Development of Chemiluminescent Immunosensor Array for GMO

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Abstract

While genetically modified organisms (GMOs) are producing in many countries, issues related to safeties of GMOs as foods for human have risen. Because of such potential problems, the development of an indication system regarding GMO content contained in foods has been required. Particularly, an immuno-chip, as widely demanded diagnostic tool for functional, structural analyses of proteins, has been investigated to simultaneously measure different analytes. We have developed methods for immobilizing antibody on glass surfaces as substrate and for generating chemiluminometric signals.

Introduction

According to the development of plant biotechnology, GMOs have been exploited and cultivated in forty ha over the world during the past several years[1]. There are GMO farm products improved in harvest, which may resolve shortage of provisions. However, the GMO products might cause potential problems, for an instance, biochemical side effects against the human health, e.g., induction of allergy and antibiotics resistance. They also cause an ecological disturbance by genetically recombinant plants to have an insecticide or pesticide resistance. Due to such potential problems, an indication system of GMO content in foods has been adopted in the world[2]. To determine GMO content, PCR and immunoassay have been used as the analytical tools that can be utilized only within laboratory. Recently, immunosensors based on a glass or silicon as substrate have been developed to quantify them in the field, such as quarantine station in port and food warehouse, where specimen is provided. In this study, we have produced GMO marker proteins[3] and constructed an immunosensor system generating a chemiluminometric signal proportional to the analyte concentration.

Materials and Methods

Production of GMO proteins. NPTII and EPSPS gene were extracted from plants and amplified using polymerase chain reaction (PCR). This were then inserted into pET28a vector using T4 DNA ligase. After transforming it within *E. coli* cells to produce recombinant proteins in a large scale, the cells were inoculated on LB medium and cultured at 37℃ and consecutively at 15℃[4]. The cells were collected by centrifugation and lysed by sonication. To obtain soluble proteins, it was purified on an affinity column after centrifugation. Purified proteins were characterized comparing to each reference protein and used for the production of specific antibodies by immunizing rabbits.

Construction of analytical system. Glass slides were cleaned in a mixture of 11.6 M hydrochloric acid and 100% methanol, and then in 18 M sulfuric acid. After thoroughly washing with de-ionized water, the glass slide was treated with 3-aminopropyltriethoxysilane (APTES) or polyethylenimine (PEI) solution. This was reacted with glutaraldehyde (GA), washed with de-ionized water, and subsequently dried[5,6]. A capture antibody was immobilized onto the prepared glass slide in a pattern using a capillary gap. After incubating in a box maintaining 100% relative humidity, residual surfaces were treated with a blocking agent (e.g., casein) and subsequently dried. Antigen was reacted with the antibody bound on the glass surface to form antigen-antibody complexes. After washing, a detection antibody-streptavidin conjugate was incubated. The chip surfaces were cleaned by washing and biotin-Horseradish peroxidase (HRP) conjugate was then reacted. After rinsing the surfaces, the luminol solution were added and consequent chemiluminescent signal was measured[7].

Results and Discussion

Production of GMO marker proteins and specific antibodies. Genes coding the target proteins were inserted into vectors and the resulting recombinant DNAs were incorporated into *E. coli*. The target proteins from recombinant organisms were produced in large quantities by IPTG induction. After the affinity purification, each target protein were

recovered in high purity (higher than 95% based on the area of protein bands stained). The recombinant proteins were characterized using specific antibodies toward their binding affinities and structural conformation. The antibodies specifically bound to each target locating at positions of 29 kDa for NPTII and 50 kDa for EPSPS on Western blotting.

Antibody productions were performed by immunizing animal (e.g., rabbit) with the recombinant proteins. The antisera collected from the animal were utilized for the test of discrimination between GMO and non-GMO prepared in this laboratory. The constitutive proteins contained in each organism were analyzed by PAGE and then Western blotting employing the antisera. Although the various proteins were present regardless of the genetic modification, we were able to specifically detect GMO plants using the antisera to the NPTII and EPSPS proteins, respectively. From these results, the production of recombinant proteins identical to each reference was successful and they can be used as marker proteins of GMOs.

Analytical performance of immunosensor. To develop an analytical system, an alternative protein such as human serum albumin (HSA) or myoglobin was adopted as model analyte. To prepare solid substrate for a capture antibody, a glass slide was cleaned and modified with an agent either APTES or PEI that provided a good reproducibility. After forming immune complexes in a sandwich type, a chemiluminescent signal from HRP was generated and measured using a detector (photodiode in this study) in proportion to the analyte concentration. We are currently constructing an immunosensor array to simultaneously measure multiple analytes and this would offer us to specifically monitor proteins, for an instance, GMO marker proteins.

References

1. Elke A., Ferruccio G., Petra H., Hans P., Guy Van D. E. (2002), "Analytical methods for detection and determination of genetically modified organism in agricultural crops and plant-derived food products", *European Food Research Technology* **214**, 3-26.
2. Regal, Philip J. (1995), International biosafety: A global imperative, *The Scientist* **9**, 13-20.

3. Rogan G. J., Dudin Y. A., Lee T. C., Magin K. M., Astwood J. D., Bhakta N. S., Leach J. N., Sanders P. R., Fuchs R. L. (1999), "Immunodiagnostic methods for detection of 5-enolpyruvylshikimate-3-phosphate synthase in Roundup Ready soybean", *Food control* **10**, 407-414.
4. Sambrook J., Fritsch E. F., and Maniatis T. (1989), Molecular cloning : second edition.
5. Catherine M. Halliwell (2001), "A Factorial Analysis of Silanization Conditions for the Immobilization of Oligonucleotides on Glass Surfaces", *Analytical Chemistry* **73**, 2476-2483.
6. Julia Y., Richard D., Martin B., Thomas L., Jenny E. (2003), "Microfluidic enzyme immunosensors with immobilized protein A and G using chemiluminescence detection", *Biosensors and Bioelectronics* **00**, 1-14.
7. Kenji H. (1996), "Highly sensitive chemiluminescence flow injection analysis system using microbial peroxidase and a photodiode detector", *Analytica chimica acta.* **329**, 127-134.