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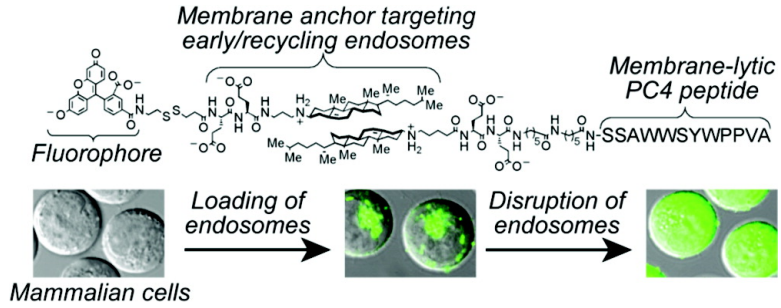

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Selective Disruption of Early/Recycling Endosomes: Release of Disulfide-Linked Cargo Mediated by a *N*-Alkyl-3β-Cholesterylamine-Capped Peptide

Qi Sun,[†] Sutang Cai,[‡] and Blake R. Peterson*[,§]

Integrative Biosciences and Chemistry Graduate Programs, The Pennsylvania State University, University Park, Pennsylvania 16802, and Department of Medicinal Chemistry, The University of Kansas, Lawrence, Kansas 66045

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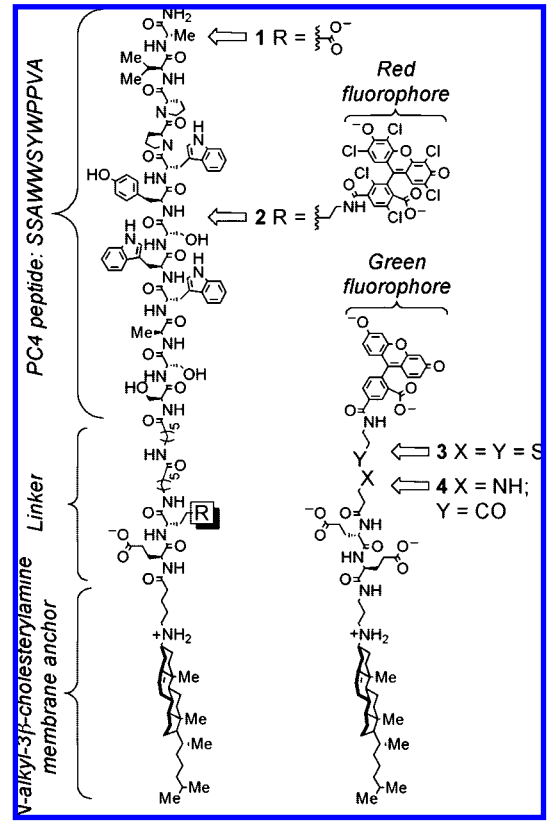
Receptor-mediated endocytosis (RME) is a major mechanism of uptake of impermeant molecules by mammalian cells.[1] In this process, extracellular ligands bind cell surface receptors that cluster in dynamic regions of cellular plasma membranes. By actively pinching off to form intracellular vesicles, these membrane regions are internalized, encapsulating ligand−receptor complexes in the cytoplasm. These vesicles fuse and form early (primary/sorting) endosomes that are acidified (pH $\approx$ 6) by the activation of proton pumps, conditions that generally promote the dissociation of receptors from bound ligands. Free receptors often cycle back to the cell surface, generally via subsequent trafficking through related recycling endosomes (also termed the endocytic recycling compartment).[2] In contrast, free ligands are typically directed to more acidic late endosomes and lysosomes (pH $\approx$ 5), where hydrolases and other enzymes promote their degradation. Some viruses and other intracellular pathogens exploit RME to enter cells, but these organisms avoid degradation in lysosomes by expressing pH-dependent fusogenic proteins that disrupt endosomal membranes.[3] To escape entrapment within these membranes and gain access to the cytosol, Semliki Forest virus disrupts early endosomes whereas influenza virus disrupts late endosomes during the course of infection.[3]

We report here the synthesis of novel compounds designed to enable membrane-bound disulfide-linked cargo to selectively escape from early/recycling endosomes of living mammalian cells. Because these endosomes are less acidic and less hydrolytically active than late endosomes/lysosomes, this approach may be advantageous when compared to delivery methods that penetrate deeper into the endosomal system. To selectively deliver compounds into early/recycling endosomes, we synthesized four derivatives of the dynamic membrane anchor *N*-alkyl-3β-cholesterylamine[4,5] (**1**−**4**). Two of these compounds (**1**, and red fluorescent **2**) incorporate PC4, a pH-dependent membrane-lytic dodecapeptide previously reported[6] by Weber to disrupt membranes of liposomes. Two others comprise the green fluorophore 5-carboxyfluorescein linked through disulfide (**3**) and amide (**4**) bonds. The unmodified PC4 peptide with the amino acid sequence AcNH(SSAWWSYWPPVA)CONH$_2$ (**5**) was additionally prepared as a control.

