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^{15}N-Labeled 5S RNA. Identification of Uridine Base Pairs in *Escherichia coli* 5S RNA by ^{1}H–^{15}N Multiple Quantum NMR†

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ABSTRACT: *Escherichia coli* 5S RNA labeled with ^{15}N at N3 of the uridines was isolated from the Sϕ-187 uracil auxotroph grown on a minimal medium supplemented with [3-^{15}N]uracil. ^{1}H–^{15}N multiple quantum filtered and 2D chemical shift correlated spectra gave resonances for the uridine imino ^{1}H–^{15}N units whose protons were exchanging slowly with solvent. Peaks with ^{1}H/^{15}N shifts at 11.6/154.8, 11.7/155.0, 11.8/155.5, 12.1/155.0, and 12.2/155.0 ppm were assigned to GU interactions. Two labile high-field AU resonances at 12.6/156.8 and 12.8/157.3 ppm typical of AU pairs in a shielded environment at the end of a helix were seen. Intense AU signals were also found at 13.4/158.5 and 13.6/159.2 ppm where ^{1}H–^{15}N units in normal Watson–Crick pairs resonate. ^{1}H resonances at 10.6 and 13.8 ppm were too weak, presumably because of exchange with water, to give peaks in chemical shift correlated spectra. ^{1}H chemical shifts suggest that the resonance at 13.8 ppm represents a labile AU pair, while the resonance at 10.6 ppm is typical of a tertiary interaction between U and a tightly bound water or a phosphate residue. The NMR data are consistent with proposed secondary structures for 5S RNA.

The small ribosomal ribonucleic acid 5S RNA is a necessary constituent of the large ribosomal subunit (Gould et al., 1970). Although little is known in detail about the specific functions and interactions of 5S RNA with other molecules in the ribosome, there are indications that 5S RNA is located near the ribosomal binding site of tRNA (Roberts, 1972; Soffer, 1974). A considerable effort has been devoted toward elucidation of the secondary and tertiary structure of 5S RNA and its interactions with other constituents in order to evaluate its role in the ribosome during translation. ^{1}H NMR spectroscopy has been widely used to study the structure of 5S RNA. The majority of these investigations focused on the uridine and guanosine imino resonances, with an emphasis on NOE studies to assign chemical shifts of nearest neighbors (Chan & Marshall, 1986; Chang & Marshall, 1986; Lee & Marshall, 1987). Other factors that influence the specta, such as ionic strength and temperature, were also used to assign resonances (Leontis & Moore, 1986b; Leontis et al., 1986).

^{1}H NMR spectra of 5S RNAs have been hampered by the complexity of the imino region between 9 and 15 ppm and by line broadening. As a result, some recent work utilized fragments obtained from limited nuclease digests (Li & Marshall, 1986; Leontis & Moore, 1986a) to simplify the spectra. Spectral editing techniques based on ^{1}H–^{15}N heteronuclear correlations (Griffey et al., 1985; Griffey & Redfield, 1987) with isotopically enriched molecules have also been employed. Some GU base pairs were identified in a uniformly ^{15}N-labeled fragment (Kime, 1984) by difference decoupling. Recently, ^{1}H–^{15}N spectra were also reported for a partially labeled 5S fragment (Gewirth et al., 1987). Unfortunately, resolution in difference decoupling experiments is inherently poor because of limitations imposed by the magnitude of the one-bond ^{1}H–^{15}N coupling constant, and it is not possible to resolve closely spaced resonances. We now report a 2D ^{1}H/^{15}N chemical shift correlation study with *Escherichia coli* 5S RNA labeled specifically with ^{15}N at N3 of uridine which allowed selective detection of hydrogen-bonded uridine imino protons in slow exchange with solvent without complications from ^{1}H/^{15}N couplings. Of particular interest is the identification of five resonances attributed to G–U interactions.

