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Application of protein-coupled liposomes to effective affinity screening from phage library

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Abstract

For effective screening by biopanning, we propose a new affinity screening method utilizing protein-coupled liposomes (proteoliposomes) as adsorbents. With multilamellar vesicles (MLVs) composed of dipalmitoylphosphatidylcholine (DPPC): dicetylphosphate (DCP) = 10: 1 (molar ratio), adsorption of nonspecific phage VCSM13 to the liposomes without any blocking was comparable to that on polystyrene tube wall coated with blocking protein. Phages displaying octapeptides specific to an anti-peptide antibody against a peptide antigen (FVNQHLCK) were screened from an octapeptide-displayed phage library by biopanning utilizing liposomes coupled with the antibody (AB-MLVs) or a conventional immunotube coated with the antibody (AB-tube). After four rounds of biopanning, all selected phages displayed homological peptides to the antigen peptide by use of AB-MLVs, while only 15% of the selected phages displayed homological peptides in the conventional biopanning. The octapeptide selected by AB-MLVs against the anti-peptide antibody showed comparable binding affinity, which were determined by the competitive ELISA and an immunoaffinity chromatography, to that of the peptide antigen. Thus, protein-coupled liposomes are useful as adsorbents for screening from combinatorial phage libraries.

Keywords: Phage display; Biopanning; Proteoliposome; Liposome; Affinity screening; Random peptide library

1. Introduction

In recent years, a large number of combinatorial libraries have been produced in genetic engineering fields for several purposes, such as design of new functional proteins, analysis of specific interactions of biomolecules and screening of specific antibodies against target molecules [1–3]. Among them, combinatorial libraries displayed by filamentous M13 phage containing phagemid vectors are the most common system because of effective infection and release properties of M13 phage and easy rescue of phagemid vectors with helper phage. In this system, however, biopanning procedures, which consist of adsorption of phages in a library to a target molecule, recovery of the adsorbed phages, infection and amplification of the phages in host cells and phagemid rescue, have to be repeated several times to obtain desirable candidates, because an initial number of phages displaying candidate molecules is small, and recovered phages contain many fakes nonspecifically adsorbed after the panning procedures. Therefore, for effective screening of target phages in panning procedures, highly specific characteristics for adsorption of target phages and a low degree of nonspecific adsorption of other phages are required.

In biopanning procedures, plastic microplates, tubes and microbeads are widely used for adsorption of target molecules and phage screening. However, the following factors in these plastic adsorbents may lead to inefficient outputs. (1) The surface area available for adsorption, and thus the adsorption capacity, is limited by geometrical shape. (2) Denaturation of target molecules on plastic surface may cause incorrect recognition of the molecules by phages. (3) Blocking procedures are essential to reduce nonspecific adsorption of phages. (4) Some phages may be specifically adsorbed on blocking agents.

Protein-coupled liposomes (proteoliposomes) have been used for drug and gene delivery, liposome immunoassay and artificial models of cells [4–6]. To solve the above problems in biopaninng procedures, we propose a biopanning method of phage-displayed libraries utilizing proteoliposomes as ad-

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sorbents. Proteoliposomes have the following characteristics. (1) The surface area available for adsorption can be controlled by the size and amount of liposomes used. (2) Proteins may be coupled on the surface of phospholipid membrane with minimum denaturation, and membrane-bound proteins are also effectively incorporated into the membrane. Jespersen et al. [7] successfully selected monoclonal antibodies against membrane receptors by biopanning utilizing proteoliposome-immobilized tube. (3) The hydrophilic nature of the surface of phospholipid membrane can reduce nonspecific adsorption of phages without blocking operations. (4) A phage library should contain very few phages specific to the components in liposome membrane, because they would be adsorbed specifically on *Escherichia coli* membrane before their secretion.

Screening from peptide libraries displayed by phages could be a promising way to obtain affinity ligands and antagonists against target proteins and to clarify sequential epitopes of antibodies, if the intrinsic interaction between the targets and peptides can be measured. In the present study, we investigated adsorption characteristics of nonspecific M13 phages to liposomes of different compositions, and compared the performances of biopanning utilizing liposomes coupled with an anti-peptide antibody and plastic tubes coated with the antibody by screening antigen peptides from an octapeptide-displayed phage library.

