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Fabrication of micromagnetic beads with molecular recognition/electron-transfer peptides for the sensing of ovalbumin

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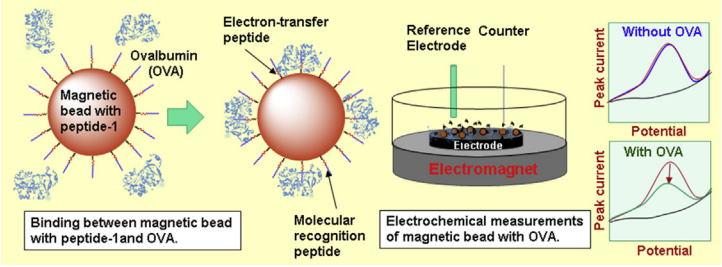
HIGHLIGHTS

- Ovalbumin recognition/electron-transfer peptides were immobilized on magnetic beads.
- The accumulation of the protein through the peptides on the beads caused the change of electrode response.
- The magnetic beads could be reused for sensing of ovalbumin.

GRAPHICAL ABSTRACT



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ABSTRACT

Electrochemical sensing of ovalbumin (OVA) was performed using magnetic beads with OVA recognition (RNRCKGTDVQAW)/electron-transfer (YYYYC) peptides. The focus of this study was to construct a highly sensitive and regenerative tool for OVA detection based on the interaction between a protein and peptide-1(RNRCKGTDVQAWYYYYC). The peptide-1 was introduced to the bead through four types of cross-linking reagents. Magnetic beads of different sizes with *N*-(6-maleimidocaproyloxy)sulfosuccinimide (Sulfo-EMCS) were also prepared. An oxidation peak due to tyrosine residues at 0.65 V depended on the distance of the electron-transfer peptide from the bead surface and on the surface area of the magnetic beads that contacted the electrode surface. The response of the electro-transfer peptide moiety was decreased because the protein was accumulated via the recognition peptide on the beads. When using Sulfo-EMCS and beads that were 6.0–6.9 μm in diameter, the calibration curve of OVA was linear and ranged from 8.0×10^{-13} to 2.0×10^{-11} M. To regenerate the magnetic beads, the measurements were achieved after removal of the OVA using a denaturing reagent. When OVA was added to fetal bovine serum containing a complex matrix, OVA was recovered at a rate of 98–100%. Consequently, these magnetic beads could be a powerful tool for the sensing of OVA in real samples.

1. Introduction

Ovalbumin (OVA), which is one of glycoproteins in egg white, has often been used as a model for biomarkers and in the study of allergies. The specific glycoproteins that are biomarkers for bladder [1], ovarian [2], prostate [3], breast [4], and hepatocellular cancers [5] are significant in this field of medicine. Monitoring these glycoproteins has been performed in serums in the diagnoses of cancer. In addition, information for allergies was also examined

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using an ovalbumin-induced murine model of asthma [6–8]. Hilmenyuk et al. reported the effects of advanced glycation end products derived from OVA used as a model food allergen on dendritic cells, which included their immunostimulatory capacity and the T-cell response compared with regular OVA [9]. Therefore, a number of OVA assays in biological and food samples have been proposed. For example, an immunoassay using surface-enhanced Raman scattering labels for the selective localization of ovalbumin in paint cross-sections was proposed based on gold nanoparticles functionalized with Nile blue A [10]. To measure the presence of egg allergen residues in a wine matrix, a label-free SPR-based immunoassay for tracing egg-derived fining agent residues via the sensing of OVA was developed [11]. Moreover, electrospray ionization-mass spectrometry and ELISA were used to determine the presence of OVA before and after thermal treatment in pasta [12]. Shah et al. developed an electrochemical method for detection of glycan and protein epitopes of OVA in serum [13].

On the other hand, various biological processes such as DNA repair, replication, and gene-expression are based on protein-peptide interactions [14]. The binding also controls the processes for signal transduction and protein trafficking [15]. When a peptide is combined with a target protein domain, a disorder-to-order transition often occurs due to the binding [16]. Accordingly, this interaction can be applied for the monitoring of protein-peptide binding and for the sensing of protein. We suggested a new peptide probe wherein an electron-transfer peptide was coupled with a peptide-recognizing OVA for the detection of protein [17]. In this process, the amino acid sequence (237–251) that constitutes OVA interacts against the amino acid sequence (RNRNRCKGTDVQAW, 112–123) of lysozyme [18]. The sequence YYYYC that was an electron-transfer peptide was introduced to the site of either the N- or C-terminals. Because the electron-transfer peptide moiety was covered with OVA, the OVA could be detected using the change in the electrode response. The homogeneous sensing level of OVA was 10^{-12} M, and this level of selectivity was due to the interaction between OVA and the molecular recognition peptide. Because the most excellent detection level of OVA is 10^{-14} M [19], an improvement in sensitivity is needed.

