pH 6.80, 0.10 M phosphate, 0.15 M KCl buffer). (A) For alanine t-RNA at 273 mμ; the solid circles represent data obtained at 273 mμ by cooling from 90° to the temperature shown. (B) For tyrosine t-RNA at 276 mμ; the solid circles represent data obtained at 276 mμ by cooling from 90° to the temperature shown.

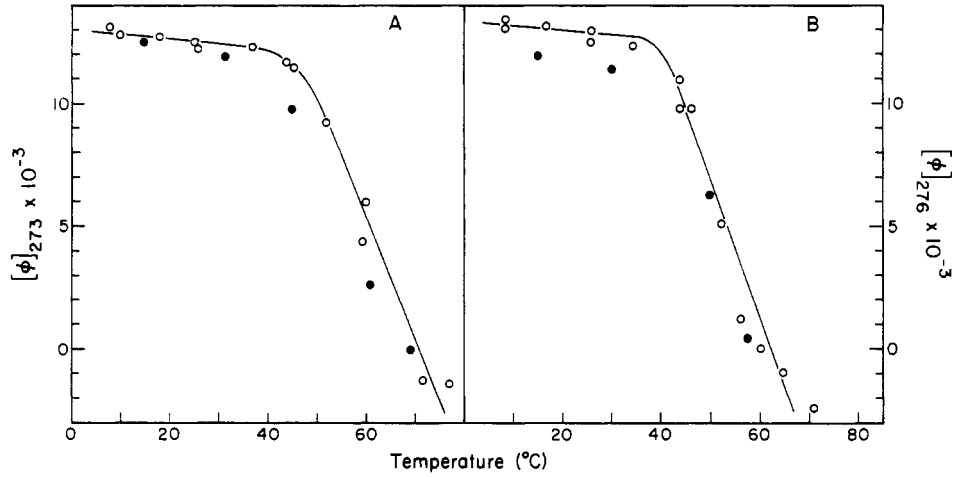


FIGURE 4: Curve of mean residue rotation *vs.* temperature (pH 6.75, 0.10 M phosphate, 0.15 M KCl buffer). (A) For alanine t-RNA in the presence of 0.003 M $MgCl_2 \cdot 6H_2O$ at 273 mμ. The solid circles represent data obtained at 273 mμ by cooling from 90° to the temperature shown. (B) For tyrosine t-RNA in the presence of 0.003 M $MgCl_2 \cdot 6H_2O$ at 276 mμ. The solid circles represent data obtained at 276 mμ by cooling from 90° to the temperature shown.

in the absence of $Mg^{2+}$ ion and Figure 2B being similar data in the presence of $Mg^{2+}$ ion. Here again, Cotton effects are observed and have maxima at 276 and 248 mμ at low temperature (8° in the absence of $Mg^{2+}$ ion, 5–40° in the presence of $Mg^{2+}$ ion). These maxima are shifted to 290 and ~250 mμ at high temperature (90°). The solid circles in Figure 2 are reversibility points obtained in the same way as those in Figure 1. The shift of the Cotton effect maxima and decrease in magnitude of the rotation, as a function of temperature, are seen to be reversible for tyrosine t-RNA.

Figure 3 shows the variation of the mean residue rotation, $[\phi]$, at the positive Cotton effect maximum (273 mμ for alanine t-RNA, and 276 mμ for tyrosine t-RNA) with temperature for solutions of the two t-RNA's in the absence of $Mg^{2+}$ ion. It is seen that the curves in Figure 3 are quite similar. Two distinct melting regions are apparent (one below ~30° having less of a slope than the one above 30°) in both Figures 3A and B. Study of the curves indicates that the transition of alanine t-RNA has a slightly larger slope and slightly higher melting temperature than the tyrosine t-RNA temperature transition. Solid circles represent reversibility data obtained by cooling to the temperature indicated from about 90°. These temperature transition curves are seen to be reversible.

