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Signal amplification in electrochemical detection of buckwheat allergenic protein using field effect transistor biosensor by introduction of anionic surfactant

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ABSTRACT

Food allergens, especially buckwheat proteins, sometimes induce anaphylactic shock in patients after ingestion. Development of a simple and rapid screening method based on a field effect transistor (FET) biosensor for food allergens in food facilities or products is in demand. In this study, we achieved the FET detection of a buckwheat allergenic protein (BWp16), which is not charged enough to be electrically detected by FET biosensors, by introducing additional negative charges from anionic surfactants to the target proteins. A change in the FET characteristics reflecting surface potential caused by the adsorption of target charged proteins was observed when the target sample was coupled with the anionic surfactant (sodium dodecyl sulfate; SDS), while no significant response was detected without any surfactant treatment. It was suggested that the surfactant conjugated with the protein could be useful for the charge amplification of the target proteins. The surface plasmon resonance analysis revealed that the SDS-coupled proteins were successfully captured by the receptors immobilized on the sensing surface. Additionally, we obtained the FET responses at various concentrations of BWp16 ranging from 1 ng/mL to 10 μg/mL. These results suggest that a signal amplification method for FET biosensing is useful for allergen detection in the food industry.

1. Introduction

People all over the world, irrespective of age or sex, suffer from food allergies. From the standpoint of food manufacturers, the management of allergenic substances in foods is important because food allergies cause many kinds of skin, respiratory, and digestive symptoms [1]. In a serious case, an immediate hypersensitivity reaction including anaphylaxis may cause a reduction in blood pressure or unconsciousness, resulting in a life-threatening condition. Although buckwheat flour products are familiar to people in many countries especially East Asia and Europe, the buckwheat protein, BWp16, can cause an acute allergic reaction [2]. BWp16 is known as a major allergen leading to anaphylaxis because it shows pepsin resistance [3,4]. The protein remains in food even after cooking, thus determination of a trace amount of the protein is of great importance in the manufacturing process. In Japan, Food Allergen Labeling Regulations require mandatory labeling of seven allergens including buckwheat proteins contained in foods 10 μg/g or more [5], thus the manufacturing control of allergens has strict regulations to ensure food safety.

Screening tests that evaluate if manufacturing lines have low concentrations of allergen proteins remaining have been widely applied in the food manufacturing process [6]. However, an existing screening test based on a highly sensitive enzyme linked immunosorbent assay (ELISA) is limited by its requirement for multiple steps, an appropriately labeled secondary antibody and the necessary optical equipment [7]. To overcome these drawbacks, a simple label-free allergen detection technique for food manufacturing lines is desirable. A field effect transistor (FET) biosensor is a promising platform because it can be used in mass production and for large-scale integration. FET biosensors may directly detect the intrinsic charge of proteins captured by immobilized receptors [8,9], suggesting that it has the potential to eliminate some of the steps involved in conventional allergen detection processes. We developed an FET biosensor with high chemical durability by modifying its surface with self-assembled monolayers [10], and adapted the sensors for use in various healthcare fields by employing different probe molecules [11–13].

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The magnitude of the FET response primarily depends on the total amount of protein charges, thus introduction of extra charges by supplementation of additives is an effective procedure to amplify the FET signals for sensitive detection [14,15]. However, these methods are limited by the synthesis or preparation of sophisticated nano-amplifiers. An anionic surfactant, sodium dodecyl sulfate (SDS), which is widely used as a chemical reagent for polyacrylamide gel electrophoresis (PAGE), is known to couple at a constant rate with soluble proteins, suggesting that the additional charges can be successfully introduced to target proteins regardless of their charge properties. In this study, we propose the use of a charge-amplification technique using SDS surfactant to cover target proteins with anionic charges, for sensitive detection by FET of buckwheat allergenic protein (Fig. 1a).

