SOME OPTICAL PROPERTIES OF 5S-RNA FROM E. COLI

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We have previously shown (1,2) that heat or urea treatment in the absence of Mg^{++} results in the conversion of 5S RNA obtained by our preparative methods (3,2) into a mixture of 2 components called A and B. These components can be separated from each other by chromatography on methylated serum albumin-silicic acid (MASA) columns or by filtration through Sephadex G-100. The B component is more strongly adsorbed on MASA and less retarded on G-100 than the native material. The B form is also devoid of affinity for the 5S RNA binding site on the 50S ribosomal subunit. Although the A component cannot readily be distinguished from the native material by chromatography or gel filtration, a difference can be demonstrated in the reduced affinity of the former for the 5S RNA binding site on reconstituted ribosomes. The B form heated in the presence of Mg^{++} regains the affinity for the 5S RNA binding site and the chromatographic behaviour of the A form. We wish here to report on some of the optical properties of these various forms of 5S RNA.

Hypochromicity studies These studies were conducted using a Cary 11 spectrophotometer equipped with jacketed cuvettes. All samples were dissolved in SSC (0.15M NaCl; 0.015M Na Citrate pH 7.0). The results of this study are summarized in Table 1. The temperature profile is presented in

Fig. 1. The profile for the native form shows a biphasic shape which is greatly reduced in the B form. The A form closely follows the profile of the native form up to approximately 45° where it rises more rapidly than the native to join the curve of the B form. At room temperature the B form is

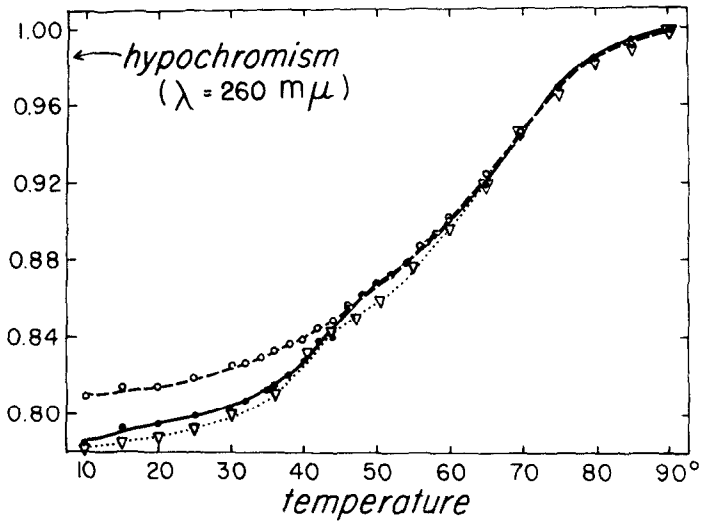

Figure 1. Hypochromism of various forms of 5S RNA. Hypochromism is calculated as indicated in Table I; other details in text. ∇.....∇, native 5S RNA; • ----- •, A Form; o ——— o, B Form.

hyperchromic with respect to both native and A forms both on the basis of phosphate analysis and on the basis of absorption normalized to 90°. Furthermore, we can detect a progressive hypochromism when the B form is heated to 50° in the spectrophotometer cuvette in the presence of 0.01M Mg^{++}. This change is complete in 15' and no further hypochromism is observed when the same sample is then heated to 55°.

Following the treatment of Boedtker and Kelling (4) and assuming that the hypochromism due to single stranded portions of all forms is 0.075, one calculates that the fractional base pairing in the native and A forms is 0.64, equivalent to 38-39 base pairs while in the B form it is 0.51, equivalent to 31 base pairs.

ORD Studies This portion of the investigation was carried out using a Cary 60 spectropolarimeter. All samples were dissolved in 0.15M NaCl and examined at room temperature (21-22°). Solvent baselines were recorded before and after each sample curve had been recorded. Concentrations were calculated using the $\epsilon_{(P)}$ values given in Table I and data are expressed as mean residue rotation.

The ORD curves for the three forms of 5S RNA are presented in Fig. 2. The principal features are (a) the close correspondence of the wavelength of the crossover of the Cotton effect in all three forms (native, 266mμ; A and B, 265mμ); (b) the appearance of a broadening of the positive limb of the Cotton effect in the B form with a slight red-shift of the peak as a consequence; and (c) the generalized increment in positive rotation which increases with decreasing wavelength as one passes from the native to the A and B forms. This latter effect results in a reduction in the assymetry of the Cotton effect so apparent in the native form, and suggests a substantial change in the rotatory power in the wavelength range below 220mμ. These changes are quite distinct from those reported by Adams, et al., (5) for the denaturation of yeast $tRNA_3^{leu}$.

