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Magnetic beads modified with an electron-transfer carbohydrate-mimetic peptide for sensing of a galactose-dependent protein

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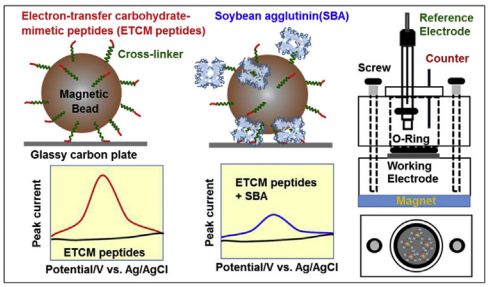
HIGHLIGHTS

- Label-free sensing of a protein was performed using carbohydrate-mimetic/electron-transfer peptides.
- Magnetic beads with the peptide were fabricated as a sensing tool.
- The detection was achieved based on the changes in peak current of the peptide.

GRAPHICAL ABSTRACT



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ABSTRACT

For use in the voltammetric sensing of galactose-dependent proteins, we modified magnetic beads with a peptide that had both electroactive- and molecular recognition properties. The peptide consisted of a YXY sequence and behaved as an electron-transfer carbohydrate-mimetic peptide that would combine with proteins. With this tool, the protein could be detected via a label-free system. We synthesized several penta- and hexa-peptides with a cysteine residue on the C-terminals to examine the properties of peptides. These peptides contained amino acid residues (X) of alanine, serine, or tyrosine. The peptides were immobilized on magnetic beads via *N*-(8-maleimidocapryloxy) succinimide. Soybean agglutinin(SBA), the *in vivo* function of which has been well established in animals, was selected as a model protein. The protein was detected via the changes in electrode response due to the oxidation of tyrosine residues from the phenol group to quinone. As a result, SBA was selectively accumulated on the beads modified with YYYYC. The calibration curve of SBA was linear and ranged from 2.5×10^{-12} to 1.0×10^{-10} M. With this system, SBA was recovered in human serum at values that ranged from 98 to 103%. Furthermore, the beads with peptides were regenerated five times using a protein denaturant. Accordingly, this electrochemical system was simple and could be rapidly applied to the detection of galactose-recognition proteins.

1. Introduction

Interactions between proteins and carbohydrates participate in various reaction processes that are related to signal transmission *in vivo* [1], recognition for cell-cell binding [2], and virus infection

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[3]. The interactions are controlled by molecular morphology that is based on the binding sites of target proteins [4]. Molecular recognition is a function of hydrogen bonding, van der Waals forces, and hydrophobicity [5,6]. Therefore, protein-carbohydrate binding can be analyzed using nuclear magnetic resonance (NMR) [7], quartz crystal microbalance [8], computer simulation [9], and mass spectrometry [10]. Examples of peptides that can now be constructed based on the advances achieved in the techniques for peptide synthesis include cyclic peptides, branched peptides, peptides with special amino acids, and peptides with more than 30 amino acid residues. The N- and C-terminals of peptides are modified in various ways, and peptides can be produced in large amounts. Several peptides function as alternative ligands in carbohydrate-binding proteins, and peptides bind with proteins due to the interactions mentioned above. Carbohydrate-mimetic peptides inhibit carbohydrate-protein interactions. The first report of carbohydrate-mimetic peptides established how MYWYPY inhibited the binding of methyl α-D-mannopyranoside to concanavalin A (ConA) [11]. This phenomenon suggested that the peptide interacted with the protein at or near the carbohydrate binding site. The interaction is initiated when a tyrosine residue with a phenolic hydroxy group, or moiety interacts with the protein instead of the ring of a carbohydrate. The binding constant of DVFYPYPYASGS to ConA is reported to be 2.2×10^4 l/mol, which is equal to that of α-ManOMe [12]. The peptide was suppressed by the ConA-induced T-cell proliferation and reacted with *anti*-α-ManOMe polyclonal antibodies. The carbohydrate-mimetic peptides act as immunogens to produce anti-carbohydrate antibodies [13,14]. Therefore, peptides are significant in the treatment of disease and in the development of vaccines. For example, identification and characterization of peptide mimics of *N*-acetylglucosaminyl-β1-4-*N*-acetylmuramyl-alanyl-d-isoglutamine (GMDP) have demonstrated adjuvant activities. These mimics were accomplished using screening phage display libraries of hexa- and penta-decapeptides with monoclonal antibody generated against GMDP [15]. Peng et al. developed a protective monoclonal antibody that binds a phase of the I-specific epitope on PI- lipopolysaccharide (LPS), and in the process they identified a protective carbohydrate-mimetic peptide of PI PI-LPS [16]. Fukuda et al. screened the phage library using anti-carbohydrate antibodies and lectins to research carbohydrate-mimetic peptides [17]. In addition, a carbohydrate-mimetic peptide (IFLLWQR) that targeted the properties of annexin indicated a specific tumor-targeting activity and became a clinically relevant vehicle for anti-cancer drugs [18]. Makhoul's group synthesized peptides that induce broad-spectrum tumor-associated carbohydrate antigens-reactive antibodies [19]. The bioactive solution conformation of the carbohydrate-mimetic MDWNMHAA to Shigella flexneri Y that binds to its complementary antibody was measured via saturation transfer difference-NMR spectroscopy [20]. Then, molecular dynamics simulation was carried out to obtain a picture of the conformational flexibility and to establish the possibilities for bound ligand conformations.

