Multimer Forms of Eukaryotic 5.8S Ribosomal RNA†

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ABSTRACT: Ribosomal 5.8S RNA from rat or chicken liver or yeast cells can be converted to dimer or multimer forms by heating at appropriate temperatures in 0.15 M salt (0.15 M NaCl, 0.001 M EDTA, 0.001 M Tris-HCl, pH 7.5) buffer, a conversion that can be prevented by heating at still higher temperatures. This interaction is dependent on both the incubation temperature and RNA concentrations; at concentrations above 0.8 mg/mL and a temperature of 67.5 °C, over 80% of rat liver 5.8 S rRNA is converted to two dimer forms and at 60 °C small amounts of trimer and tetramer. Higher RNA concentrations and a lower optimum temperature of incubation (60 °C) are required to form dimers from chicken 5.8S rRNA efficiently and only small amounts of yeast 5.8S RNA dimer were observed. Similarly, rat 5.8S RNA dimers are stable to temperatures 5 °C higher than dimers from chicken; the dissociation temperatures were 55 and 50 °C, respectively. Since the nucleotide sequence of rat 5.8S rRNA differs from chicken 5.8S rRNA in only three positions, nucleotides in the 3′- or 5′-terminal sequences, and since these regions are thought to be base paired but melted at temperatures above 60 °C, the results suggest that 5.8S rRNA dimerizes through self-complementarity in its terminal sequences. These studies also show that under commonly used extraction conditions dimers may form which, on electrophoresis, migrate as an 8S RNA component.

The large subunit of eukaryotic ribosomes contains two low molecular weight RNAs, 5S and 5.8S rRNA; 5.8S rRNA is hydrogen bonded to the high molecular weight RNA component (Pene et al., 1968; Weinberg & Penman, 1968), the 28S rRNA molecule in mammals. The primary nucleotide sequence of 5.8 S rRNAs from a number of different species have been determined (Rubin, 1973; Nazar et al., 1975; Khan & Maden, 1976b; Nazar & Roy, 1978); all are very similar in size (157–160 nucleotides), contain modified nucleotides, and show a high degree of sequence homology. For example, in the course of evolution, 75% of the sequence has been retained between yeast and man (Nazar et al., 1975). Based on maximized base pairing, a common model for the secondary structure of free 5.8S rRNA ("burp gun model") was proposed (Nazar et al., 1975) which has been generally consistent with physicochemical studies utilizing limited nuclease digestion (Khan & Maden, 1976b) or thermal denaturation and ethidium bromide probing techniques (Van et al., 1977).

The 5.8S rRNA and the high molecular weight ribosomal RNAs are cotranscribed in the nucleolus as part of a much larger precursor molecule, the 45S rRNA in mammals (see reviews by Grierson et al., 1970; Choi et al., 1975). Recently, kinetic and direct chemical evidence (Helser & McLaughlin, 1975; Trapman et al., 1975; Khan & Maden, 1976a) indicates that 5.8S rRNA is immediately derived from a slightly larger transient precursor: an approximately 7S RNA molecule in yeast as determined by gel electrophoresis (Helser & McLaughlin, 1975) and 10S RNA in man as determined by sucrose gradient centrifugation (Khan & Maden, 1976a). In the present study we have observed other intermediate sized RNA components by the same fractionation techniques which contain the 5.8S RNA sequence. Further analysis indicated that these were not precursors but multimer forms of 5.8S rRNA arising from intermolecular base pairing. As has previously been found for tRNA (Yang et al., 1972; Rordorf & Kearns, 1976), the interconversion of multimers presents a useful model system to study RNA–RNA interactions and the secondary structure of free 5.8S rRNA in solution.

Materials and Methods

Unlabeled 5.8S ribosomal RNA was prepared from whole cell RNA. The cellular RNA was extracted directly from rat or chicken liver or yeast cells with a phenol–$NaDodSO_4$ buffer at 65 °C and the low molecular weight (4–10S) fraction was prepared by sucrose density centrifugation (Ro-Choi et al., 1970). The 5.8-S rRNA was purified from this fraction by either one-dimensional polyacrylamide gel slabs (Nazar et al., 1975) or in some cases by two-dimensional polyacrylamide gel electrophoresis (Reddy et al., 1974). ^{32}P-labeled low molecular weight RNA was prepared from Novikoff hepatoma ascites cells (rat origin) as previously described (Reddy et al., 1974).

