STRUCTURAL ANALYSIS OF THE A AND B CONFORMERS OF *ESCHERICHIA COLI* 5 S RIBOSOMAL RNA BY INFRARED SPECTROSCOPY

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1. Introduction

Rapid progress is being made in elucidating the structure of pro- and eukaryotic 5 S RNAs using different physical, biochemical and sequence analysis approaches (reviewed in [1]). It seems to be evident from comparative sequence studies [1–5] that a general base pairing scheme of the type first proposed in [2] is valid for eukaryotic 5 S RNAs and that a general secondary structure of the type first proposed in [3] extended by few base pairs is the structural basis for prokaryotic 5 S RNAs. Experimental evidence supporting these basic secondary structures is now manifold and derives, e.g., from such powerful specific techniques as high-resolution ^{1}H NMR spectroscopy [6,7] and slow tritium exchange studies [8]. Summarizing data from optical ([6,9] and references within), infrared [10], Raman [11–13], and ^{1}H NMR spectroscopy [6,7] it became obvious that both for prokaryotic and eukaryotic 5 S RNA molecules in solution a highly ordered secondary/tertiary structure exists with an amount of about 35–42 base pairs (58–70% of all nucleotides are base-paired) in the presence of stabilizing ions.

Here, we report the results of infrared spectroscopic studies of the A and B conformers of *Escherichia coli* 5 S RNA. Within the experimental error of about ± 10% we have estimated for both 5 S RNA conformers at 4 mM Mg^{2+}, 25 GC and 10 AU base pairs. These data are in a rather strong contradiction to the much higher amount of base pairing (36 GC and 20 AU) reported in [14]. Despite the similarity in the GC and AU base pairing content of the A and B conformer of *E. coli* 5 S RNA the infrared spectra

and the infrared thermal melting curves reveal particular differences between both conformers at ≲70°C which may be useful with respect to the analysis of the intricate problem of the A-to-B conformational transition(s).

2. Materials and methods

5 S RNA from ribosomes of *E. coli* MRE 600 was purified essentially as in [15]. To obtain pure A and B forms, native 5 S RNA dissolved in 10 mM sodium acetate (pH 5.0), 0.75 M NaCl, 1% methanol was incubated for 5 min at 70°C. After rapid cooling to 4°C the specimen was applied to a column (2.5 × 100 cm) with Biogel P-100 (Bio-Rad, USA). Two peaks of absorbance corresponding to the A and B forms of 5 S RNA [16] were recovered and used in the optical measurements. The purity was checked electrophoretically. Lyophilized 5 S RNA preparations were dissolved in the appropriate volume of ^{2}H$_2$O for the infrared measurements such that the final concentration was about 1% (w/v) in 5 mM phosphate buffer ('pH 7.0') and 4 mM Mg^{2+}. The 5 S RNA concentration was measured directly in the infrared cells using the molar extinction coefficient for both forms of 7.82 × 10^5 l . M^{-1} . cm^{-1} at 260 nm and 20°C [14].

The infrared spectra were measured with a Perkin-Elmer M-180 Spectrophotometer at a spectral slit width of 2 cm^{-1}. The 5 S RNA solutions were placed in CaF$_2$ cells with optical pathlength of 80 or 90 μm. Cells 1% thinner than the sample cells, were used to compensate carefully the solvent absorption in the reference beam. The cells were installed in thermo-

statted holders (accuracy of the temperature measurements controlled by a thermocouple was $\pm 0.3°C$). The amount of GC and AU base pairs was estimated as in [17] using our set of basic spectra and computer evaluation of the infrared spectra.

Until now the agreement between the experimental infrared spectrum of RNA and the spectrum calculated from a set of catalog spectra has not been satisfying [18]. Therefore, we have used a simplified calculation procedure selecting the 1575 cm^{-1} band and the 1620 cm^{-1} band for the determination of the GC and AU base pairing content, respectively. The following equation was used for the estimation of the number of base pairs:

$$\left(\frac{\epsilon_{95}-\epsilon_{20}}{\Delta\epsilon_{AU}}\right)_{\nu} = f_A + \left(\frac{\Delta\epsilon\,(GC)}{\Delta\epsilon\,(AU)}\right)_{\nu} f_G$$

where $f_A^{1/2}$ and $f_G^{1/2}$ are the mole fractions of adenine and guanine in paired states, and $\epsilon_{\nu 95}$ and $\epsilon_{\nu 20}$ are the molar extinction coefficients of 5 S RNA at 95°C and 20°C, respectively. The $\Delta\epsilon_{\nu}$ values are defined by the equation:

$$\Delta\epsilon_{\nu} = \epsilon_{\nu 95} - \epsilon_{\nu 20}$$

where $\epsilon_{95°}$ and $\epsilon_{20°}$ correspond to the values of single stranded polynucleotides and double-stranded poly-(GC) and poly(AU), respectively.

