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Evaluation of Base-Pairing Schemes for E. coli 5S RNA by 400 MHz Proton Nuclear Magnetic Resonance Spectroscopy

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SUMMARY

The number of base pairs in the denatured "B" form of E. coli 5S RNA has been determined directly from 400 MHz high resolution proton nuclear magnetic resonance spectroscopy. The experimental NMR spectrum from -11.6 to -14.5 ppm from a sodium 2,2-dimethyl-2-silapentane sulfonate reference can be simulated by a theoretical spectrum consisting of 33 Lorentzian lines of equal width (corresponding to 33 base pairs) at 26°C. This result is inconsistent with previously proposed secondary structures of 17 and 23 base pairs, but is readily adapted to the Luoma-Marshall cloverleaf secondary structure.

INTRODUCTION

The primary nucleotide sequences of approximately fifty prokaryotic and eukaryotic 5S RNA's are known (1). Although many possible secondary base-pairing schemes for these relatively small RNA molecules have been proposed, no single secondary structural model is universally accepted. Of the many proposed secondary structures for prokaryotic 5S RNA (2), the cloverleaf model (3,4) is most consistent with the fraction of paired bases (from optical absorbance (5), Raman (3,6), and infra-red (2) data), and the locations of single-stranded segments (from enzymatic cleavage (7) and chemical modification (8) data) in the molecule. Moreover, the cloverleaf model can be adapted to all the 70-odd known primary nucleotide sequences for prokaryotic 5S and 5.8S

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RNA, as well as eukaryotic 5S RNA. However, additional direct determination of the extent of base-pairing in 5S RNA's is required in order to discriminate between the various secondary structural models.

Based on the previous success for NMR determination of the number of base pairs in a large number of transfer-RNA's (9,10), we have analyzed proposed secondary structures of E. coli 5S RNA. In this approach, the integrated intensity of the hydrogen-bonded region of the proton NMR spectrum (based on one assumed resonance between -11 and -15 ppm from sodium 2,2-dimethyl-2-silapentane sulfonate (DSS) for each GC or AU base pair) is compared to the number of base pairs predicted by a given secondary structural model. For Saccharomyces cerevisiae (yeast) 5S RNA, this method gave a number of base pairs in good agreement with that determined from optical absorbance, and both determinations were in agreement with the number of base pairs predicted by the cloverleaf model for eukaryotic 5S RNA (4,11).

There are two chromatographically distinguishable conformational forms of E. coli 5S RNA: a native "A" form and a denatured "B" form (7,12). The "B" form may be generated in vitro either by heating to 65°C in the absence of Mg^{++} and then re-cooling, or by addition of 7M urea (7,12). One speculation is that the "B" form may occur physiologically via an induced conformational change in 5S RNA during protein synthesis in prokaryotes (13). The two previously proposed secondary structures for the E. coli 5S RNA "B" form predict 17 (13) and 23 (14) base pairs, respectively. In this communication, we present NMR evidence that the E. coli 5S "B" form contains at least 33 base pairs, and we offer a cloverleaf secondary structural model that is consistent with this base pair number as well as with chemical modification and enzymatic cleavage results.

MATERIALS AND METHODS

E. coli 5S RNA used in this study was isolated from commercial E. coli transfer-RNA (Sigma) containing significant amounts of 5S RNA and ribosomal

RNA's. 5S RNA was separated from other RNA's by chromatography on Sephadex G-75 at 25°C, using buffer containing 10 mM phosphate, pH 7.0, 100 mM NaCl, and 10 mM $MgCl_2$. The 5S RNA fractions were pooled; 2.5 volumes of cold ethanol added, and the RNA allowed to precipitate at -20°C. This RNA was then desalted on a Sephadex G-25 column and lyophilized. Purified RNA was stored at -20°C and tested for homogeneity using 10% gel slab electrophoresis by the method of Rubin (15); under these conditions the 5S RNA migrated as a single band.

