Biologically active amines play major roles in the regulation of movement and are implicated in the pathophysiology of Parkinson’s and Huntington’s diseases, psychosis, and drug addiction. Fluorescent biosensors based on the biological macromolecule receptor are useful tools for investigating the function of biologically active amines. Our strategy of the stepwise molding using a ribonucleopeptide (RNP) framework provides fluorescent biosensors with a variety of binding and optical characteristics for small molecules. Here we report fluorescent RNP sensors for histamine with a variety of binding and signal-transducing characteristics. Combination of RNA subunits of histamine-binding RNP receptors obtained by in vitro selection and a Rev peptide modified with 7-methoxycoumarin-3-carboxylic acid afforded a fluorescent RNP sensor with distinct signaling characteristics in the changes of fluorescence emission intensity upon binding to histamine. The fluorescent histamine sensor showed distinct selectivity for histamine over structurally related histamine analogs, such as imidazole, ethylamine and L-histidine.

Key words: ribonucleopeptide, aptamer, fluorescent biosensor, biological active amine, in vitro selection

Biologically active amines, such as dopamine, histamine, and serotonin, are pivotal molecules in the central nervous system. These amines play major roles in the regulation of movement and are implicated in the pathophysiology of Parkinson’s and Huntington’s diseases, psychosis, and drug addiction. Pharmacotherapy of some major disorders of the central nervous system is based on targeting these biologically active amines. In the process of discovery and evaluation of new central nervous system drugs against these diseases, there is a persistent demand for availability of suitable analytical techniques that enable a highly sensitive detection of biologically active amines. Fluorescent biosensors based on the biological macromolecule receptor would serve as useful tools for investigating the function of biologically active amines in the living cell. However, fluorescent biosensors synthesized by the chemical modification of the ligand binding protein domains with fluorophores are not always guaranteed to execute the expected optical signals.

We have reported a strategy that enables isolation of fluorescent ribonucleopeptide (RNP) sensors with a variety of binding and signal-transducing characteristics, i.e., a high signal-to-noise ratio, various wavelengths and concentration ranges for the ligand detection. The strategy would provide ideal fluorescent RNP sensors for sensing biologically active amines. Fluorescent RNP sensors for adenosine 5'-triphosphate (ATP) were obtained in a stepwise manner. As the first step to construct a fluorescent RNP sensor for ATP, an RNA-derived RNP pool was constructed by a structure-based design of the Rev Responsive Element (RRE)-HIV Rev peptide complex appended with a randomized nucleotides region, which would provide a ligand binding site, next to the RRE segment. In vitro selection of the RNA-derived pool of RNP afforded ATP-binding RNP receptors with high selectivity and affinity. As the second step, ATP-binding RNP receptors were converted to a pool of fluorescent ATP-binding RNP receptors by chemically modifying with a fluorophore at the N-terminal of the Rev peptide with suitable optical characteristics. From the pool of fluorescent ATP-binding RNP receptors, it was possible to select a fluorescent ATP sensor with desired sensing characteristics.

Here we report fluorescent RNP sensors for histamine, one of the biologically active amines, with a variety of affinities and optical sensing characteristics (Fig. 1). By utilizing the RNA subunits of histamine-binding RNPs obtained by in vitro selection and a Rev peptide modified with a fluorophore, a histamine-binding fluorescent RNP library was constructed. Fluorescent histamine sensors with desired optical and binding properties were screened from this library. A fluorescent RNP sensor for histamine modified with 7-methoxycoumarin-3-carboxylic acid showed a change in the fluorescence emission intensity upon binding to histamine. Furthermore, the histamine sensor showed distinct selectivity over histamine analogs, such as imidazole, ethylamine and L-histidine.

**Fig. 1.** A strategy to obtain RNP fluorescent sensors specific for histamine. Combination of the RNA subunits of the histamine-binding RNP receptor and a fluorophore-modified Rev peptide provided a histamine RNP fluorescent sensor.
RNP receptors for histamine were isolated from an RNA-derived RNP pool by the in vitro selection method as previously reported\cite{9, 13}. In each round of the selection, an RNP pool was incubated with an immobilized histamine-agarose resin and the resin-bound fraction of RNP was recovered by a specific elution with free histamine. A negative selection step using a histidine-agarose resin was incorporated after the 10th round of the selection cycle. The resin-bound RNA fractions were collected, reverse transcribed and applied to successive PCR amplification (RT-PCR) to generate new DNA pools. The DNA templates were transcribed and the resulting RNA pools were subjected to the next round of selection in the presence of the Rev peptide. After 15 rounds of iterative selection, RNAs were converted to complementary DNA for the sequencing analysis. From the result of DNA sequencing analysis, three dominant DNA sequences were observed in the 15th pool (Fig. 2). These sequences in the RNA subunit were predicted to form the histamine-binding site within the RNP receptor. Nucleotide lengths of H01, H02 and H05 were 23, 38 and 30 nt, respectively. An obvious consensus sequence was not found in these three sequences.