Materials and Methods

The *E. coli* Sϕ-187 uracil-requiring auxotroph (Griffey et al., 1982) was grown to late log phase in a minimal medium supplemented with 4 μg/mL casamino acids, 40 μg/mL thymidine, 40 μg/mL cytosine, and 18 μg/mL [3-^{15}N]uracil. A soluble RNA fraction was obtained by phenol extraction and 2-propanol precipitation as previously described (Griffey et al., 1985). Crude RNA was fractionated by chromatography on DEAE-Sephadex A-50, pH 7.5, and fractions containing activity for $tRNA_2^{Tyr}$ were purified by chromatography on BD-cellulose (Nishimura, 1971). The first sharp peak to elute from BD-celluose was homogeneous 5S RNA, as determined by polyacrylamide gel electrophoresis (Barrell, 1971). NMR samples were prepared by dissolving 8 mg of 5S [3-^{15}N]RNA in 350 μL of 10 mM phosphate buffer, pH 6.0, containing 5% deuterium oxide, 10 mM magnesium chloride, 100 mM po-

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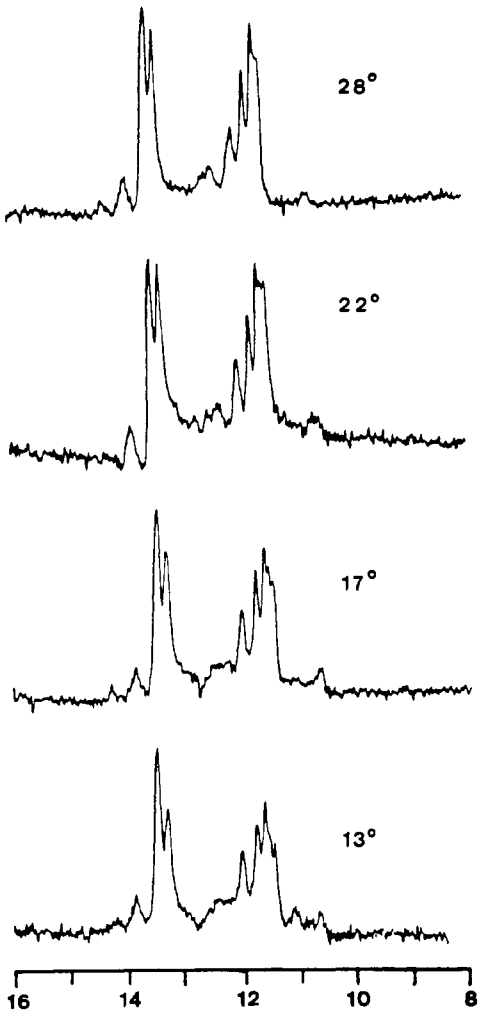

FIGURE 1: ^1H–^{15}N multiple quantum filtered 500-MHz ^1H NMR spectra of [3-^{15}N]uridine-labeled *E. coli* 5S RNA; 2800 transients were acquired at each temperature.

tassium chloride, and 0.5 mM EDTA.

A Varian XL-400 spectrometer was used for the 400-MHz 2D NMR experiment. The spectrometer was equipped for dual broad-band operation, and a ^1H observe/^{15}N decouple probe. For ^1H–^{15}N experiments, bilevel decoupling was utilized with a ^{15}N 90° pulse width of 50 μs and $\gamma H_2/2\pi$ = 2000 Hz for ^{15}N decoupling during acquisition. The ^1H transmitter was attenuated by 3 dB, resulting in a 90° pulse width of 28 μs. ^{15}N chemical shifts were referenced to the frequency of a saturated ammonium nitrate solution, δ = 20.68 (Levy & Lichter, 1979). ^1H–^{15}N multiple quantum filtered ^1H NMR spectra were obtained on a home-built 500-MHz NMR spectrometer as previously described (Roy et al., 1982). The refocused, FES, version of the ^1H–^{15}N multiple quantum pulse sequence was used for 2D chemical shift correlation and ^1H–^{15}N multiple quantum filtered experiments (Griffey et al., 1985; Bax et al., 1983). Pulse and receiver phases were cycled to produce pure absorption phase spectra (States et al., 1983). The modified "1-2-1" solvent suppression sequence 1-Δ-1-τ-1-Δ-1, was used for both the 180° and 90° ^1H pulses in the 400-MHz experiment (Starcuk & Sklenar, 1983). Each of the "1" pulses was of equal phase and length. The τ delay was 10 μs. The interpulse delay Δ was approximately $1/2\Delta\omega$, where $\Delta\omega$ is the difference between the frequencies of the proton transmitter at 12.2 ppm and the water signal at 4.8 ppm. The value of Δ was adjusted for maximal suppression of water. Modified "JR" solvent suppression pulses were used

