Selective Recognition and Detection of Biomacromolecules Utilizing Chemical Property of Amino Acid or Peptide

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Recently, the fluorometric detection of biomacromolecules is attracting much attention. In this study, we report the development of two new techniques utilizing chemical property of amino acid or peptide. Namely, 1) fluorescence assay for serine/threonine kinase activity, and 2) “turn-on” fluorescent probes for protein labeling, which could be useful for bio-imaging.

To develop the novel kinase assay, we utilized the chemical reactivity of phosphorylated serine or threonine. Phosphorylated peptide on resin was successfully labeled fluorescently via base-mediated β-elimination, followed by Michael addition with novel coumarin derivatives. Protein kinase A and casein kinase I activities were detectable with our method. Also, this method was proved to be applicable for kinase inhibitor screening.

For the development of novel protein labeling technique, the selective interaction between “His-tag (His$_6$)” and “metal ion-NTA (nitrilotriacetic acid) complex” was utilized. This interaction is very popular for protein purification and immobilization. We designed fluorescent probes which were composed of fluorophore and Ni$^{2+}$ or Co$^{2+}$-NTA complex. These probes were found to be weakly fluorescent as expected. When His-tag peptide was added, these probes became brightly fluorescent. On the other hand, these probes remained non-fluorescent by the addition of angiotensin I (H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-OH). These probes would become powerful tools for the bio-imaging of target protein.