^{32}P-labeled RNAs were characterized by two-dimensional fingerprinting techniques (Sanger et al., 1965). The purified RNA was digested with T_1 ribonuclease and the resulting oligonucleotides were fractionated by electrophoresis on cellulose-acetate at pH 3.5 and then on DEAE paper in 7% formic acid.

To study the formation of 5.8S RNA multimers, appropriate amounts of 5.8S rRNA were dissolved in 0.15 M salt buffer (0.15 M NaCl, 0.001 M EDTA, 0.001 M Tris-HCl, pH 7.5), heated in sealed capillaries at various temperatures for 5 min, and cooled rapidly in a 22.5 °C water bath. Each sample was analyzed by electrophoresis on 10% polyacrylamide disc gels (6 × 80 mm) and the RNA fractions were quantitated by staining the gels with methylene blue and scanning them using a Beckman ACTA-III spectrophotometer at 600 nm.

The thermal stability of 5.8S rRNA dimers was determined by heating aliquots over a 50 °C temperature range. Dimers were prepared by heating appropriate concentrations of 5.8S rRNA (0.7 or 2.0 mg/mL for rat or chicken 5.8S RNA, respectively) at optimum temperatures (67.5 and 60 °C, respectively) in 0.15 M salt buffer for 5 min, followed by cooling to 22.5 °C and diluting tenfold with water. Aliquots, sealed in glass capillaries, were heated at 5 °C increments for 5 min,

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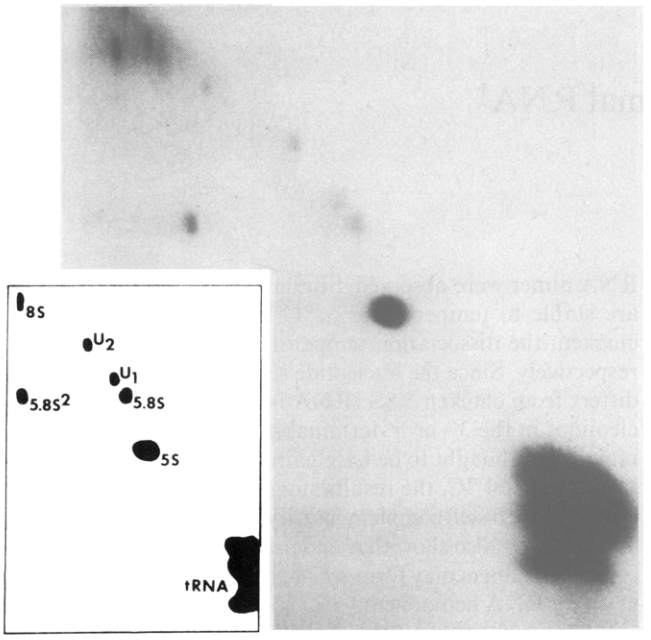

FIGURE 1: A two-dimensional polyacrylamide gel fractionation of the 4–10S RNA fraction from rat liver cells. Electrophoresis was from right to left on a 10% gel run in buffer containing 0.04 M Tris-acetate, 0.002 M EDTA, and 0.02 M sodium acetate, pH 7.2, and from top to bottom on a 12% gel slab run in 0.025 M citric acid and 6 M urea, pH 3.3.

