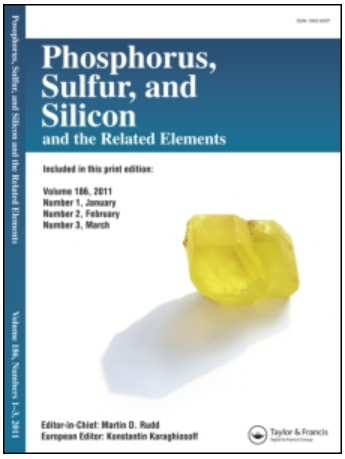

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NMR Spectroscopic Investigations of Phosphorothioate Containing RNAS

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Chirally pure *pro*R(p) phosphorothioates have been introduced 5′ to each adenine residue in the conserved core of the RNA binding site for ribosomal protein S8. These backbone modifications prevent Mg^{2+} coordination and assembly of secondary and tertiary structure elements in the core. Cd^{2+} was found to restore most of the spectral properties characteristic of the native Mg^{2+}-bound RNA binding site presumably through coordination to one or more phosphorothioate groups.

Keywords: phosphorothioate; RNA structure; Mg^{2+} binding; cadmium

INTRODUCTION

Phosphorothioates are frequently employed to probe the catalytic activity of ribonucleic acid (RNA) enzymes and to stereospecifically identify regions of the RNA phosphate backbone important for metal ion and protein binding.[1] Sulfur substitution of the phosphoryl oxygen can perturb ligand binding by disrupting hydrogen bond interactions with a protein or by eliminating the ability of a particular position to coordinate an essential metal ion. The phosphorothioate is not expected to introduce significant structural changes and indeed studies of stereospecific sulfur substitutions in DNA oligonucleotides indicate that phosphorothioate modification does not affect the global molecular structure.[2,3]

RESULTS

We are interested in addressing two questions. First, can the modified spectral properties of the phosphorothioates be used to help characterize divalent metal ion binding sites in RNA molecules? Second, what, if any, are the structural consequences of incorporating phosphorothioates into the RNA backbone? To address these questions,

we have used the RNA binding site for *E. coli* ribosomal protein S8 (Figure 1). Phosphorothioate containing RNA molecules were prepared using T7 RNA polymerase by substituting 5'–α_SATP for 5'–ATP in the transcription reaction. The *pro*S(p) isomer of 5'–α_SATP was synthesized enzymatically using adenylate and pyruvate kinases.[4] Since the T7 RNA polymerase incorporates the 5'–α_SATP with inversion of configuration, each phosphorothioate in the purified RNA molecule is the *pro*R(p) configuration (Figure 1C) and a phosphorothioate is located immediately 5' to each adenine nucleotide.

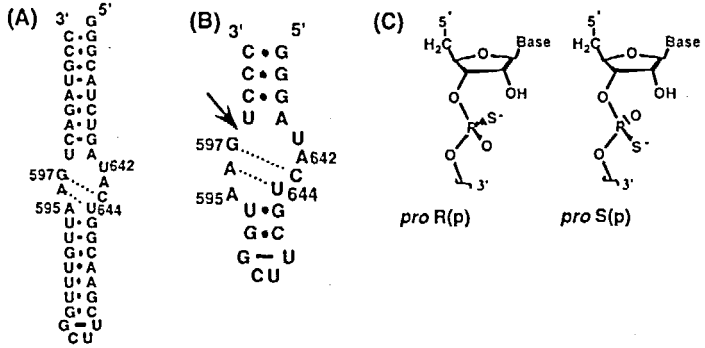

FIGURE 1. (A) RNA I, RNA binding site for *E. coli* ribosomal protein S8, (B) RNA II, hairpin containing the phylogenetically conserved core of the RNA binding site for S8. Dotted lines in B indicate base pairs formed upon Mg^{2+} binding. The sulfur substituted phosphorus atoms correspond to the positions 5' to each of the adenine nucleotides. (C) The *pro* R(p) and *pro* S(p) stereoisomers for a non-bridging phosphorothioate linkage. The *pro*R(p) configuration points toward the major groove of the RNA helix and most often is the stereoisomer that interferes with protein and metal ion binding.

The binding site for S8 is composed of a helix interrupted by an asymmetric core of nine phylogenetically conserved nucleotides (Figure 1A). NMR studies focusing on the core were used to identify a Mg^{2+} binding pocket and indicate the formation of a G•C base pair and an A-(A•U) base triple upon Mg^{2+} binding.[5] The binding of S8 to the RNA requires the presence of Mg^{2+}, but it is not clear if the function of Mg^{2+} is to stabilize a specific RNA fold or simply to bridge protein-RNA interactions.

