Base Pairing in *Bacillus subtilis* Ribosomal 5S RNA As Measured by Ultraviolet Absorption and Fourier-Transform Infrared Spectrometry†

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ABSTRACT: Ultraviolet (260 and 280 nm) and Fourier-transform infrared (FT-IR) spectra of *Bacillus subtilis* ribosomal 5S RNA have been acquired between 20 and 90 °C. In the presence of added Mg^{2+}, the average UV melting midpoint, T_m, is 60 (A_{260}) or 62 °C (A_{280}), resolving into two components (T_m = 54 and 68 °C). In the presence of 10 mM Mg^{2+}, the normalized A_{260} increases by about 5%, and the average T_m increases to 70 °C (A_{260} or A_{280}), resolving into components at 63 and 73 °C at 260 nm but not resolved at 280 nm. From the difference of the 5S RNA FT-IR spectra between 90 and 30 °C, the number of base pairs in *B. subtilis* 5S RNA was determined by the procedure outlined in the accompanying paper [Li, S.-J., Burkey, K. O., Luoma, G. A., Alben, J. O., & Marshall, A. G. (1984) *Biochemistry* (preceding paper in this issue)]. Addition of 10 mM Mg^{2+} increases the number of A-U pairs by 1 (from 11 to 12) and the number of G-C pairs by 2 (from 15 to 17). FT-IR melting curve midpoints show that addition of Mg^{2+} increases the melting point for both A-U and G-C pairs in *B. subtilis* 5S RNA. The A-U pairs melt before G-C pairs (56 vs. 64 °C) in the absence of Mg^{2+}, but both types of pairs melt at the same temperature (67 vs. 70 °C) in the presence of Mg^{2+}. Both UV and FT-IR results thus indicate that Mg^{2+} produces only a slight increase in base stacking and base pairing in *B. subtilis* 5S RNA but Mg^{2+} significantly increases the stability of the less stable base pairs (principally A-U pairs). Among three proposed universal secondary base-pairing schemes for ribosomal 5S RNAs, the Studnicka and Luoma–Marshall models best account for the observed high total number of base pairs, and the latter more closely predicts the high proportion of G-C pairs inferred from the present UV and IR hyperchromism results.

In the preceding paper (Li et al., 1984b), ultraviolet hyperchromism and Fourier-transform infrared (FT-IR) spectra were analyzed to give estimates for the total number and type (A-U, G-C, and G-U) of base pairs in *eukaryotic* ribosomal 5S RNA (wheat germ). In this paper, we present a similar analysis for base pairing in a *prokaryotic* 5S RNA from *Bacillus subtilis*.

Materials and Methods

5S RNA. *B. subtilis* 5S RNA was isolated as described elsewhere (Li et al., 1984a). The basic three-step procedure consisted of phenol/sodium dodecyl sulfate (SDS) extraction, followed by DE-32 ion-exchange chromatography and large-scale gel filtration chromatography on Sephadex G-75.

Preparation of 5S RNA Samples. 5S RNA samples were prepared as previously reported (Burkey et al., 1983), except that during dialysis, one change of buffer was used and about 50 mg of Chelex (mesh 50–100, Sigma) was added to the dialysis buffer when preparing samples without $MgCl_2$.

Ultraviolet Spectrometry. UV melting profiles at 260 or 280 nm were obtained on a Beckman DU-8 spectrophotometer with the T_m Compuset accessory, in single-beam mode. A_{260} or A_{280} was recorded at 1 °C intervals from 25 to 95 °C, with a temperature ramp of 1 °C/min. UV absorbance vs. wavelength at 25 or 85 °C was measured on a wavelength-scan Compuset. Initial A_{260} was 0.72 ($-Mg^{2+}$) or 0.68 ($+Mg^{2+}$), with A_{280} = 0.36 ($-Mg^{2+}$) or 0.28 ($+Mg^{2+}$), corresponding to concentrations of ca. 0.03 mg/mL.

Fourier-Transform Infrared Spectrometry. All measurements followed the procedures of the preceding paper. Sample concentration (from A_{260}) was 0.76 mM. At that concentration, the proton FT-NMR spectrum (work in progress) is well resolved, showing no evidence of aggregation.