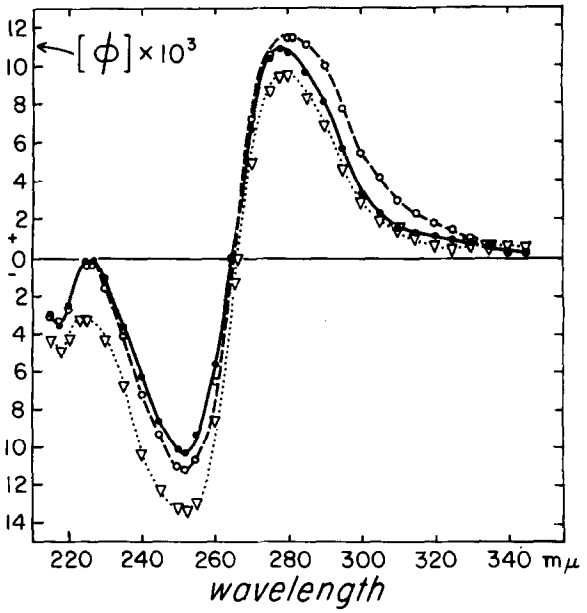

Figure 2. Optical rotatory dispersion of various forms of 5S RNA. Details of measurements in text. ▽·····▽, native 5S RNA; ● ----- ●, A Form; o ——— o, B Form.

CD Studies These investigations were carried out with the CD attachment for the Cary 60 spectropolarimeter. All samples were dissolved in 0.15M NaCl and spectra were recorded at room temperature. The absorbance of the solutions was adjusted to 1.0 - 1.6 for each of the wavelength ranges studied. Concentration was calculated as noted above and the data are presented as $[\epsilon_L - \epsilon_R]_{(P)}$. The results of this study are summarized in Fig. 3. One notes in the CD curve for the native form a weak negative band with a maximum value at 297 mμ. A similar band has been reported by Sarkar, Wells, and Yang (6) for other RNAs. The main positive band has a maximum value similar to many other RNAs which have been examined and has a wavelength of maximum dichroism at 265 mμ consistent with the crossover wavelength for the ORD data.

The differences between the CD spectrum for the native form and those for the A and B forms are, within the wavelength range reported, greater in the region on the long wavelength side of the maximum. In the case of the A form, there is a slight change in the shape of the curve and a concomitant

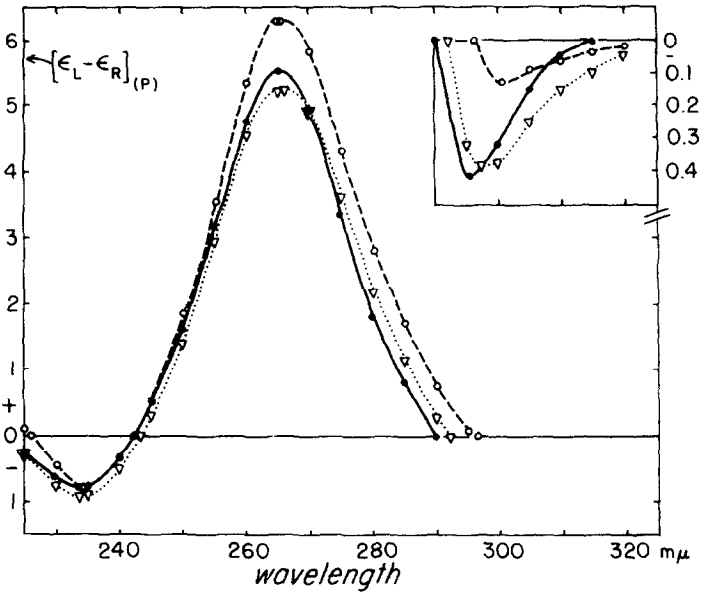

Figure 3. Circular dichroism of various forms of 5S RNA. Details of measurements in text. ∇.....∇, native 5S RNA; • ----- •, A Form; o ——— o, B Form. For the inset figure the wavelength scale of the main figure applies while the Δε scale is given on the righthand ordinate of the inset.