When added to mammalian cells, derivatives of *N*-alkyl-3β-cholesterylamine have been shown to become avidly incorporated in cellular plasma membranes and engage a membrane trafficking pathway that involves rapid cycling between the cell surface and intracellular endosomes, similar to many natural cell surface receptors.[4] The partitioning of these compounds between the plasma membrane and endosomes is affected by the structure of the linker region proximal to the membrane anchor.[7] In **1**−**4**, the glutamic acid residue(s) in this region were installed to enhance the localization of these compounds



in endosomes compared to the plasma membrane. Because early/recycling endosomes are thought to be oxidizing,[8] we hypothesized that the disulfide of **3** should be relatively stable in these compartments. However, if **3** were exposed to reduced glutathione (**GSH**), a thiol present at mM concentrations in the cytosol, this functional group would be cleaved (Figure 1).[9] Correspondingly, disruption of early/recycling endosomes loaded with **3** by compounds **1** or **2** was proposed as a mechanism to enable **GSH** to access these compartments, reduce the disulfide of **3**, and release the soluble fluorophore **6** into the cytoplasm and nucleus of cells (Figure 1).

Confocal laser scanning microscopy was employed to examine the subcellular localization of fluorescent compounds added to living mammalian cells. In Chinese hamster ovary (CHO) cells, compound **3** was found to become localized in defined intracellular compartments that reside outside of the cell nucleus (Figure 2). These compartments were identified as early/recycling endosomes by essentially complete intracellular colocalization with red fluorescent transferrin protein, a highly selective marker.[10] As a control, cells were similarly treated with red fluorescent DiI-labeled low density lipoprotein (LDL), a protein that selectively accumulates in late endosomes and lysosomes.[11]

[†] Integrative Biosciences Graduate Program, Penn State University.
[‡] Chemistry Graduate Program, Penn State University.
[§] The University of Kansas.

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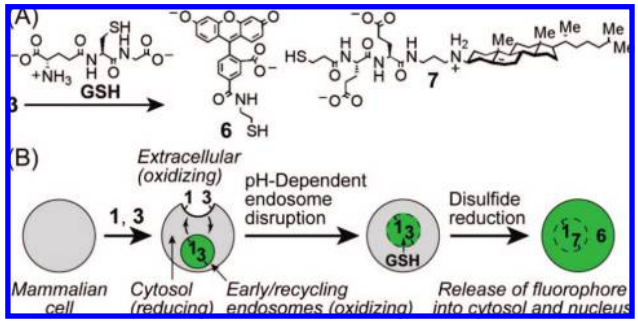

Figure 1. Strategy for the selective release of disulfide-tethered cargo from membranes of early/recycling endosomes mediated by **1** or **2**: (A) products of cleavage of **3** by glutathione; (B) mechanism of release of fluorophore **6** into the cytosol and nucleus of mammalian cells.

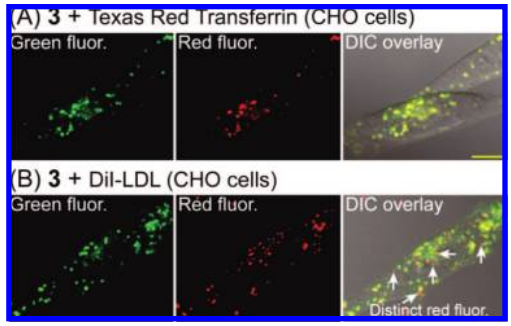

Figure 2. Confocal laser scanning and differential interference contrast (DIC) micrographs of living CHO cells treated with green fluorescent **3** (5 μM) for 12 h followed by (A) red fluorescent Texas Red transferrin (500 nM) or (B) DiI-LDL (8 nM) for 5 min. Colocalization of red and green fluorescence is shown as yellow pixels in the DIC overlay images. Arrows point to distinct red fluorescence. Scale bar = 10 μm.

Treatment with DiI-LDL revealed distinct red fluorescence, establishing that in this cell line the *N*-alkyl-3β-cholesterylamine membrane anchor promotes delivery of the fluorophore of **3** to early/recycling endosomes with a high level of specificity.

Compared to **3** alone, living cells treated with both **3** and **1** (or **2**) showed a strikingly different pattern of intracellular fluorescence (Figure 3). When combined with **1** or **2**, the green fluorescence of **3** was released from entrapment in early/recycling endosomes and fluorescence was observed in the cytosol and nucleus. As shown in Figure 3, this release of fluorescent cargo from endosomal membranes was effective in both adherent cells (CHO) and suspension cells (human Jurkat lymphocytes). Consistent with the model shown in Figure 1, replacement of the disulfide of **3** with the amide bond of **4** blocked release of the fluorophore (Figure 3C,I). The red fluorescence of **2** allowed visualization of the linked PC4 peptide in early/recycling endosomes (see the Supporting Information and Figure 3E,F). Colocalization of **1** or **2** with **3** in these compartments was required to promote efficient cargo release; little effect was observed with the unmodified PC4 peptide (**5**). To investigate the importance of endosomal acidity on the function of the PC4 peptide,[6] we increased endosomal pH by adding chloroquine[12] and bafilomycin A1[13] (Supporting Information). These compounds blocked release of the fluorophore (Figure 3, compare panels E and F), consistent with the pH-dependent membrane-lytic activity of PC4. Because the acidity of endosomes is required for efficient membrane disruption, deleterious effects of **1** and **2** on the plasma membrane, which is surrounded by media of pH 7.4, should be limited. Consistent with this idea, assays of cellular viability (Figure 3K) revealed that **1** is nontoxic under conditions