2. Experimental

2.1. Materials

Dipalmitoylphosphatidylcholine (DPPC) (Nacalai Tesque, Kyoto, Japan), dipalmitoylphosphatidylethanolamine (DPPE) (Avanti polar lipid, AL, USA), cholesterol (Chol) (Wako, Osaka, Japan), dicetylphosphate (DCP) (Sigma Aldrich, St Louis, MO, USA), stearylamine (SA) (Sigma Aldrich) and N-succinimidyl 4-(*p*-maleimidophenyl)-butyrate (SMPB) (Pierce, Rockford, IL, USA) were used for liposome preparation. 2-Iminothiolane·HCl (Pierce) was used for immobilization of antibody on phospholipid membrane. An octapeptide (FVNQHLCK), which corresponds to a part of the N-terminal region of insulin B chain, was synthesized and used for immunization to rabbits [8]. Polyclonal anti-peptide antibody against the antigen peptide was purified from rabbit antisera with an affinity column coupled with the peptide. It could recognize not only the antigen octapeptide but also bovine insulin. Polystyrene tube used for conventional biopanning (Immunotube, Type 44474) was purchased from Nalge Nunc International (Rochester, NY, USA), and Blocking solution (Nacalai Tesque) was used for blocking. Bovine insulin was conjugated with bovine serum albumin via glutaraldehyde crosslinking (Insulin-BSA) and used for competitive enzyme-linked immunosorbent assay (ELISA). All other chemicals used were of reagent grade.

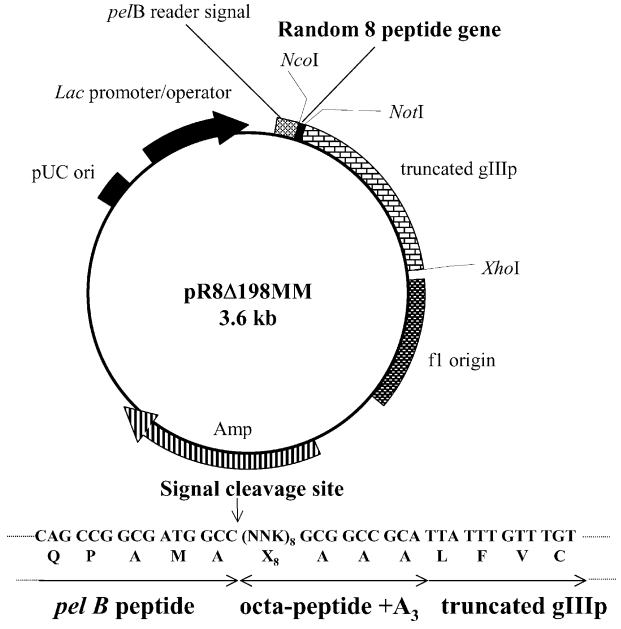

Fig. 1. Construction of gene for octapeptide-displayed phage library and signal cleavage site of fusion protein.

2.2. E. coli strain

E. coli XL1-Blue, which has the Tet^R F plasmid and the chromosomal genotype containing *endA1*, *hsdR17*, *supE44*, *thi-1*, *recA1*, *gyrA96* and *relA1*, was purchased from Stratagene (CA, USA) and was used as a host for vector constructions and generation of peptide-displayed M13 phage particles.

2.3. Vector constructions and transformation

Fig. 1 schematically shows construction of an expression vector pR8Δ198MM for preparation of a random octapeptide-displayed phage library and its signal cleavage site. The DNA fragment which contains ribosome binding site (RBS), *pelB* reader peptide including *Nco*I restriction site, and cloning sites (*Not*I and *Xho*I sites) for insertion of truncated gIIIp gene (amino acid position 198-406) was amplified by PCR. The amplified fragment was inserted at the position between *Xba*I and *Bam*HI site of pT7Blue phagemid vector. The pT7Blue derivative, pT7MM was digested with *Not*I and *Xho*I. The truncated gIIIp gene was amplified from M13mp19 vector as a template DNA with primers (5′-CTCTCTGCGGCCGCATTATTTGTTTGTGAATATCAAGGCC-3′ and 5′-CTCCTCGAGTTAAGACTCCTTATTACGCAGTATGTT-3′). After cleavage of the fragment and pT7MM with *Not*I and *Xho*I, they were ligated by T4 DNA ligase (pΔ198MM). The random octapeptide genes were prepared by one cycle PCR with two types of synthesized single strand DNA coding 5′-CGGCCATGGCC(NNK)$_8$GCGGCCGCTTACGCCAAGCTCTAAT-3′and 5′-GTATTAGAGCTTGGCGTAAGCGGCCGC-3′,