Magnetic beads modified with amino or carboxyl groups are significant as a scaffold of the reaction between biomolecules. The merits of using magnetic beads are as follows. Biological molecules such as enzymes/antibody [20], peptides [21] and carbohydrates [22] can easily be immobilized via conjugation with the functional groups on the bead surface. Construction of the magnetic beads is simple because unreacted molecules that are not immobilized on the beads can be removed by magnetic separation. In addition, the target molecules are trapped due to an interaction with the molecular recognition probe on the magnetic beads, and the binding reduces the influence of the sample matrix. When target molecules are accumulated on the beads, enhanced sensing and improved selectivity are achieved.

In the present study, magnetic beads wherein OVA recognition (RNRCKGTDVQAW)/electron-transfer (YYYYC) peptides were conjugated via a cross-linking agent were fabricated for the sensing of OVA. The focus of that study was to develop a highly sensitive and regenerative tool for OVA detection based on the selective interaction between the protein and RNRCKGTDVQAWYYYYC (peptide-1). A principle for the sensing of OVA using magnetic beads with molecular recognition/electron-transfer peptides is presented in Fig. 1. In this example, a molecular recognition peptide is immobilized on the magnetic beads, and then the beads can accumulate target proteins that are collected from the solution via a magnet. The magnetic beads are loaded onto a glassy carbon plate at the bottom of a voltammetric measurement vessel, and the supernatant is added to the cavity. Because the electron-transfer peptide in the C-terminal of peptide-1 is covered with OVA, the peak current changes depending on the concentration of the OVA. The peptide probe is immobilized on the magnetic beads via four types of cross-linking agents with differing lengths of ethylene chains. The length may influence the binding between the OVA and the peptide probes on the beads. In addition, the contact of the electron-transfer peptide to the electrode surface depends on the distance of the ethylene chain from the bead surface. Then, it is expected that the electrode response will be controlled by the diameter of the magnetic bead. Accordingly, magnetic beads with four different sizes against a cross-linking agent are used to examine the affect. To investigate whether the magnetic beads can be reused, the protein is removed from the beads via a denaturant. Furthermore, we performed recovery experiments of OVA to fetal bovine serum to evaluate the usefulness of this procedure. When a peptide that recognizes a specific biomarker is immobilized on magnetic beads, it is possible to use the beads as a tool for the sensing of a biomarker.

2. Experimental

2.1. Apparatus

All voltammograms were measured using an ALS electrochemical analyzer Model 610D. A glassy carbon plate (2 × 25 × 25 mm, Model no. 012087, BAS) that was cut in half served as the working electrode. To polish the electrode, 1.0-, 0.3-, and 0.05- μm alumina (Baikowski International Corp., Charlotte, NC) was used in succession. An Ag/AgCl electrode (sat. NaCl, Model no. 11-2020, BAS) served as the reference, and a platinum wire was used as the counter electrode. All potentials were compared to an Ag/AgCl electrode. Using a UV mini-1240 Shimadzu Visible Spectrophotometer, the absorption spectra of the peptide probes were measured.

2.2. Reagents

Magnetic beads (diameter 2.0–2.9 μm, 1.04×10^9 bead per mL, 2.5 w/v%, AM20; diameter 4.0–4.9 μm, 2.02×10^8 beads per mL, 2.5 w/v%, AM40; diameter 6.0–6.9 μm, 3.04×10^7 beads per mL, 1.0 w/v%, AM60; and diameter 8.0–9.9 μm, 6.24×10^6 beads per mL, 1.0 w/v%, AM80) were supplied by Spherotech, Inc. The peptide-1 samples, RGGRLCYCRRRFCVCVGRYYYYC, and YYYYC, were synthesized by the Life Technologies Corporation, which also synthesized each peptide probe using FMOC solid-phase technology. Electrospray ionization tandem mass spectrometry was used to confirm each of the peptide probes. The mass spectrum for peptide-1 is shown in Fig. S1. The molecular weight of the peptide was measured using the signals $[M + 5H]^{5+}$, $[M + 4H]^{4+}$, and $[M + 3H]^{3+}$. Based on these signals, the molecular weight of peptide-1 was 2189.46 Da. The spectrum of YYYYC as an electron-transfer peptide (molecular weight: 773.8) was measured using the same procedure, which is not shown. The purities estimated from the peak areas of HPLC were 93 and 90%. The *N*-(4-Maleimidobutyryloxy) sulfosuccinimide, sodium salt (Sulfo-GMBS), *N*-(6-maleimidocaproyloxy)sulfosuccinimide, sodium salt (Sulfo-EMCS), *N*-(8-maleimidocapryloxy) sulfosuccinimide, sodium salt (Sulfo-HMCS), and *N*-(11-Maleimidoundecanoyloxy) sulfosuccinimide, sodium salt (Sulfo-KMUS) that were used as cross-linkers were purchased from Dojindo Molecular Technologies, Inc. Avidin, bovine serum albumin, α-casein, concanavalin A, fetal calf serum, lysozyme, ovalbumin, soybean agglutinin, transferrin, and wheat germ agglutinin were supplied by Sigma-Aldrich. All reagents used were of analytical reagent grade. To immobilize a cross-linker and the peptide on the magnetic beads, phosphate