We have used the following $\Delta\epsilon_{\nu}$ values in this procedure:

$\Delta\epsilon\,(GC)_{1575\,cm^{-1}} = 369\ 1.M^{-1}.cm^{-1};$
$\Delta\epsilon\,(AU)_{1575\,cm^{-1}} = \ \ 80\ 1.M^{-1}.cm^{-1};$
$\Delta\epsilon\,(AU)_{1620\,cm^{-1}} = 542\ 1.M^{-1}.cm^{-1};$
$\Delta\epsilon\,(GC)_{1620\,cm^{-1}} = \ \ 50\ 1.M^{-1}.cm^{-1}.$

Details will be described elsewhere (S. Yu. V., H. F., S. B., in preparation). The results of the simplified calculation procedure agree with those of the more extended procedures [17,19] within an error of about 10%.

The infrared spectra and infrared thermal denaturation curves presented are the averages of three independent measurements.

3. Results and discussion

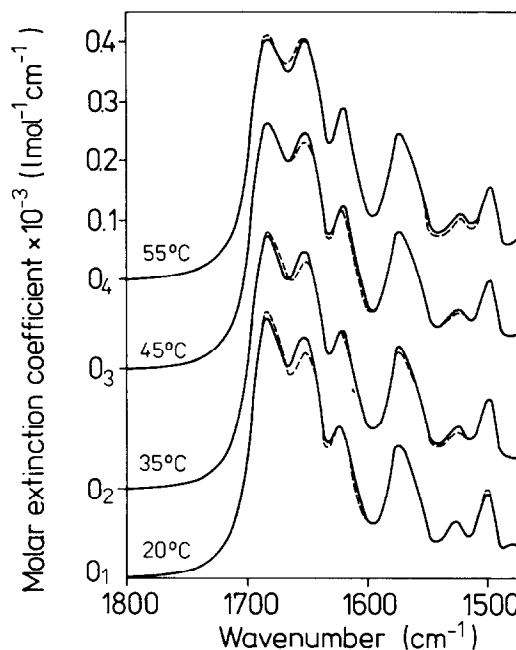Fig.1 shows the infrared spectra of the A and B

Fig.1. Infrared spectra of the A (——) and B (– – –) conformer of E. coli 5 S RNA at 20,35,45 and 55°C; 5 mM sodium phosphate buffer ('pH 7.0'), 0.4 mM Mg^{2+}.
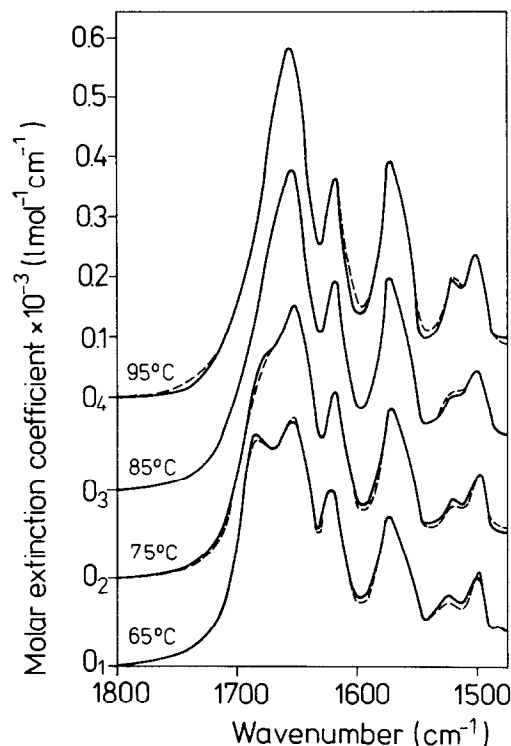

Fig.2. Infrared spectra of the A and B conformer of E. coli 5 S RNA at 65,75,85 and 95°C; conditions and symbols as in fig.1.

