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Screening of PC and PMMA-binding peptides for site-specific immobilization of proteins

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ABSTRACT

In the present study, we used proteomic research technology to develop a method for the screening and evaluation of material-binding peptides for protein immobilization. Using this screening method, soluble *Escherichia coli* proteins that preferentially adsorbed onto polycarbonate (PC) and poly(methylmethacrylate) (PMMA) as model plastic materials were first isolated and identified by 2-dimensional electrophoresis (2DE) combined with peptide mass fingerprinting (PMF). The genes of identified protein candidates (ELN, MLT, OMP, and BIF) that exhibited a hexahistidine tag (6×His-tag) were over-expressed by *E. coli* BL21 (DE3), and the proteins were purified by IMAC affinity chromatography. The candidates for PC and PMMA-binding peptides were isolated from peptide fragments from affinity protein candidates, which were digested with trypsin and chymotrypsin. Consequently, 5 candidates for the PC-binding peptide and 2 candidates for the PMMA-binding peptide were successfully identified by MALDI-TOF MS. All of the peptides identified were introduced to the C-terminus of glutathione S-transferase (GST) as a model protein for immobilization. Adsorption of peptide-fused and wild-type GSTs onto the plastic surfaces was directly monitored using a quartz crystal microbalance (QCM) device. Consequently, genetic fusion of PC-MLT8 and PC-OMP6 as PC-binders and PM-OMP25 as a PMMA-binder significantly enhanced the adsorption rates of GST, achieving an adsorption density that was more than 10 times higher than that of wild-type GST. Furthermore, the residual activity levels of GST-PC-OMP6 and GST-PM-OMP25 in the adsorption state were 2 times higher than that of wild-type GST. Thus, the PC and PMMA-binding peptides identified in this study, namely PC-OMP6 and PM-OMP25, were considerably useful for site-specific immobilization of proteins, while maintaining a higher adsorption density and residual activity levels. The method demonstrated in this study will be applicable to the isolation of a variety of material-binding peptides against the surfaces of unique materials.

1. Introduction

Recently, immobilization technologies of functional proteins onto the surfaces of organic and inorganic solid materials have been recognized to be important for the construction of protein-based nano-reactors, micro-fluidics, and microarray chips. Although a variety of immobilization methods including physical adsorption, chemical coupling, encapsulation, and others have been proposed, there is still much room for improvement to achieve higher density and biological activity of proteins immobilized to the greatest possible extent (Zhang et al., 2005; Butterfield et al., 2001; Lutz et al., 1990; Nakanishi et al., 2008; Rusmini et al., 2007; Hwang et al., 2007).

Universal affinity peptides for purification, detection, and immobilization such as 6×His tag, FLAG-tag, c-myc-tag, and Strep-tag, are now essential for biochemical research in laboratories (Wang et al., 2001; Carlsson et al., 2000; Hedhammar et al., 2005). Their performance strongly depends on the density and stability of ligand molecules such as Ni-NTA, monoclonal antibody, and streptavidin that were introduced on the surfaces of solid supports. In particular, when the ligand molecules are proteins, not only their density but also their residual activity in an immobilized state directly affect the density of target proteins that are genetically fused with affinity peptide tags.

However, several peptides that directly recognize the surfaces of solid materials have been isolated, and they are expected to be useful as alternative affinity peptide tags for direct immobilization of target proteins onto solid support surfaces (Sarikaya et al., 2003; Shiba, 2010). This research group has also developed and characterized affinity peptide tags that directly recognize the surface of hydrophilic polystyrene (PS-tag). Genetic fusion of the PS-tag

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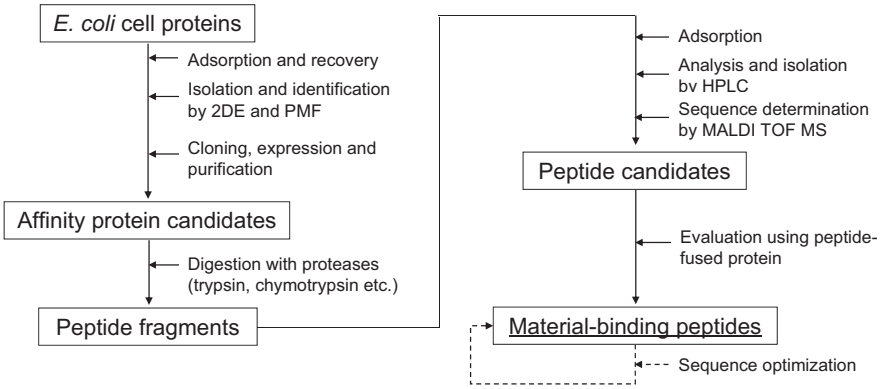

Fig. 1. Screening procedure of material-binding peptides developed in this study.

to several target proteins such as glutathione S-transferase (GST), *o*-acetylserine sulhydrylase A (OASS A), and single-chain Fv antibodies (scFv) resulted in site-specific immobilization of proteins with a much higher density and residual activity (Kumada et al., 2006, 2007, 2009a, 2009b, 2010a, 2010b).

