Mutation analysis of the base-pair connecting two functional modules in the DSL ribozyme

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ABSTRACT

The class DSL ribozyme is one of artificial RNA enzymes generated by module-based molecular design. In the structure of this ribozyme, two most important functional modules are connected by a U-A base-pair. We have examined the possible importance of this base-pair by site-directed mutation experiments using the DSL-1S ribozyme and its derivative possessing altered modular organization. The analysis indicated that the DSL-1S ribozyme preferred U-A pair at the positions whereas the derivative preferred A-U pair.

INTRODUCTION

Recent progress of structural biology and bioinformatics has revealed that many proteins and functional RNAs possess modular organization in which structurally defined modules are assembled to constitute highly complex structures. If such modular organization of natural proteins and RNAs is regarded as a principle to generate functional biomacromolecules, a module-based design should be an attractive approach in creating artificial proteins and RNAs. Indeed, several non-natural proteins and RNAs have been successfully designed by assembling defined modular units and examined their functions.¹⁻⁴

The class DSL ribozyme is an artificial RNA enzyme that has been generated through the module-based approach.⁵ Catalytic ability of this ribozyme, which 10³-fold accelerates the template-directed RNA-RNA ligation, depends on its modular structure.² The DSL ribozyme consists of four RNA modules that are substrate and catalytic modules, and two clamp modules that appropriately assemble the substrate and catalytic modules (Fig. 1). Owing to such modular architecture, the DSL ribozyme is suitable for modular engineering through addition, deletion or replacement of functional modules.²⁻⁴

In the modular structure of the DSL ribozyme, the two most important structural units, which are the catalytic module and 11ntR motif of the upper clamp module, are connected by a U-A base-pair between the positions 55 and 75 (Fig. 1). In this study, we have investigated the functional importance of the U55-A75 base-pair because its tolerance to a base-pair substitution is a factor to be considered when we apply modular engineering to the catalytic module or the 11ntR motif.

MATERIALS AND METHODS

Activity assays were performed essentially as described previously but the following modifications were introduced.² Instead of radioisotope labeling, fluorescent labeling was employed in this study. The 3' ends of ribozyme RNAs were labeled with BODIPY fluorophore and extents of the ligation reactions were quantified with Bio-Rad PhorosFx MolecularImager.² Reaction conditions used for the ribozyme activity assay are as follows: 30 mM Tris-Cl (pH 7.5), 50 or 200 mM MgCl₂, 30 °C, 3 μM substrate RNA, 0.5 μM ribozyme RNA.

RESULTS AND DISCUSSION

In the previous studies to elucidate structure-function relationship of the DSL ribozyme, many base-substitution experiments have been performed.²⁻⁴ However, mutation analysis of U55-A75 has not been done despite its possible importance. We substituted U55-A75 of the DSL-1S ribozyme, the most active clone of the class DSL ribozyme, with other base-pairs. The resulting mutants (termed C55G75, A55U75, G55C75) were tested for their catalytic activity in the presence of 50 mM MgCl₂ (Fig. 2). The original DSL-1S with U55-A75 pair was most active (kₐᵇₛ = 0.14 min⁻¹) and the observed rate constants of the A55U75 mutant (0.11 min⁻¹) and G55C75 mutant (0.053 min⁻¹) were
Fig. 2 Time courses of ligation reactions by the DSL-1S and its mutants.

1.3- and 2.6-fold smaller than that of the original ribozyme. In contrast to the two mutants, the C55G75 mutant was considerably less active as its $k_{\text{obs}}$ (0.013 min$^{-1}$) was 11-fold smaller than that of the DSL-1S.

To further investigate the importance of the 55-75 base-pair in modular organization of the DSL ribozyme, we performed modular engineering of the upper clamp module. This module consists of two structural motifs that are GAAA tetraloop and its specific receptor motif termed 11ntR, both of which were rationally installed in P1 and P3 regions, respectively. Based on an analogous modular engineering of tectoRNA by Jaeger et al., we designed and prepared a variant DSL possessing 11ntR in P1 region and GAAA loop in P3 region (Fig. 3).

As the resulting variant (termed invDSL) was hardly active in the presence of 50 mM Mg$^{2+}$, its activity was assayed in the presence of 200 mM Mg$^{2+}$ where the structure of the ribozyme was stabilized and the final product yield became acceptable (Fig. 4). However, $k_{\text{obs}}$ of the invDSL (0.18 hr$^{-1}$) with 200 mM Mg$^{2+}$ was still nearly 50-fold smaller than that of the original DSL with 50 mM Mg$^{2+}$, indicating that the DSL ribozyme is not highly tolerant to exchange of the loop and receptor motifs in the upper clamp module.

Employing the invDSL ribozyme, we again examined the effects of a base-pair substitution at the positions corresponding to U55 and A75 of the DSL-1S. The activities of three base-substitution mutants (invDSL-CG, invDSL-AU, and invDSL-GC) and a variant having GUGA loop (invDSL-GUGA) were examined. Inconsistent with the result from the DSL-1S ribozyme, substitution of the

Fig. 3 The upper clamp modules of the DSL-1S and invDSL ribozymes.

Fig. 4 Time courses of ligation reactions by the invDSL and its mutants.

original U-A pair with A-U pair significantly improved the reaction rate because its $k_{\text{obs}}$ (0.82 hr$^{-1}$) was 4.6-fold higher than that of the parental invDSL. The invDSL-GC mutant ($k_{\text{obs}}$ = 0.079 hr$^{-1}$) was less active than the parental invDSL and reduction of the activity (2.3-fold) was comparable to that observed between the DSL-1S ribozyme and its G55C75 mutant (2.6-fold). Substitution of the original U-A pair with a C-G pair, which severely reduced the activity of the DSL-1S, resulted in a loss of detectable activity.

CONCLUSION

Through mutation analysis of the DSL-1S and its invDSL variant, we have investigated the importance of the base-pair between the positions 55 and 75. In the DSL-1S ribozyme, the original U-A pair was most active. However, in the invDSL variant ribozyme derived from the DSL-1S by exchanging the GAAA loop and its receptor motifs, a mutant having A-U pair was more active than the parental invDSL. The result suggests the importance of the base-pair between the positions 55 and 75 in optimizing the active structure of the class DSL ribozyme.

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REFERENCES