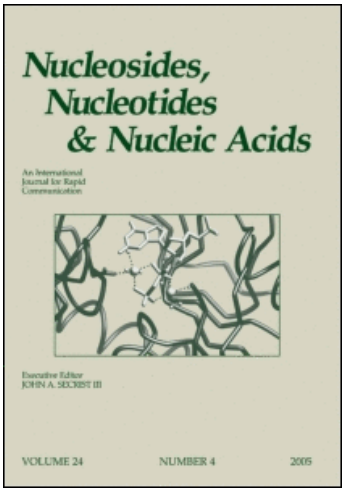

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Nucleoside Modifications Affect the Structure and Stability of the Anticodon of tRNA^{Lys,3}

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NUCLEOSIDE MODIFICATIONS AFFECT THE STRUCTURE AND STABILITY OF THE ANTICODON OF $tRNA^{Lys,3}$

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Abstract: NMR spectroscopy was used to determine the solution structures of RNA oligonucleotides comprising the anticodon domain of $tRNA^{Lys,3}$. The structural effects of the pseudouridine modification at position 39 were investigated and are well correlated with changes in thermodynamic parameters. The loop conformation differs from that seen in $tRNA^{Phe}$ and provides an explanation of the critical role of modification in this tRNA.

Introduction

In addition to the normal RNA and protein interactions important for a tRNA molecule in protein synthesis, human $tRNA^{Lys,3}$, is the primer for HIV reverse transcriptase and plays a fundamental role in the HIV-1 virus life cycle. The extensively modified anticodon of $tRNA^{Lys,3}$ has been shown to bind HIV-1 reverse transcriptase in a modification dependent manner[1,2]. In vivo and in vitro studies have shown that efficient elongation during reverse transcription requires a modification dependent interaction between an A-rich loop (bases 162-167) in the HIV-1 genome and the modified anticodon of $tRNA^{Lys,3}$ [3].

Unlike $tRNA^{Phe}$, the unmodified anticodon stem-loop for $tRNA^{Lys,3}$ does not bind to programmed ribosomes[4] suggesting that partial or full modification is required for any biological activity and that modification in this tRNA may lead to a major structural reorientation. Our initial step toward understanding the structure-function relationships in $tRNA^{Lys,3}$ was to structurally characterize the unmodified anticodon and the effects of the simple modification, pseudouridine. We have used NMR to determine the high resolution structures of an unmodified 17-mer RNA oligonucleotide comprising the anticodon stem-loop sequence of human $tRNA^{Lys,3}$ and an 17-mer oligonucleotide that has the naturally occurring pseudouridine at position 39.

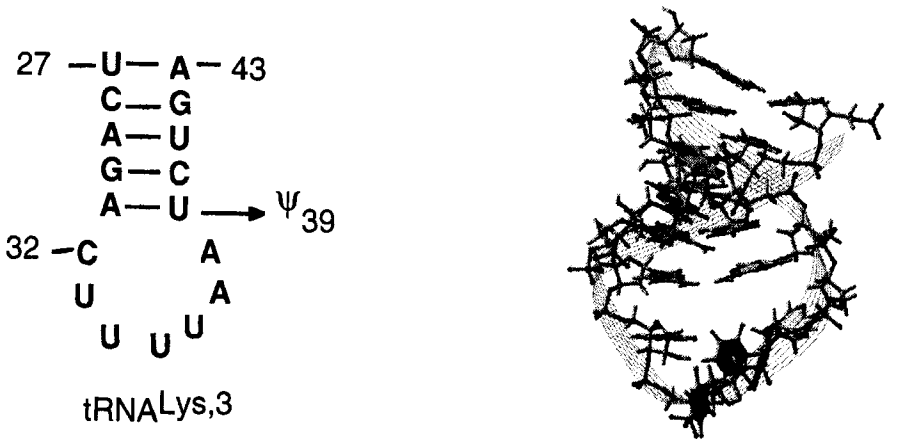

FIG. 1. **Left:** Secondary Structure of the $tRNA^{Lys,3}$ anticodon domain, and **Right:** NMR structure.

Results and Discussion

^{1}H-^{13}C HMQC spectra for $tRNA^{Lys,3}$-ψ39 confirms formation of an A^{+}-C base pair with protonation at N1 as evidenced by a 6 ppm change in ^{13}C chemical shift for the C2 of A38 upon decreasing the pH from 7.4 to 5.4 [5]. The three anticodon bases (U34-U36) have significant 2'-endo character as indicated by large sugar coupling constants and strong intranucleotide H6-H2' relative to H6-H3' NOEs. In addition to the dynamic character of the anticodon residues, the NOE data indicates that there is no base stacking at the U34-U35 step. The ^{31}P NMR spectrum also provides evidence that the loop is dynamic rather than consisting of two rigid 5' and 3' domains as seen for $tRNA^{Phe}$. All ^{31}P chemical shifts are within a narrow 0.88 ppm range that is typical of an RNA molecule with no sharp turns in the phosphodiester backbone. In contrast, the ^{31}P NMR spectrum of yeast $tRNA_i^{Met}$ has a single downfield resonance for the phosphate between residues 33 and 34 shifted 1 ppm downfield from the stem region phosphates[6].

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