conformers of *E. coli* 5 S RNA over 20–55°C, and fig.2 shows those over 65–95°C. The 20°C spectra exhibit a pronounced difference between the A and B form at around 1650 cm^{-1} and smaller differences in other part of the spectra. The characteristic difference of the absorption band(s) around 1650 cm^{-1} practically disappears at about 55°C. The infrared spectral characteristics of the B form at 20°C are converted to those of the A form at 20°C by heating the B form in solution to 65°C and recooling to 20°C (not shown) in accordance with the data about the conversion of the B form into the A form in the presence of Mg^{2+} [20]. At $\gtrsim$70°C the infrared spectra of the A and B conformer of *E. coli* 5 S RNA are identical within the experimental error (cf. fig.2) and exhibit identical melting behaviour (cf. fig.3). Obviously the more stable helical parts of the secondary structures of the A and B forms are identical. Further support for this finding arises from calorimetric and optical melting data (V. V. F., S. V. M., unpublished).

The differences between the infrared spectra of the A and B conformers seen at lower temperatures (fig.1,3) on the other hand reflect structural differ-

ences on the level of the less stable secondary structural elements and/or the tertiary structure.

Using the infrared difference spectra (95°C–20°C) and the above calculation procedure we have determined the overall extent of base pairing both for the A and B conformer of *E. coli* 5 S RNA in 5 mM phosphate buffer ('pH 7.0'), 0.4 mM Mg^{2+} to 25 ± 3 GC and 10 ± 1 AU base pair. In this connection we have to remember that the determination of GU base pairs by infrared (and Raman) spectroscopy is still an unresolved problem [21]. The GU infrared spectral characteristics introduce, by the overlapping the GC and AU characteristics, some uncertainty into the quantitative determination of specific base pairs. Nevertheless the discrepancy between our results and the infrared data for *E. coli* 5 S RNA in [14] is outside the overall limit of error of about ± 10%. The most plausible explanation for the over-estimation of the base pairing contents in [14] appears to be an incorrect compensation of the ^2H_2O infrared absorption according to the presented spectra.

We have used the stepwise melting of base pairs as estimated from the infrared melting curves at 1620 cm^{-1} and 1575 cm^{-1} (fig.3) for the identification of corresponding structural elements of *E. coli* 5 S RNA (table 1). This approach is bound to be a first approximation for three reasons:

(i) The selected temperature interval of 5°C is rather coarse in comparison to the relatively sharp melting transitions at 4 mM Mg^{2+} solvent conditions.

(ii) The melting of GU base pairs, stacking interactions, triple helices or other non-Watson–Crick type base-pairing interactions may contribute to changes of the infrared spectra at lower temperatures; consequently the estimation of molten base pairs may be influenced by these effects.

(iii) Weak base pairing interactions belonging to different structural elements may melt at low temperatures which cannot be correlated.

However, the results shown in table 1 support a Fox–Woese type secondary structure as shown in fig.4 extended by additional base pairs. The presented base-pairing scheme seems to be more compatible with the A conformer of *E. coli* 5 S RNA according to RNase cleavage points ([1] and references cited therein) and kethoxal modification studies [22]. It fits a general base-pairing scheme for prokaryotic 5 S RNA based on our comparative sequence studies [5]. Probably the intermediate state for both the A and B conformers of *E. coli* 5 S RNA (at about 70°C
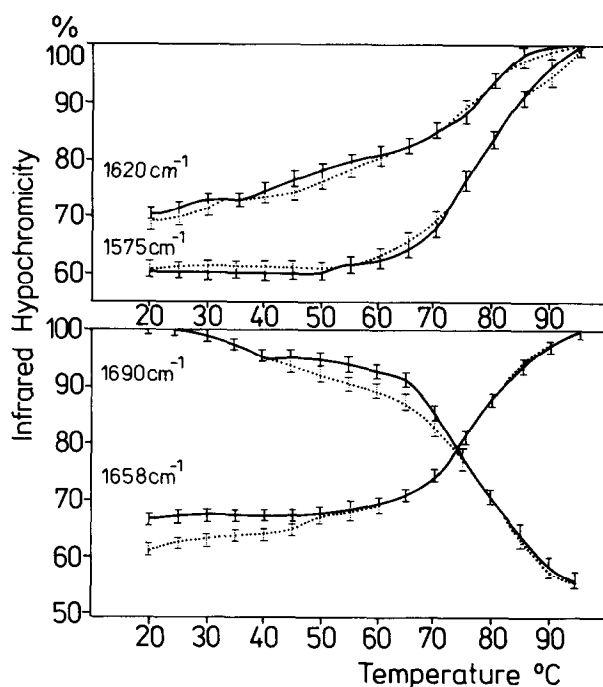

Fig.3. Infrared thermal melting curves of the A (——) and B (– – –) conformer at selected wave-numbers; conditions as in fig.1.