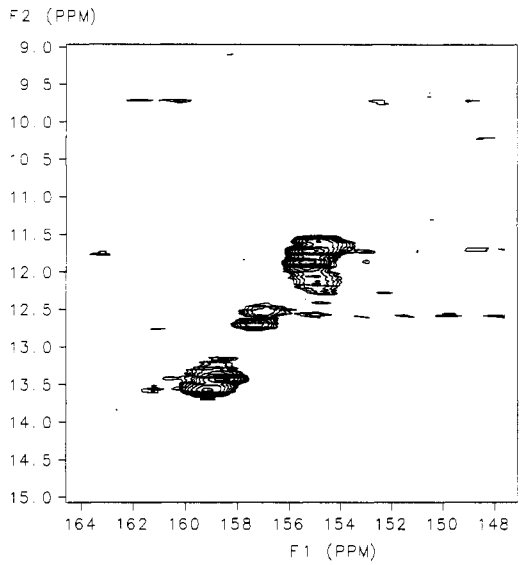

FIGURE 2: ^1H–^{15}N 2D FES spectrum at 400 MHz of [3-^{15}N]-uridine-labeled *E. coli* 5S RNA. The spectrum was acquired at 30 °C. 3000 transients were acquired for each t_1 increment. Two data sets of 64 t_1 increments were transformed as a hypercomplex pair. 2048 points were acquired in t_2, and the t_1 dimension was zero-filled to 256 points prior to transformation.

for ^1H excitation at 500 MHz (Roy et al., 1982). The interpulse delay was $1/4\Delta\omega$ for the 90° pulse and $1/2\Delta\omega$ for the 180° pulse. These values were also optimized as described for the 1-2-1 sequence.

RESULTS

^1H–^{15}N multiple quantum filtered ^1H NMR spectra of 5S RNA specifically labeled at N3 of all of the uridine residues are shown in Figure 1. As seen in the spectra, the major features persist from 13 to 28 °C over a fairly broad range of temperatures. As expected for a molecule this size, the line widths did not narrow noticeably on warming to 28 °C. Five resolved resonances of good intensity, which have chemical shifts in the range expected for uridine imino protons in G-U base pairs (Griffey et al., 1982), were seen between 11.0 and 12.2 ppm. Intense resonances were also seen at 13.4 and 13.6 ppm. The ^1H chemical shifts for these signals are in the range expected for uridine imino protons in Watson–Crick A–U interactions in double-stranded RNA (Robillard & Reid, 1979). Both sets of resonances had full intensity over the temperature range studied, indicating that they were in a stable portion of the molecule protected from exchange with solvent (Reid, 1981). Additional resonances of lower intensity were observed at 13.8 and 10.6 ppm, along with unresolved peaks of low intensity near 12.5 ppm. The chemical shift of the resonance at 13.8 ppm is characteristic of a uridine imino proton in an A-U base pair, whereas the resonance at 10.6 ppm is in the region where non-base-paired imino protons are found (Robillard & Reid, 1979; Griffey et al., 1985). Typically, signals in this region arise from protons hydrogen bonded to sugar hydroxyls, phosphate oxygens, or tightly coordinated structural water molecules.

The ^1H–^{15}N 2D FES spectrum shown in Figure 2 was acquired at 30 °C and showed nine resolved peaks for ^1H–^{15}N units in labeled 5S RNA. The ^1H and ^{15}N chemical shifts for each of the resolved peaks are listed in Table I. Five peaks were seen with ^1H chemical shifts between 11.0 and 12.2 ppm. The ^{15}N chemical shifts of these peaks were clustered within a 1 ppm range centered at 155 ppm and were similar to those for G-U base pairs found in tRNA (Griffey et al., 1983a,b).