Figure 4 represents temperature transition data for the two t-RNA's in the presence of $Mg^{2+}$ ion. It can be seen that the transition curves of Figure 4 are much sharper than those of Figure 3, and that

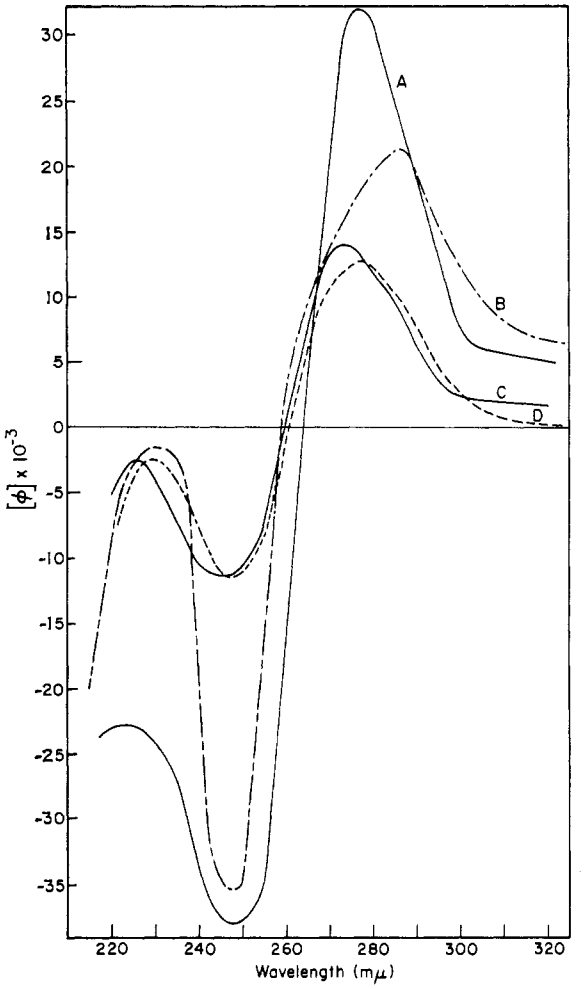


FIGURE 5: Optical rotatory dispersion curves (A) of poly-(G-C) double-stranded helix, from data of Sarkar and Yang (1965a) (pH 7.5, temperature 27–80°). (B) Of poly-(A-U) double-stranded helix, from data of Sarkar and Yang (1965b) (pH 7.5, temperature 27°). (C) Of alanine t-RNA (pH 6.80, 0.10 M phosphate, and 0.15 M KCl). This curve represents the ORD at 8.0° in the absence of $Mg^{2+}$ ion or at 5–45° in the presence of 0.003 M $Mg^{2+}$ ion. (D) Of tyrosine t-RNA (pH 6.80, 0.10 M phosphate and 0.15 M KCl). This curve represents the ORD at 8.0° in the absence of $Mg^{2+}$ ion and at 5–40° in the presence of 0.003 M $Mg^{2+}$ ion.

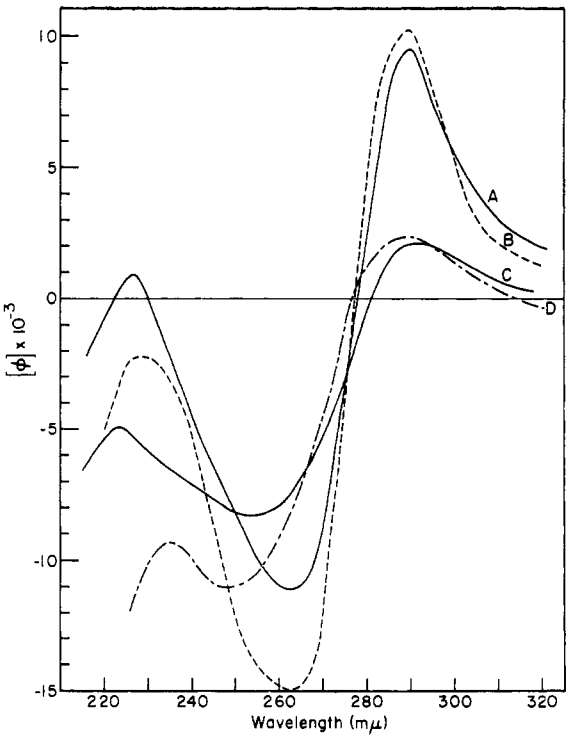


FIGURE 6: Optical rotatory dispersion curves (A and B) calculated from dinucleotide stacking interactions alone (pH 7.0, 25°) for alanine t-RNA and for tyrosine t-RNA, respectively. (C) Observed for alanine t-RNA (pH 6.80, 0.10 M phosphate, 0.15 M KCl buffer) at 90° for solutions with and without 0.003 M $Mg^{2+}$ ion. (D) Observed for tyrosine t-RNA (pH 6.80, 0.10 M phosphate, 0.15 M KCl buffer) at 90° for solutions with and without 0.003 $Mg^{2+}$ ion.

the temperature transition occurs at higher temperatures in the presence of $Mg^{2+}$ ion. Also, the transition seems to occur with very little change in $[\phi]$ below ~40°. It should be noted that the alanine t-RNA curve in Figure 4A has a slightly sharper slope and slightly higher melting temperature than the curve for tyrosine t-RNA in Figure 4B.

