Title of Project : **Generation of Minimal Peptide Catalysts Based on the Macrocyclic Scaffold**

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【Purpose and Background of the Research】

Protein enzymes play the central role in catalyzing chemical reactions in cellular systems, and residues responsible for catalysis or/and binding to target molecules in any of enzymes are set in the three-dimensional space. However, we do not well understand how such complex proteins have evolved to the present forms. For instance, one of the shortest enzymes, 4-oxalocrotonate tautomerase yet has the length of 62 residues; therefore a chance to evolve such an enzyme is one out of 10^{80} possibility, which is extremely rare chance. On the other hand, RNA catalysts that are responsible for catalytic function before advent protein enzymes might have evolved a protein synthesis machinery, primitive ribosome. It is well known that the modern translation machinery requires many protein factors/enzymes for efficient translation. This indicates that the primitive ribosome would not be able to synthesize long peptide molecules, probably their length is limited to 20–30 residues at most.

There were many attempts to generate shorter enzymes using the secondary structural modules of alpha-helices or/and beta-sheets *in silico* or *in vitro*, so-called de novo protein design. Although there are some successes, their protein lengths are not less than 50 residues. Upon considering the elongation capability of the primitive ribosome, this would be difficult to achieve synthesis of such long peptides. Thus, the above successes do not necessarily reveal how the primitive protein enzymes evolved or what minimal length of proteins to be catalytic.

We here aim at generating libraries of short peptides with highly constrained scaffolds and seeking catalytically active molecules by the selection strategy. Each specific aims set in this program are follows:
① Construction of macrocyclic peptides that generate 3D space
② Selection of catalytically active species, pepzymes
③ Studies on the mechanisms of individual pepzymes and their structural engineering

【Research Methods】

To achieve the goals of ①–③, we will utilize our knowledge of the RaPID system devised in our laboratory to design the experiments of construction of macrocyclic libraries and selections. In the aim ①, we will construct the macrocyclic peptides constrained with a mono, bi, and tri-cyclic structures. In the aim ②, we will select *cis*-active species for four independent reactions. In the aim ③, the mechanistic studies on the individual clones for the reactions will be conducted and based on the knowledge we will engineer the *cis*-acting pepzymes to the trans-acting pepzymes.

【Expected Research Achievements and Scientific Significance】

We expect that the outcomes of this research program will provide a hint of "the origin of protein catalysts". Also, such pepzymes can be a new tool for chemical biology.

【Publications Relevant to the Project】

・ K. Yamagata, Y. Goto, H. Nishimasu, J. Morimoto, R. Ishitani, N. Dohmae, N. Takeda, R. Nagai, I. Komuro, H. Suga, O. Nureki "Structural basis for potent inhibition of SIRT2 deacetylase by a macrocyclic peptide inducing dynamic structural change" **Structure** *22*, 345-352 (2014).
・ Y. Tanaka, C.J. Hipolito, A.D. Maturana, K. Ito, T. Kuroda, T. Higuchi, T. Katoh, H.E. Kato, M. Hattori, K. Kumazaki, T. Tsukazaki, R. Ishitani, H. Suga*, O. Nureki "Structural basis for the drug extrusion mechanism by a MATE multidrug transporter" **Nature** *496*, 247-51 (2013).

【Term of Project】 FY2014-2018

【Budget Allocation】 140,000 Thousand Yen

【Homepage Address and Other Contact Information】

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