Most material-binding peptides reported so far were isolated from random peptide libraries in which genetically engineered phage particles or *Escherichia coli* cells displayed oligo peptides on their surfaces. Although biopanning using such peptide libraries is becoming a standard procedure for identification of peptides that bind to target substances such as antibodies, enzymes, receptors, and others, this method often makes it difficult to isolate material-binding peptides, mainly due to the following 3 reasons: (1) non-specific and multipoint interaction of phage particle or cells, and/or different growth rates of each clone prohibit efficient fine screening; (2) the material-binding peptides often have lower sequence-specific motifs compared to protein-binding affinity peptides; and, (3) there are no guidelines as to how many rounds of biopanning should be performed to isolate material-binding peptides. Therefore, even when complicated and laborious biopanning procedures are repeated many times, the amino acid sequences of the peptides finally obtained often lack pattern and tendency. When polystyrene-binding peptides (PS-tag) were isolated from a random peptide display *E. coli* cell library by this research group, the biopanning procedure had to be repeated 10 times. Consequently, there was no typical motif or tendency with the amino acid sequences of the isolated peptides. All the peptides selected were then genetically fused with glutathione S-transferase (GST), and the adsorption properties of peptide-GST fusion proteins had to be characterized in order to determine which peptide possessed the desired binding affinity for the surface of polystyrene.

Because of the situation described above, we developed and demonstrated a new screening method to identify the amino acid sequences of material-binding peptides by a combination of proteome analysis technologies such as 2-dimensional electrophoresis (2DE), MALDI-TOF MS and HPLC. Fig. 1 schematically shows the comprehensive screening procedure in our method. *E. coli* cells contain more than 4000 kinds of proteins that possess different and unique amino acid sequences. Some of them may preferentially adsorb onto the surfaces of certain materials with a relatively strong binding affinity. In our screening method, proteins that are preferentially adsorbed onto the target materials with a strong binding affinity were first isolated from the soluble fraction of *E. coli* cell lysate and identified by 2DE followed by peptide mass fingerprinting using MALDI-TOF MS. The proteins that over-expressed and purified were digested with trypsin or chymotrypsin, and the peptide fragments that maintained a high binding affinity were identified by HPLC followed by MALDI-TOF MS. Finally, the adsorption and residual activity levels of peptide-fused proteins were compared in order to evaluate the usefulness of the identified peptides as affinity peptide tags for protein immobilization. *E. coli* internal proteins as a protein source were used in this study due to the following reasons. (1) *E. coli* proteins with few post-translational processing such as glycosylation can be easily identified by peptide mass fingerprinting method. (2) Genome database as well as protein database is well-studied and established. Therefore, the genes identified from 2D-gel can be easily cloned into the expression vector. (3) The genes of E. coli proteins can be easily expressed in the original host cell without codon optimization.

In this study, polycarbonate (PC) and poly(methyl methacrylate) (PMMA) were used as model solid materials, and the PC-binding and PMMA-binding peptides were screened by the method described above.

2. Materials and methods

2.1. Materials

Cube-shaped plastic grains made of polycarbonate (PC) and poly (methylmethacrylate) (PMMA) were purchased from Teijin (#AD-5503) and Asahi-Kasei Chemicals (#70NH), respectively. Packed columns of affinity chromatography resins, HisTrap HP, and GSTrap HP were purchased from GE Healthcare. The octadecylsilane (ODS) column, 5C18-AR-II for HPLC analysis was from Nacalai Tesque. The expression vector pET 22b from Merk was used for expression of cloned affinity protein candidates. Other chemicals and materials obtained from Nacalai Tesque, Wako Pure Chemicals, and Sigma–Aldrich were of reagent grade unless otherwise specified.

2.2. Extraction of soluble protein from E. coli

E. coli BL21 (DE3) was used as a model host strain in this study. A single colony was inoculated into 20 ml of LB medium in a 100-ml flask, and the flask was shaken at 37 °C overnight. Then, a portion of the culture was added to 100 ml of 2×YT medium in a 500 ml shake flask to obtain a final OD_{600} of 0.1. The flask was shaken at 200 rpm and 37 °C for 7 h. The cells were harvested by centrifugation at 10,000 × *g*, and the supernatant was removed. Pellets were suspended with 10 ml of Bugbuster™ protein extraction reagent (Merk) containing 5 μl of benzonase nuclease (Merk) and 100 μl of protease inhibitor cocktail (Nacalai), and the mixture was incubated at 37 °C for 30 min. After centrifugation for 20 min at 10,000 × *g*, the supernatant was recovered as an *E. coli* protein sample and stored at −20 °C until use.