Development of activatable fluorescence probe for carboxypeptidase activity to visualize cancer

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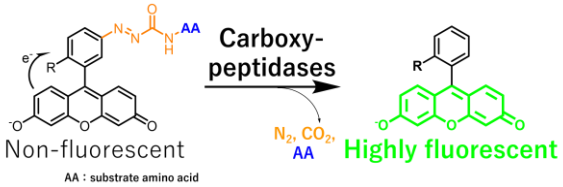
[Introduction] Carboxypeptidases (CPs) are enzymes that are up-regulated in some cancer cells, thus fluorescence detection of a specific CP activity allows visualization of cancer. However, only a few activatable fluorescent probes targeting CPs activities have been developed, as it is difficult to translate the chemical reaction from amide to carboxylate by CPs into the change in fluorescence intensity. In this study, we aimed to develop new activatable fluorescent probes that can detect CP activities with high sensitivity in live cells.

[Molecular design] We designed and synthesized molecules in which an azoformyl (AF) group and a substrate amino acid were introduced into the benzene moiety of fluorescein scaffold. The probe is weakly fluorescent before reaction with CP due to photoinduced electron transfer from fluorophore to the benzene moiety, but is converted to strongly fluorescent scaffold fluorophore upon reaction with CP.

[PSMA probe][1)] We developed an activatable fluorescent probe, 5GluAF-2MeTG, which targets prostate cancer-specific membrane antigen (PSMA), a CP that is upregulated in prostate cancer, by introducing glutamate (Glu) as a substrate amino acid into membrane-permeable TokyoGreen (TG) scaffold. 5GluAF-2MeTG successfully visualized the tumor sites in resected specimens from prostate cancer patients, including milli-meter size tumors that are difficult to detect with the naked eye.

[CPM probe][2)] By incorporating basic amino acid (arginine; Arg) as a substrate amino acid into fluorescein diacetate (FDA) scaffold, we developed a fluorescent probe 5ArgAF-FDA with improved intracellular retention that targets Carboxypeptidase M (CPM), which is upregulated in breast cancer. By using 5ArgAF-FDA, we succeeded in visualizing the different CPM activity of different cell lines at the single cell level.

[Conclusion] We established a molecular design strategy of activatable fluorescent probes that can detect CPs with high sensitivity and succeeded in developing activatable fluorescent probes that are useful for cancer imaging.



1) M. Kawatani, *J. Am. Chem. Soc.* **2019**, *141*, 10409. 2) H. Iwaki, *Anal. Chem.* **2021**, *93*, 3470.