Co-expression of zinc binding motif and GFP as a cellular indicator of metal ions mobility

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Abstract
A significant role of zinc-binding motifs on metal mobility in Escherichia coli was explored using a chimeric metal-binding green fluorescent protein (GFP) as an intracellular zinc indicator. Investigation was initiated by co-transformation and co-expression of two chimeric genes encoding the chimeric GFP carrying hexahistidine (His6GFP) and the zinc-binding motif fused to outer membrane protein A (OmpA) in E. coli strain TG1. The presence of these two genes was confirmed by restriction endonucleases analysis. Co-expression of the two recombinant proteins exhibited cellular fluorescence activity and enhanced metal-binding capability of the engineered cells. Incorporation of the zinc-binding motif onto the membrane resulted in 60-fold more binding capability to zinc ions than those of the control cells. The high affinity to metal ions of the bacterial surface influenced influx of metal ions to the cells. This may affect the essential ions for triggering important cell metabolism. A declining of fluorescent intensity of GFP has been detected on the cell expressed of zinc binding motif. Meanwhile, balancing of metal homeostasis due to the presence of cytoplasmic chimeric His6GFP enhanced the fluorescent emission. These findings provide the first evidence of real-time monitoring of intracellular mobility of zinc by autofluorescent proteins.

Keywords: chimeric green fluorescent protein, zinc ions, co-transformation, co-expression, metal mobility