Table 1

Analysis of the infrared thermal denaturation curves of *E. coli* 5 S RNA (tentative assignment to the base pairing scheme shown in fig.3)

Temperature interval ($^{\circ}$C)	No. molten base pairs		Corresponding structural elements
	AU	GC	
20–65	4	3– 4	Weak secondary and tertiary structure base pairs, e.g., A_{73}/U_{103}, $G_{75}GUA_{78}/C_{37}CAU_{40}$, helix C, GU base pairs, non-Watson–Crick base-pairing interactions
65–75	2–3	5– 6	Helix C, segment b and other GC base pairs in the branching region I, G_{79}/C_{97}
75–85	2–3	9–10	Helix A and B (the more stable base pairs drawn in fig.3)
85–95	1	5	Stable part of helix E

under the selected solvent conditions) is characterized by the secondary structure with helices A, B and E. The particular structure of the A and B conformer may be folded at lower temperatures from this intermediate state with variations in the most unstable secondary and/or tertiary structural elements depending on the solvent composition and annealing conditions [23].
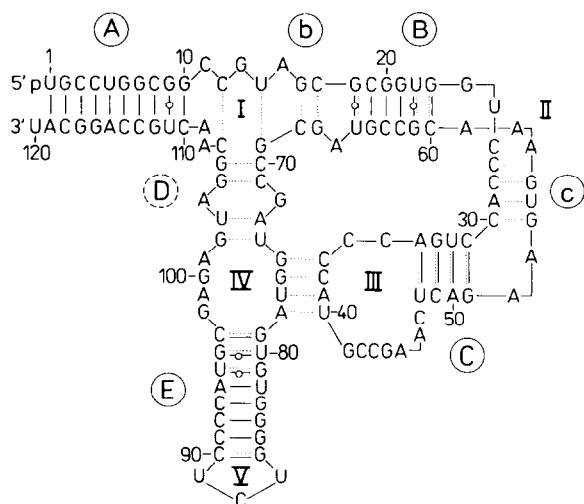

Fig.4. Proposed base-pairing scheme of *E. coli* 5 S RNA adapted to the proposed generalized base-pairing scheme of prokaryotic 5 S RNAs [5]: (——) more stable base pairs; (⁻⁻⁻⁻) less stable base pairs; (. . .) weak secondary/tertiary structure base pairs; (A–E) helices A–E, respectively; (b,c) extended parts of helices B and C; (I–V) loop regions.

The characteristic changes between 20°C and 55°C in the infrared absorption band at 1650 cm^{-1} of the B form (fig.1) resemble the infrared spectral shifts which were observed during the melting of triple helical polynucleotides, especially of poly(CGC) [24]. This may be an indication that few triple helical structural elements, e.g., CGC and UAU, are present preferably in the B form. We may propose that intricate interactions between the highly conserved purine stretch (sequence positions G_{106}–G_{98}), the pyrimidine-rich stretch (sequence positions C_{35}–C_{47}) and the nucleotide stretch ranging from G_{69}–A_{78} should be very important for the A-to-B form transition of *E. coli* 5 S RNA (and vice versa). In agreement with this assumption, kethoxal modification studies [22] point to different accessibility of guanine residues between the A and B conformers especially in these regions of the *E. coli* 5 S RNA molecule.

Fig.4 shows one possible base-pairing arrangement of the mentioned segments (for the A form) including the disturbed D helix and the parallel tertiary base pairing between $C_{37}CAU_{40}$ and $G_{75}GUA_{78}$. Our proposal for this structural feature arises mainly from the analogy to the eukaryotic 5 S RNAs [5] and from the possibility of verifying the proposed base pairs within molecular models of 5 S RNA molecules (S. B., H. F., H. W., submitted). Other tertiary base-pairing interactions cannot be excluded, e.g., an antiparallel base-pairing between $A_{73}UGG_{76}$ and $U_{40}ACC_{37}$ as considered on the basis of slow tritium exchange results on

E. coli 5 S RNA [8]. Very recently a cross-linking between G_{41} and G_{72} was reported for this molecule [25] supporting the suggested tertiary interactions between two of the three mentioned nucleotide stretches.

Acknowledgements

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