Results and Discussion

A_{260} UV Hyperchromism. Figure 1 shows the UV absorbance melting profiles, normalized to room-temperature A_{260} (or A_{280}), for *B. subtilis* 5S RNA in the absence and presence of 10 mM Mg^{2+}. The original UV hyperchromism = A_{260}(90 °C) − A_{260}(25 °C) increases from 0.19 in the absence of Mg^{2+} to 0.22 in the presence of 10 mM Mg^{2+}. On the basis of a value of 0.30 for hyperchromism from maximal double-stranded stacking (i.e., maximal base pairing) and a 10% minimal contribution from single-stranded stacking (Boedtker & Kelling, 1967), an upper-limit base-pair number is $(0.22 - 0.022)/0.30 \times 100 = 66\%$ of the 116 bases, or about *38 base pairs* (Table I) in the presence of Mg^{2+}.

In the presence of Mg^{2+}, the A_{260} melting midpoint (T_m = 70 °C) for *B. subtilis* 5S RNA is 4–5 °C lower than that for $tRNA^{Phe}$ and about the same as that for wheat germ 5S RNA (see preceding paper). As shown by Ohta et al. (1983), the barely discernible stepwise character of UV melting curves such as Figure 1 can be magnified by plotting dA_{260}/dT vs. T for the same data. For example, the A_{260} melting curve derivative (not shown) for *B. subtilis* 5S RNA is resolved into two distinct components with T_m = 54 and 68 °C (no Mg^{2+}) and T_m = 63 and 73 °C (10 mM Mg^{2+}).

It is also clear from Figure 1 that Mg^{2+} increases the A_{260} of *B. subtilis* 5S RNA by about 5% [i.e., approximately the same as for yeast 5S RNA (Luoma et al., 1980)]. The corresponding increase for wheat germ 5S RNA is about 1%. Thus, although all three 5S RNAs appear to have the same number of base pairs once Mg^{2+} is present, Mg^{2+} appears to be more necessary for maximal secondary base pairing in *B. subtilis* and yeast than in wheat germ.

A_{280} UV Hyperchromism. A_{280} hyperchromism largely reflects unstacking of G-C pairs (Fresco et al., 1963; Van et al., 1977). For *B. subtilis* 5S RNA, T_m increases from 62 to

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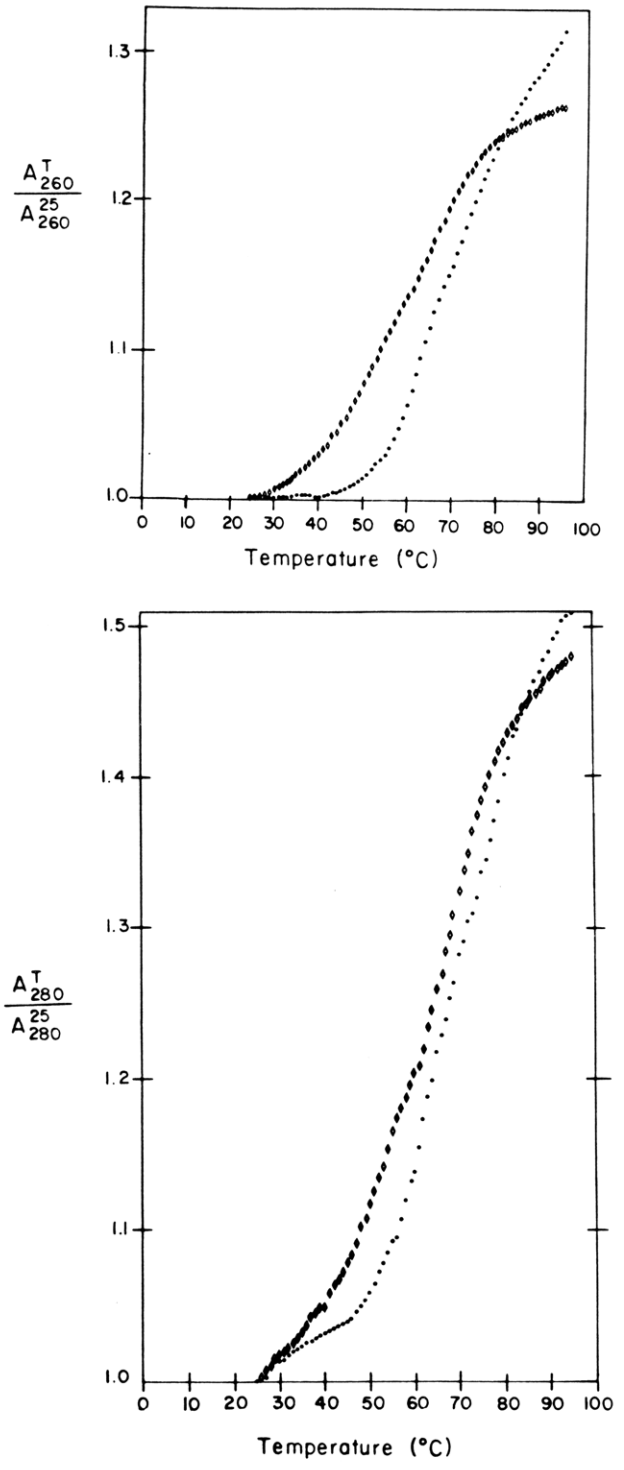

