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HWGMWSY, an unanticipated polystyrene binding peptide from random phage display libraries

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ABSTRACT

Phage display is a powerful technique for the discovery of peptide ligands that bind to various targets; however, ambiguous results often appear. Peptide HWGMWSY has been isolated repeatedly in our laboratory and by other research groups dealing with different protein and nonprotein targets, which led to a hypothesis that it may be a target-unrelated peptide interacting with polystyrene plastic surfaces. We compared binding properties and amplification rate of phage clone displaying the peptide HWGMWSY, a previously confirmed plastic binding clone WHWRLPS, and a control phage clone ASVQERK. An enzyme-linked immunosorbent assay and a phage elution assay confirmed that phage clone HWGMWSY binds to polystyrene. Surface plasmon resonance measurements on the other hand excluded the possibility of binding to bovine serum albumin, a common blocking agent in phage display experiments. Amplification rates of the above-noted phage clones were not statistically different. We therefore conclude that phage clone HWGMWSY was isolated in different selection procedures as a result of its affinity to polystyrene.

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Phage display is a commonly used technique for the discovery of peptide ligands that bind to various targets. Despite the simplicity and usefulness of this technique phage display can often lead to ambiguous results. Phage clones displaying peptides that are unrelated to the target and have no actual affinity are sometimes selected for instead of clones that display high-affinity binders. Some peptides can bind to the components of the screening system (selection-related target-unrelated peptides), like a blocking agent or a solid surface, and thus these selection-related peptides are selected but do not display affinity for the target molecule. In addition, propagation-related target-unrelated peptides are selected because some phage clones propagate faster than others due to a propagation advantage. Target-unrelated peptides of any kind are false positives and should be distinguished from true binders. Several peptides with affinity to different components of the screening system [1,2] and faster propagating phage clones [3,4] have already been characterized and reported. Peptide HWGMWSY has been isolated repeatedly in our laboratory in standard biopanning experiments [5] on ghrelin and β2-glycoprotein I and showed apparent affinity toward these targets. To our surprise, a detailed database search revealed that the same clone had been isolated by other research groups (Table 1), raising suspicions that it may be a target-unrelated peptide. Because it is highly unlikely that peptide HWGMWSY interacts with a wide variety of apparently unrelated targets, we investigated the binding and propagation properties of the corresponding phage clone. Our results show that HWGMWSY is a target-unrelated peptide that binds to polystyrene.

Materials and methods

Phage target binding assay

Ghrelin (Bachem, Bubendorf, Switzerland) or β2-glycoprotein I was dissolved in PBS[1] (0.1 mg/mL) and 100 μL of this solution was applied to MaxiSorp microtiter wells (Nunc, Roskilde, Denmark) and incubated overnight at 4 °C with gentle agitation. The wells were blocked with 280 μL of 1% bovine serum albumin (BSA) for 1 h at room temperature and washed three times with 0.05% PBS with Tween 20 (PBST). Ten microliters of amplified phage clone Pb1 (displaying peptide HWGMWSY) from Ph.D.-7 library (New England Biolabs, Ipswich, USA) was diluted with 90 μL of PBS, and 100 μL of this phage suspension was added to the wells. Next, an enzyme-linked immunosorbent assay (ELISA) was performed as previously described [12].

Binding to polystyrene

Binding affinity to plastic was investigated as previously described [13]. Polystyrene microtiter wells were blocked with

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[1] *Abbreviations used:* BSA, bovine serum albumin; NFM, nonfat milk; PBS, phosphate-buffered saline; PBST, PBS with Tween 20.

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Table 1
Biopanning experiments resulting in the isolation of the HWGMWSY displaying phage clone.

Target molecule	Binding to the target molecule	References
Bovine serum albumin	NA[a]	[6]
Chromatin high mobility group protein 1	Yes	[7]
Monoclonal antibody 8C11	NA	[8]
Helix 9 of 16S rRNA of *Pseudomonas aureginosa*	Yes	[9]
SPARC (osteonectin)	Yes	[10]
Monoclonal antibody HmenB13	NA	[11]
Ghrelin	Yes	Our work
β2-glycoprotein	Yes	Our work

[a] Binding studies for phage clone HWGMWSY were not available in the publication.

280 μL of 1% nonfat milk (NFM), 1% BSA, or PBS for 2 h. The amount of 5×10^9 pfu of phages Pb1, Pb2 (displaying peptide WHWRLPS), and Co1 (displaying peptide ASVQERK) from library Ph.D.-7 was diluted in the corresponding blocking buffers and added to the wells. Following a 1-h incubation, the wells were washed five times with 200 μL of the matching buffer. Monoclonal HRP conjugated-anti-M13 antibodies (GE Healthcare, Little Chalfont, UK) diluted 1:5000 were added to the wells and after 1 h the wells were washed five times with 200 μL of PBS. Reagent TMB (Thermo Scientific, Rockford, USA) was used for detection and the absorbance at 450 nm was read using a Tecan GENios (Tecan, Grödig, Austria) microtiter plate reader.