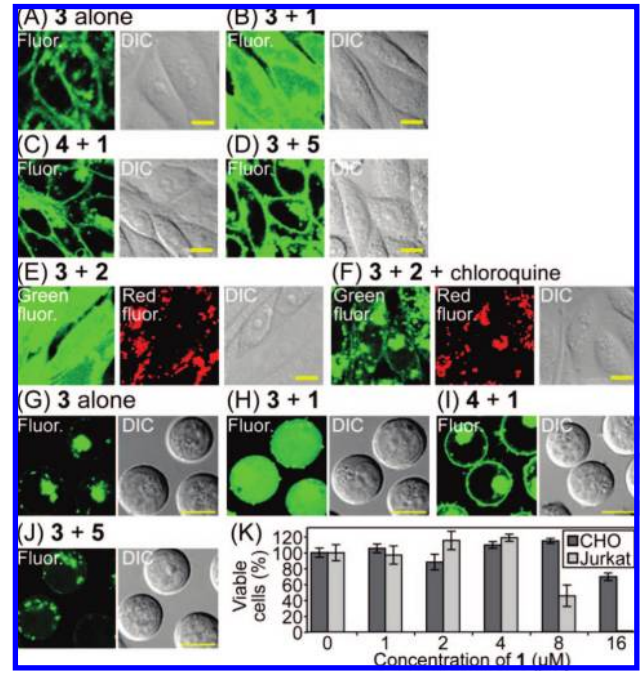

Figure 3. (A−J) Confocal fluorescence and DIC micrographs of living cells treated with fluorescent probes. (A−F) CHO cells were treated with **3** or **4** (5 μM) and **1**, **2**, or **5** (8 μM) for 24 h. In panel F, [chloroquine] = 5 μM. In panels G−J, Jurkat lymphocytes were treated with **3** or **4** (2.5 μM) and **1** or **5** (2 μM) for 12 h. Scale bars = 10 μm. (K) Toxicity to CHO and Jurkat cells after incubation with **1** for 48 h at 37 °C.

that disrupt early/recycling endosomes in CHO cells (8 μM) or Jurkat lymphocytes (2 μM, see the Supporting Information).

Although other synthetic vehicles that disrupt endosomes have been reported,[14] the ability of *N*-alkyl-3β-cholesterylamines to specifically target a subset of relatively nonhydrolytic early/recycling endosomes and release disulfide-linked cargo from these compartments may be advantageous for a variety of cellular delivery applications.

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Supporting Information Available: Supporting figures, experimental procedures, and compound characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Conner, S. D.; Schmid, S. L. *Nature* **2003**, *422*, 37–44.
(2) Maxfield, F. R.; McGraw, T. E. *Nat. Rev. Mol. Cell. Biol.* **2004**, *5*, 121–132.
(3) Lakadamyali, M.; Rust, M. J.; Zhuang, X. *Microbes Infect.* **2004**, *6*, 929–936.
(4) Peterson, B. R. *Org. Biomol. Chem.* **2005**, *3*, 3607–3612.
(5) Boonyarattanakalin, S.; Hu, J.; Dykstra-Rummel, S. A.; August, A.; Peterson, B. R. *J. Am. Chem. Soc.* **2007**, *129*, 268–269.
(6) Hirosue, S.; Weber, T. *Biochemistry* **2006**, *45*, 6476–6487.
(7) Boonyarattanakalin, S.; Martin, S. E.; Dykstra, S. A.; Peterson, B. R. *J. Am. Chem. Soc.* **2004**, *126*, 16379–16386.
(8) Austin, C. D.; Wen, X.; Gazzard, L.; Nelson, C.; Scheller, R. H.; Scales, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 17987–17992.
(9) Saito, G.; Swanson, J. A.; Lee, K. D. *Adv. Drug Deliv. Rev.* **2003**, *55*, 199–215.
(10) Sheff, D.; Pelletier, L.; O'Connell, C. B.; Warren, G.; Mellman, I. *J. Cell. Biol.* **2002**, *156*, 797–804.
(11) Ghosh, R. N.; Gelman, D. L.; Maxfield, F. R. *J. Cell Sci.* **1994**, *107*, 2177–2189.
(12) Adachi, K.; Ichinose, T.; Takizawa, N.; Watanabe, K.; Kitazato, K.; Kobayashi, N. *Arch. Virol.* **2007**, *152*, 2217–2224.
(13) Yoshimori, T.; Yamamoto, A.; Moriyama, Y.; Futai, M.; Tashiro, Y. *J. Biol. Chem.* **1991**, *266*, 17707–17712.
(14) Wolff, J. A.; Rozema, D. B. *Mol. Ther.* **2008**, *16*, 8–15.

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