FIGURE 1: Normalized A_{260} (top) and A_{280} (bottom) hyperchromism for *B. subtilis* 5S RNA in the absence (◇) and presence (●) of 10 mM Mg^{2+}.

Table I: Number of Secondary Base Pairs in *B. subtilis* 5S RNA[a]

source	A-U	G-C	G-U	total
cloverleaf model	10	20	4	34
revised Fox–Woese model	6	17	2	25
Studnicka et al.–Nishikawa–Takemura model	7	23	4	34
UV hyperchromism (+Mg^{2+})	17	21(GC + GU)		38
FT-IR				
–Mg^{2+}	11	15	(10)	26 (36)
+Mg^{2+}	12	17	(8)	29 (37)

[a] Precision of FT-IR A-U and G-C values is ±10%; G-U estimates are much less precise (see text).

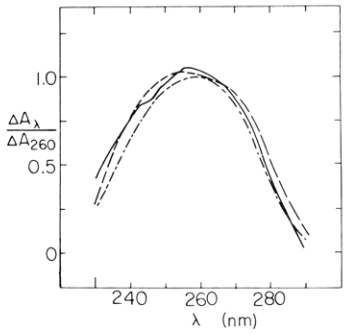

FIGURE 2: UV hyperchromism spectrum for *B. subtilis* 5S RNA (—) compared to curves calculated from standard spectra for 50 (–·–) or 60% G-C pairs (––).

70 °C and the melting changes from the biphasic to monophasic on addition of 10mM Mg^{2+}. Thus, the effect of Mg^{2+} appears to be to increase the base stacking and stability of the less stable segments, without much effect on other already highly stacked and stable segments. Similar effects have been observed from differential scanning calorimetry of $tRNA^{Val_1}$ (Privalov et al., 1975) and *Escherichia coli* 5S RNA (Matveev et al., 1982).

Hyperchromism vs. Wavelength—G-C/A-U Ratio. Figure 2 shows the UV hyperchromism spectrum of *B. subtilis* 5S RNA (solid line), compared to spectra simulated from 50:50 and 60:40 relative contributions from G-C and A-U base pairs (Fresco et al., 1963; Luoma et al., 1980). As can be seen from the simulations, the best match for the experimental curve is found to be 55 ± 5% G-C pairs (Table I). In this estimate, single-stranded stacking, G-U pairs, nearest-neighbor effects, and end-of-helix effects are not considered.

