【Grant-in-Aid for Specially Promoted Research】
Science and Engineering (Chemistry)

Title of Project : Non-standard peptide-probe discovery

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Research Area : Biomolecular Science
Keyword : Natural product organic chemistry, Bioactive compounds, Biopolymers, Biosynthesis, Chemical biology

【Purpose and Background of the Research】

The genetic code is the law of translation, where genetic information encoded in RNA is translated to amino acid sequence. The code consists of tri-nucleotide sequences, so-called codons, assigning to particular amino acids. In cells or in ordinary cell-free translation systems originating from prokaryotes or eukaryotes, the usage of amino acids is generally restricted to 20 proteinogenic (standard) kinds, and thus the expressed peptides are composed of only such monomers. However, we recently devised a new means to reprogram the genetic code, which allows us to express "non-standard" peptides containing multiple non-proteinogenic amino acids.

To execute the genetic code reprogramming, we developed an artificial RNA enzyme (ribozyme), referred to as "flexizyme" capable of aminoacylating tRNAs. The most notable feature of flexizyme is its versatility; it is able to charge virtually any α-amino acids, including those with non-proteinogenic sidechain, *D*-a-amino acids, *N*-alkyl-amino acids, β-amino acids, and even hydroxy onto tRNAs bearing various anticodons. We thus integrated this unique enzyme system with a reconstituted cell-free transcription-translation system, from which certain amino acids (occasionally their cognate aminoacyl-tRNA synthetases) were withdrawn to vacant the corresponding codons, referred to as *w*PURE system (*w* stands *withdrawn* and PURE stands Protein translation Using Recombinant Elements). By the integration of these two systems, any desired amino and hydroxy acids can be reassigned to the vacant codons, and we have recently showed that a wide variety of non-standard peptides can be expressed from mRNAs under the reprogrammed genetic code in the *w*PURE system, referred to as Random Peptide Integrated Discovery (RaPID) system.

【Research Methods】

To achieve our goals, we plan the following research specific aims, and accordingly we designed the research methods: ❶ Ribosomal expression of amphiphilic cyclic non-standard peptides; ❷ Ribosomal expression of (poly)ketide-containing peptides; ❸ Integration of the above non-standard peptides with RaPID system; ❹ Discovery of biological probes (therapeutic agents) based on non-standard peptides.

【Expected Research Achievements and Scientific Significance】

The proposed project will utilize this RaPID system and accelerate the discovery of natural product-like cyclic peptides that agonize or antagonize biological functions in cells and potentially in human as new therapeutic agents. We are hoping that our research proposed in this application opens a new frontier of chemical biology and biotechnology using non-standard peptides.

【Publications Relevant to the Project】

・ "Structural basis of specific tRNA aminoacylation by a small in vitro selected ribozyme" H. Xiao, H. Murakami, H. Suga, A. R. Ferre-D'Amare **Nature** *454*, 358-361 (2008).

・ "Reprogramming the initiation event in translation for the synthesis of physiologically stable cyclic peptides" Y. Goto, A. Ohta, Y. Sako, Y. Yamagishi, H. Murakami, H. Suga **ACS Chemical Biology** *3*, 120-129 (2008).

・ "Messenger RNA-directed incorporation of multiple N-methyl-amino acids into linear and cyclic peptides" T. Kawakami, H. Murakami, H. Suga **Chemistry & Biology** *15*, 32-42 (2008).

・ "A highly flexible tRNA aminoacylation tool for non-natural polypeptide synthesis" H. Murakami, A. Ohta, H. Ashigai, H. Suga **Nature Methods** *3,* 357-359 (2006).

【Term of Project】 FY2009-2013

【Budget Allocation】 406,700 Thousand Yen

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