Genetically encoded fluorescent biosensors for citrate

Yuki Honda

Hydrogen Production Research Division, International Institute for Carbon-Neutral Energy Research (WPI-I2CNER), Kyushu University

Many researchers have developed a number of genetically encoded biosensors based on green fluorescent proteins (GFP) for real-time detection of small molecules or ions in living cells. Citric acid, or its salt form citrate, is a key metabolic intermediate playing important roles in living cells since the biomolecule exhibits regulatory functions for the metabolic processes and the fatty acid synthesis. Since estimation of citrate concentration in urine is of considerable value in diagnosis of certain diseases, such as kidney stones and prostate cancer, qualitative and quantitative analysis of citrate is also of importance in medical field. We developed new GFP-based fluorescent biosensors for citrate by fusing the GFP mutant and a bacterial citrate sensor protein CitA, which can bind to citrate with high specificity. A series of the chimeric proteins were generated by inserting the GFP mutant into the periplasmic domain of CitA. Among these proteins, we selected one candidate named CF98 and tested its molecular and physicochemical properties *in vitro*. The ratiometric fluorescent signal change was observed in CF98: upon addition of citrate, the excitation peak at 504 nm increased proportionally to the decrease in the peak at 413 nm, suitable for build-in quantitative estimation of the binding compound. We confirmed that CF98 can be used for detecting citrate *in vitro* at millimolar levels in the range of 0.1 to 50 mM with high selectivity; even in the presence of other organic acids such as isocitrate and malate, the fluorescence intensity of CF98 remains unaffected. We finally demonstrated the *in vivo* applicability of CF98 to estimation of the intracellular citrate concentration in *Escherichia coli* co-expressing the genes encoding CF98 and the citrate carrier CitT. The newly generated GFP-vade biosensor CF98 is a simple and specific tool for citrate detection *in vitro* and a non-destructive, non-invasive, and genetically-encodable tool for real-time measurement of intracellular citrate concentration *in vivo*.