Phosphorothioate substitution 5' to each adenine in the S8-RNA binding site appears to inhibit Mg^{2+} binding in the core while Mg^{2+} binding at other sites is maintained. Figure 2 shows the imino region of the substituted and unsubstituted forms

of RNA II as a function of Mg^{2+} concentration. The shift of the G597 N1-H resonance and the presence of U644 N3-H resonance in spectra of the unsubstituted RNA are indicative of Mg^{2+}.[6] The substituted RNA shows no indication of the U644 N3-H resonance and only a broadened G597 N1-H resonance, even at 20 mM Mg^{2+} (not shown). G645 participates in a G•U wobble base pair that also binds Mg^{2+}. The upfield shift of the G645 N1-H resonance from 10.5 to 10.0 ppm is characteristic of Mg^{2+} binding at this site. Although the G645 N1-H resonance is broad, none of the phosphorothioates disrupt Mg^{2+} binding at the G•U base pair. This is consistent with expectations for Mg^{2+} binding at this site since coordination occurs in the major groove and should not involve any of the phosphorothioate positions.[7]

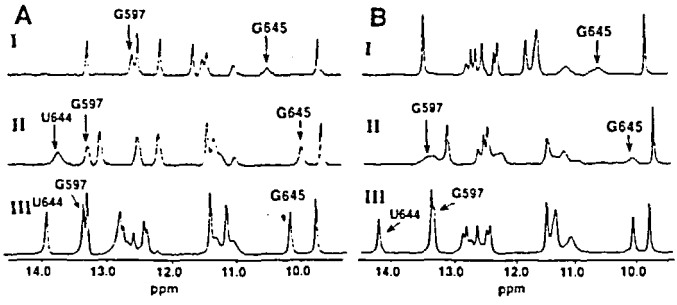

FIGURE 2. Imino ^1H region of (A) the phosphate and (B) the phosphorothioate forms of the RNA II hairpin (≈1.0 mM) and containing (I) no divalent metal ions, (II) 5 mM Mg^{2+}, and (III) 10 mM Cd^{2+}. The spectra were recorded at 15 °C and the RNA samples were annealed once before acquisition.

The phosphorothioate anion binds the Mg^{2+} poorly but has the potential to interact strongly with the "soft" metal Cd^{2+} and thus promote folding of the core. Figure 2 shows that Cd^{2+} binds to both the substituted and unsubstituted forms of RNA II. Although Cd^{2+} does not reproduce the effect of Mg^{2+} at all sites in RNA II, the metal ion sites in the core and at the G•U base pair appear to accommodate Cd^{2+} and Mg^{2+} in similar fashions. Cd^{2+} rescue of the imino spectrum of the phosphorothioate RNA indicates that at least one of the four substituted phosphoryl oxygens participates in Mg^{2+} coordination.

The ^{31}P NMR spectrum provides additional evidence for destabilization of the core and inhibition of Mg^{2+} binding by the phosphorothioate substitution. Two of the four ^{31}P resonances corresponding to phosphorothioate positions are broadened by

chemical exchange in the absence of Mg^{2+}. In the presence of Mg^{2+}, one of those two resonances shifts down field and sharpens while the other remains broad. Only minor changes are observed in the cluster of phosphate ^{31}P resonances with addition of Mg^{2+}. Comparison of the phosphorothioate and all phosphate ^{31}P spectra also suggests that phosphorothioate substitution may locally perturb the structure of the phosphate backbone in the Mg^{2+} free state. The ^{31}P chemical shifts differ for positions adjacent to the core (U594, C599, G639, and G645), but those distal to the core are unchanged.

The affinity of phosphorothioate substituted RNA I for protein S8 was tested using filter binding assays. The phosphorothioate substitution weakens binding only slightly (< 2x) relative to all phosphate RNA I which has a $K_a \approx 1.5 \times 10^{7}\ M^{-1}$ (R.A. Zimmermann, personal communication). Complex formation was also tested in the presence of Cd^{2+} and was found to inhibit complex formation between S8 and all phosphate RNA I. The filter binding assays together with the divalent metal ion binding data suggest that Mg^{2+} stabilization of 2° and 3° structure elements in the core is not required for protein binding.

Thus, phosphorothioate substitutions described above indicate that the *pro*R(p) oxygen 5' to at least one adenine in the core participates directly in Mg^{2+} coordination. Cd^{2+} rescue of the imino spectrum (which is characteristic of the core secondary structure) negates the alternative possibility that the phosphorothioate groups introduce gross structural changes that prevent folding of the core even in the presence of Mg^{2+}.

Acknowledgments

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References

[1] O. Heidenreich, W. Pieken. and F. Eckstein, *FASEB J.*, **7,** 90 (1993).
[2] C. González. W. Stec, A. Kobylanska, R. I. Hogrefe, M. Reynolds, and T. L. James, *Biochemistry,* **33,** 11062 (1994).
[3] W.B.T. Cruse, S.A. Salisbury, T. Brown. F. Eckstein, and 0. Kennard, *J. Mol. Biol.* **192,** 891 (1991).
[4] F. Eckstein and R.S. Goody, *Biochemistry* **15,** 1685 (1976).
[5] K. Kalurachchi, K. Uma. R.A. Zimmermann, and E.P. Nikonowicz, *Proc. Natl. Acad. Sci. USA,* **94,** 2139 (1997).
[6] K. Kalurachchi, K. and E.P. Nikonowicz, *J. Mol. Biol.*, **280,** 639 (1998).
[7] F.H.-T. Allain and G. Varani, *Nucleic Acids Res.*, **23,** 341 (1995).