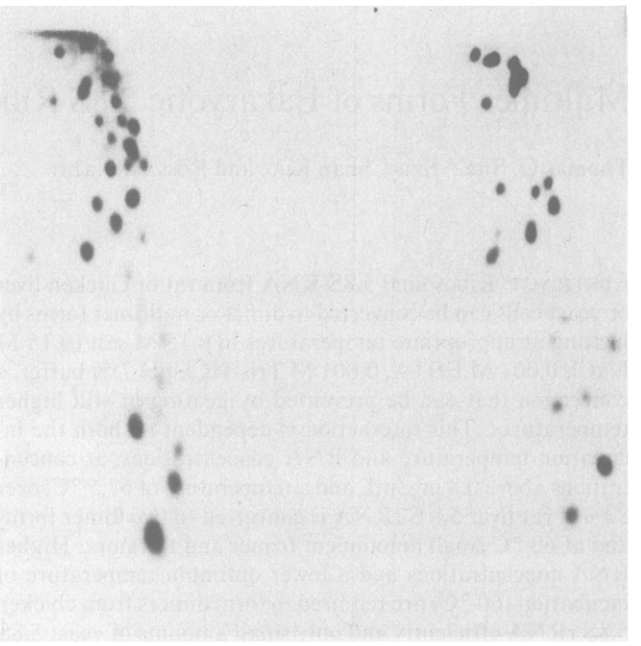

FIGURE 2: Autoradiographs of two-dimensional fractionations of T_1 RNase digests of ^{32}P-labeled Novikoff hepatoma 8S RNA (left) and 5.8S rRNA (right). Electrophoresis was from right to left on cellulose-acetate at pH 3.5 and from top to bottom on DEAE paper in 7% formic acid.

quickly cooled in an ice bath, and analyzed by polyacrylamide gel electrophoresis as described above.

Results

When rat whole cell RNA was extracted at 65 °C and the 4–10S fraction was separated by two-dimensional polyacrylamide gel electrophoresis, an 8S RNA component was observed to undergo an unusual mobility change in the second dimension; about 25% of the RNA remained as an 8S component, while the mobility of the other 75% increased significantly and ran as 5.8S rRNA (Figure 1). When both components, labeled in vivo with [^{32}P]orthophosphate, were further examined by fingerprint analysis using T_1 RNase digestion (Sanger et al., 1965), the faster migrating 5.8S^2 component (Figure 1) was found to be 5.8S rRNA, while the slower 8S component (Figure 1) was found to be an entirely unrelated molecule with no resemblance to 5.8S RNA (Figure 2). Since the second dimension of the gel system contained 6 M urea to disrupt base pairing, these results were interpreted to indicate that the original 8S RNA material in the first dimension was a mixture of a unique 8S RNA molecule and a 5.8S rRNA dimer. Both the electrophoretic mobilities and the fingerprint analysis of the original 8S band were consistent with this interpretation. Whole cell 4–10S RNA that was heated and quick cooled before being layered on the gel did not contain the 5.8S RNA dimer.

Studies on tRNA dimers have previously yielded valuable information about their secondary structures (Loehr & Keller, 1968; Yang et al., 1972; Rordorf & Kearns, 1976). In search of similar information about 5.8S rRNA, we have examined the conditions under which 5.8S RNA multimers form and compared this phenomenon in RNAs of different origin. Figure 3 shows the different gel scans which were obtained with rat liver 5.8S RNA under varying RNA concentrations. At very low concentrations or in the absence of heating, 5.8S rRNA was present only as a monomer. When moderate concentrations of RNA (0.13 mg/mL) were incubated in 0.15 M salt buffer for 5 min at 65 °C, about 40% of the 5.8S RNA was present in dimer form (Figure 3). Most of the dimer was present as a single major peak; however, a slightly faster migrating form was also present as an adjacent minor peak or shoulder. At a still higher RNA concentration (0.63 mg/mL) more dimer was formed which now accounted for about 72% (Table I) of the 5.8S rRNA on the gel. Furthermore, at 60 °C small amounts of even higher multimer forms were observed (Figure 3 and Table II). When the mobilities of these bands were plotted against the log of the molecular weight, a linear relationship was observed. This indicated that the slowest migrating multimers were most likely trimers and tetramers. This formation of dimers at relatively low RNA concentrations and the existence of trimers and tetramers at moderate concentrations emphasizes the unusual efficiency in this RNA–RNA interaction.

When chicken 5.8S rRNA was studied using the same incubation conditions (0.15 M salt buffer, 65 °C for 5 min), similar results were obtained with two quantitative exceptions. The minor dimer component was more prevalent representing as much as 30% of the dimer fraction and at RNA concentrations of 0.1 mg and 1.0 mg/mL, somewhat less dimerization occurred, 17 and 38%, respectively (Table I). Under these

TABLE I: The Effect of RNA Concentration on Dimer Formation.[a]

source of RNA	concn (mg/mL)	% dimer
rat 5.8 S	0.63	72
	0.13	40
chicken 5.8S	1	38
	0.1	17
yeast 5.8S	11	15
	2.2	1

[a] The RNA was heated to 65 °C for 5 min and then placed in a 22.5 °C water bath. The % dimer represents the sum of the major and minor dimer forms.