Phage elution assay

Phage clones Pb1, Pb2, and Co1 (1×10^{10} pfu) were diluted in 100 μL PBS and incubated in microtiter wells for 2 h with gentle shaking at room temperature. The wells were washed five times with 200 μL 0.1% PBST; the plate was incubated for 5 min with PBST with vigorous shaking, for each wash. Next, the remaining phages were eluted with pH 2.2 glycine–HCl buffer. The concentration of phage in the eluate was determined by titration as previously described [14].

Phage amplification rate

An overnight culture of *E. coli ER2738* was diluted 1:100 in 100 mL of LB medium containing 0.02 mg/mL tetracycline and put in a 500-mL culture flask. The culture was shaken for 15 min in an incubator at 37 °C. Next, the culture was split into two aliquots of 30 mL each in 500 mL Erlenmeyer flasks and 1×10^8 pfu of phage Pb1 or Ph.D.-7 was added. During the incubation, 500-μL samples were withdrawn with a sterile pipette every 30 min (sample No. 1 was taken immediately after addition of the phage). The samples were centrifuged at 9300*g* twice using an Eppendorf 5415 R centrifuge, and the number of phage in the supernatant was determined by titration.

Surface plasmon resonance

The binding of the phages to BSA was monitored using the Biacore T100 system (Biacore, Uppsala, Sweden). BSA was immobilized on a CM5 sensor chip (BR-1005–30) to 5500 RU. Phage stocks were prepared in 0.1% PBST (1×10^9 pfu/μL) and 25 μL of phage suspension was injected for each assay. The association was monitored for 300 s and the dissociation for 180 s. After every assay, the sensor chip was regenerated with 10 mM NaOH. All the steps were performed using a flow rate of 5 μL/min at 25 °C with 0.1% PBST as the running buffer.

Results

Peptide HWGMWSY has been isolated in biopanning experiments by several research groups using unrelated targets (Table 1). The same peptide was enriched in our laboratory after three rounds of specific and nonspecific selection on the functionally and structurally unrelated proteins ghrelin and β2-glycoprotein I. Although a phage target-binding assay (ELISA) indicated the affinity of HWGMWSY to both targets, significant background binding was also observed (data not shown). To evaluate the binding affinity to polystyrene, we compared the binding properties of HWGMWSY (Pb1) with those of the phage clone displaying peptide WHWRLPS (Pb2), a previously known plastic-binding peptide [4,15], and a control phage clone displaying peptide ASVQERK (Co1). We compared the effects of different blocking agents and washing conditions on the binding of phage to polystyrene microtiter wells as previously described [13]. An increased binding to plastic was observed when microtiter wells were unblocked (blocked only with PBS), but not when BSA or NFM were used for blocking (Fig. 1). Regardless of the applied washing procedure, the absorbance was always higher for Pb1 and Pb2 compared to Co1, indicating that Pb1 has a similar affinity for plastic as Pb2.

Secondary antibodies, like any other protein, adsorb to some extent to the unblocked plastic, resulting in a false increase in the signal. To uncouple the signal yielded by bound phages and that resulting from plastic adsorption of antibodies, another ELISA was performed where the surface was blocked with NFM after the binding of phage particles. As expected, our previous result was confirmed (Fig. 2), as the observed signal was significantly higher for Pb1 and Pb2 (two-sided *t* test, α 0.05). A similar binding pattern was observed with all polystyrene microtiter plates used. This was further confirmed when bound phages were eluted with acidic buffer and the number of phage particles was determined by titration (Fig. 3). Because the same number of each phage was used in the experiments, we conclude that the increased plastic binding is a result of the displayed peptide.

We also investigated the effect of BSA on plastic binding. When the wells were blocked with increasing concentrations of BSA, the plastic binding of all three selected clones was inhibited in a dose-dependent manner, indicating that the clones bind to polystyrene but not to BSA (Fig. 4). This is in agreement with our surface plasmon resonance measurements (data not shown), where Pb1 and Pb2 showed only background binding to a BSA sensor chip. The results also demonstrate that a higher concentration of BSA (approximately 10 times higher) was needed to reduce the plastic binding of Pb1 and Pb2 as compared to Co1 (Fig. 4). This again indicates that clone Pb1 has affinity for polystyrene.

Peptide HAIYPRH [4] from the Ph.D.-7 library has been found to possess a mutation that results in a propagation advantage, enabling this phage to overtake the phage pool during amplification. To exclude this as a possibility for the selection of clone Pb1, we compared its amplification rate with the whole phage library. We found no statistically significant difference between the amplification rates of phage Pb1 and the phage library (data not shown).

Discussion

Target-unrelated peptides are sometimes selected in biopanning experiments even though they have no actual affinity for the target. A phage clone displaying the peptide HWGMWSY (Pb1) has been selected from the phage display library Ph.D.-7 by different research groups using various targets. Although affinity for certain targets has been confirmed (Table 1), it is unlikely that this peptide has affinity for all structurally and functionally unrelated targets. Due to the presence of aromatic tryptophan residues, we suspected that HWGMWSY might be a plastic-binding peptide.

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