## Discussion

In order to account for the data of Figures 1–4, it must first be pointed out that the t-RNA's remain monomeric, *i.e.*, they do not aggregate in solution, under conditions employed here. This statement is strongly supported by osmotic pressure and sedimentation equilibrium experiments carried out by Lindahl *et al.* (1965) on unfractionated t-RNA under the same pH and salt conditions as used here, but in the absence of $Mg^{2+}$ ion. Therefore, it seems reasonable to assign the curves of Figures 1–4 to intramolecular interactions. No molecular weight data are available as yet in the presence of $Mg^{2+}$ ion.

Some insight into the origin of the low-temperature ORD behavior of both alanine and tyrosine t-RNA can be gained by comparison with ORD data obtained from various model systems. Such a comparison is begun with Figure 5. Curve 5A is a reproduction of the ORD data of Sarkar and Yang (1965a) for the poly-(G-C) double-stranded helix. These data were obtained with poly-(G-C) prepared according to the procedure of Haselkorn and Fox (1965), for which there is good evidence that little intramolecular G-G hydrogen bonding is present, but that the double strand consists only of G-C interactions (Pochon and Michelson, 1965). Curve 5B is a reproduction

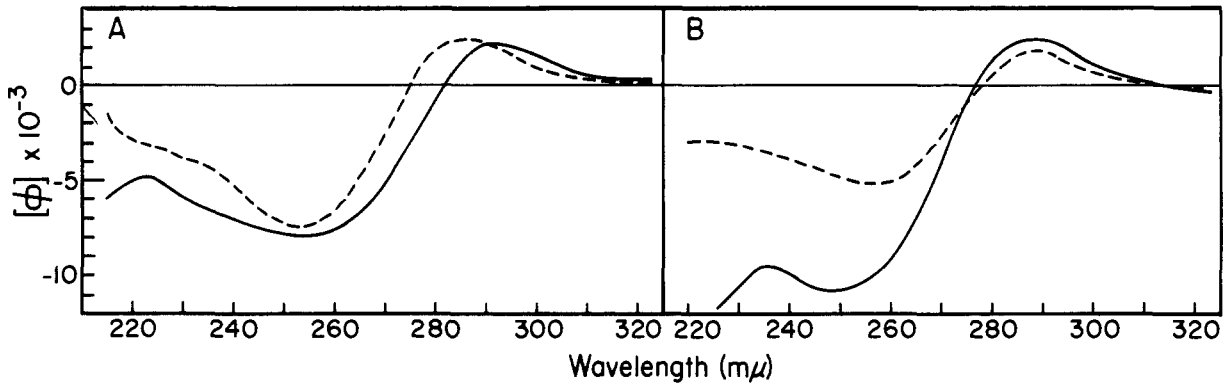


FIGURE 7: The solid lines represent the optical rotatory dispersion at 90° of (A) alanine t-RNA (same as curve 6C) and of (B) tyrosine t-RNA (same as curve 7D). The dotted line in (A) represents the theoretical optical rotatory dispersion curve for a mixture of mononucleotides having the composition of alanine t-RNA (pH 7.0, 25°). The dotted line in (B) represents the theoretical optical rotatory dispersion curve for a mixture of mononucleotides having the composition of tyrosine t-RNA (pH 7.0, 25°).