TABLE II: The Effect of Temperature on Multimer Formation of 5.8S rRNA.[a]

source of RNA	temp (°C)	% multimers: dimer (major, minor)	trimer	tetramer
rat 5.8S (1.4 mg/mL)	50	16, 7	4	0
	60	49, 0	5	3
	70	53, 0	0	0
chicken 5.8S (3 mg/mL)	50	9, 19	7	5
	60	40, 13	3	2
	70	24, 4	3	1

[a] The RNA was heated at the indicated temperature for 5 min and then placed in a 22.5 °C water bath. The minor dimer is the dimer form with the fastest migration rate on the gels.

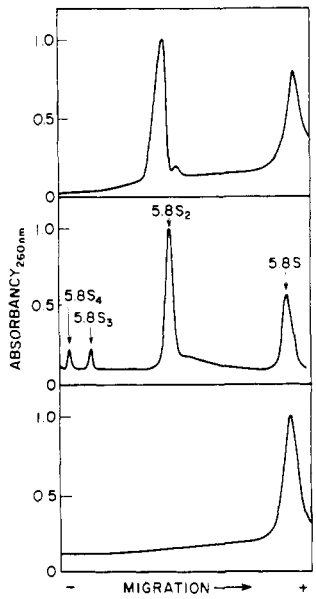

FIGURE 3: Effect of RNA concentration on the dimerization of rat liver 5.8S rRNA. Appropriate amounts of RNA were dissolved in 0.15 M salt buffer and incubated for 5 min, and the products were fractionated by electrophoresis on 10% polyacrylamide gels. Gel scans for RNA concentrations of 0.13 mg/mL (65 °C) and 1.4 mg/mL (60 °C) and an unheated control (lower) are shown.

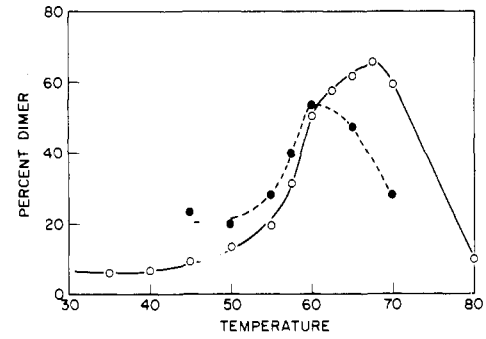

FIGURE 4: Effect of temperature on the dimerization of rat (open circles) and chicken (closed circles) liver 5.8S rRNA. Appropriate amounts of RNA were dissolved in 0.15 M salt buffer; aliquots were incubated for 5 min at the indicated temperatures and analyzed by polyacrylamide gel electrophoresis as shown in Figure 3. Each point which is the average of two experiments includes the minor dimer.

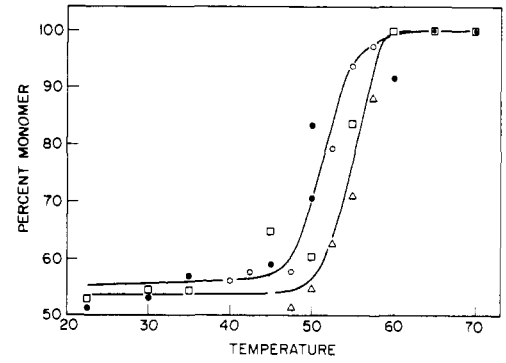

FIGURE 5: Effect of temperature on rat and chicken 5.8S rRNA dimers. Dimers were prepared in 0.15 M salt buffer and aliquots were diluted tenfold with water and incubated for 5 min at the indicated temperatures and analyzed by polyacrylamide gel electrophoresis as shown in Figure 3. Data for two different dimer preparations are presented for each RNA: chicken 5.8S rRNA (open and closed circles); 5.8S rRNA (squares and triangles).

conditions the dimerization reaction was less favored for chicken 5.8S RNA.

The amount of dimer which formed was also dependent on the incubation temperature. The data in Figure 4 and Table II shows the effect of temperature on the amount of rat and chicken dimers which form. For rat liver 5.8S rRNA, the optimum incubation temperature was about 67.5 °C. As expected, at lower temperatures, less or no dimer was formed. Surprisingly, at higher temperatures there was also less dimerization; the intermolecular reaction probably competes with an intramolecular reaction which is favored at high temperatures. The formation of multimers under a variety of conditions is summarized in Table II.

