【Grant-in-Aid for Scientific Research(S)】
Science and Engineering (Chemistry)

Title of Project： Creation of Bright Near-Infrared Bioluminescent Probes and Their Application to Tumor Imaging

Koji Suzuki
(Keio University, Faculty of Science and Technology, Professor)

Research Area：Analytical Chemistry
Keyword：Molecular Imaging, Bioluminescence, Chemiluminescence, Protein Probe, Perfectly-matched biomolecular probe

【Purpose and Background of the Research】

The imaging of cells and living organisms with molecular probes is expected to play an important role in medical diagnostics, treatment and drug design. Especially the development of an imaging tool for the detection of small tumors in the early developing stage of cancer in humans is desired. In order to develop a useful molecular in vivo applicable probe for cancer within a short time span, the effective collaboration between researchers in the fields of synthetic chemistry, analytical chemistry, biochemistry, and clinical chemistry is of high importance.

In this project, collaborative research for the development of a useful near-infrared (NIR) bioluminescent molecular probe system will be performed, where a chemistry research team will target the chemical synthesis of new small molecule luciferin substrates, and a biochemistry research group will deal with protein mutation to create a designed matching luciferase enzyme. Although at present molecular imaging is normally performed with fluorescent probes, there are some unfavorable features, such as autofluorescence caused by the required excitation light. Bioluminescent probes allow circumventing this problem, and therefore, are able to realize higher sensitivity compared to fluorescent probes.

Moreover, the NIR spectral range (NIR window for bioanalysis: 650-900 nm) with high tissue penetration is advantageous for optical imaging from areas located deep inside a living organism. In this research, based on the concept of a "perfectly-matched biomolecular probe" consisting of a synthetic small luciferin molecule and a protein mutant luciferase enzyme fitting three-dimensionally well to each other, a high-intensity near-infrared bioluminescent molecular probe system for cancer cell imaging will be developed.

【Research Methods】

By designing and preparing various synthetic luciferin derivatives, factors influencing the brightness and the degree of red-shift in the bioluminescence spectra will be evaluated (correlation of molecular structure and bioluminescent property) in order to achieve excellent bioluminescence properties (maximum bioluminescence wavelength and high brightness in the NIR region). Additionally, an approach connecting fluorescent and bioluminescent dyes to undergo RET (resonance energy transfer) will also result in NIR emitting luciferins.

A highly luminescent probe system with a variant of luciferase can be designed by systematic mutagenesis of the catalytic center of the enzyme, leading to a greatly improved enzymatic reaction rate. In this research, an evaluation method efficiently allowing to isolate a mutant resulting in remarkably high luminescence will be established. Amino acid variations are specifically introduced at the amino acid residues near the substrate binding center of the luciferase. In this case, about 20 amino acids can be changed at random. Consequently, a large number of luciferase mutants can be expressed in E. coli bacteria, followed by high throughput screening of the colonies with a special CCD camera. By optimizing this experimental system, conditions efficiently resulting in mutants with remarkably increased luminescence brightness will lead to an optimal luciferase enzyme for the synthetic luciferin molecules.

【Expected Research Achievements and Scientific Significance】

In the case where a high-intensity near-infrared bioluminescent molecular probe system is produced, it will not only be useful for the detection of biological substances in very small quantities, but also for bioluminescence imaging from deep locations of living organism. This will contribute to the acceleration of cancer research and brain research, among others.

【Publications Relevant to the Project】

1. “A Novel Luciferin-Based Bright Chemiluminescent Probe for the Detection of Reactive Oxygen Species”, M. Sekiya, K. Umezawa, A. Sato, D. Citterio, K. Suzuki, ***Chem. Commun.***, 21, 3047-3049 (2009).

2. “Bright, Color-Tunable Fluorescent Dyes in the Vis/NIR Region: Establishment of New “Tailor-Made” Multicolor Fluorophores Based on Borondipyrromethene”, K. Umezawa, A. Matsui, Y. Nakamura, D. Citterio, K. Suzuki, ***Eur.J. Chem.***, 15, 1096-1106 (2009).

【Term of Project】FY2012-2016
【Budget Allocation】157,400 Thousand Yen

【Homepage Address and Other Contact Information】

http://suzuki-lab.applc.keio.ac.jp/
suzuki@applc.keio.ac.jp