THE ORDERED STRUCTURE OF 5S RNA

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Single stranded RNA molecules are believed to be composed of short DNA-like double stranded helical segments separated by single stranded regions consisting of both stacked and unstacked sequences. Although the three dimensional structure is not known for any RNA molecule yet, the ellucidation of the primary sequence of several transfer RNA's (Holley et al., 1965; Zachau et al., 1966; Madison et al., 1966; and Raj Bhandary et al., 1967) and of 5S E. coli ribosomal RNA (Brownlee et al., 1967) has evoked a number of suggestions for possible conformations of these molecules. While direct proof, for or against the proposed structures, is still awaited one can judge the potential validity of some models by their concurrence with predictions from measurements of their optical and hydrodynamic properties. Such studies have already been reported for yeast transfer RNA, and were instrumental in showing that the average spatial deployment of these molecules is fairly symmetrical, (Fresco et al., 1966) that t-RNA probably has a tertiary as well as a secondary structure, (Adams et al., 1966) and that the double helical content is 60 ± 5%. (Boedtker, 1967)

We wish to report some optical absorbance and sedimentation studies of 5S RNA which offer some insight into its general three dimensional structure.

5S RNA was either isolated from E.coli B cells according to the procedure of Comb and Zehavi-Willner, (Comb and Zehavi-Willner, 1967) or it was purchased from Menotomy Lab., Boston, Mass. where the identical isolation procedure was followed. Its homogeneity and molecular integrity was checked by measuring its sedimentation constant in 1M HCHO after prior reaction in 1M HCHO for 15 minutes at 63°. HCHO reacted 5S RNA sediments as a single component with an $s_{20,w}$ of 3.7S.

The double helical content of 5S RNA was estimated by measuring the spectrum at 15 and 85° both before and after reaction with HCHO, following the procedure previously described, (Boedtker, 1967) From the results, shown in Fig. 1, the hypochromicity, H, de fined as 1 -(absorbance at 15°/absorbance at 85°)$_{255\ m\mu}$, is calculated as 0.21 for unreacted 5S RNA and 0.075 for its HCHO derivative. By making the simplest assumption, namely that the total hypochromicity, H_T, is a linear combination of the hypochromicity of the fraction of bases in double stranded regions, $H_{d.s.}$ and of the fraction in single stranded regions, $H_{s.s.}$, the double helical content can be calculated from the experimental data, using a value of 0.30 for $H_{d.s.}$, obtained from studies of complexes between homopolynucleotides. Thus, if $H_T = (1-x)H_{s.s.} + xH_{d.s.}$, the fraction of bases, x, in double stranded helices in 5S RNA is equal to 0.63.

To ascertain that the observed hypochromicity of HCHO reacted 5S RNA is due to single stranded base stacking and not to

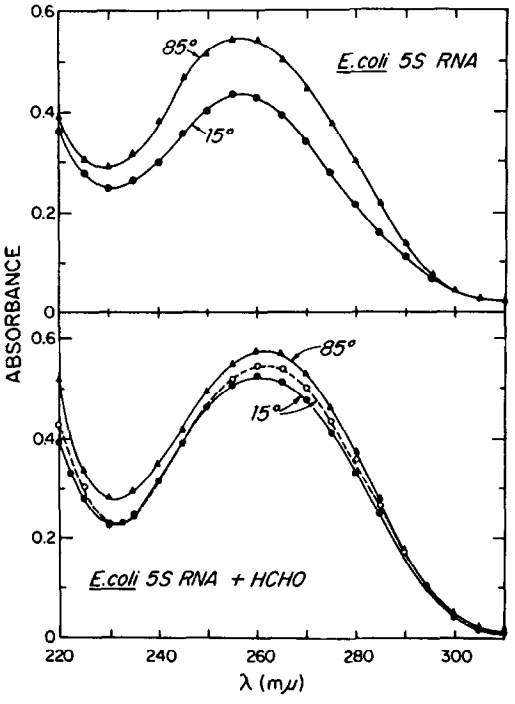

Figure 1: Absorption spectra of 5S E.coli RNA before and after reaction with 1M HCHO for 15 min. at 85° in 0.09M Na_2HPO_4 + 0.01M NaH_2PO_4. (●) Original spectrum at 15°; (▲) spectrum at 85°; and (0) spectrum at 15° after heating to 85°.

residual hydrogen bonding, the relative absorbance-temperature profile was measured. The results, shown in Fig. 2A, clearly indicate that HCHO reacted RNA has the non-cooperative melting profile typical of single stranded stacked structures such as poly A at neutral pH. (Stevens and Rosenfeld, 1966)

The contribution of single stranded stacked nucleotides, to the hypochromicity of 5S RNA can be estimated as 0.028 $((1-x)H_{s.s.})$ from the formaldehyde results. One may ask if this is representative of the actual stacking in unreacted RNA. Two methods were used. One was to compare the hypochromicity of unreacted RNA in

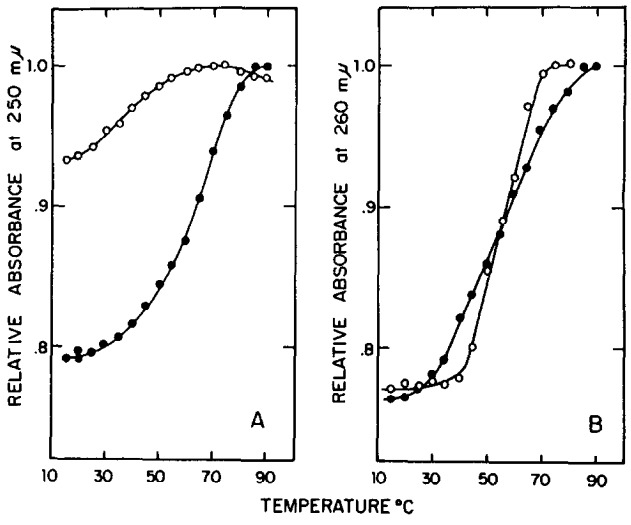

Figure 2: Relative absorbance as a function of temperature of 5S E.coli RNA.
A. 5S RNA before (●) and after (○) reaction with 1M HCHO for 15 min. at 85° in 0.09M Na_2HPO_4 + 0.01M NaH_2PO_4.
B. 5S RNA in 0.05M Na_2HPO_4 + 0.05M NaH_2PO_4; (●) control; (○) in 50% methanol.