The data in Figure 4 also shows that the optimum temperature for dimer formation varies with the 5.8S RNA sequence. The optimum temperature for chicken liver 5.8S RNA dimers was 60 °C, 7.5 °C lower than rat liver RNA. Sequence analyses (Nazar et al., 1975; Kahn & Maden, 1976b; Sitz et al., 1977) indicate that the chicken 5.8S rRNA sequence differs in only three positions from the rat RNA sequence; there is a single purine substitution in the 5′ end of the molecule and both a pyrimidine substitution and a single nucleotide deletion in the 3′ region of the sequence. The lower optimum temperature for chicken dimer formation is presumably due to these slight differences. Dimers can also be formed with yeast 5.8S rRNA (Table I); however, the yields were significantly lower.

The dimers were further characterized by an examination of their thermal stability. Rat liver 5.8S RNA dimers were formed at 67.5 °C, diluted tenfold with autoclaved water, and heated at various temperatures. As shown in Figure 5, 50% of the dimers had dissociated at 55 °C. Chicken 5.8S RNA dimers, formed at 60 °C and heated under identical conditions, were somewhat less stable; 50% of the dimers were dissociated at about 50 °C, 5 °C lower than the rat RNA complex. The difference in the dissociation temperature was presumably

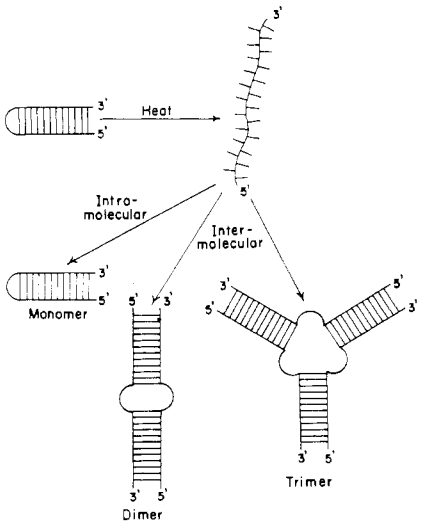

FIGURE 6: Proposed model for the formation of 5.8S rRNA multimers.

again due to the slight sequence differences at the 5′ and 3′ termini of the RNA molecules.

Discussion

This study shows that, under conditions commonly used to extract RNA, dimers of 5.8S rRNA can form, which on polyacrylamide gel electrophoresis migrate with an unrelated 8S RNA molecule. In studies on the synthesis of 5.8S rRNA it is important that this mixture not be confused with transient precursors to 5.8S RNA which have been identified as an 8–10S molecule.

While RNA dimers have previously been observed with tRNA (Loehr & Keller, 1968), dimers of rat 5.8S RNA could be observed at much lower RNA concentrations (0.1 vs. 15–40 mg/mL). Apparently, 5.8S RNA molecules have an even greater ability to pack into stable multimer structures. The influence of temperature and RNA concentration on the formation of these stable multimers indicates two essential features of the reaction. Since the RNA must be heated some melting or rearrangement of the secondary structure must occur for dimer formation provided the RNA concentration is high enough. The multimer profiles on gels are probably the result of two competing reactions (Figure 6): an intramolecular refolding of the 5.8S RNA to form monomers and an intermolecular base-pairing reaction between part of, or the same complimentary regions of two or more 5.8S RNA molecules to form multimers. The probability of intramolecular base pairing is determined by the secondary structure of 5.8S rRNA and is not concentration dependent. The probability of intermolecular base pairing, on the other hand, is concentration dependent and, therefore, this reaction is favored at higher RNA concentrations.

The fact that dimer formation does not occur at high incubation temperatures was surprising but is still compatible with this model. At higher than optimum incubation temperatures the competing or yet another intramolecular base-pairing reaction appears to be favored, i.e., the reaction has an increasingly high conformational free energy with increasing temperature.

