Native Zinc Catalyzes Selective and Traceless Release of Small Molecules in β-Cells

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ABSTRACT: The loss of insulin-producing β-cells is the central pathological event in type 1 and 2 diabetes, which has led to efforts to identify molecules to promote β-cell proliferation, protection, and imaging. However, the lack of β-cell specificity of these molecules jeopardizes their therapeutic potential. A general platform for selective release of small-molecule cargoes in β-cells over other islet cells ex vivo or other cell-types in an organismal context will be immensely valuable in advancing diabetes research and therapeutic development. Here, we leverage the unusually high Zn(II) concentration in β-cells to develop a Zn(II)-based prodrug system to selectively and tracelessly deliver bioactive small molecules and fluorophores to β-cells. The Zn(II)-targeting mechanism enriches the inactive cargo in β-cells as compared to other pancreatic cells; importantly, Zn(II)-mediated hydrolysis triggers cargo activation. This prodrug system, with modular components that allow for fine-tuning selectivity, should enable the safer and more effective targeting of β-cells.

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reside. For most cargoes (e.g., small-molecule inducers of β-cell proliferation), both the activation mechanism and the escape from insulin granules are critical—selective activation prevents proliferation in off-target tissues, whereas the exit from the granules ensures that the small molecule reaches its protein targets. Third, the hydrolytic mechanism allows traceless release of the cargo in its native form, without any modifications, allowing ZnPDs to be developed for small molecules that cannot tolerate modifications without a loss of activity. Fourth, several Zn(II) ligands exist with a wide variety of affinities ranging from pM to mM, allowing the precise fine-tuning of β-cell specificity.28 Finally, although the aqueous Zn(II) ion is not a potent Lewis acid, multiple tridentate coordinating ligands exist that can be placed proximally to the scissile bond of a ZnPD, thereby facilitating a high effective molarity of Zn(II) with an available coordination site and potent Lewis acidity.

Before constructing ZnPDs, we first determined the rate of Zn(II)-mediated hydrolysis in β-cells compared to other islet cells. We employed a previously reported reaction-based probe, DA-ZP1, a PET-based zinc sensor that requires Zn(II)-mediated unmasking of DA-ZP1 to turn-on fluorescence (Figure 1A) in a way that is selective for Zn(II) over other biologically relevant metal ions.27 DA-ZP1’s activity in β-cells has not been demonstrated. To confirm that the unmasking of DA-ZP1 fluorescence in cells was not catalyzed by an esterase and that proximally located dipicolyl ligands are necessary for hydrolytic cleavage, we tested the compound DA-FC, which lacks the Zn(II)-binding dipicolyl moieties. As expected, DA-FC did not fluoresce in the presence of Zn(II) (Figure 1B).27 To demonstrate that the high intracellular Zn(II) concentrations could release fluorescent cargo selectively in β-cells, we incubated DA-ZP1 in a β-cell line (INS-1E), an α-cell line (αTC1.6), a ductal cell line (PANC-1), and cells of other lineages (Figures 1C, D and S1A). After a 1 h incubation, the β-cells were highly fluorescent compared to the other cell types, with minimal background fluorescence at concentrations much greater than the K_d of DA-ZP1 (0.6 nM).26 β-Cells treated with the control compound DA-FC were not fluorescent. Finally, we monitored the kinetics of fluorescence release in β-cells at various concentrations of DA-ZP1 (n = 4). See also Figure S3. Human pancreatic donor information is available in Table S1. (G) Dispersed human islets were stained, and DA-ZP1+ and DA-ZP1– cells were collected after FACS (n = 4). Representative images show C-peptide (green) and glucagon (red) staining in unsorted, DA-ZP1+, DA-ZP1– cell populations. Nuclei stained with DAPI (blue).

Figure 1. (A) Zn(II)-mediated unmasking of DA-ZP1 releases active fluorophore ZP1. (B) Structure of DA-FC and graph of Zn(II)-mediated fluorescence release of DA-ZP1 and DA-FC. (C) Selective unmasking of DA-ZP1 fluorescence in INS-1E cells compared to other cell types. (D) Representative images of DA-ZP1- or DA-FC-treated cells under the FITC channel (top) measuring ZP1 release, DAPI staining (middle), and the overlay (bottom). (E) Representative confocal images of dissociated human islets treated with DA-ZP1 followed by immunostaining for C-peptide. (F) Quantification of dispersed human islets treated with DA-ZP1 (n = 4). See also Figure S3. Human pancreatic donor information is available in Table S1. (G) Dispersed human islets were stained, and DA-ZP1+ and DA-ZP1– cells were collected after FACS (n = 4). Representative images show C-peptide (green) and glucagon (red) staining in unsorted, DA-ZP1+, DA-ZP1– cell populations. Nuclei stained with DAPI (blue).
and S4C). ZnPD1, lacking electron-withdrawing groups, released rhodamine urea in a Zn(II)-dependent fashion in PBS (Figure S4A), which was also confirmed by liquid chromatography−mass spectrometry (LC-MS) (Figure S4D). Removing the Zn(II)-binding ligand from ZnPD1 (ZnPD1ctrl, Figure S4E) prevented the release of rhodamine urea (Figure S4F), again confirming the proximity effect. We then demonstrated dose-dependent release of rhodamine urea in INS-1E cells at low micromolar concentrations (Figure S5).

