Development of ribonucleopeptide-based fluorescent sensors for biologically active amines based on the stepwise molding strategy

Kazuki Tainaka¹, Tetsuya Hasegawa², Masatora Fukuda³, Shun Nakano¹, Nobutaka Fujieda³ and Takashi Mori¹,²

¹Institute of Advanced Energy, ²Institute of Sustainability Science, ³Pioneering Research Unit for Next Generation, Kyoto University, Uji, Kyoto 611-0011, Japan

ABSTRACT

General strategy for the development of fluorescent biosensors as a tracer of ‘key’ molecule in the cellular system would provide important breakthroughs for ubiquitous applications in the field of diagnosis and pharmacology in addition to our understanding of cellular events. The sophisticated design of fluorescent biosensors based on the organic synthesis is one of the promising approaches, but this type of biosensors frequently fail to maintain their performance in the cellular environment despite of laborious protocols. Another procedure for simultaneous preparation of a wide variety of fluorescent biosensors for the optical monitoring of a target molecule represents an especially attractive alternative. In our continuous efforts, we have recently developed a conceptually new strategy for coincidental production of fluorescent biosensors with diverse functions based on a framework of ribonucleopeptide (RNP). RNP-based fluorescent sensors were fabricated with a combination of in vitro selection method and a successive modification of the peptide of RNP with a fluorophore. Each RNP composed of a ligand-binding RNA subunit and a fluorophore-tagged peptide motif facilitated the fluorometric detection of biologically active amines with a unique binding affinity and an inherent fluorescent signal.

INTRODUCTION

Biologically active amines, such as dopamine, histamine, and serotonin, have a close relevance to the regulation of movement in the central nervous system and are presumably associated with the pathophysiology of Parkinson’s and Huntington’s diseases, psychosis, and drug addiction [1]. Quantitative and high-sensitive diagnostic methods for biologically active amines were persistently required for exploration of a new class of drugs against such neurological disorders.

Our strategy for the stepwise molding based on a framework of ribonucleopeptide (RNP) provides simultaneously a wide variety of fluorescent biosensors with diverse functions, i.e., high signal-to-noise ratios, different wavelengths and various concentration ranges for the ligand detection [2]. In the first step to fabricate RNP-based fluorescent sensors, an RNA-derived RNP pool was prepared by a structure-based design of the Rev Responsive Element (RRE)-HIV Rev peptide complex [3] appended with a randomized nucleotides region as a ligand binding domain, adjacent to the RRE segment. In vitro selection method [4] was applied to a randomized nucleotides region to afford a series of ATP-binding RNP receptors with high selectivity and affinity [5]. In the second step, ATP-binding RNP receptors were successfully functionalized as ATP-binding fluorescent sensors by the chemical modification of the N-terminal of the Rev peptide with a various kind of fluorophore. RNP-based fluorescent sensors are also applicable to the other class of small molecules, such as biologically active amines.

According to stepwise molding strategy of RNP-based fluorescent sensors, dopamine-selective RNP sensors, which exhibited unique specificity to the dopamine
compared with other dopamine derivatives, have been
developed [6]. Herein we report a series of fluorescent RNP
sensors for other biologically active amines, histamine and
serotonin with diverse functions. RNA subunits as a ligand
binding domain were prepared by in vitro selection method,
as previously reported [2, 5]. Rev peptide motifs as a
function of optical response were afforded by the
fluorescent labeling to the N-terminal of peptide. Amino-
selective RNP fluorescent sensors were conveniently
screened by the RNP pool composed of RNA subunits and
Rev peptide motifs, and as a result, displayed expedient
optical and binding properties.

RESULTS AND DISCUSSION

RNP receptors for histamine and serotonin were isolated
from an RNP library by in vitro selection method as
previously reported [2, 5]. In each round of selection, an
RNP pool was incubated with immobilized amines-agarose
resin. After removal of unbound RNP spieces, bound RNP
spieces were recovered by specific elution of free substrate.
An ethanalamine- and n-propylamine-agarose resin was
utilized in the negative selection step of histamine and
serotonin, respectively. The bound RNA 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
several rounds of iterative selection, substrate-selective
RNA receptors were collected.

In order to convert the amine-binding RNP receptor to
fluorescent amine sensors, a various kind of fluorophores
were introduced to the N-terminal of the Rev peptide.
Generally, fluorescent properties of fluorophore could be
modulated dramatically by the local hydrophobicity,
polarity, and viscosity around fluorophore or the distance
from a specific quencher of the fluorophore. Especially, it
is accepted that guanine residue in RNA acts as a
remarkable quencher for various fluorophores due to its
lower oxidation potential. A ligand-binding pocket in RNA
subunit is presumably located adjacent to the N-terminal
of the Rev peptide. Therefore, it is expected that a ligand-
binding event would affect intensively the fluorescent
signal of labeled fluorophore due to drastic change of its
local arrangement. Actually, fluorescent RNP sensors for
histamine and serotonin showed a variety of binding and
signal-transducing characteristics.

CONCLUSION

In this study, we developed a wide variety of fluorescent
biosensors for histamine and serotonin with diverse
functions based on a stepwise molding strategy. Each RNP
composed of a ligand-binding RNA subunit and a
fluorophore-tagged peptide motif facilitated the
fluorometric detection of target amines with a unique
binding affinity and an inherent fluorescent signal.

REFERENCES

2. Hagihara, M., Fukuda, M., Hasegawa, T., Morii, T.
3. Battiste, J. L., Mao, H., Rao, N. S., Tan, R., Muhandiram,
   D. R., Kay, L. E., Frankel, A. D., Williamson, J. R.
   818-822.

*Corresponding Author. E-mail: t-morii@iae.kyoto-u.ac.jp
(Font: Times or Times New Roman 10pt).