The comparison of 5.8S rRNA dimers of divergent origins suggests that 5.8S RNAs interact through their 5′- and 3′-terminal sequences. As observed earlier, sequence analyses indicate that chicken 5.8S rRNA differs in only three positions from rat 5.8S rRNA, and yet the optimum incubation temperature for rat 5.8S RNA dimers was 7.5 °C higher and the thermal stability was about 5 °C greater. Also, higher concentrations of chicken 5.8S rRNA (2–3 mg/mL) were required to obtain significant amounts of dimer. In the generalized model ("burp gun" model) for 5.8S rRNA (Nazar et al., 1975) the 5′- and 3′-terminal sequences are thought to be base paired. Since the sequence changes are restricted to the 5′- and 3′-terminal regions, it is attractive to postulate that part, or all, of these complimentary sequences are involved in multimer formation (Figure 7). Earlier studies on the secondary structure of 5.8S RNA using thermal denaturation techniques (Van et al., 1977) indicated that many of the base-paired regions of the molecule would not be denatured under temperature and salt conditions at which dimers form. Base pairing between the two termini, however, was thought to be among the regions which would denature under these conditions. Furthermore, yeast 5.8S rRNA which forms less stable dimers at lower temperatures apparently also contains more weakly paired termini (Van et al., 1977). Therefore, both the nucleotide sequences and the thermal denaturation studies support the interactions suggested in Figure 6.

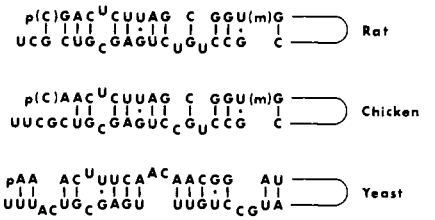

FIGURE 7: A comparison of possible intramolecular base pairing between the termini of rat (upper), chicken (middle), and yeast (lower) 5.8S rRNA. The rat 5.8S sequence is from Nazar et al. (1975), the chicken sequence is from Kahn & Maden (1976b) and Sitz et al. (1977), and the yeast sequence is from Rubin (1973).

Although the multimers of 5.8S rRNA are not physiologically important and their structure requires further study, they remain as an interesting model for RNA–RNA interaction and for the elucidation of 5.8S rRNA structure in solution. The present study provides further evidence that the 3′ and 5′ termini of free 5.8S RNA interact in solution.

References

Choi, Y. C., Nazar, R. N., & Busch, H. (1975) in *the Cell Nucleus* (Busch, H., Ed.) Vol. III, p 109, Academic Press, New York, N.Y.

Grierson, D., Rogers, M. E., Sartirana, M. L., & Loening, V. E. (1970) *Cold Spring Harbor Symp. Quant. Biol. 35,* 589–597.

Helser, T. L., & McLaughlin, C. S. (1975) *J. Biol. Chem. 250,* 2003–2007.

Khan, M. S. N., & Maden, B. E. H. (1976a) *FEBS Lett. 61,* 10–13.

Khan, M. S. N., & Maden, B. E. H. (1976b) *FEBS Lett. 72,* 105–110.

Khan, M. S. N., & Maden, B. E. H. (1977) *Nucleic Acid Res. 4,* 2495–2505.

Loehr, J. S., & Keller, E. B. (1968) *Proc. Natl. Acad. Sci. U.S.A. 61,* 1115–1122.

Nazar, R. N., & Roy, K. L. (1978) *J. Biol. Chem. 253,* 395–399.

Nazar, R. N., Sitz, T. O., & Busch, H. (1975) *J. Biol. Chem. 250,* 8591–8597.

Pene, J. J., Knight, J. E., & Darnell, J. (1968) *J. Mol. Biol. 33,* 609–623.

Reddy, R., Sitz, T. O., Ro-Choi, T. S., & Busch, H. (1974) *Biochem. Biophys. Res. Commun. 56,* 1017–1022.
Ro-Choi, T. S., Moriyama, Y., Choi, Y. C., & Busch, H. (1970) *J. Biol. Chem. 245,* 1970–1977.
Rordorf, B. F., & Kearns, D. R. (1976) *Biochemistry 15,* 3320–3330.
Rubin, G. M. (1973) *J. Biol. Chem. 248,* 3860–3875.
Sanger, F., Brownlee, G. G., & Barrell, B. G. (1965) *J. Mol. Biol. 91,* 235–256.
Sitz, T. O., Kuo, S. C., & Nazar, R. N. (1977) Abstracts of the 174th National Meeting of the American Chemical Society, BIOL 164.
Trapman, J., Delange, P., & Planta, R. J. (1975) *FEBS Lett. 57,* 26–30.
Van, N. T., Nazar, R. N., & Sitz, T. O. (1977) *Biochemistry 16,* 3754–3759.
Weinberg, R. A., & Penman, S. (1968) *J. Mol. Biol. 38,* 289–304.
Yang, S. K., Söll, D. G., & Crothers, D. M. (1972) *Biochemistry 12,* 2311–2320.