from Sarkar and Yang (1965b) for the ORD behavior of the poly-(A-U) double-stranded helix. Curve 5C represents the low-temperature ORD behavior of alanine t-RNA (both in the absence and presence of $Mg^{2+}$ ion) and curve 5D is the low-temperature ORD behavior of tyrosine t-RNA (both in the absence and presence of $Mg^{2+}$ ion). All these data pertain to the wavelength range of 320–210 mμ. The poly-(G-C) and poly-(A-U) data were obtained at neutral pH as were those for the t-RNA's. The similarity in curves A, C, and D, especially with respect to the positions of the Cotton effect maxima (276 and 247 mμ for poly-(G-C); 273 and 247 mμ for alanine t-RNA; 276 and 248 mμ for tyrosine t-RNA), and the over-all shapes of the curves should be noted. The poly-(A-U) curve B is quite different from the other three curves, since its Cotton effect maxima occur at 286 and 249 mμ. Although there are not yet any experimental data on proper model systems to indicate that a G-C hydrogen-bonded pair would behave the same way in t-RNA as it does in poly-(G-C), the similarity of the ORD curves of the t-RNA's to that for poly-(G-C) seems to strongly suggest that regions of the t-RNA molecules are quite similar in structure to poly-(G-C). Specifically, it seems that alanine and tyrosine t-RNA have a high degree of G-C pairing (by means of intramolecular hydrogen bonding) at low temperatures. If the t-RNA's would have had a significant amount of intramolecular A-U hydrogen bonding the positive maximum in their ORD curves would be shifted to higher wavelength, *i.e.*, toward the 286-mμ maximum of poly-(A-U). This is not the case, *i.e.*, the positive maximum for both t-RNA's occurs quite near the 276-mμ maximum of poly-(G-C). It is seen, however, that the positive maximum for tyrosine t-RNA is at a somewhat higher wavelength than that for alanine t-RNA. Therefore, *assuming* that an A-U hydrogen-bonded pair in t-RNA would behave optically like one in poly-(A-U), we suggest that both of the t-RNA's have little A-U pairing, and that tyrosine t-RNA has somewhat more A-U hydrogen bonding than alanine t-RNA. This result is consistent with the base composition data of these compounds (Holley *et al.*, 1965; Madison *et al.*, 1966).

It might be thought possible to account for the ORD data for the t-RNA's on the basis of dinucleotide stacking interactions alone, *i.e.*, single-strand stacking with no hydrogen bonding. In order to test this idea, we make use of the data of Warshaw and Tinoco (1965) and Warshaw (1966) for the stacking of the 16 dinucleoside phosphates. These data were obtained at 25° at neutral pH in 0.1 M salt (including 0.08 M $KClO_4$) in the 320- to 210-mμ wavelength range. Using these data, it is possible to calculate a theoretical ORD curve for both alanine and tyrosine t-RNA (since their base sequence is known) by means of the formalism of Cantor and Tinoco (1965). Such a theoretical curve would represent the ORD of the t-RNA's at 25° in the absence of any G-C or A-U hydrogen bonds. The results of this calculation are shown in Figures 6A and B for alanine and tyrosine t-RNA, respectively. In this calculation the "rare" bases were assumed to behave as their structural analogs (*i.e.*, I, MeI, MeG, and DiMeG replaced by G, and ψ and T by U). Since dihydro-U does not absorb in the 260-mμ region, it was assumed to be a blank. It should be noted that these calculated curves show Cotton effects with positive and negative maxima at 290 and 260 mμ, respectively. Curves 6A and B may be compared to our data at 25° and 0.15 M KCl (Figures 1 and 2) since I. Tinoco, Jr., C. R. Cantor, and R. Jaskunas (private communication, 1965) have shown that the stacking interactions in ApA and CpC are independent of salt concentration; we *assume* this to be true for all 16 base pairs. Comparison of curves 6A and B with the low-temperature data in Figures 1 and 2 (especially Figures 1B and 2B) clearly indicates that there are marked differences in the