neutral buffers to that in 50% methanol-buffer solutions, in which single stranded stacked structures are unstable. The other was to measure the hypochromicity as a function of cation concentration and separate the cation dependent (double-stranded) from the cation independent parts of the absorbance-temperature profiles. Results obtained for the determination of the hypochromicity in 50% methanol are shown in Fig. 2B. The total hypochromicity of 5S RNA is essentially the same in phosphate buffer and in 50% methanol-phosphate buffer mixtures, and the stability of the double stranded regions in unchanged, the midpoint of the transition being the same in both cases. However the transition is considerably narrower in 50% methanol. Assuming that the hypochromicity of completely double

stranded RNA is the same in 50% methanol as in aqueous solution, the fraction of bases in double stranded regions is found to be 0.73.

A third estimate of the double helical content of 5S RNA obtained by measuring the absorbance-temperature profiles as a function of Na^+ and Mg^{++} concentration. The results, summarized in Table I, show that the hypochromicity due to double stranded

TABLE I

HYPOCHROMICITY OF 5S RNA AS A FUNCTION OF CATION CONCENTRATION

	T_M	H_T	H_D
NaCl:			
1.0 M	66	.205	.20
0.1 M	54	.225	.20
0.01 M	49.5	.25	.19
$MgCl_2$:			
10 mM	77	.22	.19
1.0 mM	76	.22	.18
0.1 mM	74	.23	.18

H_T is the total hypochromicity observed between 20 and 90°

H_{CD} is the cation dependent part of the hypochromicity

(cation-dependent) stacked bases is 0.19 ± 0.01. This would be consistent with a double helical content of 0.63 ± 0.03. Thus three independent absorbance measurements indicate that at least three fifths of the bases in 5S RNA are arranged in DNA-like

double stranded regions.

To obtain information about the spatial arrangement of these helical regions, sedimentation-velocity measurements were carried out at several Na^+ and Mg^{++} concentrations. The results, given in Table II, show that the sedimentation constant is surprisingly

TABLE II

SEDIMENTATION CONSTANT OF 5S RNA

AT VARIOUS CATIONS CONCENTRATIONS

0.01 M Tris HCl, pH 7.6, 0.15 M NaCl

$[Mg^{++}]$	$S_{20,w}$
0.0	4.69
0.1 mM	4.75
1.0 mM	4.75
10.0 mM	4.75

0.01 M Tris HCl, pH 7.6

$[Na^+]$	
0	4.50
0.001 M	4.56
0.01 M	4.55
0.15 M	4.69
0.50 M	4.50

constant, being equal to 4.5 to 4.8S under all salt conditions studied. Such insensitivity to counterion concentration is indicative of a rather rigid three dimensional structure. Moreover,

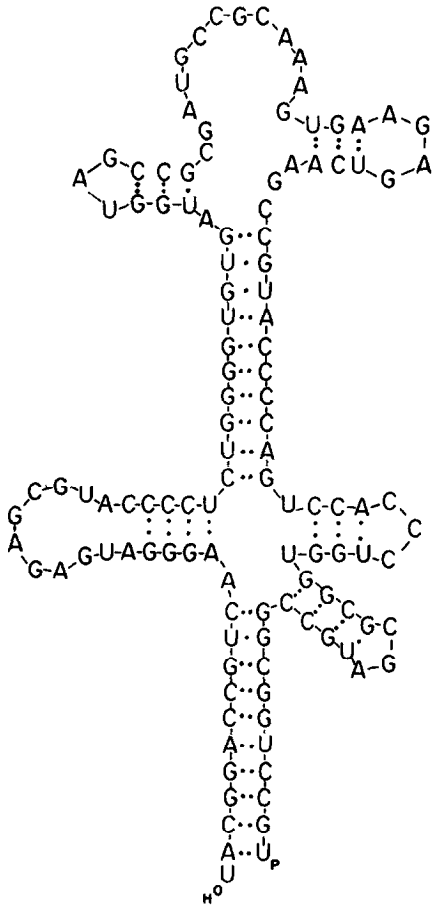

Figure 3: A possible three dimensional structure of 5S RNA.
(:) represent Watson-Crick hydrogen bonded base pairs;
(·) represent pairing between guanine and uracil.

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the $s_{20,w}$ values are lower than would be expected if 5S RNA had as symmetrical a conformation as 4S transfer RNA. This increased asymmetry had previously been suggested by Comb and Zehavi-Willner as a result of the much greater dependence of sedimentation constant on concentration which they observed for 5S RNA than for 4S RNA. (Comb and Zehavi-Willner, 1967)

A possible three dimensional structure of 5S RNA with a heli-

cal content of 0.62 and asymmetry indicated by the results just presented is shown in Fig. 3. Although there are several alternative ways of arranging the shorter (4 base pairs or less) double stranded regions, the ability of this arrangement of the two longer regions to confer the predicted helicity and asymmetry to the structure is self evident. In addition, the resemblance to the cloverleaf model for transfer RNA is obvious and may explain why 5S RNA can compete for one of the 4S ribosome binding sites. (Comb and Sarkar, 1967)

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