where N is dA, dT, dG or dC; K is dT or dG. The pΔ198MM and random octapeptide genes were digested by *Nco*I and *Not*I for directional insertion. The pR8Δ198MM was constructed by ligation of the digested pΔ198MM and the random octapeptide genes. The pR8Δ198MM was transformed into *E. coli XL1-Blue* by electroporation (2.5 kV, 25 μF and 200 Ω, GENE PULSER, Bio-Rad, CA, USA) and 1.2×10^5 numbers of independent colonies were obtained. The $(NNK)_8$ sequence on pR8Δ198MM was translated to random octapeptides, since the host *XL1-Blue* is characterized as the suppressed strain of the amber stop codon (TAG) [9].

2.4. Cultivation and generation of octapeptide-displayed M13 phages encapsulating phagemid DNA

The transformant was inoculated in 50 ml of a 2 × YT medium (yeast extract 10 g/l, trypton 16 g/l, NaCl 5 g/l, pH 7.0) containing 50 μg/ml ampicillin (Amp) and 12.5 μg/ml tetracycline (Tet) in a 500 ml-flask and shaken at 37 °C at 250 rpm. At OD_{600} of 0.5, helper phage VCSM13 (Stratagene) was added at multiplicity of infection (MOI) of 20, and the culture cells were incubated for 30 min at 37 °C without shaking. Cells were harvested by centrifugation at 5000 × *g* for 10 min and transferred to a fresh culture medium 2 × YT containing Amp and Tet and 70 μg/ml kanamycin (Kan). The cells were shaken again for 12–16 h under the condition stated above. After centrifugation twice at 9000 × *g* for 15 min, the supernatant containing octapeptide-displayed M13 phages was recovered as an octapeptide phage library (R8Δ198MM) and stored at 4 °C until use. If necessary, phages were concentrated by PEG precipitation or diluted with PBS (NaCl 16 g/l, KCl 0.2 g/l, $Na_2HPO_412H_2O$ 2.9 g/l, $KH_2PO_4$0.2 g/l, pH 7.2). The phage titer (colony forming unit) in the supernatant was determined from the number of colonies of precultured and infected *XL1-Blue* cells on 2 × YT selective agar plate containing Amp and Tet. Helper phage VCSM13 used as a model of nonspecific phage was prepared by a standard protocol provided from Stratagene.

2.5. Preparation of activated liposomes and immobilization of antibody to liposomes

DPPE was activated to *N*-[4-(*p*-maleimidophenyl) butyryl] dipalmitoylphosphatidylethanolamine (MPB-DPPE) by SMBP as previously reported [10]. The lipid mixture containing DPPC (10 μmol) and other lipids (1–10 μmol) in chloroform was dried to a thin lipid film on the inside wall of a pear-shaped flask (50 ml) using a rotary evaporator above 42 °C under reduced pressure. For protein coupling on liposomes, 0.5 μmol MPB-DPPE was added in the lipid mixture. The lipid film was dried overnight under reduced pressure in a desiccator. Then, 3 ml of PBS was added, and the flask was shaken at around 50 °C to peel off the lipid film and to form multilamellar vesicles (MLVs). Activated liposomes prepared were purified twice by centrifugation for 2 min at 15,000 × *g* and washing with PBS at 4 °C. To prepare antibody-coupled liposomes, the affinity purified anti-peptide antibody dissolved in PBS was mixed with the activated liposome suspension to give the final concentrations of 200–300 μg-antibody/ml and ca. 2 μmol-DPPC/ml, and 2-iminothiolane-HCl, which conjugates antibody molecules to activated liposomes through sulfhydryl groups, was added in five-fold excess of antibody. The mixture was incubated with gentle stirring for 12–16 h at room temperature. By removing uncoupled antibody three times by centrifugation for 2 min at 23,500 × g and washing with PBS at 4 °C, antibody-coupled liposomes were obtained. Pellet was resuspended in PBS and stored at 4 °C until use. The concentrations of DPPC and antibody in liposome suspensions were measured after addition of 10–100 μl of 10% deoxycholic acid sodium salt to 100 μl of sample. The concentrations of DPPC and coupled antibody in liposome suspensions were determined by the cholineoxidase method using phospholipids C-test Wako (Wako) and by DC-protein assay (Bio-Rad), respectively. The average diameters of antibody-coupled liposomes were measured by the differential light scattering analysis (DLS 7000, Photal Otsuka Electronics, Osaka, Japan). The number of vesicles was counted by use of a hemocytometer with microscope. Antibody-coupled liposomes obtained were stored at 4 °C for at least 1 month without loss of binding activity.

