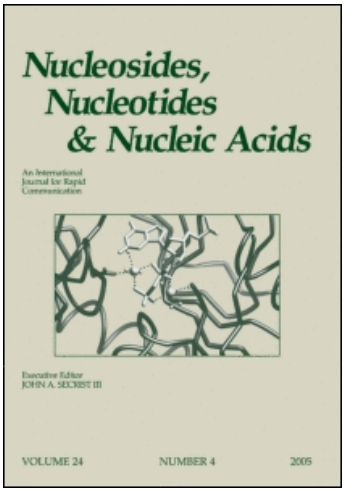

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Structural Studies of 2-Thiouridine in RNA

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STRUCTURAL STUDIES OF 2-THIOURIDINE IN RNA

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Abstract: A pentamer RNA sequence , Gs^{2}UUUC, and a s^{2}U containing 14-mer RNA tetraloop hairpin were synthesized and characterized by NMR and by UV melting studies. These oligonucleotides were used as models to understand the effect of 2-thiouridine substitution on RNA structure and the potential for stabilization of tRNA codon-anticodon interactions through s^{2}U-34 modification.The magnitude of the effect of s^{2}U in our model system is comparable to the 20 ^{o}C stabilization reported for 2-thiolation in a codon-anticodon model system composed of two tRNAs with complementary anticodon sequences.

Introduction

2-Thiouridine (s^{2}U) and its 5-modified derivatives are commonly found at the wobble position 34 in the anticodon of tRNAs[1]. For s^{2}U itself, it has been shown that sulfur substitution substantially stabilizes the 3'-endo sugar conformation at the nucleoside and dinucleotide level[2,3]. As part of a project to investigate the influence of RNA modification on codon-anticodon interactions, we synthesized the RNA sequence Gs^{2}UUUC as a minimal anticodon model. The central pyrimidines form the anticodon trinucleotide of tRNA^{Lys} and the GCs were added to impart reasonable stability for a duplex formed with the complementary strand $G_mA_mA_mA_mC_m$. The 2'-O-methyl complement was chosen to add stability and reduce aggregation at NMR concentrations[4].

In order to extend the pentamer duplex model to a system that better mimics the tRNA codon-anticodon interaction, an RNA tetraloop hairpin model was investigated. The tetraloop model shown in Figure 2 contains the UUU•AAA base-pairing interaction of the tRNA^{Lys} anticodon with only a single flanking GC. The 3' end adopts a single stranded stacked conformation similar to the 3' side of the anticodon loop of tRNA. UV monitored thermal melting of the hairpin and temperature dependent NMR of the imino protons involved in base-pairing were used to determine the effect of s^{2}U on RNA stability.

Methods

Synthesis and purification of s^2U containing RNA. The protected phosphoramidite of s^2U was synthesized as described previously and incorporated into RNA oligonucleotides using the modified tert-butyl hydroperoxide oxidation protocol[5]. No protecting groups on the base were required. Deprotection and purification of the product oligonucleotides were carried out following standard protocols for RNA[6].

UV Melting Curves and NMR. Measurements of RNA duplex stability were made by UV absorbance vs temperature studies at 260 nm in a 10 mm cell for the tetraloop and at 270 or 280 nm in a 1 mm cell for the duplexes. Samples were dissolved in 25 mM phosphate buffer, pH 7.0, containing either 0.1 M or 1M NaCl and 0.05 mM EDTA. NMR experiments were done on a Varian Unity 500 in the 0.1 M NaCl buffer.

Results and Discussion

Figure 1 shows the UV trace for the RNA pentamer duplexes and the RNA tetraloop models containing either U or s^2U at tRNA position 34. For the pentamer duplex system, replacement of U with s^2U results in a 11.7 °C increase in the T_m from 19.0 to 30.7 °C. The comparison of the hairpin tetraloop shows a comparable increase in the T_m for s^2U substitution where the T_m increases by 12.8 °C. This dramatic increase in RNA stability has been previously reported for two tRNAs with complementary anticodons[7]. Our results for the tetraloop hairpin and for the s^2U containing pentamer duplexes indicate that Grosjean's T-jump results may be typical of what one might expect for s^2U stabilization of codon-anticodon recognition during protein synthesis.