FT-IR Spectra. Determination of the number of A-U and G-C base pairs in RNA from FT-IR data has produced results inconsistent with other techniques, other laboratories, and other RNA species (see preceding paper). In early experiments with dispersive IR spectrometers, IR spectral data points were sampled at intervals of approximately 4 cm^{-1} in the range 1600–1700 cm^{-1} (Thomas, 1969). Schernau & Ackermann (1977) fitted a linear combination of the spectra of poly(rA)·poly(rU), poly(rG)·poly(rC), 5′–3′ ApA, 5′-3′ UpU, 5′-3′ CpC, and 5′-GMP directly to the RNA spectrum in the 1570–1670-cm^{-1} region. Deviations from a best fit straight line for the region above 1670 cm^{-1} were attributed to insensitivity of the IR frequency and intensity to the vertical-stacking interactions of the base residues, to the length of the helical regions, and to the base sequence. Still, the deduced base-pair number of 56 for *E. coli* 5S RNA in the presence of Mg^{2+} (Appel et al., 1979) was unrealistically high. Böhm et al. (1981) introduced the idea of fitting the RNA difference spectrum (90 minus 20 °C) to the IR hyperchromism spectra for A-U and G-C pairs in 1:1 mixtures of complementary homopolymers, on the basis of the intensities of the 1575- and 1620-cm^{-1} peaks. Burkey et al. (1983) improved the reliability of the base-pair estimate by introducing FT-IR in place of dispersive IR and by basing the calculation on all spectral intensity data within ±5 cm^{-1} of the four largest peaks in the difference spectrum, namely, peaks at 1574, 1618, 1660, and 1688 cm^{-1}.

Figure 3 shows the FT-IR extinction coefficient spectra of *B. subtilis* 5S RNA in the absence of Mg^{2+} at 90 (Figure 3A) and at 30 °C (Figure 3B) and the difference of these two spectra, also defined as "base-pair spectrum" (Figure 3C). Analogous spectra are shown in Figure 4 for *B. subtilis* 5S RNA in the presence of 10 mM Mg^{2+}. G-C, A-U, and G-U base pairs were determined by successive fits to the peaks at

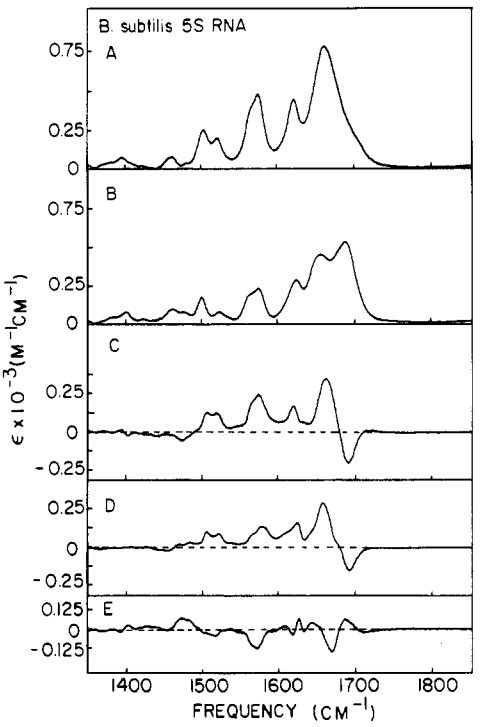

FIGURE 3: FT-IR spectra of *B. subtilis* 5S RNA in the absence of Mg^{2+}: (A) FT-IR spectrum of *B. subtilis* 5S RNA at 90 °C; (B) FT-IR spectrum of *B. subtilis* 5S RNA at 30 °C; (C) difference spectrum, or base-pair spectrum, (A) – (B), representing the infrared intensity due to base pairing of the RNA bases; (D) simulation of (C), computed from the reference A-U and G-C base-pair spectra with base-pair numbers listed in Table I (14 A-U and 17 G-C pairs); (E) difference spectrum, (D) – (C), representing the difference between experimental and simulated base-pair content.