2.6. Adsorption of nonspecific M13 phage on liposomes made of different lipid compositions

Nonspecific phage (VCSM13) was added in a micro-centrifuge tube (1.5 ml) containing MLVs (200 nmol-DPPC/ml) made of different compositions of lipids in PBS + 10% (v/v) Blocking solution or an Immunotube containing PBS + 10% (v/v) Blocking solution to form 1 ml of a suspension of 2.6×10^9 plaque forming unit (pfu)/ml. The mixture was incubated for 30 min at 25 °C. In the case of MLVs, they were centrifuged at 20,000 × *g* for 2 min at 4 °C, and 990 μl of the supernatant was carefully discarded. The pellet was resuspended in 1 ml of fresh PBS. The centrifugation and washing operations were repeated 10 times. To reduce phages nonspecifically adsorbed on the tube wall, at the tenth washing operation, the suspended liposomes in PBS were transferred in a new micro-centrifuge tube and centrifuged. The Immunotube was washed 10 times with PBS containing 0.05% Tween 20 (PBST). In both cases using the MLVs and Immunotube, adsorbed phages were eluted by addition of 200 μl of 0.1N HCl after the removal of the supernatant. Then, the titer (plaque forming unit) of eluted phage (VCSM13) in the solution neutralized with 100 μl of 2 M Tris–HCl buffer (pH 8.0) was determined by use of precultured *XL-1Blue* dispersed in 3.5 ml of 2 × YT soft agar and selective agar plate containing Tet. After incubation at 37 °C for 16 h, numbers of plaque were counted and averaged in duplicate experiments.

2.7. Biopanning selection of phages displaying peptides specific to anti-peptide antibody from library

In this study, two types of panning strategies were used in liposome biopanning. (Strategy L1) MLVs coupled with 10 μg of the antibody were used as adsorbents for each panning round. (Strategy L2) MLVs coupled with 100 μg of the antibody were used at first round, and 10 μg-antibody for successive rounds. In conventional method using Immunotube, the wall of Immunotube was coated with antibody molecules by use of 1 ml of PBS containing 25 μg/ml of the anti-peptide antibody. After 10 times washing with PBS, it was blocked with Blocking solution and used for panning.

A solution (1 ml) containing octapeptide-displayed M13 phages (R8Δ198MM, about 1–4 × 10^8 cfu/ml) in 10% (v/v) Blocking solution was incubated with the antibody-coupled MLVs for 30 min at 25 °C. Centrifugation and washing steps were repeated 10 times in the same way described above, and adsorbed phages were eluted with 200 μl of 0.1N HCl. After neutralization with 2M Tris–HCl, they were infected into precultured *XL1-Blue*. Infected cells grew up to OD 0.5–1.0 in 2 × YT (Amp, Tet). After superinfection of VCSM13 (MOI = 20), recovered phages were produced in fresh 2 × YT medium (Amp, Tet, Kan). The supernatant containing phages was recovered. The obtained phages were concentrated by PEG precipitation, and used for the second round of selection. This panning operation was repeated four times. The initial and recovered phage titers (colony forming unit) in each panning round were determined in duplicate with precultured *XL1-Blue* and selective plates containing Amp and Tet.

2.8. DNA sequencing

After infection of recovered phages to *XL1-Blue* cells, a portion of cells were inoculated on 2 × YT selective agar plate (Amp, Tet) to form a single colony. The 16–20 colonies were randomly chosen, and their DNA sequences coding octapeptide were analyzed. They were determined by a genetic analyzer (310 Genetic Analyzer, Applied Biosystems, CA, USA) according to standard protocol from the supplier, after PCR with a primer (5′-CCCCCCCCCTCTAGAAATAATTTTGTTTAA-3′) and Big dye terminator cycle sequencing kit (Applied Biosystems).