NMR Spectra of Imino Protons in the RNA Hairpin. Figure 2 shows the downfield region of the NMR spectrum of the tetraloop hairpins as a function of temperature. The spectra were acquired using a binomial read pulse which is non-saturating[8]. A comparison of the two sets of spectra show that the s^2U modified hairpin is substantially stabilized compared to the unmodified molecule. All of the imino resonances in the s^2U hairpin persist at higher temperature than in the unmodified system and they have narrower linewidths indicating increased stability and slower exchange with solvent water[9]. The imino resonance of s^2U-10 is shifted downfield compared to U-10 due to its increased acidity and stronger H-bonding. The base pairs adjacent to U10 are also affected with G9 showing the greatest degree of stabilization while U11 is also substantially stabilized even though the imino resonance is shifted upfield upon s^2U substitution at the neighboring site.

In conclusion, substitution of U with s^2U results in dramatic stabilization of RNA. The stabilization is likely due to a combination of properties which result when the C2-carbonyl of uridine is replaced by thiocarbonyl. The pKa is lowered by approximately 0.5 pH units resulting in a stronger hydrogen bond,[10] the 3'-endo sugar conformation is stabilized

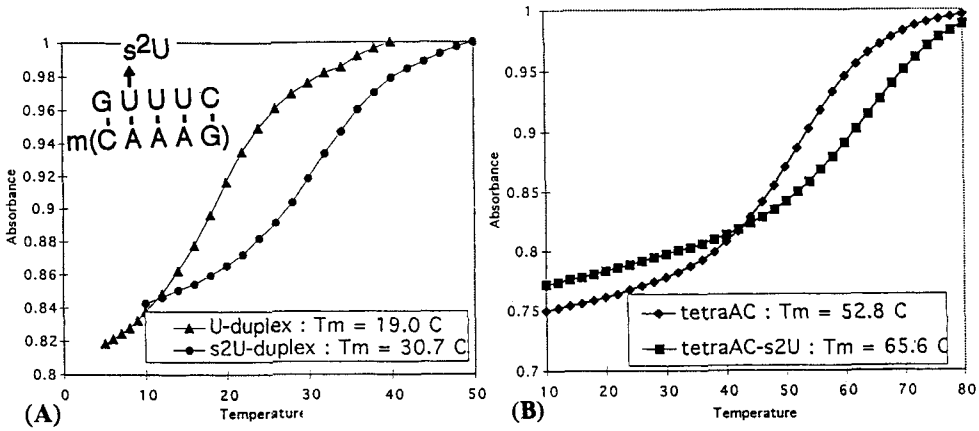

FIG. 1. (**A**) UV T_m Curves for U duplex at 270 nm and s^2U duplex at 280 nm at 1M NaCl, RNA concentrations are approximately 0.1 mM; (**B**) T_m Curves for U and s^2U RNA tetraloops at 260 nm, RNA concentrations are approximately .005 mM.

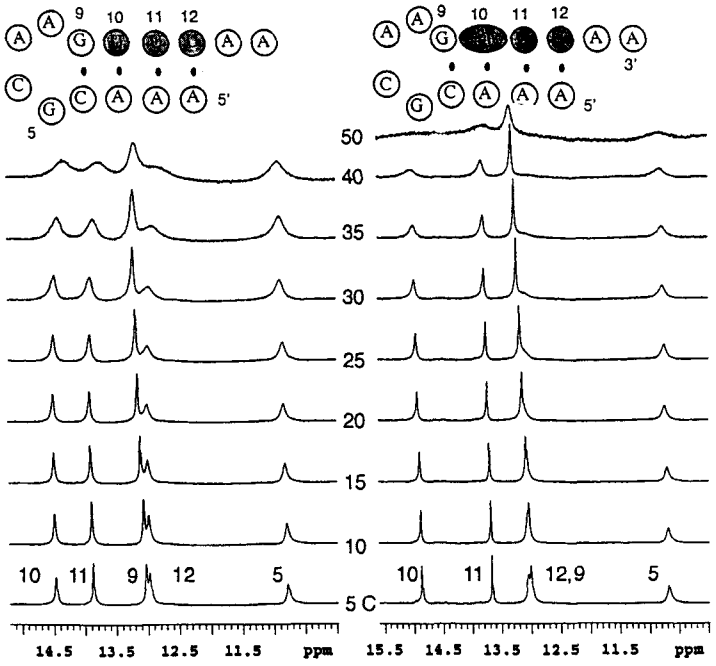

FIG. 2. NMR imino region as function of temperature for RNA tetraloop hairpins.

through a steric interaction with the sugar 2'-OH that promotes an A-form geometry,[2] and finally the s^2U base should stack better due to the highly polarizable sulfur group. NMR studies to determine the three-dimensional structure of the tetraloop hairpin are in progress in our laboratory and should help us to better describe the structural interactions that result in such dramatic duplex stabilization.

Acknowledgment

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