Fig. 2. Nucleotide sequences obtained for the randomized region of histamine-binding RNP receptors.

In order to convert the histamine-binding RNP receptor to fluorescent histamine sensors, the Rev peptides modified at the N-terminal with fluorophore, 7-methoxycoumarin-3-carboxylic acid (7mC-Rev), 1-pyrenesulfonyl chloride (Pyr-Rev), 6-fluorescein carboxylic acid NHS ester (6FAM-Rev) and 4-fluoro-7-nitrobenz-2-oxa-1,3-diazole (NBD-Rev) were prepared. Each fluorophore-modified Rev peptide, 7mC-Rev, Pyr-Rev, 6FAM-Rev, and NBD-Rev was complexed with the RNA subunit of the histamine-binding RNP receptor. The fluorescence intensities in the absence and presence of histamine (10, 100 µM and 1 mM) of the fluorescent RNP were evaluated. Relative ratios of fluorescence intensity ($I/I_0$) in the absence ($I_0$) and the presence ($I$) of histamine for fluorescent RNPs with 7mC-Rev, Pyr-Rev, 6FAM-Rev and NBD-Rev monitored at 390, 390, 535 and 535 nm, respectively, were summarized in Fig. 3. The RNP receptor with fluorophore-modified Rev peptide showed an increase in the fluorescence emission intensity upon addition of histamine, as observed in the case of the fluorescent RNP sensor for ATP\cite{9}. H05 RNA complexed with 7mC-Rev, 6FAM-Rev and NBD-Rev recorded the $I/I_0$ value of 1.68, 1.24 and 1.23, respectively (Fig. 3 panel a, c, d). When Pyr-modified Rev was complexed with H05 RNA, changes in the fluorescence intensity of the fluorescent RNPs were quite small upon addition of histamine. In the cases of H05 RNA derived fluorescent RNPs with 7mC-Rev, 6FAM-Rev, and NBD-Rev, fluorescence intensity changes were observed mainly in the presence of 100 µM to 1 mM histamine. These results suggest that the dissociation constant ($K_D$) of H05 RNA and histamine is more than 100 µM.

![Fig. 3. Relative fluorescence intensity changes ($I/I_0$) of RNPs with existence of 10 µM (White bar), 100 µM (gray bar) and 1 mM (black bar) histamine are shown in the bar graphs for (a) 7mC-Rev RNP, (b) Pyr-Rev RNP, (c) 6FAM-Rev RNP, and (d) NBD-Rev RNP.](image)

The characteristics of histamine-binding fluorescent RNP H05/7mC-Rev were further investigated in detail. Figure 4 shows the titration curve of H05/7mC-Rev by increasing concentrations of histamine as judged by relative fluorescence intensity changes. A nonlinear regression analysis of the titration curve yielded a
dissociation constant of 502.5 µM for the complex of H05/7mC-Rev and histamine. Observed relative fluorescence intensity change ($I/I_0$) was 2.28 at the saturation of histamine binding to H05/7mC-Rev.

![Fig. 4. Direct titration of a fluorescent RNP complex (1 µM) of the H05 RNA subunit and 7mC-Rev (H05/7mC-Rev) with histamine (1, 3, 10, 30, 100, 300 µM, 1, 3, 10, 30 mM).](image)

The presence of a carboxyl group at the α position inhibited the formation of a stable ligand-fluorescent RNP complex.

In conclusion, fluorescent histamine sensors were successfully constructed by utilizing the modular strategy for tailoring fluorescent RNP sensor. Three kinds of RNA sequences were obtained for the RNA subunit of histamine-binding RNP receptor by in vitro selection. Combination of the RNA subunit derived from the histamine-binding RNP and the fluorescent Rev peptides afforded fluorescent RNP sensors that showed distinct changes in the fluorescence emission intensities upon binding to histamine. The fluorescent histamine sensors H05/7mC-Rev revealed a distinct selectivity for histamine over the structurally related analogs of histamine, such as imidazole, ethylamine and L-histidine. It would be possible to construct RNP-based fluorescent biosensors for other biologically active amines.

References


(Received May 1, 2009; Accepted July 1, 2009)