The "B" form of E. coli 5S RNA was produced by dissolving approximately 10 mg of RNA powder in 0.3 ml 10 mM phosphate, pH 6.5, containing 100 mM NaCl and 15 mM ethylenediamminetetraacetic acid (EDTA). This solution was then heated to 65°C for 5 min and cooled slowly to 25°C; this cycle was repeated at least five times. The resulting sample was dialyzed against 100 volumes of 10 mM phosphate buffer, pH 7.0, containing 100 mM NaCl and 1 mM EDTA at 4°C for at least 12 hr. The sample was then transferred to a 5 mm NMR tube fitted with a vortex plug and 5% D_2O added v/v.

Proton NMR spectra were obtained on a Bruker WH-400 FT-NMR spectrometer at the University of British Columbia. Suppression of the strong solvent resonance was accomplished using a low power excitation pulse (~333 μsec) centered at (for example) 3020 Hz downfield from the H_2O peak (at 26°C). Typical spectral parameters were: 4K f.i.d. data set, 340 msec acquisition time, ± 6000 Hz spectral width using quadrature detection with phase alternation sequence; 250 msec delay between successive acquisitions, and exponential apodization equivalent to 3 Hz line broadening.

RESULTS

Figure 1 (top) depicts the hydrogen-bonded region of the 400 MHz proton NMR spectrum of the "B" form of E. coli 5S RNA. A previous investigation of the solution structure of this RNA was hampered by poor resolution, due both to lower magnetic field strength (^1H Larmor frequency of 300 MHz) and

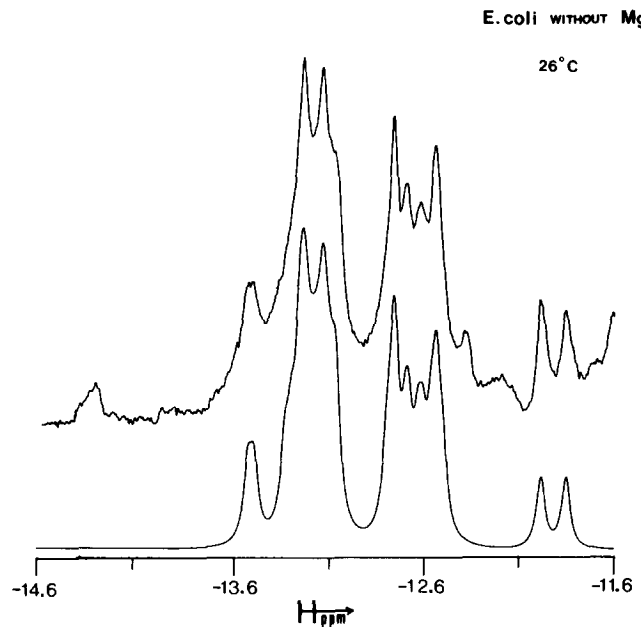

Figure 1: 400 MHz ^{1}H-magnetic resonance spectrum of the denatured (B form) of E. coli 5S RNA at 26°C (upper curve), and theoretical spectrum (bottom curve) constructed from a sum of 31 Lorentzian lines of equal intensity and linewidth (30 hertz at half maximum height). Chemical shifts are referenced to DSS at 0 ppm. Buffer contained 10 mM phosphate, pH 6.5, 100 mM NaCl and 1 mM EDTA.

to probable aggregation in the sample at high (56 mg/ml) RNA concentration (16). However, under the present conditions, well-resolved resonances are easily distinguished (Fig. 1, top). This experimental spectrum was simulated by summing Lorentzian lines, each with the same full width at half-maximum height of 30 Hz, assuming that each of the two resonances near -11.9 ppm represents a single proton intensity (Fig. 1, bottom). The minimum number of Lorentzians needed to simulate the experimental 5S RNA spectrum between -11.6 and -14.5 ppm at 26°C was 31. Experimentally observed resonances at -12.4 ppm and -14.4 ppm were not included in the simulation, since these resonances "melt" upon increasing the temperature and do not represent unit proton intensity even at 26°C (17). Including these resonances would increase the number of protons in this spectral region to at least 33: in other words, the "B" form of E. coli 5S RNA must contain at least 33 base pairs.