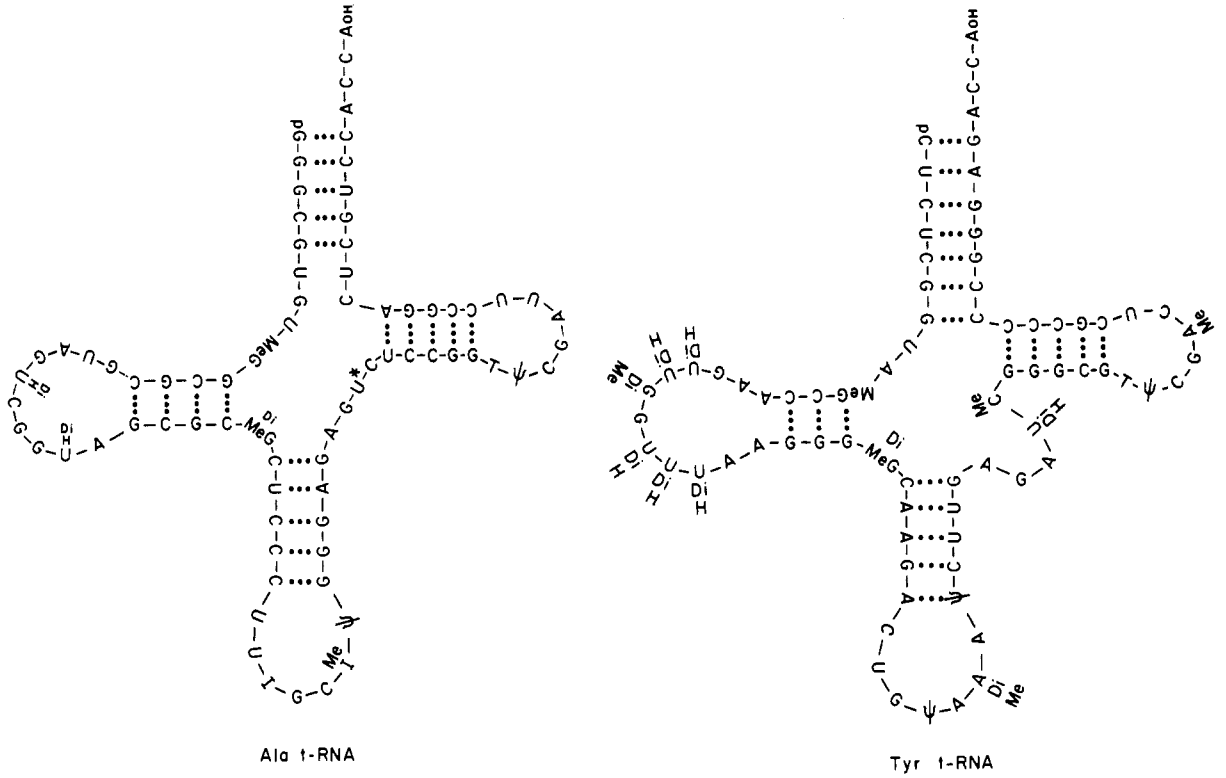


FIGURE 8: Proposed possible conformations for yeast alanine t-RNA and yeast tyrosine t-RNA (Holley *et al.*, 1965; Madison *et al.*, 1966).

shapes and positions of the Cotton effects. The experimental curves (below 10° in the absence of $Mg^{2+}$ ion, and at 5-40° in the presence of $Mg^{2+}$) show maxima at 273–276 and 247–248 mμ compared to 290 and 260 mμ for the calculated curves in Figure 6. Therefore, it seems quite clear that the low-temperature behavior of alanine and tyrosine t-RNA cannot arise solely from stacking interactions, but must have intramolecular hydrogen bonding, as discussed above. Since curves 6A and B resemble curves 6C and D, the high-temperature (90°) curves for alanine and tyrosine t-RNA, respectively, especially with respect to the positions of the maxima, much of the previously proposed intramolecular hydrogen bonding has melted out by 90°, there being only a small degree of stacking left at the high temperature. The large difference in magnitude between the calculated single-strand curves (6A and B) and the experimental curves 6C and D indicates that there is a decrease in stacking between 25 and 90°.

The large decrease in the stacking interactions with increasing temperature is clearly demonstrated by the curves in Figure 7. Figure 7A shows a comparison between the ORD curve of alanine t-RNA at 90° and an ORD curve computed from the ORD data for a mixture of mononucleotides having the composition of alanine t-RNA. Figure 7B shows similar data for tyrosine t-RNA. The data for the mononucleotides (320–210 mμ) were obtained from Warshaw and Tinoco (1965), and Warshaw (1966), and pertain to pH 7.0 at 25° in 0.1 M salt. In this calculation it was again assumed that the "rare" bases behaved as the structural analogs (see above) and that dihydro-U was a blank. Comparison of the two curves in Figure 7A illustrates clearly that essentially all of the stacking interactions of the intact alanine t-RNA are disrupted at 90° (similarly for tyrosine t-RNA in Figure 7B).

