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Short communication

Immunomagnetic separation of human myeloperoxidase using an antibody-mimicking peptide identified by phage display

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ABSTRACT

Phage display biopanning is a powerful *in vitro* selection process for screening and identifying peptides that bind to a target protein of interest. With the aim of replacing antibodies in immuno-diagnostic applications, we identified peptides whose binding characteristics mimicked those of anti-human myeloperoxidase (hMPO), a biomarker for acute cardiac diseases. Based on ELISA results from four phage clones, we selected and chemically synthesized a 12-mer peptide (SYIEPPERHRHR). Quartz crystal microbalance and surface plasmon resonance analyses revealed that the molar binding equilibrium ratio of the synthesized peptide was 0.023, approximately 43-fold lower than that of the anti-hMPO antibody. The dissociation constant (K_d) was 57 nM, which was comparable to that of the native antibody (83 nM). Next, we biotinylated the peptide at its N-terminus and attached the biotinylated peptide to the surface of streptavidin-coated magnetic particles to assess its ability to selectively capture hMPO. The binding equilibrium data were similar to the previous analyses; specifically, around 0.021 mol peptide bound to 1 mol of hMPO. Antigen capture was found to be selective and to be relatively little influenced by the presence of human serum albumin (HSA), an abundant constituent of serum. Our work demonstrates the potential of immunomagnetic isolation to achieve selective capture of a low-concentration antigen from complex solutions such as serum.

1. Introduction

In antigen immuno-diagnostics, the whole antibody molecule is frequently used as a ligand in the formation of antigen-antibody complexes. Since whole antibodies are expensive, delicate, and technically challenging to work with, much research has focused on finding smaller, more robust, and more economical peptides as a replacement to whole antibodies (Ruigrok et al., 2011; Wu et al., 2016; Morton et al., 2013). It is a challenging task to isolate or detect an antigen present at a very low concentration in a complex and heterogeneous mixture of proteins, such as serum. To solve this problem, one approach is to remove the abundant background proteins to obtain a more pure and concentrated antigen solution before exposing the mixture to antibodies for capture. Another possible solution is a single-step isolation procedure using a solid matrix such as magnetic particles (MPs) coated with an immunological ligand; this process has been termed immunomagnetic separation (Hien et al., 2012; Morton et al., 2013; Prioult et al., 2000).

Human myeloperoxidase (hMPO) is released by activated neutrophils and displays powerful pro-oxidative and pro-inflammatory properties. Moreover, hMPO has been validated as a biomarker for acute coronary syndrome and as a risk marker for clinical diagnostics (Peacock et al., 2011; Sawicki et al., 2011; Ahmad and Sharma, 2012). However, for immuno-diagnostic detection of serum hMPO, an anti-MPO antibody or an equivalent detection reagent is necessary.

This study consisted of two sequential steps. First, using hMPO as a model antigen, we used a phage display technique with biopanning to identify antibody-mimicking peptides with high binding affinity to hMPO. Phage display can generate a library of peptides (or motifs) with a range of affinities for a target biomolecule; subsequent biopanning then allows rapid screening for the peptides with the highest binding affinities from the library phage pool (Hamzeh-Mivehroud et al., 2013). Once an optimum clone is identified, its amino acid sequence can be obtained and a peptide can be chemically synthesized. For potential replacement of the native anti-hMPO antibody in immuno-diagnostics/separation, the binding affinity of the peptide to hMPO is evaluated and compared with that of the native anti-hMPO antibody (Hamzeh-Mivehroud et al., 2013; Chan et al., 2014; Stewart et al., 2012). In the second step, the peptides were immobilized to the surface of MPs. The ligand-

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decorated MPs were then allowed to react with the antigen, hMPO. The capture (*i.e.* immunobinding) efficiency was then evaluated to assess the potential of the immunomagnetic separation technique (Safarik and Safarikova, 2004; Marsillach et al., 2011). Our results demonstrate the technical feasibility of our robust, cost-effective, and single-step isolation procedure of hMPO from complex solutions such as serum.

2. Materials and methods

2.1. Identification and synthesis of peptide ligands by phage display and biopanning

Peptide ligands were identified and synthesized as previously described (Hien et al., 2012), with only minor modifications. Briefly, the Ph.D.-12 Phage Display Peptide Library Kit and *Escherichia coli* ER2738 host cells were obtained from New England Biolabs, Inc. (Beverly, MA, USA). Myeloperoxidase from human leukocytes (hMPO), purchased from Sigma Aldrich (M6908), was immobilized to epoxy-functionalized MPs (Dynabeads® M-270 from Invitrogen Dynals AS, Oslo, Norway), thus generating an affinity surface for attracting phage clones with affinity to hMPO. Biopanning and amplification were performed as described (Hien et al., 2012). The affinity of each phage clone screened was evaluated by sandwich phage ELISA. The HRP/anti-M13 monoclonal conjugate (27942101) was from GE Healthcare, the anti-MPO antibody was from Abcam [4A4] (ab10165, Cambridge, UK), and the hMPO ELISA kit was from Ab Frontier, Inc. (Seoul, Korea). Phage clone DNA sequencing was performed by Bioneer, Inc. (Daejeon, Korea); N-terminally biotinylated 12-mer peptides were synthesized at Peptron, Inc. (Daejeon, Korea).

