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. While the total concentration of intracellular Zn\(^{2+}\) is at \(\mu\)M level, the labile Zn\(^{2+}\) concentration ([Zn\(^{2+}\)]) is regulated at a much lower level (pM–nM). 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\(^{2+}\) (\(K_d = 0.24 \mu\)M). 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.

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.