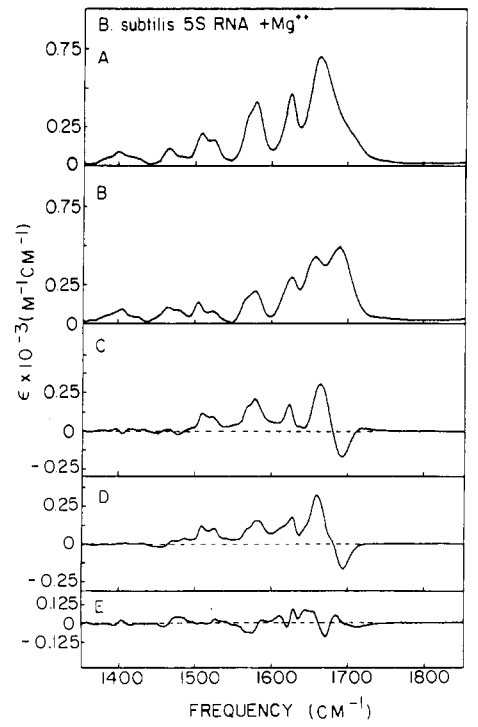

FIGURE 4: FT-IR spectra of *B. subtilis* 5S RNA in the presence of Mg^{2+}: (A–C) and (E) as in Figure 4; (D) simulation of (C), computed from the reference A-U and G-C base-pair spectra with base-pair numbers listed in Table I.

1505, 1620, and 1575 cm^{-1} (see preceding paper). In this data reduction, the G-C base-pair estimate should be the most precise, since the IR intensity at 1505 cm^{-1} should be affected only by G-C stacking. The G-U estimate is likely to be highly

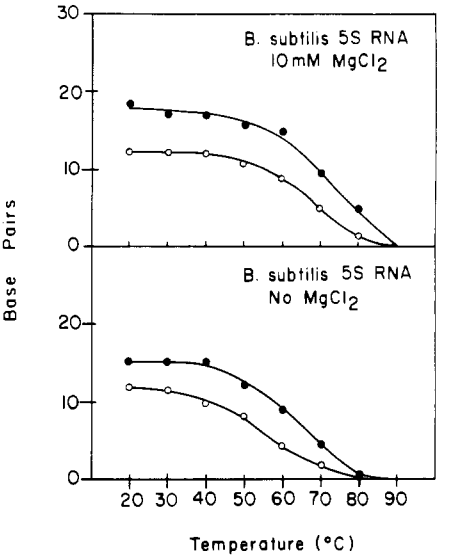

FIGURE 5: Number of A-U (○) and G-C (●) base pairs of *B. subtilis* 5S RNA as a function of temperature: (A) in the presence of Mg^{2+}; (B) in the absence of Mg^{2+}.

Table II: Melting Midpoints (°C) of *B. subtilis* 5S RNA

	type of base pair	$-Mg^{2+}$	$+Mg^{2+}$
UV hyperchromism	A-U, G-C, G-U	61 (54 and 68)	70 (63 and 73)
FT-IR	A-U	56	67
	G-C	64	70

imprecise (±50%), since it is based on previously estimated G-C and A-U base-pair numbers and on the (untested) assumption that G-C and G-U exhibit the same hyperchromism at 1575 cm^{-1} (see preceding paper). Although the absolute numbers of A-U and G-C base pairs obtained by FT-IR and UV differ (Table I), it is encouraging to find that the ratio, G-C/(A-U + G-C) = 0.55 from UV hyperchromism, closely matches the analogous ratio of 17/(12 + 17) = 0.59 from FT-IR (see Table I) for *B. subtilis* 5S RNA in the presence of 10 mM Mg^{2+}. Finally, Table I shows that addition of 10 mM Mg^{2+} increases the number of A-U pairs by one (from 11 to 12) and the number of G-C pairs by two (from 15 to 17), much as for eukaryotic wheat germ 5S RNA (see preceding paper).

FT-IR Melting Profiles. Figure 5 shows the melting of the G-C and A-U base pairs in *B. subtilis* 5S RNA, determined from FT-IR extinction coefficients at 1505 and 1620 cm^{-1}. The melting midpoint (Table II) increases from 56 to 67 °C for A-U pairs but only from 64 to 70 °C for G-C pairs on addition of 10 mM Mg^{2+}. Thus, the stabilizing effect of Mg^{2+} acts principally on A-U pairs, probably because the G-C-rich regions are already highly stable even before addition of Mg^{2+}. The opposite is true for wheat germ 5S RNA, presumably because G-C pairs are more uniformly dispersed in that case.