Table I: ^1H and ^{15}N Chemical Shifts for *E. coli* 5S RNA

^1H ppm	^{15}N ppm	assignment
10.6		
11.6	154.8	G-U
11.7	155.0	G-U
11.8	155.5	G-U
12.1	155.0	G-U
12.2	155.0	G-U
12.6	156.8	A-U
12.8	157.3	A-U
13.4	158.5	A-U
13.6	159.2	A-U
13.8		A-U

Two strong peaks were observed with $^1H/^{15}N$ chemical shifts at 13.4/158.5 and 13.6/159.2 ppm which were assigned to A-U base pairs.

The 2D FES spectrum also had well-defined peaks of somewhat lower intensity at 12.6/156.8 and 12.8/157.3 ppm. The ^1H shifts are between those typically observed for uridine imino signals in AU and GU pairs. In some cases, an imino proton resonance in an AU pair at the end of a helix is shifted upfield by approximately 1 ppm into the region near 12.5 ppm (Griffey et al., 1983a). The upfield shift may, in part, result from distortions of normal helix geometry which weaken the hydrogen bonds. Typically, terminal base pairs are susceptible to fraying, and the intensity of the imino resonances is reduced because of exchange with solvent (Griffey et al., 1983a; Griffey & Redfield, 1987; Hyde & Reid, 1985).

Discussion

Optimal pairing of complementary bases results in the secondary model initially proposed by Fox and Woese (1975) and subsequently refined by Gewirth and Moore (1987) shown in Figure 3. This structure agrees with most of the studies published to date, including recent NMR experiments (Gewirth et al., 1987; Kime & Moore, 1983a). ^1H NOEs between bases in adjacent pairs gave nearest-neighbor patterns consistent with the sequences of helical regions in the proposed model (Kime & Moore, 1983a), and studies of fragments also support the structure, although some of the predicted secondary interactions are missing.

Our results with native 5S RNA are in agreement with the most recent secondary structure. Most notably, five resonances that would be expected for G-U base pairs imbedded in helical regions (G9U111, G18U65, U22G60, U80G96, and G81U95) were present in ^1H–^{15}N multiple quantum filtered and ^1H–^{15}N 2D FES spectra. A sixth possible pair, U74G102, is located at the end of a short helix containing an AG mismatch. One expects that this interaction is less stable than the others and, if present, was not seen, presumably because of rapid exchange. Previous studies tentatively identified only four G-U interactions (Gewirth et al., 1987; Kime & Moore, 1983b; Leontis & Moore, 1986a).

The five G-U resonances in our sample arise from stable interactions as evidenced by the intenstiy of the signals in the 2D spectrum at 30 °C. In contrast, Moore and co-workers (Gewirth et al., 1987) found only the two GU imino protons, assigned to U80G96 and G81U95, at 30 °C in a reconstituted fragment composed of bases 1–11 and 71–120 which was expected to contain three G-U base pairs. A third GU resonance, thought to be G9U111, was only seen at 10 °C. It is reasonable that the G-U pair near the deletion in the fragment would be more susceptible to exchange, and it is also possible that the failure to detect the predicted number of G-U base pairs in their fragments is a result of increased exchange due to helix fraying.