Stereodynamics of Dimer Segments of RNA in Aqueous Solution†

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ABSTRACT: Arguments are presented which show that conformations II and III proposed by Lee and Tinoco [Lee, C. H., and Tinoco, I., Jr. (1977), *Biochemistry 16,* 5403] for ribodinucleoside monophosphates in aqueous solution are untenable. It has been shown that ribodinucleoside monophosphates exist in aqueous solution as an equilibrium blend of the classically recognized right-handed stack (g^-g^-), loop stack (g^+g^+), skewed (g^+t), and extended arrays. In order to determine the effect of ϵA base on the conformer distribution in the equilibrium blend, detailed ring-current calculations were performed and the isoshielding curves for ϵA were derived. Use of these curves vis-a-vis dimerization shift data indicates that introduction of ϵA perturbs the equilibrium blend which causes an increase in the population of skewed (g^+t) arrays.

Several theoretical calculations (Perahia et al., 1974; Kim et al., 1973; Govil, 1976) and X-ray diffraction studies (Hingerty et al., 1975; Seeman et al., 1976; Rubin et al., 1972) have shown that ribodinucleoside monophosphates (see Figure 1 for nomenclature) principally display two stacked conformations. In the right-handed stacked conformation, χ_1 and χ_2 are anti, the ribose rings are ^{3}E, ψ_1 and $\psi_2 \simeq 60°$, $\phi_1' \simeq 210°$, $\phi \simeq 180°$, $\omega' \simeq 290°$, and $\omega \simeq 290°$. In the left-handed loop structure the conformational details are same as above, except $\omega' \simeq 80°$ and $\omega \simeq 80°$. NMR studies from this laboratory and that of Danyluk (Lee et al., 1976; Ezra et al., 1977, Cheng and Sarma, 1977a,b; Dhingra and Sarma, 1978a,b) agree with these theoretical projections and solid-state findings. However, recently, Lee and Tinoco (1977) have suggested the existence of three different stacked conformations in aqueous solutions for dinucleoside monophosphates which differ in the magnitude of ω', ω, ϕ', and one of the sugar geometries. Their conformation I is same as the traditional right-handed stack. The conformation II differs from the loop structure in the magnitude of ω' and ω, which are reported to be 30° and 100°, respectively. Conformation III is a new stacked one in which χ_1 is anti, $\chi_2 = 100°$, the sugar of the Np- residue is ^{2}E and that of the -pN residue is ^{3}E, $\phi_1' = 260°$, ψ_1 and $\psi_2 = 60°$, $\phi = 180°$, $\omega' = 50°$, and $\omega = 220°$.

Untenability of Conformation III. In addition to the unusual torsion angles in conformation III, one notices that ^{3}E sugar pucker is coupled to a high value of χ_2 (100°). In view of this, we have been compelled to examine the feasibility of this conformation vis-a-vis the NMR data. From the torsion angles supplied by Lee and Tinoco (1977) and from the known bond lengths and bond angles of nucleic acid structures from X-ray data (Hingerty et al., 1975; Seeman et al., 1976; Rubin et al., 1972), we have derived the atomic coordinates for conformation III. These coordinates were used to construct the perspective of conformation III (Figure 2) using ORTEP II. In developing our arguments, we have used ApA as an example, and it should be emphasized that the nature of the base does not affect the arguments presented. A comparison of Figure 2 with that of conformation III in Figure 7 of Lee and Tinoco (1977) shows important differences. In order to check the validity of their NMR arguments, as well as to check the extent of base parellelism and overlap, we have calculated the cylindrical coordinates z, ρ_6, and ρ_5 for ApA in conformation III. In Table I are given the cylindrical coordinates z, ρ_5, and ρ_6 for the Np residue from the center of the five- and six-membered rings of the -pN residue. In Table II are given the same coordinates for the -pN residue with respect to the Np- residue. Inspection of the magnitude of z for the base carbons, H-2 and H-8 clearly indicates no base parellelism and substantial tilt between the bases. The magnitude of ρ_5 and ρ_6 for base carbons and base protons further reveals no overlap between the bases.

The argument advocated by Lee and Tinoco (1977) for the presence of conformation III is the observation of upfield dimerization shifts for H-5′, H-5″, and H-4′ of the Np- residue by the -pN residue. Inspection of Table I clearly shows that the magnitudes of cylindrical coordinates for H-4′, H-5′ and H-5″

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