2.9. Binding affinity of selected peptides against anti-peptide antibody in competitive ELISA

Two kinds of octapeptides (FVNQDTTC and HVCQPLLL), which showed the highest homology to the antigen peptide sequence in biopanning by use of liposomes and Immunotube, were synthesized (BEX Corporation, Tokyo, Japan) and were used in determination of the binding affinity by competitive ELISA. A microtiter plate was incubated with 100 μl of 10 μg/ml insulin-BSA in PBS to immobilize insulin molecules on its surface of solid. After washing with PBS, the plate was blocked with four times diluted Blocking solution. After washing, the sample solutions containing 5 μg/ml of the anti-peptide antibody, different concentrations of the octapeptide and 10% Blocking solution were incubated at room temperature for 1 h. After washing, 100 μl of 2000 times diluted HRP-conjugated anti-rabbit IgG antibody (goat) solution was added and incubated for 1 h at room temperature. After washing, color was developed by addition of 100 μl of substrate solution composed of 3 mg/ml 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonicacid) diammonium salt (ABTS) and 0.01% H_2O_2 in 0.1 M citrate buffer. The absorbance of developed signal was detected at 405 nm by a microplate reader (Model 550, Bio-Rad). The values of IC_{50} were determined from duplicate experiments as the peptide concentrations showing 50% inhibition of the interaction between the antibody and insulin-BSA.

2.10. Preparation of peptide-coupled column and adsorption of anti-peptide antibody in affinity chromatography

The synthesized peptide (FVNQDTTC or HVCQPLLL) or the antigen peptide (4.5 mg) was coupled with 1 g of epoxy-activated Sepharose 6B (GE Health Care) according to the protocol provided from the supplier. The peptide-coupled Sepharose 6B was packed in an adsorption column of 1.5 cm i.d., and was equilibrated with PBS. A solution of anti-peptide antibody purified with the antigen peptide-coupled column was applied to the peptide-coupled columns at a flow rate of 1.5 ml/min. After washing with PBS, adsorbed antibody was eluted by 0.1N HCl (pH 1.0). After neutralization of recovered antibody with 2 M phosphate buffer (pH 7.2), the recovered amount of antibody was determined by DC-protein assay.

3. Results and discussion

3.1. Effects of lipid composition of liposomes on adsorption of nonspecific M13 phage

To reduce adsorption of nonspecific M13 phage in biopanning, we studied the effects of lipid compositions of liposomes on adsorption of M13 phage. Four types of MLVs were prepared; MLVs composed of (a) DPPC:Chol:DCP = 10:10:1, (b) DPPC:DCP = 10:1, (c) DPPC:Chol:SA = 10:10:1 and (d) DPPC:SA = 10:1. Liposomes (a) and (b) have negative charges on the surface of vesicles, and the others have positive charges. Cholesterol was utilized to increase the membrane stability and fluidity of the liposomes at room temperature. Liposomes (c) did not disperse well in PBS and were not used for measurement of phage adsorption. The adsorption characteristics of these liposomes were studied under the same procedures for biopanning by use of nonspecific phage VCSM13 (2.6 × 10^9 pfu/ml), and the recovered phage titer was determined. As shown in Fig. 2, the liposomes (b) DPPC:DCP = 10:1 showed significantly

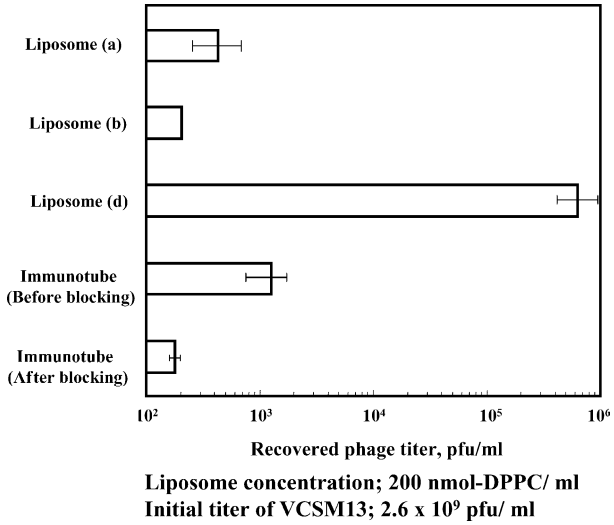

Fig. 2. Adsorption of nonspecific M13 phage on liposomes and Immunotube.

low adsorption of nonspecific phage among other types of liposomes. These results indicate that nonspecific adsorption of M13 phage to the liposomes is mainly caused by electrostatic interactions between the surface charges, because major surface proteins of M13 phage are negatively charged at neutral pH [11,12]. Without any blocking operations, nonspecific adsorption of M13 phage to the liposomes (b) can be reduced to the adsorption level comparable with the Immunotube coated with blocking reagent.

