Specific recognition of the basic N peptide by an RNA

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ABSTRACT

Artificial binding model systems with sufficient specificity are indispensable for study and understanding of living systems. Here, we report a small hairpinloop RNA that interacts with a basic peptide derived from bacteriophage lambda N protein, as a new protein-RNA binding model. The RNA was selected in vitro from a hairpinloop RNA library and recognized the target N peptide more specifically than the boxB RNA, the naturally occurring N protein binder.

INTRODUCTION

Specific and tight binding of biomolecules is requisite to strict regulation of biological systems. Although electrostatic interaction is certainly indispensable for a tight binding, nonspecific attractive force between positive charges on the amino acid side chains and negative charges on the nucleotide backbone is always accompanied by the electrostatic interaction between a basic protein and a nucleic acid. Sometimes this intrinsically nonspecific interaction is the dominant force even in a 'sequence specific' interaction.

In this study, we designed an in vitro selection procedure to decrease the energetic contribution of the electrostatic interaction in the total binding energy and to increase the contribution of hydrogen bonding and π-π stacking for obtaining an RNA that is not the most forceful binder but one with sufficient selectivity. A highly positive charged peptide (Tat peptide: RKKRRQRRR) was used as a pseudo-target molecule for the negative selection and RNA molecules with significant affinity to the pseudo-target by the nonspecific electrostatic interaction were removed from a hairpinloop RNA library with a 7 nts random region. After the negative selection, hairpinloop RNAs that specifically bound to a model peptide of lambda N protein (N peptide: MDAQTRRRRRAEKQAQWKAA) were selected. As a result, a new thermostable hairpinloop RNA motif (N binding thermo-stable RNA hairpin: NTS RNA) that binds to the N peptide was revealed after three cycles of the selection. Based on kinetic and thermodynamic data, it is apparent that the NTS RNA binds to the N peptide with superior specificity to the boxB RNA, the naturally occurring partner of the lambda N protein.

MATERIALS AND METHODS

All DNA and RNA samples were chemically synthesized by the phosphoramidite method. Peptides were synthesized with an Fmoc strategy on N-α-9-furoylmethoxy-carbonyl-super acid labile polystyrene resin, producing an amide end at the carboxy terminus. After cleavage from the resin and deblocking, the synthesized DNA, RNA and peptides were purified by HPLC on a C-18 column. For in vitro selection, immobilized target peptides were prepared on an avidin agarose support. After the selection, nucleotide sequences of the selected clones were resolved by dyeoxy termination method. RNA-peptide interaction was analyzed by surface plasmon resonance (SPR) with immobilized RNAs and free peptides. All experiments were carried out in a buffer containing 10mM Na₂HPO₄ (pH 7.0), 100mM NaCl, and 1mM Na₂EDTA at 25 °C.

RESULTS AND DISCUSSION

The peptide binding property of RNAs in the initial library before the selection (Generation 0; G0) and in the library after three cycles of the selection (G3) were examined. The library RNA was passed through the affinity columns and the amount of bound and retained RNA in the columns was determined. As shown in Figure

![Figure 1 Binding properties of the hairpinloop RNA libraries to N peptide, Tat peptide and glycine (Gly) immobilized columns. The amount of the bound RNA in the library RNA was calculated from the amount of the eluted RNA. The white and black bars indicate the G0 and Tat-G3 libraries, respectively.](https://academic.oup.com/nass/article-abstract/49/1/353/1070642/Specific-recognition-of-the-basic-N-peptide-by-an?content-type=image%2Fjpg)
1. the N peptide binding RNAs were concentrated during the selection; 45.3% of the total amount of RNAs in the Tat–G3 library was bound to the N column, whereas only 27.3% of the RNAs in the G0 library bound to the N column. On the other hand, the amount of Tat peptide binding RNAs (nonspecific binders) decreased in the Tat–G3 library from 46.0% (G0) to 38.0%. In other words, it was proved that specific binders to the N peptide were selectively concentrated in the Tat–G3 library. Similar results were obtained from the experiments that mimicked the selection procedures. After the negative selection procedure, more than 70% of RNAs in the Tat–G3 library bound to the N column, suggesting the specific binding of RNAs in the Tat–G3 library to the N peptide. No such concentration of specific binder could be seen for the library before the selection (data not shown).

The nucleotide sequences of 20 clones from the Tat–G3 library were compared with that of the natural boxB hairpinloop RNA. The sequence of the clones resembled each other and a consensus sequence with a pentaloop, 5'–GGYRRRC–3' (loop region was underlined), was revealed. This consensus sequence was distinct from the natural boxB RNA sequence, 5’–UGAARAA–3’. Only a transversion of the closing base pair from U/A to A/U leads to a drastic decrease in the binding constant of the N peptide–boxB RNA complex both in vitro and in vivo. Also, purine residues at the center of the boxB RNA are indispensable for the highly ordered folding of the hairpin structure and binding to N peptide. Consequently, the consensus sequence obtained from the Tat–G3 library should fold into a novel structure that is different from the structure of the natural boxB. Because a nucleic acid with a hairpinloop structure has complementary sequences at both its ends, it may interact with another molecule to form a duplex with an internal loop. However, the UV melting curves for two RNA samples with different concentrations, at 5 μM and 50 μM, did not change for both RNAs, and therefore the new RNA motif should fold into a hairpinloop structure as well as the boxB RNA. The structural transition of the RNA was observed in the higher temperature region above 80°C, denoting an extraordinarily stable hairpinloop structure of the RNA (N binding thermo-stable RNA hairpin: NTS RNA).

The affinity of the N peptide binding RNAs to the N peptide was evaluated by SPR. As shown in Figure 2a, the boxB RNA binds rapidly to both peptides, N (black line) and Tat (gray line). The binding constants (Ka) were calculated by the curve fitting method and it was appeared that the Ka values to N peptide and Tat peptide of boxB RNA differ only within one order of magnitude. On the other hand, as shown in Figure 2b, the response unit (RU) of NTS binding to N peptide increased more slowly than in the case of the boxB RNA. The Ka value of the NTS binding is three times smaller than that of the boxB RNA binding, indeed. However, the binding of the NTS RNA to the Tat peptide (Figure 2b, gray line) is more drastically decreased, indicating the NTS RNA discriminated two highly basic peptides, the Tat and N peptides. This result agrees with the expectation from the binding experiments using affinity columns for the Tat–G3 library before cloning and the NTS RNA would be an appropriate representative of the library. The results from the SPR experiments suggested that this novel model RNA interacts with the N peptide with a higher specificity than the boxB RNA. After all, a novel protein–RNA recognition system with superior specificity to the naturally occurring one could be established.

CONCLUSION

In this study, a new functional thermo-stable hairpinloop RNA (NTS RNA) was obtained by a designed in vitro selection procedure to pick the RNA with high selectivity for the basic N peptide binding. The new specific peptide-RNA binding model system is constructed by enough small components, and thus the system should be useful for detailed investigation about molecular recognition.

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REFERENCES