Figures 1 and 2 show quite dramatically the shift in appearance of the ORD curves for alanine and tyrosine t-RNA from ones that closely resemble the ORD of poly-(G-C) to ones that are similar to dinucleotide stacking single-strand ORD curves (Figure 6A and B). The difference in magnitude between the calculated (6A and B) and experimental (6C and D) curves of Figure 6 has been shown to be due primarily to disruption of neighbor–neighbor stacking, since these two sets of curves pertain to 25 and 90°, respectively. The data of Figure 7 support this conclusion. In preparing the calculated curves of Figure 6 it was necessary to use 25° (rather than 90°) data for the dinucleotides since the measurements have thus far been carried out only at 25° (Warshaw and Tinoco, 1965; Warshaw, 1966). However, approximately the same maximum wavelength and magnitude of ORD, computed in Figure 6 (6A and 6B), were obtained by Fasman *et al.* (1965) for a solution of t-RNA's at 20° having stacked bases but no hydrogen bonding, the latter having been removed by treatment with formalde-

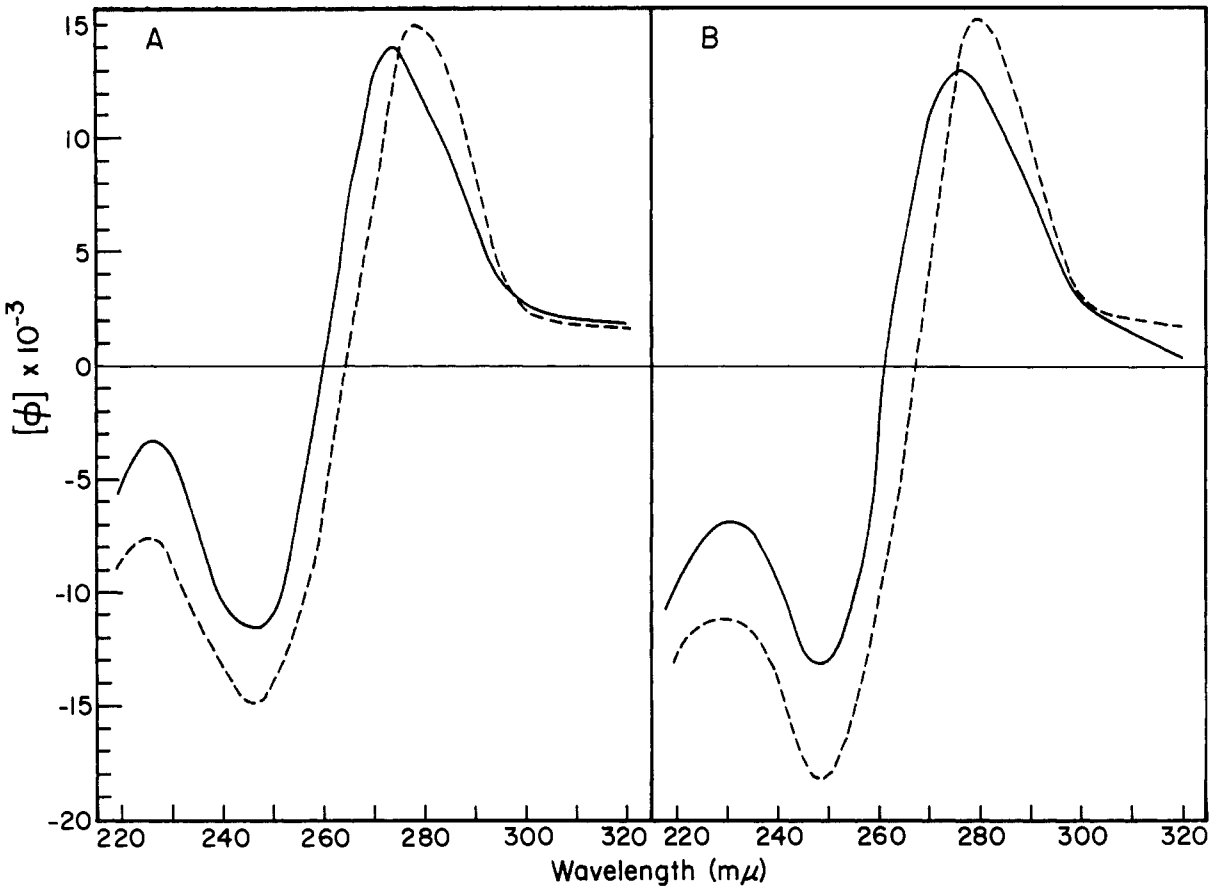


FIGURE 9: (A) The solid line is the same as curve 5C. The dashed line represents the optical rotatory dispersion calculated for alanine t-RNA assuming the conformation of alanine t-RNA shown in Figure 8. The calculated curve includes both dinucleotide stacking and hydrogen bonding (pH 7.0, 25°) as proposed in the assumed conformation. (B) The solid line is the same as Figure 5D. The dashed line represents the optical rotatory dispersion calculated for tyrosine t-RNA assuming the conformation of tyrosine shown in Figure 8. The calculated curve includes both dinucleotide stacking and hydrogen bonding (pH 7.0, 25°) as proposed in the assumed conformation.