3.2. Adsorption characteristics of antibody-coupled MLVs and antibody-coated Immunotube

Activated liposomes composed of DPPC:DCP:MPB-DPPE = 20:2:1 (in molar ratio) were coupled with the anti-peptide antibody via iminothiolane as described in Section 2.5.

Table 1 shows the characteristics of antibody-coupled MLVs (AB-MLVs) prepared for panning operation. When 10 μg of the antibody was used, it was immobilized on the surface of 1.9×10^6 vesicles of MLVs, which total surface-area, calculated from the average diameter, was 1.8 cm^2, 38% of the surface area of immunotube.

Fig. 3 shows phage titers recovered from the library (R8Δ198MM) with use of AB-MLVs or antibody-coated tube (AB-Tube). MLVs (DPPC/DCP) showed low titer of

Table 1
Characteristics of MLVs coupled with 10 μg of antibody

Coupled antibody	67 pmol (10 μg)
Phospholipid	170 nmol-DPPC
Number of vesicles	1.9×10^6
Average diameter	5.6 ± 1.0 μm
Antibody molecule on a vesicle	2.0×10^7
Surface area	1.8 cm^2 (tube 4.8 cm^2)
Immobilized density of antibody	36 pmol/cm^2

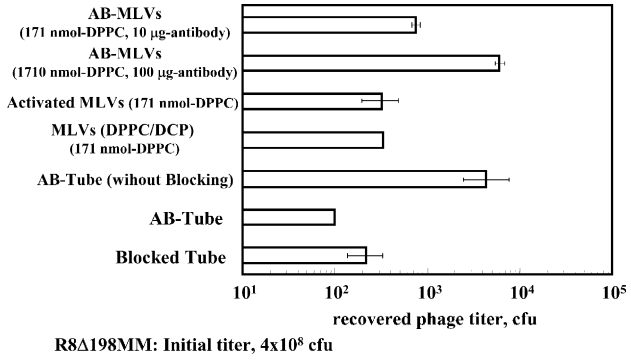

Fig. 3. Adsorption characteristics of phages displaying peptide library on MLVs and Immunotube.

adsorption against not only nonspecific phage (VCSM13) but also the phage library. The presence of MPB-DPPE in vesicles (activated MLVs) did not affect the adsorbed titer. In spite of the smaller surface area, the number of phages recovered from the library with AB-MLVs containing 10 μg of the antibody was about 10 times that with AB-Tube. By increasing the amount of the antibody to 100 μg, the number of recovered phages increased about 10 times. These results may indicate that recognition of peptides displayed on phages by the antibody coupled on MLVs is more specific and natural than by the antibody adsorbed on the wall of immunotube.

3.3. Biopanning selection of phages displaying specific peptides from library

Fig. 4(a) shows initial (closed keys) and recovered (open keys) phage titers of each round and Fig. 4(b) shows the recovery, which is calculated from the ratio of recovered titer to initial titer. In biopanning with Strategy L1 and Strategy L2, the recoveries were higher than those with AB-Tube in successive rounds.

After four rounds of biopanning, amino acid sequences displayed by 16–20 colonies recovered from the library were determined by DNA sequencing. Table 2 shows sequences of peptides which have more than two amino acids at the same position with the antigen octapeptide (FVNQHLCK). If octapeptides, which have overlapped sequences to that of the antigen peptide at least at two positions, are defined as homological peptides, with Strategy L1, all recovered phages after four rounds displayed homological peptides, especially in the N-terminal region from one to three or four positions. With Strategy L2, another peptide showing homology at five and six positions (VKCDHLTR) was obtained with 40% probability, together with similar peptides obtained with Strategy L1. With AB-Tube only three peptides showed homological sequences among 20 colonies.

