A Fluorescent Probe to Detect Cytosolic and Nuclear O-GlcNAcase

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O-GlcNAcase, which catalyzes the removal of the β-O-GlcNAc moiety from Ser and Thr residues of proteins, is involved in the dynamic cycling of protein O-GlcNAcylation in conjunction with the action of O-GlcNAc transferase, which is responsible for attachment of the GlcNAc monosaccharide to Ser and Thr side chains. Dysregulation of protein O-GlcNAcylation is closely related to the pathogenesis of diverse human diseases. Owing to the pathopysiological importance of O-GlcNAcase, five fluorogenic probes (β-GlcNAc-FC, β-GlcNPr-FC, β-GlcNBu-FC, β-GlcNVa-FC and β-GlcNAc-Bn-FC) for the detection of cellular O-GlcNAcase were synthesized (Figure 1). Among these probes, β-GlcNAc-FC was found to be the best fluorogenic probe for O-GlcNAcase. In addition, β-GlcNAc-FC was able to detect cytosolic and nuclear O-GlcNAcase in cells. The cell study using this probe revealed that O-GlcNAcase activity in AGS cells (human gastric adenocarcinoma cells) is higher than those in Capan-1 (human pancreatic adenocarcinoma cells) and HaCaT cells (human keratinocyte cells). In addition, fluorescence signals arising from β-GlcNAc-FC were very low in cells cultured under glucose deprivation conditions and gradually increased when the concentration of glucose in culture media increased up to 10 mM. As a result, β-GlcNAc-FC will be employed as a fluorogenic probe to determine the level of O-GlcNAcase activity in live cells.

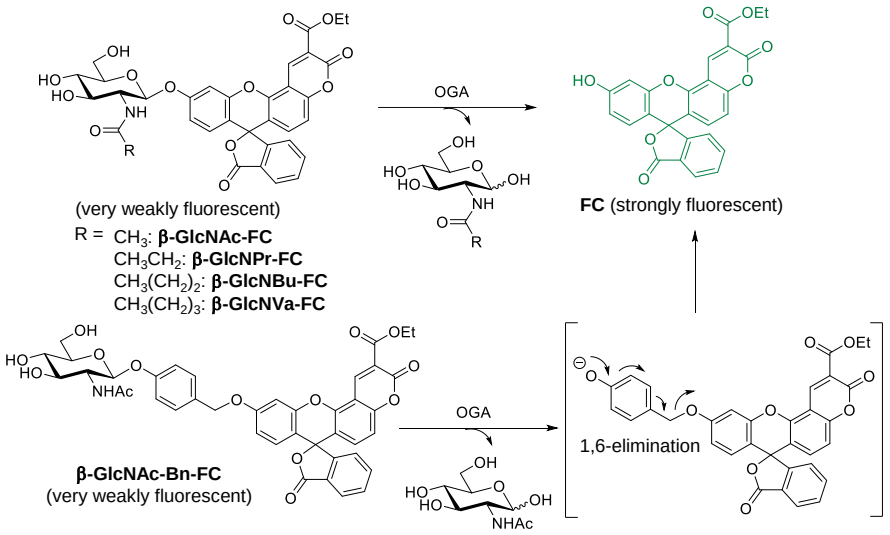

Figure 1. Fluorescence response of fluorogenic probes to O-GlcNAcase.