Unfortunately, the low fluorescence intensity of rhodamine urea prevented us from performing cell-selectivity studies through fluorescence, although we did confirm selective enrichment by extracting the compound from the cells followed by LC-MS analysis (Figure 2B).

To demonstrate that this prodrug system is generalizable to other cargo types and hydrolyzable bonds, we used a boron dipyrromethene (BODIPY)-based caged probe, where the meso-carboxyl moiety of BODIPY was caged with a self-immolative linker (ZnPD4, Figure 2C).33 Upon Zn(II) binding, the carbamate linkage undergoes hydrolysis and triggers self-immolation, yielding the native fluorophore (Figure S6A). With ZnPD4, we observed selective fluorescence emission in only INS-1E cells but no other cell lines (Figure 2D and E), with dose and kinetic studies showing the fast turn-on of BODIPY fluorescence in INS-1E cells (Figure S6B and S6C).

After the successful, selective release of BODIPY in INS-1E cells, we used ZnPD4 to capture β-cells from dissociated human islets (Figures 2F, G and S7) and observed an enrichment of 73% in β-cell population.

We next designed ZnPD5 (Figure 3A) based on DA-ZP1 for simultaneous release of fluorescence and a small molecule (GNF-4877) that promotes pancreatic β-cell proliferation.36,37 We could follow the Zn(II)-triggered hydrolysis of ZnPD5 through a steep rise in fluorescence intensity (Figures 3A, 3B and S8A) followed by saturation, indicating reaction completion that was also confirmed using LC-MS (Figure S8B). Additionally, ZnPD5 was stable in cell culture media and hydrolyzed only in the presence of added Zn(II) (Figure S8C).

More importantly, we observed selective fluorescence emission from INS-1E cells when incubated with ZnPD5 (Figures 3C, D and S8D). The direct role of Zn(II) for ZnPD5 was confirmed by using the metal chelator TPEN, whereby INS1E cells preincubated with TPEN failed to hydrolyze ZnPD5 (Figure S8E). Next, we tested ZnPD5 in human islets and observed fluorescence release in β-cells (Figures 3E and S9A, B) as well as dose-dependent induction of proliferation (Figures 3F, S9C).
modular in nature, with a specific molecules, including Furthermore, this approach can deliver various small based prodrug system for selective and traceless cargo release in

Figure 3. (A) Structures of ZnPD5 and its Zn(II)-catalyzed release of GNF-4877. (B) Reaction kinetics of ZnPD5 with different concentrations of Zn(II) as monitored by fluorescence spectroscopy. (C) Fold change in fluorescence in INS1E cells versus other cells. (D) Representative images of ZnPD5-treated cells under the FITC channel (top), DAPI staining (middle), and the overlay (bottom). (E) Representative fluorescence images of intact human islets showing β-cell selective hydrolysis of ZnPD5 under the FITC channel (green) and C-peptide (red). Intact islet cells were treated with either DMSO (top) or 150 nM of ZnPD5 (bottom). (F) Dose-dependent induction of β-cell proliferation by ZnPD5 in human islets.

and D). The degree of proliferation induced by ZnPD5 is similar to that of GNF-4877 suggesting efficacious release of the cargo (i.e., GNF-4877).

We report the first examples of a rationally designed zinc-based prodrug system for selective and traceless cargo release in β-cells. These ZnPDs are nonenzymatic, cell-permeable, and modular in nature, with a specificity that can be fine-tuned. Furthermore, this approach can deliver various small molecules, including β-cell mitogens. Using controls lacking zinc-binding moieties (e.g., ZnPD1ctrl and DA-FC), we show that cargo release is not due to cellular esterases and con

ASSOCIATED CONTENT
Supporting Information
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Notes

The authors declare the following competing financial interest(s): Broad Institute has led PCT/US2018/028660 that claims inventions related to targeted delivery to beta cells.

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