With biopanning using AB-MLVs, the percentages of phages displaying homological peptides were much higher than that with AB-Tube from earlier stages of biopanning. For example, they were 60% and 20% after two rounds of panning with Strategy L1 and L2, respectively, while it was

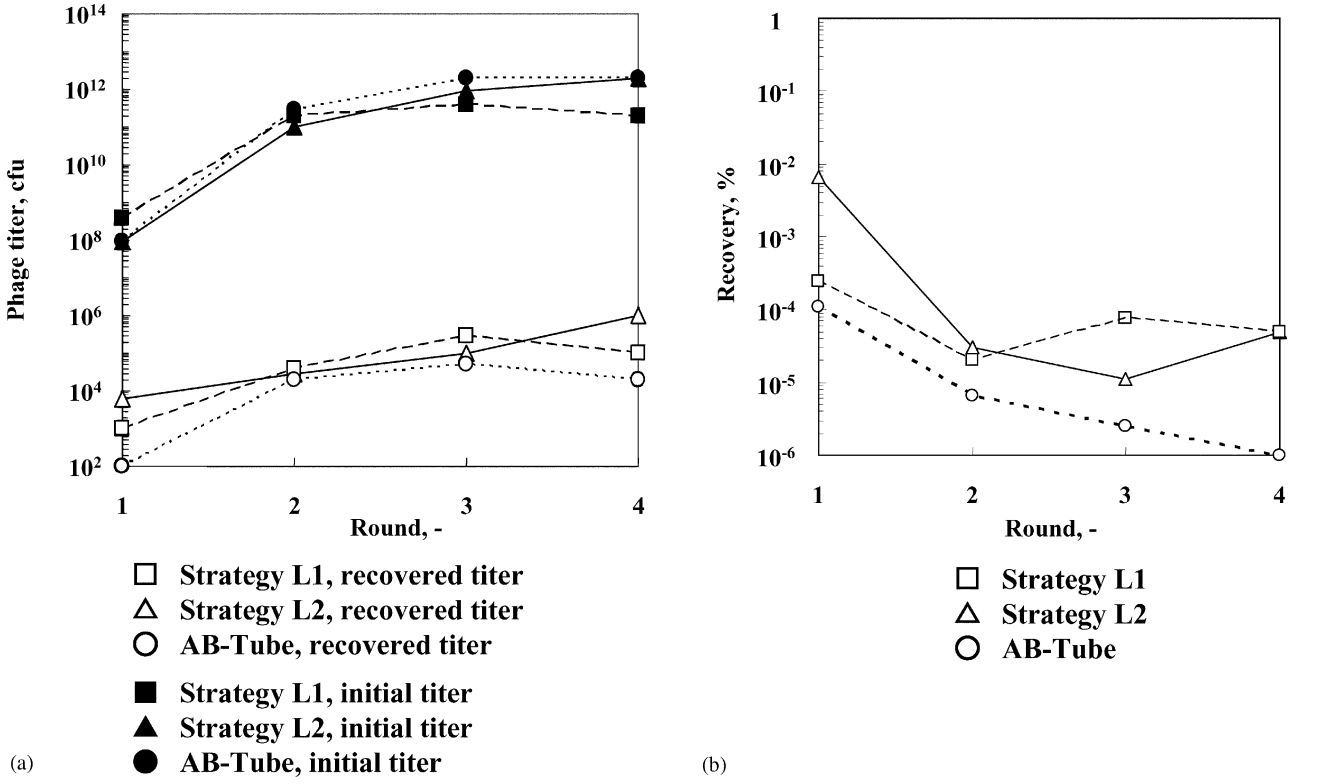

Fig. 4. Recovered phage titer and recovery in biopanning selection.

0% with use of AB-Tube. Interestingly, with Strategy L2, the recovered efficiency after four rounds was lower than that with Strategy L1. This indicates that larger surface area may increase recovery of targets showing lower affinity and/or non-specific phages from the library in the first round, although higher binding capacity is effective to select targets from large size of libraries. By use of a larger numbers of AB-MLVs, therefore, wide varieties of targets might be obtained, while the recovery of specific targets decreases. The lower recovery in the conventional biopanning using AB-Tube may be caused by slight denaturation of antibody molecules adsorbed on polystyrene wall and changes in binding characteristics. It was reported that protein immobilized on plastic plate was denatured and showed changes in binding affinity [13]. Plastic or magnetic beads are often utilized as supports to increase the concentration of target protein, but they might suffer similar problems. Thus, biopanning using protein-coupled MLVs is one of promising methods for screening of phages expressing peptides showing specific interactions.