Table I

	$H_{t(260m\mu)}$	$H_{t(280m\mu)}$	$\epsilon_{(P)260m\mu} \times 10^3$	$T_{m(260m\mu)}$	R X 10^{-40} cgs
Native	0.22	0.33	$6.90 \pm .05$*	59°	10.2
A Form	0.22	0.33	$6.92 \pm .16$	59°	11.0
B Form	0.19	0.28	$7.20 \pm .04$	61°	13.8

$H_t = 1-(A_{10°}/A_{90°})$; T_m = midpoint in the hypochromism curve. R was calculated by integration of the positive dichroic band extending from ca. 245mμ to ca. 295mμ. * ± standard error of the mean of 4 determinations; absorbance measurements made on RNA dissolved in SSC; phosphate measurements according to Lowry et al., (11).

displacement of the negative band maximum from 297 mμ to 296 mμ. The changes in the case of the B form are greater. There is an increase in the rotatory power between 250 mμ and 330 mμ amounting to approximately 20% of that of the native form and there is a marked reduction in the weak negative band. When either the A or the B forms are heated for 4 minutes at 60°C in the presence of 0.01M $MgCl_2$ in 0.01 tris-HCl, pH 6.9 and dialyzed against 0.15M NaCl, their CD curves become indistinguishable from that for the native form.

Discussion: It is clear that the native, A and B forms of 5S RNA can be distinguished on the basis of the optical properties presented here. From these differences we may infer something about the nature of the conformational changes leading to the latter two forms. The total hypochromism data suggests that there is little, if any, difference between the native and A forms in the fraction of the bases hydrogen bonded, but that the A form must have a different arrangement of these pairs which is somewhat less stable judging from the hypochromism profile. It should be recalled here that the A form has a lower affinity for the ribosome binding site than the native form (2).

The differences between the A and B form are more striking. The change in the total hypochromism is consistent with a significant reduction in the base pairing as noted above. The fact that the hypochromism of B is observed

at both 260mμ and 280mμ indicates that both A:U and G:C pairs are involved. A significant increase in the extent of single stranded regions in the B form as compared with the native and A forms is also consistent with the increased binding of B to MASA columns, and with its increased tendency to aggregate in solutions of high ionic strength or containing Mg^{++} (2). The change in the CD spectrum is most interesting and can be ascribed principally to the appearance of a broad band of positive sign centered at approximately 280 mμ which amounts to somewhat more than 20% of the rotatory power of the main positive band. This has the effect of increasing the assymetry of the positive CD band, decreasing the strength of the weak negative CD band centered near 295mμ and moving the crossover between these two bands from near 290mμ to 297mμ. The change in the long wavelength limb of the Cotton effect in the ORD is consistent with the changes seen in the CD.

The data obtained in this study are in general agreement with previously published optical data on E. coli 5S RNA (4,7) particularly insofar as they suggest a highly ordered structure for this molecule. Among the various models which have been proposed (4,7,8,9) the only one which is supported by independent chemical evidence is the model of Sanger et al., (9) in which the extent and location of base pairing have been deduced from the accessibility of internucleotide bonds to endonucleases. It has already been pointed out (4,7) that the number of base pairs in the isolated 5S RNA is most likely significantly higher than the number suggested by Sanger's model. In this connection it is worth emphasizing that the material used by Sanger's group for degradative purposes was extracted from polyacrylamide gels with a concentrated urea solution at pH 8.9. We have found (1) that such a procedure leads to the partial conversion of 5S RNA to the B form. It is therefore likely that their model more nearly represents this form rather than 5S RNA which has not been submitted to denaturing conditions.

Although our results are in general agreement with those of other authors, some differences do exist. We have repeatedly found a biphasic hypochromicity-temperature profile for the native 5S heated in the absence of Mg^{++}. This par-

ticular feature has also been noted by Cramer and Erdmann (10). It is not apparent in the results of Boedtker and Kelling (4) or of Cantor (7). Our ORD data differ from those of Cantor (7). Part of this can be explained by differences in the composition of the solutions used. However, control experiments with native 5S RNA dissolved in the medium used by Cantor (7) have failed to reconcile completely the two sets of data. Since the results presented in this note clearly establish that the different forms of 5S RNA possess different optical properties we think that meaningful comparisons between results obtained in various laboratories must include consideration of the histories of the preparations studied. In view of this, it is likely that at least a part of the discrepancies might originate from the techniques of sample preparation and handling.

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