低分子蛍光プローブを用いた酸性細胞内オルガネラにおける遊離 Zn^{2+} の可視化と定量化

Visualization and quantification of labile Zn^{2+} in the acidic subcellular compartments using a small-molecule fluorescent probe

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Zn^{2+} plays a fundamental and crucial role in all forms of life, including plants, animals, and humans. In particular, zinc is an essential element for the pancreatic β cells especially in acidic insulin granules. Zn^{2+} and insulin form a hexametric crystal structure inside insulin granules, which is involved in the insulin biosynthesis, maturation, storage and secretion process. Zn^{2+} transporters, ZIPs and ZnTs, help to maintain the homeostasis of pancreatic β cells, and their dysfunction may cause abnormal Zn^{2+} homeostasis in the pathogenesis of type 2 diabetes. Thus, targeting Zn^{2+} transporting pathways could be a potential therapeutic strategy for treating diabetes, suggesting the significance of monitoring the Zn^{2+} levels in acidic insulin granules. Nowadays, fluorescence imaging has been considered as one of the most powerful tools to visualize and quantify the labile Zn^{2+} in live cells. There have been several reports that estimated labile [Zn^{2+}] in the insulin granule to be ranging from 1 to 100 μM. However, since the fluorescence properties and zinc-binding ability of protein-based probes are greatly affected by acidic environments of the insulin granule (pH 5–6), the labile [Zn^{2+}] in the insulin granules is still controversial.

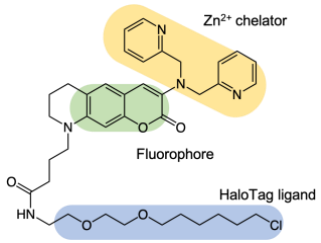

Figure 1. The structure of ZnDA-1H.

Recently, we have developed a small-molecule fluorescent probe, ZnDA-1H, which is composed of a coumarin fluorophore, dipicolyl amine as a Zn^{2+} chelator and a HaloTag ligand (Figure 1). Owing to the less pH-sensitivity of ZnDA-1H, we have achieved the quantification of labile [Zn^{2+}] in the Golgi apparatus, which is known as a slightly acidic subcellular compartment (pH 6.0–6.7).[1] In the current study, ZnDA-1H was utilized to visualize and quantify labile Zn^{2+} in the insulin granules of the pancreatic β cells, MIN6. Firstly, the K_d value of HaloTag-conjugated ZnDA-1H for Zn^{2+} was determined to be 0.86 ± 0.09 μM at pH 6.0. Thus, ZnDA-1H is expected to be a promising probe for visualizing the nM- to μM-level labile Zn^{2+} in acidic compartments. By expressing HaloTag in the insulin granules of MIN6 cells, ZnDA-1H was successfully localized into the insulin granules. Through labeling HaloTag with a zinc-insensitive fluorescent dye, HTL-TMR, and ZnDA-1H, we attempted the quantification of labile [Zn^{2+}] in the insulin granules. (Figure 2)

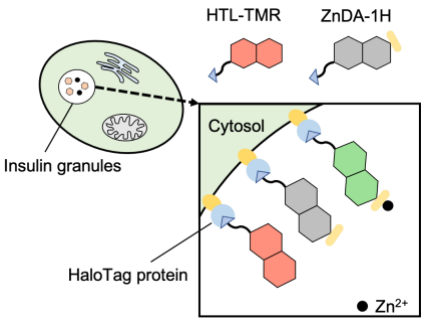

Figure 2. Illustration of organellar targeting of ZnDA-1H.

＜参考文献＞

1）Kowada, T., Watanabe, T., Amagai, Y., Liu, R., Yamada, M., Takahashi, H., Matsui, T., Inaba, K., Mizukami, S. *Cell Chem. Biol.*, **2020**, *27*, 1521–1531.

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