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Construction of a library of structurally diverse ribonucleopeptides with catalytic groups

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ABSTRACT

Functional screening of structurally diverse libraries consisting of proteins or nucleic acids is an effective method to obtain receptors or aptamers with unique molecular recognition characteristics. However, further modification of these selected receptors to exert a newly desired function is still a challenging task. We have constructed a library of structurally diverse ribonucleopeptides (RNPs) that are modified with a catalytic group, in which the catalytic group aligns with various orientations against the ATP binding pocket of RNA subunit. As a proof-of-principle, the screening of the constructed RNP library for the catalytic reaction of ester hydrolysis was successfully carried out. The size of both the substrate-binding RNA library and the catalytic group modified peptide library are independently expandable, and thus, the size of RNPs library could be enlarged by a combination of these two subunits. We anticipate that the library of functionalized and structurally diverse RNPs would be expanded for various other catalytic reactions.
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1. Introduction

Biomacromolecular receptors provide useful frameworks for constructing sensors with high specificity for a particular ligand. Efforts have been taken to construct biosensors through modification of proteins and nucleic acids receptors with appropriate fluorophores[1–5] or artificial enzymes with a catalytic group to achieve proximity effect between the substrate and the catalytic group.[6,7] However, naturally occurring receptors do not always provide the recognition characteristics for the molecule of interest, and rational design of a receptor with desired specificity is still a challenging task. A library of RNA molecules that differ in their three-dimensional structures by means of randomized nucleotide sequences has been applied for the selection of receptors for the target ligands.[8–10] Although the library-based selection offers one of the promising methods to obtain aptamers with the specific recognition characteristics for the molecule of interest, further modification of the selected aptamers to exert a newly desired function is often difficult due to the lack of their structural information. In addition, the original substrate binding characteristics of the aptamer could be impaired by the introduction of a new functional group. Synthetic functional groups were incorporated to biomacromolecules in the library by modification of proteins or nucleic acids via chemical modification or genetic mutation.[11,12] These chemical or genetic methods require laborious tasks to generate a library with limited size. Therefore, an alternative method is required to construct a large size library to increase the possibility of selecting a "hit" biomacromolecule with the function of interest.

Ribonucleopeptide (RNP) is one of the appropriate scaffolds to construct a library with structurally diverse RNA-peptide complexes. We have reported a method to obtain ATP-binding RNP receptors by in vitro selection of a library of RNP with randomized RNA sequences.[13] Because the RNA subunit works as a receptor for the substrate, the selected ATP-binding RNP receptor was further converted into a new RNP library by complexation of the RNA subunit and a library of Rev peptides, in which a peptide loop with randomized amino acid residues was incorporated at the N-terminus of Rev peptide. An ATP-binding RNP receptor selected from this peptide-based RNP library exerted higher ATP-binding specificity than the original RNP (Fig. 1a).[14] A fluorescent RNP library was also constructed by combining the RNA subunit library and a library containing fluorophore-modified Rev peptides (Fig. 1a).[15–17] The original substrate-binding ability of the RNP receptor was maintained in the selected RNP sensor even upon modification of the Rev peptide by a fluorophore. By taking advantage of the noncovalent complex formation of RNP, combination of the RNA library and the peptide library would dramatically increase the size of the RNP library. Therefore, RNP is a good candidate of scaffolds to construct a library of structurally diverse biomolecular assemblies containing a substrate-binding pocket and a synthetic functional group.

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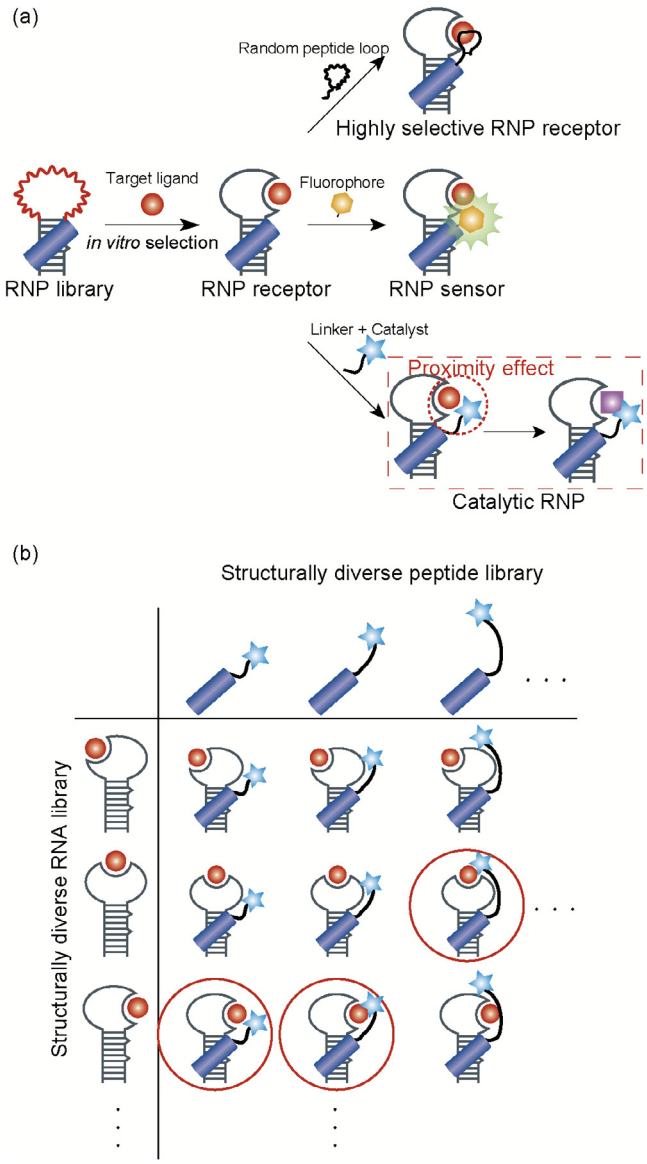