hyde. This indicates that t-RNA has stacking interactions at 25°. The validity of using only dinucleotide and not longer range stacking interactions to calculate the ORD of a polynucleotide of known base sequence is supported by the findings that the state of stacking of a given base pair, in the homologous adenylic acid series $(pA)_n$, is independent of the state of stacking of its neighbors (Brahms *et al.* (1966) and Poland *et al.* (1966)). Thus, the sum total of data shown in Figures 1, 2, and 5–7 strongly supports the idea that the alanine and tyrosine t-RNA's have both intramolecular hydrogen bonds (mostly G-C) and neighbor–neighbor base stacking at low temperature; these melt out at high temperature so that, around 90°, no hydrogen bonding and only some small amount of the dinucleotide-type stacking is left. In other words, as the temperature is increased, the partially double-stranded t-RNA is converted to a single-stranded (partially stacked) structure.

The thermal transition curves of Figures 3 and 4 are now seen to represent the transition of alanine and tyrosine t-RNA from a state where many of its bases are hydrogen bonded (mostly G-C pairing) and also its bases are stacked in the usual fashion to one where all of the intramolecular hydrogen bonding and most of the stacking is gone. It is well known that the melting temperature of poly-(G-C) is above 100° (Haselkorn and Fox, 1965; Pochon and Michelson, 1965). Figures 3 and 4 show that the melting temperature of the t-RNA's is much lower than 100° (~50° in the absence of $Mg^{2+}$ ion and ~60° in the presence of $Mg^{2+}$ ion). This behavior is to be expected since the runs of double helix in t-RNA are much shorter than in poly-(G-C). The increase in melting temperature and the sharpening of the transition in t-RNA upon the addition of $Mg^{2+}$ probably arises from the binding of $Mg^{2+}$ ions to the negatively charged phosphate groups of the backbone chain (Kotin and Lyons, 1965), removing electrostatic repulsions which tend to weaken the hydrogen bonds in the absence of $Mg^{2+}$ ion. Presumably, the electrostatic repulsions broaden the transition curve in the absence of $Mg^{2+}$

ions (*cf.* the relative steepness of the curves in Figure 3 to those in Figure 4). It is also possible that $Mg^{2+}$ ion could form intramolecular cross-links, thereby adding further conformational stability. It should be mentioned that the low-temperature region (below 30°) of curves A and B in Figure 3 may be related to a conformational rearrangement preceding the major structural transition (*i.e.*, disruption of hydrogen bonding and stacking). This low-temperature effect is masked in Figure 4 due probably to increase in over-all stability of the low-temperature conformation caused by $Mg^{2+}$ ion binding.

Recently, techniques have become available for the calculation of ORD curves of polyribonucleotides of known conformation (*i.e.*, known primary sequence, base stacking, and hydrogen bonding), from model compound data in the 320- to 210-mμ wavelength region. The calculation (Cantor *et al.*, 1966) involves the addition (at each wavelength) of the contributions of both G-C and A-U hydrogen bonding to the curve calculated for the single-strand base-stacking model. Values for the contributions made by G-C and A-U hydrogen-bonded pairs were estimated from the data of Sarkar and Yang (1965a,b). This estimate suffers from the lack of more complete model compound information since it is not yet possible to determine the contribution of a G-C base pair in other than a G-C environment, and likewise for an A-U base pair; this point is discussed in detail by Cantor *et al.* (1966). With an awareness of these limitations, we found it interesting to calculate ORD curves for the conformations of alanine and tyrosine t-RNA shown in Figure 8, these conformations having been previously proposed by Holley *et al.* (1965) and Madison *et al.* (1966). Figure 9 shows a comparison of these calculated curves with our experimental data. The agreement between experiment and calculation seems fortuitous at this time, recognizing the crudeness of the calculation. Cantor *et al.* (1966) performed a calculation on a slightly different model of alanine t-RNA (one having the maximum possible hydrogen bonding) and they observed equally good agreement with experiment. The deviations between theory and experiment in Figure 9 are consistent (*i.e.*, both calculated curves are shifted to higher wavelength and have greater magnitude than the corresponding experimental curves); these deviations may possibly be due to our inability to include tertiary structural information in the calculation. It is more likely that the observed disparity arises from the lack of better model compound data and better calculation techniques.

