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The guanosine binding mechanism of the *Tetrahymena* group I intron

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ABSTRACT

The *Tetrahymena* group I intron catalyzes self-splicing through two consecutive transesterification reactions, using a single guanosine-binding site (GBS). In this study, we constructed a model RNA that contains the GBS and a conserved guanosine nucleotide at the 3'-terminus of the intron (ωG). We determined by NMR the solution structure of this model RNA, and revealed the guanosine binding mechanism of the group I intron. The G22 residue, corresponding to ωG, participates in a base triple, G22••G3•C12, hydrogen-bonding to the major groove edge of the Watson-Crick G3•C12 pair. The G22 residue also interacts with A2, which is semi-conserved in all sequenced group I introns.

INTRODUCTION

In the self-splicing reaction performed by group I introns, two consecutive transesterification reactions involve the same guanosine-binding site (GBS) (1). In the first step of the reaction, an exogenous guanosine nucleoside is bound to GBS. Then, the 3'-OH of this guanosine nucleoside attacks the phosphate group at the 5' splice site. In the second step of the reaction, a conserved guanosine at the 3'-terminus of the intron (ωG) is bound to GBS (2, 3). Then, the 3'-hydroxyl group of the 5' exon attacks the phosphate group at the 3' splice site. The second transesterification reaction is chemically equivalent to the reverse of that of the first step.

These interactions between the guanosine moieties and GBS have been extensively investigated. The 5.0 Å crystal structure of the core region of the *Tetrahymena* group I intron was recently determined (4). Although the crystal structure revealed the geometry of the helices and the approximate position of each residue, the interactions between the ωG and the GBS have not been understood at atomic resolution. We previously designed a 31-mer model RNA (P7/P9.0/G), which consists of the P7 stem with GBS, the P9.0 stem, and the ωG residue from the *Tetrahymena* group I intron (5). The model RNA retains the same specific interactions between ωG and GBS as the intact group I intron. Thus, in this study, we designed a 22-mer model RNA (Fig. 1), which is shorter than the P7/P9.0/G RNA, and determined its tertiary structure in solution by NMR.

RESULTS AND DISCUSSION

In order to identify the interactions between ωG and GBS, we prepared the model RNA with $^{13}C/^{15}N$-labeled guanosine at position 22 (6). The amino and imino proton resonances due to G22 were observed in the HMQC spectrum of this labeled RNA in the condition of pH 6.5. The observation of these resonances indicates that these protons are involved in hydrogen bonds. NOEs from the imino proton of G22 to the protons of the G3•C12 base pair indicate the formation of a hydrogen bond between the imino proton of G22 and O6 of G3, as previously proposed (2).

The resonance due to the imino proton of G13 was not observed at pH 6.5. This result indicates that this imino proton exchanges rapidly with the solvent, H_2O. A downfield shifted resonance of this imino proton, however, was observed at pH 5.5, suggesting that a hydrogen bond is formed between C1 and G13. Furthermore, the "sequential" NOEs of C1 and G13 indicate that these bases are stacked between P7 stem and P9.0 stem. By mutational and phylogenetic analyses of the group I introns, the Watson-Crick base pairing that corresponds to C1•G13 have been shown to be conserved (7). These results suggest that C1 and G13 are base paired in the Watson-Crick manner. The sugar puckering of G22 was determined to be C2'-*endo* by measuring ^3J coupling constants.

The solution structure of the model RNA (Fig. 2) was calculated with the constraints determined by NMR. In the structures calculated with the constraints of the Watson-Crick base pair between C1 and G13, C1 is stacked on G22.

In these structures, an upfield-shifted resonance originated from H5 of C1 (4.63 ppm) can be attributed to the ring-current effect of the purine ring of G22. Although G13 stacks on C12, C1 does not stack on G3 but stacks on G22. This unusual structure of GBS bends the global structure of the model RNA.

In the majority of the calculated structures that did not violate the distance constraints more than 0.2 Å, G22 is positioned in the major groove of G3•C12 base pair. These structures indicate that G22 forms base triple with G3•C12 base pair, as previously proposed (2).

A2 is positioned under G22. And, the structure of the model RNA indicated the existence of two hydrogen bonds between G22 and A2. One is a hydrogen bond between the amino proton of A2 and O2' of G22. Another is a hydrogen bond between N7 of A2 and 2'-OH of G22. Although it is known that 2'-OH of guanosine is critical for self-splicing reactions (8), this 2'-OH is not necessary for the guanosine binding (3, 9, 10). The semi-conserved A2 residue does not contribute to the base specificity (11, 12, 13). Profenno *et al.* suggested that 2'-OH of G22 is required for folding of the proper ternary structure (14). The structure with hydrogen bonds between the sugar moiety of G22 and the base moiety of A2 could be the active conformation predicted by Profenno *et al.*. The conformation of G22 is the *anti* form around the glycosidic bond, as predicted (15).

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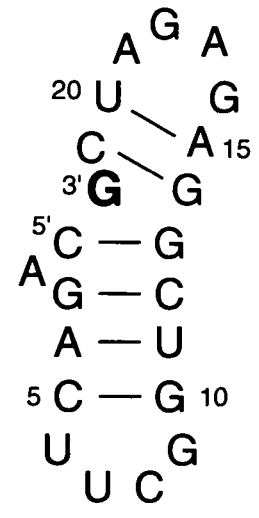

Fig. 1 Secondary structure of the model RNA

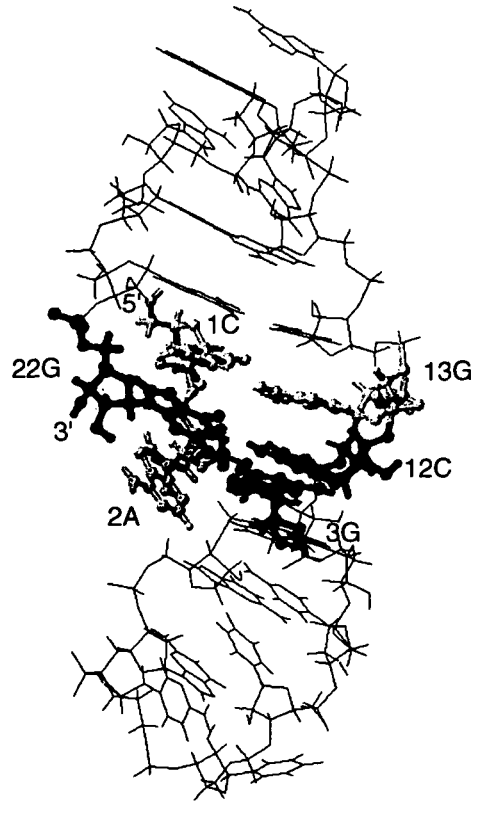

Fig. 2 The tertiary structure of the model RNA