Carbobenzoxy-capped Phe-Lys(BODIPY TMR-X-acyloxymethyl ketone(QSY7)

GB117

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Chemical name:	Carbobenzoxy-capped Phe-Lys(BODIPY TMR-X)-acyloxymethyl ketone(QSY7)	
Abbreviated name:	GB117	
Synonym:	Quenched activity-based probe (qABP)	
Agent Category:	Compounds	
Target:	Cysteine cathepsin	
Target Category:	Enzyme	
Method of detection:	Optical imaging	
Source of signal / contrast:	BODIPY TMR-X	
Activation:	Yes	
Studies:	• *In vitro*	Click on the PubChem for additional information of the relevant substances.

Background

[PubMed]

The human cysteine cathepsins have 11 members (cathepsins B, C, H, F, K, L, O, S, V, W, and X/Z) and share a conserved active site that is formed by cysteine, histidine, and asparagine residues (1-5). Cathepsins B, L, H, F, O, X/Z, and C are expressed ubiquitously, whereas the expression of cathepsins S, K, W, and V are relatively organ-limited. Cysteine cathepsins interact with other proteases (aspartic, metallo, serine, and threonine) in a cascade-like manner, involving various physiological processes, including protein

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degradation, precursor protein activation, MHC-II–mediated antigen presentation, bone remodeling, keratinocytes differentiation, hair follicle cycle, reproduction, and apoptosis (6, 7). Increased expression and activity, and relocalization to the plasma membrane of cysteine cathepsins are associated with the pathogenesis of a number of human diseases such as cancer, atherosclerosis, and neurodegenerative diseases, and changes related to cysteine cathepsins have been shown to be of diagnostic and prognostic value for the diseases (2, 8-11).

Significant efforts have been made in developing optical molecular probes of protease activity (4, 7, 12, 13). These probes are either substrate-based or activity-based, providing a readout of the enzyme activity rather than simple protein abundance. Substrate-based probes (SBPs) are designed using fluorescent peptide sequences tethered to a large polymer or dendramer backbone, and these SBPs are internally quenched by the high density of fluorophores loaded onto the backbone structure. Similar to conventional enzymatic tests, SBPs appear to be less reliable because of overlapping substrate specificities, enzymatic activity instability, and interactions with endogenous inhibitors. Activity-based probes (ABPs) label target proteases through the formation of a covalent bond with the active site cysteine. The selectivity of an ABP is controlled by both its peptide selectivity sequence and reactive functional group. The fluorescent reporter allows probe-labeled cathepsins to be directly visualized. Because ABPs tend to be small molecules, the *in vivo* half-lives of ABPs are relatively short, which results in the production of high-contrast images. A drawback of using a covalent probe is the lack of signal amplification because the target proteases are inactivated upon binding the probe. However, sufficient levels of the active proteases have been found to exist in tumor tissues, which allows the generation of reasonable contrast images using noninvasive methods (4, 7, 12).

Blum et al. synthesized a group of near-infrared fluorescent activity-based probes (NIRF-ABPs) for noninvasive optical imaging of cysteine protease activity (4, 7, 12, 14). These probes can be subgrouped into quenched ABPs (qABPs) (e.g., GB117, GB119, GB135, and GB137) and nonquenched ABPs (e.g., GB111, GB123, and GB138). In short, the probes consist of a reactive group of peptide acyloxymethyl ketone (AOMK) that targets diverse members of cysteine cathepsins. The ketone in AOMK reacts with the cysteine in the enzyme active site and produces a stable thiomethyl ketone adduct. The covalent binding involves the loss of the acyloxy group of AOMK. Thus, a probe carrying a fluorescent reporter group on its peptide scaffold and a highly efficient quenching molecule attached to the acyloxy-losing group should result in a quenched probe that only becomes fluorescent upon covalent binding with an enzyme. In addition, a spacer is designed to reduce the steric congestion between the reporter and the fluorescence quencher. Blum et al. have shown that the NIRF-ABPs are nontoxic to cells, reasonably water soluble, potentially valuable as imaging agents for disease diagnosis, and powerful tools for *in vivo* preclinical and clinical testing of small-molecule therapeutic agents. Although NIRF-ABPs present different features regarding stability, specificity, and kinetics, the quenched probes can be used to image specific protease activity at considerably earlier time points than can be used for substrate-based methods or nonquenched ABPs. However, high

levels of signal in large organs with high cathepsin activity such as liver, kidney, and spleen make activity-based imaging of specific locations within the central body cavity difficult. In this chapter, the synthesis and analytic results of quenched GB117 were introduced, which were compared with that of GB111. The quenched GB117 and its corresponding nonquenched control GB111 are the first generation of this group of NIRF-ABPs.

