Electrochemical sensing of protein using two aptamers in sandwich manner

Kazunori. Ikebukuro, Chiharu Kiyohara, Koji Sode
Department of Life Science and Biotechnology,
Tokyo University of Agriculture and Technology
Naka-machi 2-24-16, Koganei, Tokyo 184-8588, Japan

Proteome analysis is one of the most interesting issues in life science after the confirmation of human genome sequence. For it, detection of specific protein is very important and antibody would be the most powerful molecular recognition device. However antibody is not perfect one since it has several problems such as high cost and difficulty in production for small molecules. In recent years, aptamers, which are DNA and RNA molecules that can bind target molecules with high affinity and specificity were reported and those were obtained by the method called systematic evolution of ligands by exponential enrichment (SELEX)[1),2)]. Many aptamers which can recognize different proteins has already been reported, and some of them showed nanomoler level K_d and good selectivity, so that aptamers can be used as molecular recognition device instead of antibody. Aptamer has other advantages, for example, it can be easily synthesized, labeled and modified. Therefore it is one of the powerful candidate for good molecular recognition device.

Sandwich manner of protein recognition is most commonly adopted for its detection using two different antibodies, but there is no report of the protein detection in sandwich manner using aptamers so far. Then, we attempted at constructing electrochemical protein sensor system using two different aptamers which recognizes different parts of the target protein[3),4)]. In this study, we used thrombin and its aptamers for the construction as the model sensor system.

The scheme of protein detection is shown in Figure 1. Thiolated aptamer 1 was fixed at the gold electrode and then, target protein and glucose dehydrogenase labeled aptamer 2 (ap2GDH) was added and incubated for 30 min at room temperature. We used glucose dehydrogenase obtained from *Burkholdelia cepacia*[5)], which was the thermostable enzyme. Then, the response current generated by glucose addition was measured at 25˚C. In the presence of thrombin, aptamer 1-thrombin-ap2GDH complex was formed, and a response current was obtained although no current was obtained when the bovine serum albumin (BSA) was added instead of thrombin.

Figure 2 shows the calibration graph for thrombin of this sensor system. From the range of 1 µM to 10 µM, in case of BSA addition the generation of current was not observed but increased current was observed depending on the increase of concentration of thrombin. The lower detection limit was 1 µM and this sensor system did not show high sensitivity but sensitivity can be improved by choosing the enzyme for labeling carefully. Thus we have developed the electrochemical protein detection system using the two different aptamers in sandwich manner. This is the first report of the sandwich type detection system using aptamers and it was ascertained that aptamers can be used for detection instead of antibodies.

Reference

1. Ellington A. D. and Szostac J. W. (1990) In vitro selection of RNA molecules that bind specipic ligands. *Nature* 346:818-822
2. Tuerk C and Gold L. (1990) Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science.* 249:505-510
3. Bock L. C, Griffin L. C, latham J. A, Vermass E. H. and Toole J. J. (1992) Selection of single-stranded DNA molecules that bind and inhibit human thrombin. *Nature* 355: 564-566
4. Tasset D. M, Kubik M. F, Steiner W. (1997) Oligonucleotide inhibitors of human thrombin that bind distinct epitopes. *J. Mol. Biol*. 272:688-698
5. Koji Sode, Wakako Tsugawa, Tomohiko Yamazaki, Masato Watanabe, Nobuhiro Ogasawara, and Mitsuhara Tanaka (1996) A novel thermostable glucose dehydrogenase varying temperature properties by altering its quaternary structures. *Enzyme. Microb. Technol* 19: 82-85

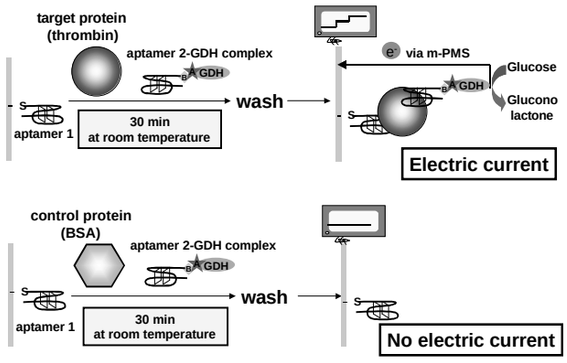

Figure 1. Scheme of the protein detection

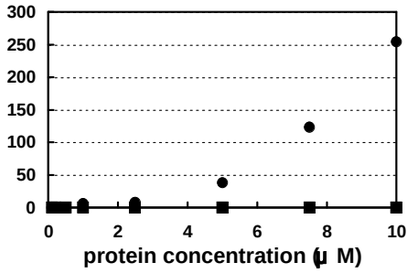

Figure 2. Calibration graph for thrombin