Development of high-affinity fluorescent probes for quantification of organellar labile Zn^{2+}

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Cellular zinc, of which homeostasis is tightly maintained by zinc transporters (ZIPs and ZnTs) and zinc buffer (metallothioneins and metalloproteins), plays several essential biological functions in a cell.[1] While the total concentration of intracellular Zn^{2+} is at μM level, the labile Zn^{2+} concentration ([Zn^{2+}]) is regulated at a much lower level (pM–nM).[2] To date, a large number of studies have devoted to the development of Zn^{2+} fluorescent probe for detecting the labile Zn^{2+} in cells. However, the subcellular [Zn^{2+}] at the organelle level has not reached a consensus, which might be because of the lack of robust probes and proper quantification methodology. In our recent study, through the combination of the rational design of a 7-aminocoumarin-based small-molecule fluorescent probe and the utilization of a protein-labeling technology, we developed a organelle-localizable green fluorescent Zn^{2+} probe ZnDA-1H, which has less-pH sensitivity and moderate affinity to Zn^{2+} (K_d = 0.24 μM).[3] By co-labeling of HaloTag with ZnDA-1H and a Zn^{2+}-insensitive red fluorescent HaloTag ligand, HTL-TMR, as a standard, quantitative imaging of [Zn^{2+}] in the Golgi apparatus was achieved.

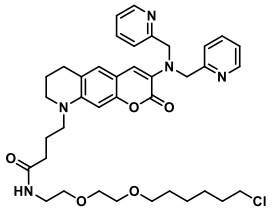

Figure 1. Structure of ZnDA-1H.

In this study, in order to achieve real-time monitoring of Zn^{2+} dynamics and quantitative mapping of [Zn^{2+}] in the other cell compartments, such as the ER and nucleus, we developed new ZnDA derivatives with higher affinity to Zn^{2+}, resulting in ZnDA-2H (K_d = 5.0 nM) and ZnDA-3H (K_d = 0.16 nM). We investigated the fluorescence enhancement mechanism of the ZnDA-probes and assumed that binding with Zn^{2+} could hinder the transition to the twisted intramolecular charge transfer (TICT) state in the excited state. Both new probes showed nearly pH-independent fluorescence at various pH (5.5–8.0), suggesting that these probes could be used for monitoring of cellular Zn^{2+} fluctuation without the serious disturbance from pH change. Furthermore, considering the specific localization ability and suitable affinity, ZnDA-3H was used for quantification of organellar [Zn^{2+}] in HeLa cells. Finally, quantitative [Zn^{2+}] mapping of the cells was achieved, and the results implied that the [Zn^{2+}] in the cytosol (0.15 nM) and nucleus (0.14 nM) are higher than the [Zn^{2+}] in the ER (13 pM) and mitochondria (24 pM). Additionally, a significant increase of [Zn^{2+}] in the ER (0.41 nM) was observed following the treatment of the ZIP7 inhibitor, and the [Zn^{2+}] was retained at a higher level more than 20 min. This result indicated that the [Zn^{2+}] in the ER is maintained at a very low level under the physiological condition and the high [Zn^{2+}] might involve in the unfolded protein response and upregulation of ER stress.

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