Design, Synthesis and Biological Application of in Vivo Imaging Probes with Tunable Chemical Switches

One of the great challenges in the post-genome era is to clarify the biological significance of intracellular molecules directly in living cells. If we can visualize a molecule in action, it is possible to acquire biological information, which is unavailable if we deal with cell homogenates. One possible approach is to design and synthesize chemical probes that can convert biological information to chemical output. Fluorescence protein labeling by synthetic probes is a powerful approach to investigate protein function and localization inside living cells. This chemistry-based technique utilizes a pair of a protein tag and its specific ligands connected to fluorophores. Its potential advantage is that various fluorescent molecules are available as labeling reagents, and the timing of protein labeling is easily controlled. Because of these characteristics, this method is attracting attention as an alternative to fluorescent proteins. On the other hand, in this labeling system, there is a problem that the fluorescence of free probes inside cells prevents the identification of labeled proteins. Thus, washing procedures are required to remove the free probes from cells. However, if the probes are not completely washed out, the remaining probes cause the reduction of the signal-to-noise ratio. As a solution to this problem, we previously developed a fluorogenic probe for labeling photoactive yellow protein (PYP) tag. PYP is a small protein (14 kDa) derived from purple bacteria, and binds to the thioester derivatives of cinnamic acid/coumarin through transthioesterification with Cys69. Novel fluorogenic probes, which possess 4-hydroxycinnamic acid or 7-dimethylaminocoumarin as a ligand scaffold, were synthesized. The labeling kinetics was significantly improved by these probes. Furthermore, no-wash labeling of intracellular proteins was successfully achieved.

Magnetic resonance imaging (MRI) is an imaging modality adequate for in vivo studies. Therefore, many scientists are interested in the development of MRI probes capable of detecting enzyme activities in vivo. Because background signal is hardly detectable, 19F-MRI probes are promising for in vivo imaging. A novel design strategy for 19F-MRI probes to detect protease activities is proposed. The design principle is based on the paramagnetic relaxation effect from Gd3+ to 19F. A peptide was synthesized, Gd-DOTA-DEVD-Tfb, attached to a Gd3+ complex at the N-terminus and a 19F-containing group at the C-terminus. The 19F-NMR transverse relaxation time (T2) of the compound was largely shortened by the paramagnetic effect of intramolecular Gd3+. The peptide was designed to have a sequence cleaved by an apoptotic protease, caspase-3. When the peptide was incubated with caspase-3, the peptide was cleaved and subsequently the Gd3+ complex and the 19F-containing group were separated from each other. T2 after cleavage, was extended to cancel the intramolecular paramagnetic interaction. T2 is a parameter that can be used to generate contrasts in MR images. Using this probe as a positive contrast agent, the probe could detect caspase-3 activity spatially from an image using 19F MRI.