2. Materials and methods

2.1. Materials

The recombinant buckwheat protein BWp16 was prepared using an *Escherichia coli* strain carrying the cDNA of the structural gene [16]. The anti-BWp16 monoclonal antibody was produced with a hybridoma clone, which was prepared from a mouse immunized with the BWp16 protein. The BWp16 protein and anti-BWp16 antibody were prepared by diluting the stock solution in phosphate buffer saline (PBS). The self-assembled monolayer (SAM) reagent, 3-aminopropyltriethoxysilane (APTES), was purchased from Sigma-Aldrich. The capping reagent, ethanolamine, was purchased from Tokyo Chemical Industry Co. (Japan). Albumin from chicken egg white (OVA) and sodium dodecyl sulfate (SDS) were purchased from Wako Pure Chemical Industries, Ltd. (Japan). Pierce™ Mouse IgG1 Fab and F(ab')$_2$ Preparation Kit and SDS-Out SDS Precipitation Kit were purchased from Thermo Scientific (US). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) was purchased from GE Healthcare (Sweden). The Biotin Labeling Kit-NH_2 and ExtrAvidin®-Peroxidase was from Dojindo Molecular Technologies (Japan) and Sigma-Aldrich Co. LLC (USA), respectively. Tris(hydroxymethyl)-aminomethane (Tris) was purchased from Nacalai Tesque, Inc. (Japan). 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) and ethylenediamine-*N*,*N*,*N*′,*N*′-tetraacetic acid (EDTA), disodium salt, dihydrate were purchased from Wako Pure Chemical Industries, Ltd. (Japan). All other materials were purchased from Kanto Chemical Co. (Japan). All the chemicals were of analytical reagent grade and used as received.

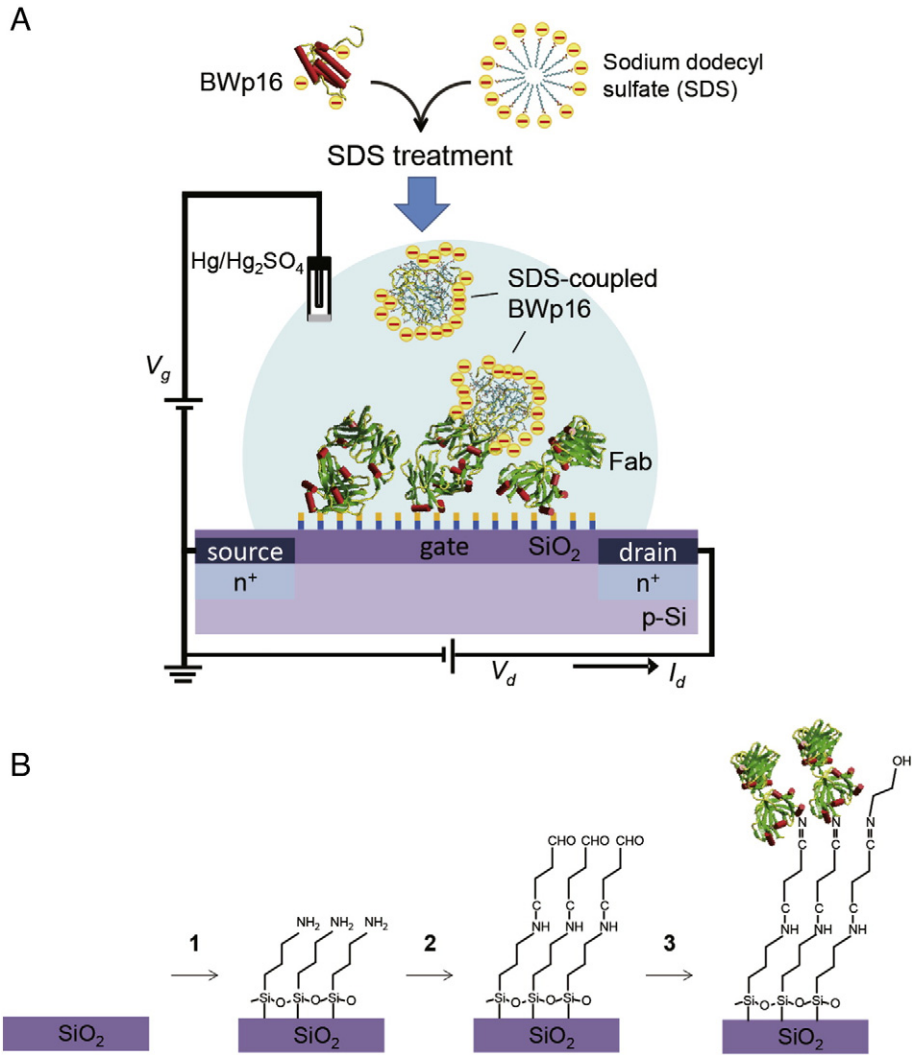

Fig. 1. Schematic illustration of the FET sensing. (a) Signal amplification for FET detection by coupling the anionic surfactant with the target buckwheat protein. The negative charges derived from the anionic surfactant, SDS, enhanced the intrinsic negative charges of the buckwheat protein BWp16. (b) Preparation process for the FET biosensor. Silanization with aminopropylsilane (1), modification with glutaraldehyde and reduction of Schiff base (2), and Fab immobilization and ethanolamine-capping treatment (3).