Related Resource Links:

1) Cysteine cathepsin imaging-related chapters in MICAD:

- Substrate-based probes: Cy5.5-PL-MPEG, Cy5.5-GHPGGPQK(Fitc)C-PL-MPEG (Cy5.5-CatK-PGC)
- Activity-based probes: GB137

2) Gene and protein information of human cathepsins

3) Cathepsin-related resource in OMIM

4) Cathepsin-related substances in PubChem

Synthesis

[PubMed]

Blum et al. described a four-step synthesis of the probes GB117 and GB111 (12). The investigators first synthesized the fully protected carbobenzoxy-capped Phe-Lys dipeptide using standard solid-phase peptide synthesis (85% purity and 99% yield relative to resin loading). The investigators then converted the dipeptide to bromo-methyl ketone (BMK, 91% yield). A subsequent conversion of the BMK *via* *N*-trityl–protected glycine yielded AOMK (25% yield). As the final step, GB117 was generated by coupling the QSY7-quenched group after removal of the trityl group and by coupling the BODIPY-TMR-X fluorophore (excitation/emission is 544/570 nm) after removal of the *t*-butoxy carbonyl–protecting group on the side chain of lysine (72% yield). In contrast to GB117, the QSY7-quenched group was not coupled for the control GB111 (58% yield). The molecular weights were 1,632.7 and 1,089.6 for GB117 and GB111, respectively (12).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Blum et al. evaluated the quenching of fluorescent signals and the effect of fluorophore attachment on the probe potency toward cathepsins (12). Attachment of the fluorophore to the P1 lysine side chain resulted in >70-fold increased fluorescent quenching for GB117 compared with the nonquenched GB111. Fluorophore attachment to the more distal positions (i.e., P2, P3, or the amino terminus) also led to sufficient quenching for imaging applications (data not shown). Because the majority of the critical substrate interactions

take place in the P2 position of the papain-family proteases, Blum et al. selected the P1 position for fluorophore attachment in developing probes. After attachment, GB111 exhibited a 100-fold loss of potency toward cathepsin L and a 30-fold loss of potency for cathepsin B when compared with its free amine intermediate (NH_2-GB111). Interestingly, replacement of the 2,6-methyl benzoyl group with the QSY7 quencher resulted in a larger decrease in the potency of GB117 toward cathepsin B than toward cathepsin L. The investigators explained this phenomenon by postulating that the QSY7 group potentially produced van der Waals clashes with the occluding loop of cathepsin B. The apparent rate constant (K_{app}) of GB111 was 2,580 ± 800 and 1,256 ± 308 for cathepsins L and B, respectively. The K_{app} of GB117 was 7,000 ± 1,200 and 275 ± 66 for cathepsins L and B, respectively. These results suggest that GB117 is more specific to cathepsin L than to cathepsin B.

Blum et al. tested the labeling of cathepsins B and L with these probes with the use of purified recombinant cathepsins in intact cells (12). Both GB111 and GB117 labeled the enzymes through a stable covalent linkage. This labeling can be specifically countered with pretreatment of the enzymes with a broad-spectrum, papain-family cysteine protease inhibitor JPM-OEt (15). However, the investigators noticed that both GB111 and GB117 also labeled other purified cathepsins (cathepsins X and S; data not shown), suggesting that these probes may label multiple targets. In intact NIH-3T3 cells, both probes have been shown to label endogenous enzyme targets after incubation of the cells with the probes. Under microscopy, incubation of the NIH-3T3 cells with GB111 resulted in an intense, nonspecific labeling of the entire cell when imaged without washing, suggesting that GB111 does not accumulate in any particular location but distributes throughout the cell. On the contrary, a unique labeling pattern for GB117 was observed, which overlapped with the signal from the acidotropic lysosomal marker Lysotracker. The cell labeling can be blocked with pretreatment of the cells with inhibitor JPM-OEt for 1 h. With extensive washing after incubation of the cells with GB111, a labeling pattern similar to that seen with GB117 was observed. GB117 had a slower rate of modification than did GB111, suggesting that extended labeling times would be required to obtain optimal signal. SDS-PAGE analysis of the extracts of wild-type, cathepsin B–deficient, and cathepsin L–deficient fibroblast cells have further confirmed that GB117 is more specific to cathepsin L than to cathepsin B. These results indicate that both probes freely penetrate cells and label a series of protease targets with a high selectivity to cathepsins L and B. GB117 is fluorescently activated upon binding to protease targets and provides sufficient signal over noise to allow direct real-time analysis of protease activity in live cells.

To further confirm the feasibility of GB117 in imaging protease activity, Blum et al. evaluated the probes using a three-dimensional MCF-10A cell model that more accurately mimics human cancer (12). The fibrocystic breast cell line MCF-10A forms highly organized three-dimensional spheroids with glandular architecture when grown on reconstituted basement membranes (or matrix), and this line is considered to be an excellent cellular model for various forms of tumor growth. After 3 days of cell growth on the matrix containing GB111, direct imaging without washing produced a bright, nonspecific, intracellular fluorescent staining. The free probe in the matrix did not show

any significant fluorescence, which suggested a quenching effect by matrix proteins. When the spheroids were washed and fixed, a specific staining pattern was observed, which was partially overlapped with immunofluorescent staining of the same fixed cells with an anti-cathepsin B antibody. Cells incubated on the matrix containing GB117 demonstrated a distinct punctate staining of the lysosomal compartments, and this staining was completely blocked by addition of the JPM-OEt, which suggested a specific staining of the active proteases. The population of active cathepsin B labeled by the probes was found predominantly at the apical pole of the cells in the acini, although the total distribution of cathepsin B protein was both apical and basal. These results further support the specific nature of the ABPs and also highlight the difference in imaging active protease populations rather than total protein levels with antibodies that recognize both precursor and active forms of proteases.