Fig. 1. (a) Schematic illustration of the stepwise molding of RNP receptors. The RNP receptor was first selected by in vitro selection of the RNP library. The resulting RNP receptor was converted to more selective RNP receptors by a suitable peptide loop containing randomized amino acid residues[14] or to fluorescent RNP sensors by fluorophore modification.[15–17] Also, an RNP library containing catalytic groups in the peptide subunit could provide catalytic RNPs. (b) Combination of a structurally diverse RNA library and a structurally diverse peptide library affords the construction of a library of RNPs, in which the catalytic group aligns with various orientations to the substrate-binding pocket.

In this study, we have constructed a library of structurally diverse RNP receptors equipped with a synthetic functional group. A series of RNA subunits obtained by the in vitro selection of ATP-binding RNP receptors[15,17] afforded a library of structurally diverse RNAs consisting of the ATP-binding region and the RRE (*R*ev *R*esponsive *E*lement) nucleotide sequence. A library of peptides was constructed by modification of the Rev peptide with catalytic groups through various peptide linkers (Fig. 1b). Combination of these two types of libraries by the complex formation between the RRE RNA and the Rev peptide generated a library of functionalized and structurally diverse RNPs, in which the catalytic group would align with various orientations against the ATP binding pocket of RNA subunits.

The functionalized and structurally diverse RNPs were expected to exhibit the ATP-binding ability. An adenosine derivative with an ester group was utilized to select the RNPs that could accelerate the ester hydrolysis reaction by the proximity effect between the ATP binding domain and the catalytic group on the peptide (Fig. 1a). Screening of the RNP library was successfully performed for the hydrolysis reaction of the ester derivative of adenosine to obtain catalytic RNPs.

2. Results and discussion

2.1. Construction of a functionalized structurally diverse RNP library

A library of structurally diverse RNPs with various geometries between the substrate binding pocket and the catalytic group was constructed by combination of the RNA subunits of ATP-binding RNP and a library of Rev peptide modified with catalytic groups. From the RNA-oriented RNP library, a series of ATP-binding RNP receptors was obtained through in vitro selection[15,17] with a variation in the RNA sequences (Fig. 1a). The nucleotide sequence of ATP-binding RNP consisted of three segments, the RRE, consensus, and variable regions (Fig. 2). The consensus region of the ATP-binding RNP receptors forms the binding pocket for the substrate adenosine.[15,17] The consensus and the RRE regions were connected through the variable region that differ in various length and sequences of the nucleotides. The structural diversity in the variable region affords diverse structures of RNPs, which in turn exhibits a variety of geometries between the ATP-binding pocket and the Rev peptide that is bound to the RRE sequence.

The N-terminus of Rev peptide in RNP can be chemically modified without diminishing the binding affinity of Rev peptide to the RRE sequence.[14–17] Judging from the structure of Rev-RRE complex,[18] the N-terminus of Rev peptide in RNP should locate closer to the binding-pocket than its C-terminus. Therefore, the N-terminus of Rev peptide is a suitable position for the modification by a catalytic group. N,N-Dimethyl-4-aminopyridine (dmap), 4,4′-dimethyl-2,2′-bipyridine (bpy) and histidine (H) were chosen as possible catalytic groups for the ester hydrolysis reaction. In addition, peptide linkers with various amino acid sequences were introduced between the catalytic groups and the N-terminus of Rev peptides. Four types of short peptide motifs were selected from known RNP structures[19–22] (Fig. 3) to achieve a structural diversity (Fig. 4). The motif pa (Fig. 3b) traverse the phosphate backbone as found in the eukaryotic ribosome structure (Fig. 3a), the motif pb (Fig. 3d) snugly fits in the major groove of RNA duplex in the Tav2b/siRNA complex (Fig. 3c), the motif pc (Fig. 3f) binds along the phosphate backbone in the complex of Mss16p DEAD-box helicase domain 2/RNA duplex (Fig. 3e), and the motif pd (Fig. 3h) passes over the phosphate backbone from the major groove to the minor groove as found in the small RNA methyltransferase HEN1 complex (Fig. 3g). Synthetic flexible linkers containing multiple repeating sequences (GGS) were also incorporated between the N-terminus of Rev peptide and the catalytic group (pe and pf). Combination of the RNA subunit library and the peptide subunit library afforded a structurally diverse RNP library with catalytic group, in which the substrate ATP resided in various orientation and distances to the catalytic group (Fig. 1b).

2.2. Design and synthesis of an ester derivative of adenosine for facile screening of catalytic RNPs

Spectroscopic monitoring of the *p*-nitrophenyl ester hydrolysis is a rapid, sensitive and quantitative way to evaluate the catalytic activity of biomacromolecules.[23,24] The *p*-nitrophenyl ester has been used as the reporter motif of substrate because it shows little or no absorbance of around 400 nm in water but provide characteristic absorbance of *p*-nitrophenolate upon hydrolysis of the

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