3.4. Binding characteristics of antibody to isolated octapeptides in competitive ELISA

Two kinds of peptides selected by AB-MLVs and AB-Tube, which showed the highest homology in each method, were synthesized and used as competitors in competitive

Table 2
Homological sequences of selected peptides against antigen octa-peptide after four times panning

		1	2	3	4	5	6	7	8	Percentage (%)
	Ll-l	F	V	N	Q	D	T	T	C	75
	Ll-2	F	V	N	V	E	Q	S	C	6
Strategy LI 100% (16/16)	Ll-3	F	V	N	H	Q	D	H	H	6
	Ll-4	F	V	N	V	S	C	P	H	6
	Ll-5	F	V	N	D	Y	P	A	R	6
	L2-1	V	K	C	D	H	L	T	R	40
Strategy L2 60% (12/20)	L2-2	F	V	N	C	V	S	P	H	15
	L2-3	F	T	N	W	C	G	G	D	5
	T-l	F	C	V	*Q*	Y	M	R	V	5
AB-Tube 15% (3/20)	T-2	H	V	C	*Q*	P	L	L	L	5
	T-3	F	E	V	I	R	Y	C	F	5
Antigen peptide		F	V	N	Q	H	L	C	K	

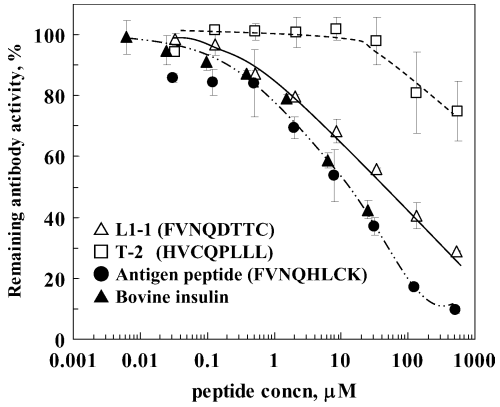

Fig. 5. Inhibition of antibody binding by homological peptides in competitive ELISA.

ELISA. Fig. 5 shows the result of competitive ELISA with the octapeptide (L1-1 or T-2), the antigen peptide or bovine insulin. The octapeptide L1-1 inhibited the interaction between antibody and Insulin-BSA with comparable affinity (IC_{50}; 50 μM) to that of the antigen peptide, while IC_{50} value of the peptide T-2 was over 1 mM. Thus, these results indicated that the target-immobilized MLVs as a support could successfully select more specific candidates with smaller number of panning rounds.

3.5. *Affinity purification of antibody by use of peptide-coupled columns*

Performances of the selected peptides as ligands in affinity chromatography were compared by use of the peptide-coupled columns. Table 3 shows the amount of recovered antibody, which was purified by the antigen peptide-coupled column, and the recovery in affinity chromatography. The recovery was naturally 100% with the antigen peptide-coupled column, and it was 60% by use of the peptide L1-1-coupled column, while it was only 9% by use of the peptide T-2-coupled column. Since the binding capacity of the L1-1 column is considered to be much higher than the amount of applied antibody, 40% of antibody molecules passing through the L1-1 column might recognize other epitopes in the antigen peptide, such as His-Leu residues in antigen peptide. Thus, as a ligand in affinity chromatography, the peptide selected by AB-MLVs was more effective than that by AB-Tube.

Table 3
Purification of anti-peptide antibody by use peptide-coupled columns

	Recovered antibody amount (μg)	Recovery (%)
Ll-l FVNQDTTC	249	60
T-2 HVCQPLLL	39	9
Antigen peptide FVNQHLCK	429	103

Applied antibody concentration, 104 μg/ml total antibody applied, 414 μg.

4. Conclusions

In this study, we clarified the characteristics of antibody-coupled liposomes as adsorbents for effective biopanning operation. Antibody-coupled MLVs consisted of DPPC:DCP:MPB-DPPE = 20:2:1 could significantly reduce the nonspecific adsorption of VCSM13 phages, and showed high specificity for biopanning from the octapeptide-displayed phage library. This screening method utilizing target proteins coupled on liposomes is very effective to select specific peptides against antibody, enzyme, and other proteins from random peptide libraries, and might be applied also to selection of monoclonal antibodies against target antigens from reconstituted antibody libraries [14,15]. For further improvements of this method, it is necessary to optimize liposome size, density of target protein on liposome membrane, total concentration of target molecules and other factor. Development of an effective B/F separation method such as magnetic separation method would make this method more effective.

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