Blum et al. concluded that qABPs can be used to dynamically image protease activity in real time. It should be possible to apply this methodology to a range of diverse cysteine proteases. The use of this quenched-probe strategy can be adapted easily to incorporate near-infrared tags that may facilitate noninvasive, whole-body imaging applications. One of the main limitations to this strategy is the lack of signal amplification, which potentially reduces the probe utility for whole-body imaging (4, 7, 12, 14).

Animal Studies

Rodents

[PubMed]

No references are currently available.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

References

1. Colbert J.D., Matthews S.P., Miller G., Watts C. *Diverse regulatory roles for lysosomal proteases in the immune response.* Eur J Immunol. 2009;39(11):2955–65. PubMed PMID: 19637232.
2. Affara N.I., Andreu P., Coussens L.M. *Delineating protease functions during cancer development.* Methods Mol Biol. 2009;539:1–32. PubMed PMID: 19377975.
3. Cox J.L. *Cystatins and cancer.* Front Biosci. 2009;14:463–74. PubMed PMID: 19273078.
4. Blum G., von Degenfeld G., Merchant M.J., Blau H.M., Bogyo M. *Noninvasive optical imaging of cysteine protease activity using fluorescently quenched activity-based probes.* Nat Chem Biol. 2007;3(10):668–77. PubMed PMID: 17828252.
5. Hang H.C., Loureiro J., Spooner E., van der Velden A.W., Kim Y.M., Pollington A.M., Maehr R., Starnbach M.N., Ploegh H.L. *Mechanism-based probe for the analysis of cathepsin cysteine proteases in living cells.* ACS Chem Biol. 2006;1(11):713–23. PubMed PMID: 17184136.
6. Palermo C., Joyce J.A. *Cysteine cathepsin proteases as pharmacological targets in cancer.* Trends Pharmacol Sci. 2008;29(1):22–8. PubMed PMID: 18037508.
7. Blum G. *Use of fluorescent imaging to investigate pathological protease activity.* Curr Opin Drug Discov Devel. 2008;11(5):708–16. PubMed PMID: 18729022.
8. Cavallo-Medved D., Rudy D., Blum G., Bogyo M., Caglic D., Sloane B.F. *Live-cell imaging demonstrates extracellular matrix degradation in association with active cathepsin B in caveolae of endothelial cells during tube formation.* Exp Cell Res. 2009;315(7):1234–46. PubMed PMID: 19331819.
9. Gounaris E., Tung C.H., Restaino C., Maehr R., Kohler R., Joyce J.A., Ploegh H.L., Barrett T.A., Weissleder R., Khazaie K. *Live imaging of cysteine-cathepsin activity reveals dynamics of focal inflammation, angiogenesis, and polyp growth.* PLoS One. 2008;3(8):e2916. PubMed PMID: 18698347.
10. Lutgens S.P., Cleutjens K.B., Daemen M.J., Heeneman S. *Cathepsin cysteine proteases in cardiovascular disease.* FASEB J. 2007;21(12):3029–41. PubMed PMID: 17522380.
11. Joyce J.A., Baruch A., Chehade K., Meyer-Morse N., Giraudo E., Tsai F.Y., Greenbaum D.C., Hager J.H., Bogyo M., Hanahan D. *Cathepsin cysteine proteases are effectors of invasive growth and angiogenesis during multistage tumorigenesis.* Cancer Cell. 2004;5(5):443–53. PubMed PMID: 15144952.
12. Blum G., Mullins S.R., Keren K., Fonovic M., Jedeszko C., Rice M.J., Sloane B.F., Bogyo M. *Dynamic imaging of protease activity with fluorescently quenched activity-based probes.* Nat Chem Biol. 2005;1(4):203–9. PubMed PMID: 16408036.
13. Yang Z., Fonovic M., Verhelst S.H., Blum G., Bogyo M. *Evaluation of alpha,beta-unsaturated ketone-based probes for papain-family cysteine proteases.* Bioorg Med Chem. 2009;17(3):1071–8. PubMed PMID: 18343672.
14. Blum G., Weimer R.M., Edgington L.E., Adams W., Bogyo M. *Comparative assessment of substrates and activity based probes as tools for non-invasive optical imaging of cysteine protease activity.* PLoS One. 2009;4(7):e6374. PubMed PMID: 19636372.

15. Schurigt U., Sevenich L., Vannier C., Gajda M., Schwinde A., Werner F., Stahl A., von Elverfeldt D., Becker A.K., Bogyo M., Peters C., Reinheckel T. *Trial of the cysteine cathepsin inhibitor JPM-OEt on early and advanced mammary cancer stages in the MMTV-PyMT-transgenic mouse model.* Biol Chem. 2008;389(8):1067–74. PubMed PMID: 18710344.