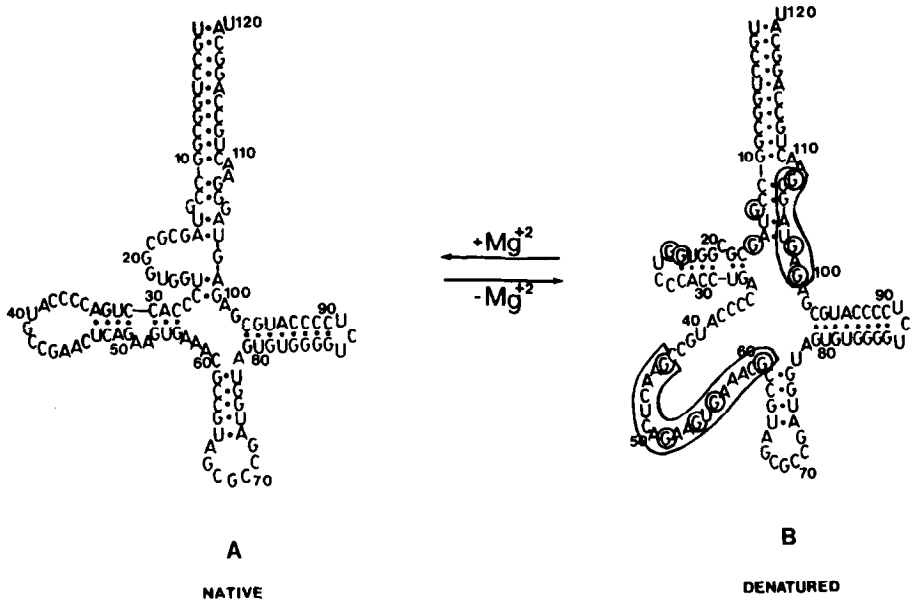

Figure 2: Schematic representation of the interconversion of the two forms of E. coli 5S RNA. The A form structure has been previously proposed (see refs 3 and 4). The B form is the proposed structure based on nmr results. Ⓖ represent guanosines modified by kethoxal and ▭ represent regions susceptible to digestion in the B form.

DISCUSSION

Two models have been proposed for the secondary structure of the "B" form of E. coli 5S RNA, predicting either 17 (13) or 23 (14) base pairs. However, both models were deduced primarily from nucleotide sequence data. That is, if Watson-Crick complementary segments were conserved in all known sequences, it was presumed that these segments were in fact base-paired in the secondary structure. Thus, neither model is based on any experimental determination of number of secondary base pairs. In contrast, the present experimental ^{1}H NMR results directly demonstrate that E. coli 5S RNA must contain at least 33 base pairs. It might be suggested that tertiary base pairs could contribute to the total base pair number; however, the number of tertiary base pairs in E. coli 5S RNA is probably quite small, based on proton and ^{31}P NMR data (17).

A previously proposed "cloverleaf" secondary structural model for the "A" form of E. coli 5S RNA (3) is consistent with the number of base pairs

from optical absorbance (5), infrared (2), and Raman (3,6) data, and also with chemical modification (8) and enzymatic cleavage (7) data. Figure 2 shows how the "A" and "B" forms of E. coli 5S RNA might interconvert. The Fig. 2 picture is consistent with the suggestion that this interconversion requires a rearrangement of base pairing involving approximately 9 ± 2 AU and GC pairs (12). The Fig. 2 model for the "B" form contains 31 secondary base pairs and is thus consistent with chemical modification and enzymatic cleavage sites shown in the Figure. Work is currently in progress to assign these resonances to specific base pairs, in order to more accurately characterize this form of 5S RNA, to facilitate understanding of its possible physiological role in prokaryotic protein synthesis.

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