By contrast, the amino acid sequence YXY (X: alanine, glutamic acid, threonine, and tyrosine) indicated selective interaction to several galactose recognition proteins, although peptides including YPY recognized ConA [21]. Pentapeptides that included the YXY sequence were designed to evaluate the interaction of a lectin to one of the glycoproteins, asialofetuin, immobilized on a surface plasmon resonance chip in the presence of the peptide. Although YYYYF-NH_2 was bound to galectin-1, YYAYF-NH_2 and YYSYY-NH_2 sequences would not combine with the lectin. Therefore, the binding of the peptide to the galectin family was controlled via the amino acid sequence. Wéber et al. used NMR to report that carbohydrate-mimetic peptide-galectin-1-asialofetuin interactions depend on the sequence of the peptide [22].

When our group developed peptides (YYYYC and YYYYYC) with cysteine residue on the C-terminals of oligotyrosine, we discovered that the peptide functioned as an electron-transfer agent [23]. In a previous study, an ovalbumin(OVA) recognition peptide that was conjugated with an electron-transfer peptide was constructed as a sensing probe for the detection of OVA [24]. Each of the probes consisted of amino acid residues and indicated superior electrode responses. To improve the sensitivity of OVA detection, we also designed molecular recognition/electron-transfer peptides immobilized on magnetic beads. When the electron-transfer peptide was modified with cellobiose, voltammetric measurements were performed using a competitive reaction to wheat germ agglutinin between the carbohydrate chains existing on the surface of human histocytic lymphoma cells and on cellobiose/electron-transfer peptide probes [25]. Based on the amino acid sequence, the electron-transfer peptide proposed in this study appears to be a carbohydrate-mimetic peptide. Since YYYYC and YYYYYC contain a YXY sequence, these peptides have significant utility as probes that can sense galactose-dependent proteins.

In the present study, electron-transfer carbohydrate-mimetic peptides immobilized on magnetic beads were fabricated to electrochemically sense a galactose-recognition protein. Soybean agglutinin (SBA) is one of the lectins that combine with *N*-acetyl-galactosamine and galactose [26,27]. SBA can distinguish between normal and malignant cells and is a powerful probe for the screening of cancers based on changes in the carbohydrate chains on a cell surface [28]. In addition, the SBA, which is a food allergen, is an important target [29]. Several studies have focused on the interesting behavior of SBA when it is applied to living organisms. Pan et al. reported that binding between SBA and carbohydrate chains expressed on intestinal epithelial cells causes anti-nutritional effects in animals [30], and the addition to SBA to living organisms has been traced to increases in aspartate amino-transferase [31]. Therefore, we selected SBA as a model protein, and a sensing system for SBA in human serum was constructed. The measurement approach follows (Fig. 1). Since the electron-transfer carbohydrate-mimetic peptides were expected to bind with SBA, the peptides were immobilized on magnetic beads via a cross-linking reagent. To confirm the selectivity of several electro-transfer peptides to SBA, we constructed pentapeptides (YXYYC) and hexapeptides (YXYYYC, YYXYYC, and YYYXYC). X represents amino acid residues such as tyrosine, alanine and serine. The properties of the peptides could have been influenced by the types of amino acid residue, the position of the residue in the sequence, and the number of amino acids. In the proposed sensing system, the magnetic beads are separated from a solution using a magnet after the beads and SBA are incubated in solution. Then, the beads are loaded to the measurement cell, and the supernatant is subsequently added. When voltammetric measurement is carried out, the peak current of a peptide is thought to decrease in the presence of SBA. The decrease in the peak current is due either to the uptake into the binding site of the carbohydrate in SBA, or to a binding with the amino acid sequence in SBA. Therefore, the contact of electron-transfer carbohydrate-mimetic peptides on the electrode surface was suppressed because peptides containing YXY were immobilized on beads covered with SBA. As a result, the voltammetric sensing of SBA was based on the binding between SBA and peptide-immobilized beads. For the sensing of a target protein, we previously used a peptide sequence that combined a molecular recognition peptide with an electron-transfer peptide. However, in the present study, a peptide indicating molecular recognition and electroactive properties was constructed to detect the target protein. One of the advantages of this new system is that it simply allows for a label-free SBA sensing system because the electron-transfer peptide is identical to a carbohydrate-mimetic peptide.

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