Secondary Structural Models. Figure 6 shows three generalized secondary base-pairing schemes for prokaryotic 5S RNA, each adapted to the *B. subtilis* 5S RNA base sequence. Model A is the highly base-paired "cloverleaf" model proposed by Luoma & Marshall (1978a,b) and can be adapted to all known prokaryotic and archebacterial and eukaryotic 5S RNAs as well as to eukaryotic 5.8S RNA. Model B is the original Fox & Woese (1975) model for prokaryotic 5S RNA, as revised by Noller et al. (1979) for Gram-negative prokaryotic 5S RNA (e.g., *E. coli*, whereas *B. subtilis* is a Gram-positive prokaryote) and by Luehrsen & Fox (1981) for eukaryotic cytoplasmic 5S RNA. Model C is a different variant

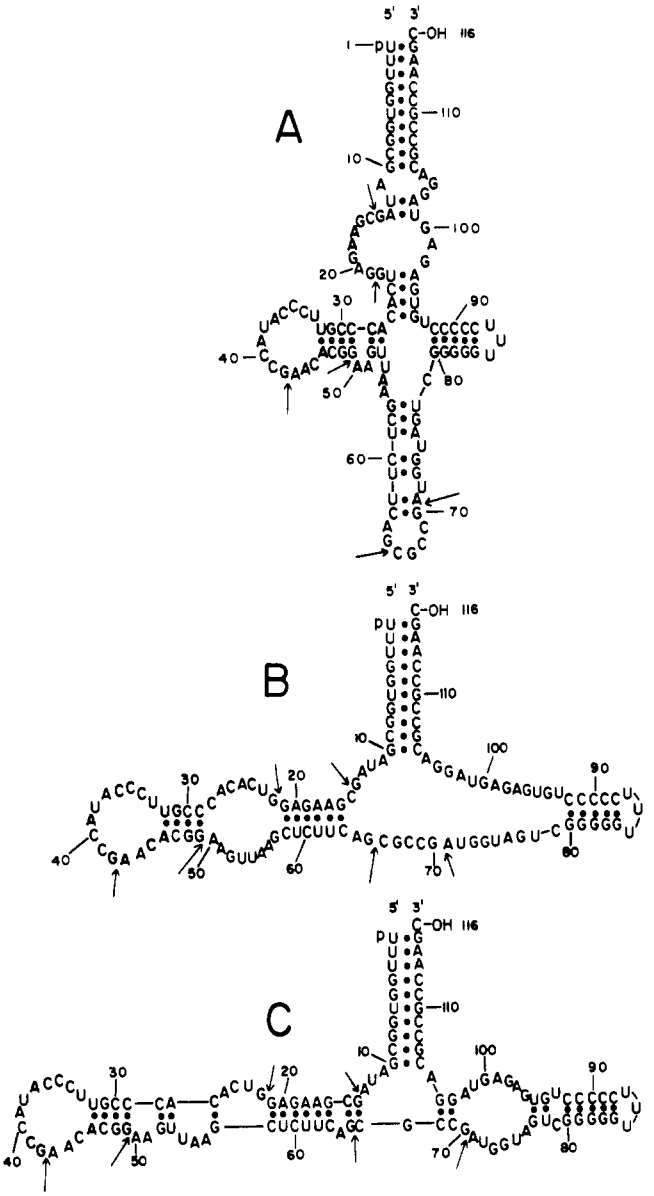

FIGURE 6: Proposed secondary structure models of *B. subtilis* 5S RNA. Sites of cleavage by T_1 RNase are shown by small arrows: (A) cloverleaf (Luoma–Marshall) model; (B) revised Fox–Woese model; (C) Studnicka et al.–Nishikawa–Takemura model.

of the Fox–Woese model exhibiting additional base pairing for prokaryotic 5S RNA (Studnicka et al., 1981) and for eukaryotic 5S RNA (Nishikawa & Takemura, 1978). The arrows inserted onto each model denote points of cleavage by T_1 RNase (Morell et al., 1967) and probably correspond to single-stranded segments. Table I lists the base-pair numbers predicted by each of the three secondary structural models, for comparison to the values deduced from UV and IR hyperchromism data. Both the cloverleaf and Studnicka models account for the high total number of base pairs, but the cloverleaf model more closely predicts the high proportion of G-C base pairs inferred from the UV and IR hyperchromism experiments. Of course, all three models are intended to account only for secondary base pairs, and there may well be a significant number of tertiary base pairs: 6 of the 27 base pairs in $tRNA^{Phe}$ are tertiary pairs (Sussman & Kim, 1976).

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