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CHARTCHALERM ISARANKURA NA AYUDHYA: ENGINEERING OF CHIMERIC PROTEIN FOR BINDING TO METAL IONS. THESIS ADVISORS: VIRAPONG PRACHAYASITTIKUL, Ph.D., LEIF BULOW, Ph.D., BYAPORN NA NAGARA, Ph.D. 163 p. ISBN 974-664-263-4.

This study presents gene construction of chimeric peptide binding to metal while providing enzymatic activity or fluorescence as taggers. Synthetic oligonucleotides encoding two and four molecules of cadmium-binding regions, previously constructed by phage display, were fused in-frame to the lactate dehydrogenase (LDH) gene from *Bacillus stearothermophilus*. A chimeric gene coding for a green fluorescent protein (GFP) with avidity to Cd ions was constructed by inserting a synthetic DNA to the green fluorescent gene from *Aequorea victoria*. A chimeric gene encoding hexahistidine and GFP was also generated via a similar manner. All the chimeric genes were subsequently expressed in *Escherichia coli*. The chimeric proteins were purified by immobilized metal (Zn^{2+}) affinity chromatography (IMAC). The purified chimeric proteins were subjected to biochemical study. Their binding capacities to ions of Cd and other related metals (Zn, Cu and Ni) were revealed as concentration dependence.

Roles of the chimeric enzyme/proteins on metals toleration and accumulation in biological system were explored. Expression of chimeric LDH enhanced toleration of *E. coli* to both Cd and Zn up to 0.4 and 0.9 mM, respectively, while the non-protective effect to metals of cell by the chimeric GFP was observed. Only cells carrying the hexahistidine GFP exhibited 1.5 fold increased in Cd accumulation compared to the others. This infers a possibility to develop this chimeric GFP for bioremediation of metals.

The potential usage of all the chimeric genomes and proteins to accommodate metal ion quantitation was explored. Quantitation of metal ions could be applied in three different approaches: codetermination of metal with the IMAC; direct exposure of metal to cadmium binding protein; and determination of metal via organism encoding chimeric GFP. On the IMAC system, unknown quantity of metal (e.g. Zn and Cu) could be immobilized. Quantitation of the immobilized metal then was tagged by the LDH activity or fluorescent emission of the chimeric protein. The revealed activity or emission denoted linear correlation to quantity of binding Zn^{2+} and Cu^{2+} ranging from 100-500 μM and 2.5 to more than 250 μM, respectively. This as well corresponded to the concentration of loaded metal concentration to the IMAC. Direct exposure of various concentrations of Cd and related metals quantitatively declined intensity of the emitting fluorescence of the chimeric cadmium-binding GFP. Linear reciprocal response was shown at the nanomolar level of Cd with a correlation coefficient of 0.99 while those of Zn, Cu and Ni were accordingly responded at the micromolar level. Quantitation of metal via the chimeric GFP-carrying *E. coli* contrarily revealed a direct raising of fluorescent intensity to the amount of Cd and Zn except Cu. Again, linear corresponding was shown at the high nanomolar to high micromolar of Cd. Such series of experimental data support a high potential to develop a metal sensor by using the purified chimeric protein or an intact cell-chip sensor from cells expressing the chimeric GFP.