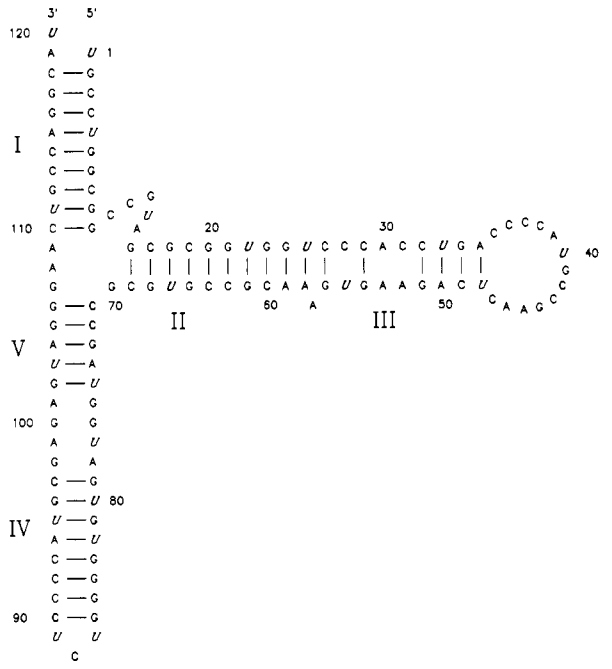

FIGURE 3: Consensus secondary structure of ribosomal 5S RNA from *E. coli* (Fox & Woese, 1975; Gewirth & Moore, 1987).

Five peaks attributable to A-U interactions were identified. The two intense resonances with $^1H/^{15}N$ chemical shifts at 13.4/158.5 and 13.6/159.2 ppm are typical of Watson–Crick base pairs (Griffey et al., 1983a). The resonances are most reasonably assigned to A-U base pairs imbedded in helical regions and may contain unresolved peaks. The most likely candidates are those flanked by G-C pairs (U5A115, U33A51, U25G28, and U82A95). Another peak tentatively assigned to an A-U interaction was found with a ^1H chemical shift of 13.8 ppm. The signal is weak and did not appear in the ^1H–^{15}N 2D FES spectrum (Figure 2).

Two other A-U resonances had slightly atypical chemical shifts. ^1H chemical shifts at 12.6 and 12.8 ppm were upfield from the range for "normal" A-U base pairs and are located in the region where most G-C imino protons resonate. Two uridine-related peaks near 12.5 ppm (Gewirth et al., 1987) were also identified in reconstituted 5S fragments containing a labeled and an unlabeled strand. Resonances with similar chemical shifts in tRNA have been attributed to base pairs at helix termini (Griffey et al., 1983a; Griffey & Redfield, 1987) and often have decreased intensity in ^1H–^{15}N multiple quantum experiments because of exchange with solvent. Moore and co-workers (Gewirth et al., 1987) also assigned the two peaks with chemical shifts near 12.5 ppm to base pairs at the end of helices in 5S RNA fragments, and our data indicate that the interactions are retained in the intact molecule as well.

An additional weak signal was detected at 10.6 ppm which was not observed in the ^1H–^{15}N 2D FES spectrum. This resonance is not associated with an A-U or G-U interaction and is most likely from an imino proton hydrogen bonded to a phosphate oxygen, a ribose oxygen, or a bridging water molecule (Davis et al., 1986).

It is important to note that the model shown in Figure 3 can be altered significantly and still be consistent with the observed NMR data. For example, the stem and helix composed of bases 16–68 have been depicted in several different arrangements which accommodate results from fragment studies (Leontis & Moore, 1986a), and most of the structures are consistent with our observations. Regardless of how this

region is arranged, one is presented with several unpaired regions or mismatches, and the total assignment of this part of the 5S RNA molecule may be further impeded if several base pairing arrangements are in equilibrium (Leontis & Moore, 1986).

In summary, many of the secondary structural features of 5S RNA predicted by the Moore model (Gewirth & Moore, 1987) shown in Figure 3 were found by $^{1}H-^{15}N$ 2D NMR. These include resonances for five stable G-U pairs located within helical regions and two peaks typical of A-U pairs at the end of a helix. Except for a resonance at 10.6 ppm, there are no peaks from uridine that can be clearly attributed to tertiary interactions. This is in contrast to tRNAs where resonances are seen for imino protons in uridine and modified uridines which form tertiary hydrogen bonds to A (reversed Hoogsteen), phosphate oxygens, and tightly bound waters (Robillard & Reid, 1979; Griffey et al., 1983a; Davis et al., 1986).

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Registry No. G, 73-40-5; U, 66-22-8; A, 73-24-5.

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