Bioluminescence for the Assay of Anti-cancer Activity

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Bioluminescence is a phenomenon in which the energy generated by a chemical reaction in a protein is emitted as light. Luminescent proteins, such as GFP (Green Fluorescent Protein), have become an important research tool in life science. Photoproteins are characterized by having a chromophore in which organic molecules and proteins are bound. Since the biological components such as calcium ions induce their luminescence, photoproteins enable high-sensitive detection of these biological components. Our research has focused on the photoprotein (pholasin) derived from a glowing bivalve mollusc (*Pholas dactylus*). Pholasin utilizes dehydrocoelenterazine (DCL) as organic substance, and emits light in the presence of reactive oxygen species (ROS). In order to detect ROS with high sensitivity by utilizing pholasin, the purpose of this research was to improve the luminescent efficiency by modifying the chemical structure of DCL. Starting from commercially available aminopyrazine (a heterocyclic aromatic compound), a versatile synthetic route was established by employing cross-coupling reactions catalysed by transition metals to yield DCL analogues. To evaluate luminescent activities of these DCL analogues, it required apo-pholasin that lacks organic substance, because natural pholasin contains natural abundant DCL, and it was difficult to obtain apo-pholasin from commercially available natural pholasin. After many trials, a baculovirus–silkworm multigene expression system afforded the best result. The obtained apo-pholasin was successfully activated with DCLs, and emitted light when a mixture of peroxidase and hydrogen peroxide was added in order to produce ROS. Throughout the investigation of structure and activity relationships, a DCL analogue afforded ten times brighter luminescence than natural substance. In conclusion, we succeeded in making the artificial pholasin, which was reconstituted with the artificially synthesized DCL derivative and the gene-expressed apo-pholasin, emitted light with higher brightness than the natural type. We now try to apply this established a high-sensitive ROS detection system to an assay system to evaluate anti-cancer activity of natural products.

Keywords (5 keywords): bioluminescence; photoprotein; reactive oxygen species; chromophore; natural products
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