2.2. Binding analysis with QCM and SPR

A quartz crystal microbalance sensor (QCM D-300; Q-sense, Sweden) equipped with a QSX 301 gold chip was used to determine the molar binding ratio of hMPO to the immobilized ligand, *i.e.* the anti-hMPO antibody or the synthesized peptide. For QCM analysis, the peptide or anti-MPO antibody was diluted in acetate buffer (pH 5.0) and immobilized on the QCM sensor surface. The QCM sensor surface was modified with 11-MUA to form a self-assembly monolayer and then activated with 75 mM of EDC and 15 mM of NHS in PBS (pH 7.4). The remaining activated NHS-ester groups were blocked with 1 M ethanolamine, and hMPO was used as the binding analyte. For comparison, the molar binding ratios of hMPO to the anti-MPO antibody and to the peptide were both measured (Bianco et al., 2013; Tlili et al., 2004; Sala et al., 2014). To study the binding kinetics and equilibrium, a surface plasmon resonance (SPR) biosensor (Biacore 3000, GE Healthcare, USA) equipped with a CM5 Sensor chip was used. The peptide and the antibody were each immobilized as a ligand, and hMPO was used as the analyte. The SPR procedure was performed as previously described (Hien et al., 2012).

2.3. Immunomagnetic separation using peptide-decorated magnetic particles

For immunomagnetic separation, the biotinylated peptide (0.75 μM) was immobilized on streptavidin-coated MPs (Dynabeads® M-280, Invitrogen Dynals AS) and incubated for 1 h at ambient temperature. To calculate the mass of the bound peptides, the free peptides in the supernatant were analyzed by the Bradford assay. After washing, the MPs were incubated with 0.1% BSA in PBS (pH 7.4) for 10 min to block nonspecific binding sites. For immuno-binding, the beads were reacted with 11.5 nM of hMPO in the same buffer for 1 h. The binding efficiency was calculated by measuring the enzyme activity of the hMPO in the supernatant. To evaluate binding selectivity, the same procedure was performed in the presence of various concentrations of human serum albumin (HSA) as an abundant background protein.

Table 1
Molar binding ratios of adsorbed hMPO to immobilized ligands, as determined by QCM. Data are reported as means of triplicate experiments.

Ligand	Molar binding ratio	Relative ratio
Anti-hMPO Ab	0.990	1.00
Peptide #2	0.0062	0.0063
Peptide #3	0.0230	0.0230
Peptide #5	0.0036	0.0036
Peptide #9	0.0057	0.0058

3. Results and discussion

3.1. Phage screening and peptide synthesis

From 5 rounds of biopanning, 9 phage clones were selected. The sequences of these clones were determined, and the binding affinity of each clone to hMPO was evaluated by sandwich ELISA. The following 4 peptides with the highest binding affinities were chemically synthesized: (#9, FIPFDPMSMRWE; #3, SYIEPPERHRHR; #2, DDAKSRQGPLFR; and #5, NVNEGKAGVTGW; in order of binding affinity, starting with the highest). No consensus amino acid sequences were observed. The molar binding affinity of each peptide to hMPO was evaluated by QCM.

3.2. QCM and SPR analysis

The molar binding ratios between the immobilized ligands (peptide or anti-MPO antibody) and hMPO were then determined. As shown in Table 1, peptide #3 showed the highest ratio (3–5 times higher than the ratios of the other peptides), although it was approximately 43-fold lower than that of the antibody. Next, SPR was used to compare the binding kinetics and equilibrium of hMPO to the immobilized ligands, *i.e.* the selected peptide #3 and the anti-hMPO antibody. The SPR sensorgram and isotherm data are shown in Fig. 1. Assuming Langmuir isotherm (Sala et al., 2014), we obtained the dissociation constants (K_d) of 83 nM and 57 nM for the antibody and peptide #3, respectively. However, the Q_{max} values for the antibody and the peptide were 2.10 and 0.12, respectively, representing an approximately 18-fold difference. These data indicate that the binding kinetics of the peptide and the antibody are similar, but that the equilibrium masses are significantly different. To explain the difference, we calculated the surface coverage by the immobilized ligands, *i.e.*, % of the SPR chip surface occupied by the antibody and peptide ligands. Since the peptide has only one amine-containing residue, its coverage was only 0.36%, compared with 10.0% with the antibody. It was approximately 28-fold difference, and we think the binding mass difference could stem from the surface coverage (or, ligand density) difference.

3.3. Immuno-magnetic separation using the peptide ligand

Next, a biotinylated version of peptide #3 was quantitatively immobilized to streptavidin-coated MPs (approximately 3.0×10^{-9} mol of immobilized peptide per ml of MP suspension) and used as a ligand for hMPO capture. The average molar binding ratio of hMPO to the biotinylated peptide was approximately 0.021 (n = 3), which was similar to the ratio obtained in the QCM analysis. The average capture yield was about 60.0%, which is comparable to the capture yield in a previous study in which antibody-coated MPs were used to capture nisin (Prioult et al., 2000). To evaluate