A fluorogenic probe to differentially monitor both O-GlcNAcase and phosphatase

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O-GlcNAc protein modification has crosstalk with protein phosphorylation. These posttranslational modifications are highly dynamic events that regulate a variety of cellular events. Because of the physiological and pathological significance of protein O-GlcNAcylation and phosphorylation, we devised the fluorogenic probe, βGlcNAc-CM-Rhod-P, to differentially monitor O-GlcNAcase (OGA) and phosphatase.[1] This probe consisted of a βGlcNAc-conjugated coumarin (βGlcNAc-CM) serving as an OGA substrate and a phosphorylated rhodol (Rhod-P) as a phosphatase substrate, two moieties which were connected by piperazine. Because the emission wavelength maxima of CM and Rhod released from the probe are greatly different (100 nm), spectral interference can be avoided. Addition of OGA to βGlcNAc-CM-Rhod-P led to formation of the GlcNAc-cleaved probe, CM-Rhod-P, and a consequent increase in the intensity of fluorescence associated with free CM. Also, it was found that treatment of the probe with phosphatase generates a dephosphorylated probe, βGlcNAc-CM-Rhod, which exhibits strong fluorescence arising from free Rhod. Furthermore, when exposed to both OGA and phosphatase, βGlcNAc-CM-Rhod-P was converted to CM-Rhod which lacked both βGlcNAc and phosphoryl groups, in conjunction with increases in the intensities of fluorescence arising from both free CM and Rhod. This probe was employed to detect activities of OGA and phosphatase in cell lysates and to fluorescently image both enzymes in cells.

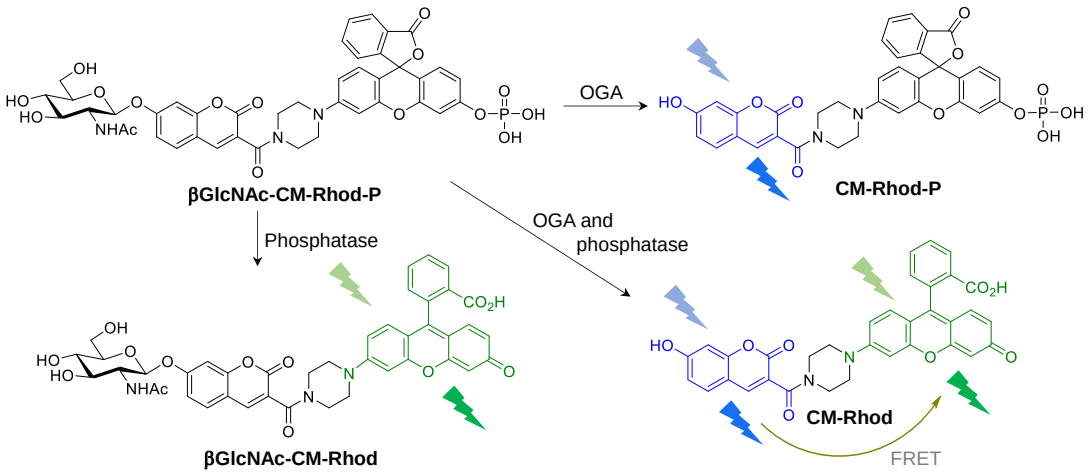

Figure 1. Fluorescence response of βGlcNAc-CM-Rhod-P to O-GlcNAcase (OGA) and phosphatase.

References

[1] Jihyeon Boo, Jongwon Lee, Young-Hyun Kim, Chang-Hee Lee, Bonsu Ku, and Injae Shin, *Front. Chem.* **2023**, *11*, 1133018.