The Rational design of allosteric biaptazyme sensors and its applications to biochip for 2-dimensional detection by fluorescence and mass spectrometry

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The fusion molecule(i.e. aptazyme) of aptamer and hammerhead ribozyme[1)] was developed as *in situ* sensor by applying FRET assay. Previously, the ribozyme conjugated with aptamer through stem II module showed a significant blank signal by the self-cleavage of the ribozyme moiety. To reduce or remove its self-cleaving activity in the absence of target molecule, rational designs were attempted for the purpose of reducing the binding affinity of the aptazyme to its substrate, while maintaining the activity of the aptazyme. Interestingly, the aptazymes which comprise the aptamer binding sites at both stem I and stem III of the ribozyme showed very low blank signals of self-cleavage and two-fold higher ribozyme activities.

The biaptazyme was applied as 2-dimensional (2-D) protein analysis tool in heterogeneous phase. In micro bead-packed chip with multi-channels, biaptazyme was introduced as ligand for capturing HCV related protein. The HCV helicase and replicase was detected by the interaction of the biaptazyme and the substrate double labeled by fluorescence and quencher, because the substrate could bind on the biaptazyme in the presence of their target protein. In addition, the proteins from 100 nM to 500 nM were analyzed through the fluorescence emitting concurrently into multi-channels. This system showed linear correlation between the fluorescence intensity and the protein concentration and also the high specificity for targaet protein. Successively, bound proteins were digested directly to peptide fragments by elution using trypsin and these peptides were spotted directly on matrix of MALDI-TOF and analyzed. Through 2-Dimensional protein

detection of fluorescence & mass spectrometry, we suggested a new detection method for analyzing the target protein more accurately and fastly without protein labeling.

References

1) Koizumi, M., Soukup, G.A., Kerr, J.N. and Breaker, R.R. (1999). Allosteric selection of ribozymes that respond to the second messengers cGMP and cAMP, Nat. Struct. Biol. 6, 1062-1071.