Properties of Reagents used in Cellular Zn^{2+} Sensing

Jeff W. Meeusen, Jamieson B. Koestler, Susan K. Kresoski, David H. Petering

University of Wisconsin – Milwaukee; Dept. Chemistry/Biochemistry

Fluorescent metal chelating agents are used to image changes in intracellular chelatable Zn^{2+} that occur as part of physiological or pathological events. Other reagents such as 2-pyridinethiol-1-oxide, (pyrithione, pyr) are employed as ionophores to deliver Zn^{2+} to cells. These compounds have been used largely in the absence of information about toxicity or capacity to alter or modulate the intracellular Zn^{2+} pools, independent of the Zn-related cellular process they are intended to observe. The present report begins an inquiry into the properties of such reagents in cultured LLC-PK_1 pig kidney cells. Experiments were performed with control cells and cells induced to express metallothionein (MT) by 24h exposure to 40ìM Zn^{2+}. A commonly employed fluorophore, N-(6-methoxy-8-quinolyl)-p-toluenesulfoamide (TSQ), forms a 2:1 complex with Zn^{2+} in a reaction that causes a dramatic increase in fluorescent emission (~490nm). In LLC-PK_1 cells, 1h exposure to 30ìM TSQ reduces cell viability by 80% (MTT assay) *only* in the presence of 15ìM Zn^{2+} in the medium. Sephadex G-75 gel filtration analysis of the cytosolic Zn^{2+} after cell exposure to 10ìM TSQ and 5ìM Zn^{2+} for 30 min, showed intracellular Zn^{2+} increased by 1.7 fold overall with extra Zn^{2+} appearing in the high molecular weight, >30kD, (HMW) (1.7x), MT (1.4x), and low molecular weight, <3kD, (LMW) (3.2x) fractions. These results imply that TSQ acts as an ionophore, its toxicity is related to the delivery of Zn^{2+} into the cell, cellular HMW and MT binding sites compete with TSQ for Zn^{2+}, and possibly about 50% of the acquired Zn^{2+} remains as $Zn(TSQ)_2$ in the LMW fraction. In turn, these results suggest that if $Zn(TSQ)_2$ is generated from the intracellular reaction of Zn^{2+} with TSQ it would be able to subsequently redistribute Zn^{2+} to other sites such as the HMW fraction. Preliminary results show that 30 min incubation of cells with 10ìM TSQ reduces the total cellular Zn^{2+} by 16%. Pyr is more toxic than TSQ, reducing cell viability by 70-80% (MTT assay) at 10ìM within 1h, with and without added Zn^{2+} in the medium. Cellular incubation with 10ìM pyr and 5ìM $^{67}Zn^{2+}$ for 30 min resulted in the transfer of 50% of the extracellular $^{67}Zn^{2+}$ into the cells and a net accumulation of Zn^{2+} in the HMW and LMW fractions. Adding ^{67}Zn-pyr_2 directly to prepared cell cytosol led to the preferential accumulation of $^{67}Zn^{2+}$ in the LMW fraction, indicating that the distribution observed in the first experiment reflected events in the intact cells not just an equilibrium distribution of Zn^{2+} in the final cell extract. Supported by NIH grants ES-04184 and ES-04026.