Design of a ribonucleopeptide biosensor

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ABSTRACT
Ribonucleopeptide receptors for ATP have been designed by using a structure-based design and in vitro selection method. The ATP binding ribonucleopeptide receptors revealed submillimolar affinity to ATP and discriminate ATP against other ribonucleotides. In this research, we have developed a simple strategy to convert the ATP-binding ribonucleopeptide receptor into a ribonucleopeptide sensor by introducing a fluorophore in the peptide subunit. Fluorophore labeled ribonucleopeptide complex showed a large change in the fluorescence intensity upon addition of ATP.

INTRODUCTION
A recently described three-dimensional structure of the ribosome provides a sense of remarkable diversity of RNA-protein complexes. We have designed a new class of scaffold for artificial receptors consisting of an RNA subunit and a peptide subunit. RNA-peptide receptors (ribonucleopeptide receptors) for small molecules were designed on the basis of the structure of Rev Responsive Element (RRE)–Rev peptide complex, because (1) RRE and Rev peptide form a specific and stable complex, (2) its 3-D structure is determined by NMR, (3) ligand binding domains can be introduced both in RRE RNA and in the Rev peptide, and (4) the ligand binding ability would be optimized by the in vitro selection method. Moreover, the ribonucleopeptide receptor can be readily functionalized by a simple chemical modification of each subunit.

RESULTS AND DISCUSSION
The RNA subunit was designed to consist of two functionally separated domains, namely a ligand-binding domain with 30 randomized nucleotides and...
an adjacent stem region that serves as the binding site for the peptide. Ribonucleopeptide receptors for ATP were isolated from a large population \((4 \times 10^3)\) of RNAs by the \textit{in vitro} selection method as previously reported [1], in each round of selection an RNA pool was incubated with immobilized ATP in the presence of the Rev peptide, washed out unbound RNA species and eluted bound RNA with ATP. The eluted fractions were collected, reverse transcribed and applied to successive PCR amplification (RT-PCR) to generate new DNA pools, DNA templates were transcribed and the resulting RNA pools were subjected to the next round of selection. After eight rounds of selection RNAs were converted to complementary DNA and sequenced. Nucleotide sequences were classified into two groups. The class I group shares a highly conserved sequence 5'-GUAGUG---GUGUG-3' in the ligand binding region. Gel mobility shift assay of the complex of the selected RNA subunit and the peptide subunit indicates that introduction of the additional ligand binding domain does not interfere the specific complex formation between the RNA subunit and the Rev peptide subunit. In order to functionalize the ATP-binding ribonucleopeptide receptor, an amine-reactive fluorophore NBD (4-fluoro-7-nitrobenz-2-oxa-1, 3-diazole) was introduced to the N-terminus of Rev peptide. Because the N-terminus of the peptide subunit was predicted to locate nearby the ligand binding region, binding of ATP might cause a local conformational perturbation to result a change in fluorescence emission character. The fluorophore labeled ribonucleopeptide showed large changes in fluorescence intensity in response to the ATP binding. Other nucleotides (UTP, CTP, GTP) failed to cause a significant fluorescent change. The method described here provides a convenient strategy to convert the ribonucleopeptide receptors to optical sensors for specific ligands.

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\text{Fig.2 Amine-reactive fluorophore used in this study}
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REFERENCES