In summing up, the authors wish to emphasize the following points. (1) It has been shown that there is intramolecular hydrogen bonding (primarily G-C type) and neighbor–neighbor base stacking in yeast alanine and yeast tyrosine t-RNA, at low temperatures, in the presence and absence of $Mg^{2+}$ ion at neutral pH. (2) Both the hydrogen bonding and base stacking in the t-RNA's are disrupted at high temperature. (3) $Mg^{2+}$ ion tends to stabilize the low-temperature conformation of the t-RNA's so that the thermal transition occurs at higher temperatures and with steeper slopes in the presence of $Mg^{2+}$ ion. (4) There appears to be less A-U hydrogen bonding than G-C hydrogen bonding in both alanine and tyrosine t-RNA. The tendency of the ORD of tyrosine t-RNA to have a positive Cotton effect maximum closer to the positive maximum of poly-(A-U) than alanine t-RNA, and the slightly lower melting temperature for tyrosine t-RNA than for alanine would indicate that tyrosine has somewhat more A-U base pairing than alanine t-RNA. This is consistent with the proposed conformations of these molecules. (5) Support can be given for previously proposed conformations of the two t-RNA's on the basis of comparisons of experimental and empirically calculated ORD curves in the 320- to 210-mμ wavelength region.

It is of interest to mention that the stacking interactions between the bases in t-RNA and other oligoribonucleotides are not primarily hydrophobic bonds, as indicated in much of the literature on this subject (Fasman *et al.*, 1964, 1965; Sarkar and Yang, 1965a,b; Kotin and Lyons, 1965). The temperature dependence of the base stacking indicates that electrostatic interactions must dominate over any hydrophobic interactions which may be present, *i.e.*, the enthalpy of formation of the stacking interaction is negative (Brahms *et al.*, 1966; Poland *et al.*, 1966). It should also be mentioned that the thermal transition curves in this paper show no biphasic character. Other workers (Fresco *et al.*, 1963) have interpreted the biphasic character of ultraviolet absorption *vs.* temperature curves in terms of A-U to G-C ratios in the hydrogen-bonded regions of the t-RNA molecules. It is possible that, due to the small relative changes in the ultraviolet absorbtion measurements as a function of temperature (compared to large relative changes in $[\phi]$ over the temperature range), experimental artifacts yielded what appeared to be biphasic curves.

The authors would suggest that the ORD curve of a polyribonucleotide in the 320–210-mμ wavelength region may be used as a tool for detecting G-C hydrogen bonding and A-U hydrogen bonding. Crude estimates could be made of the amount of G-C hydrogen bonding by comparing the peak height at 276 mμ with the 276-mμ maximum of the poly-(G-C) ORD curve (provided that the comparison was made on a mean residue rotation basis). Such an estimate would predict that roughly 40–50% of the bases in alanine and tyrosine t-RNA are involved in G-C hydrogen bonding. This agrees with the proposed conformations. A closer look at the proposed conformations shows that they are quite homologous, *i.e.*, they both have the so-called "clover leaf" shape. Madison *et al.* (1966) discuss the implications of these homologies in detail. Should other t-RNA's have similar clover leaf secondary structures, the authors would expect to observe ORD and temperature transition data similar to that presented in this paper. Some recent ORD data (Sarin *et al.*, 1966) seem to bear this out. Finally, the authors point out that the interpretation of ORD data in the 210–185-mμ wavelength region (Lamborg and Zamecnik, 1965; Sarin *et*

*al.*, 1966) involves the difficulty that the ORD behavior of few, if any, model compounds have been explored in this wavelength region. This region is not only difficult for carrying out experiments but has long been known to be an especially difficult one from a theoretical point of view (Devoe and Tinoco, 1962; Warshaw *et al.*, 1965). The authors feel more confident in attempting to interpret data in the 320–210-m$\mu$ region because of the abundance of both experimental and theoretical studies on model compounds in this region.

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