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A target-unrelated peptide in an M13 phage display library traced to an advantageous mutation in the *gene II* ribosome-binding site

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Abstract

Screening of the commercially available Ph.D.-7 phage-displayed heptapeptide library for peptides that bind immobilized Zn^{2+} resulted in the repeated selection of the peptide HAIYPRH, although binding assays indicated that HAIYPRH is not a zinc-binding peptide. HAIYPRH has also been selected in several other laboratories using completely different targets, and its ubiquity suggests that it is a target-unrelated peptide. We demonstrated that phage displaying HAIYPRH are enriched after serial amplification of the library without exposure to target. The amplification of phage displaying HAIYPRH was found to be dramatically faster than that of the library itself. DNA sequencing uncovered a mutation in the Shine–Dalgarno (SD) sequence for gIIp, a protein involved in phage replication, imparting to the SD sequence better complementarity to the 16S ribosomal RNA (rRNA). Introducing this mutation into phage lacking a displayed peptide resulted in accelerated propagation, whereas phage displaying HAIYPRH with a wild-type SD sequence were found to amplify normally. The SD mutation may alter gIIp expression and, consequently, the rate of propagation of phage. In the Ph.D.-7 library, the mutation is coincident with the displayed peptide HAIYPRH, accounting for the target-unrelated selection of this peptide in multiple reported panning experiments.

Keywords: Phage display; Phage-displayed peptides; Target-unrelated peptide; HAIYPRH; M13; Shine–Dalgarno sequence; Ribosome-binding site; *Gene II* protein

Phage-displayed random peptide libraries are powerful tools in the identification of ligands for a variety of protein and nonprotein molecules, including antibodies, cell surface receptors, enzymes, small molecules, and inorganic materials [1–5]. In phage display, a peptide or protein is genetically fused to a coat protein of a bacteriophage, resulting in the display of the foreign peptide/protein on the outer surface of each phage virion. If a library of variant sequences is appended to the coat protein, the resulting phage display library can be screened against a target in an in vitro selection process called panning. Phage that bind to the target are amplified, and subsequent rounds of panning and amplification enrich the pool in favor of the peptides that have the desired binding characteristics. Because each displayed peptide is physically linked to the gene that encodes it, identification of each selected peptide follows directly from sequencing the viral DNA.

Target-unrelated peptides are phage-displayed peptides that are enriched in the pool of phage during the rounds of panning but are not actually selected as a result of their binding to the target. Menendez and Scott recently reviewed a collection of target-unrelated peptides that have arisen in the use of phage-displayed peptide libraries for epitope mapping [6]. Because the antibody target itself is only one component of the screening system, these target-unrelated peptides have been selected as a result of their affinity for other constituents of the system such as the solid phase for affinity capture. Menendez and Scott organized several sequence motifs of target-unrelated peptides

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according to the component of the screening system to which they bind. For instance, the motifs WXXW and FHXXW have been found to bind to polystyrene even when it has been blocked [7–10]. The HPQ motif at any position in a peptide is sufficient to bind to streptavidin, which is used as the capturing agent for biotinylated antibody targets in biopanning [11–14]. Target-unrelated peptides may also be enriched due to their binding of constant regions of the screening antibody or to common contaminants in the target antibody sample such as bovine immunoglobins (see references within Ref. [6]). It can be difficult to recognize target-unrelated peptides because the binding assays used to confirm the affinity of peptides for the target often employ the same components (e.g., solid phase, capturing agent) as the initial panning experiment. However, in certain cases, target-unrelated peptides can be identified through control and binding competition experiments that demonstrate that these peptides do not actually have a strong affinity for the target [6].

The Ph.D.-7 library from New England Biolabs is a combinatorial library of random heptapeptides fused to the N terminus of the M13 minor coat protein gIIIp, which is present in five copies at one end of the phage virion [1]. The pentavalent display of each 7-mer peptide on an M13 virion does not measurably affect the infectivity of the phage. The library has a complexity in excess of 2 billion independent clones, and extensive sequencing of the naive library has demonstrated a wide diversity of sequences [15]. Although no significant positional biases for amino acid residues have been observed, arginine is found to be underrepresented due to its interference in the secretion of gIIIp [16], cysteines are extremely rare because unpaired cysteine residues interfere with phage infectivity (Ref. [17] and K. A. Noren and C. J. Noren., unpublished work), and proline occurs at roughly twice the theoretical frequency [15,17].

Target-unrelated peptides such as those described by Menendez and Scott have been selected from the Ph.D.-7 library. The peptides FHWTWYW [6] and WHWRLPS (R. Hirschfeld and C. J. Noren, unpublished work) have been found to bind to polystyrene. Although not binders of polystyrene itself, the peptides FHEFWPT and FHENWPS have been shown to bind to bovine serum albumin (BSA),[1] which is commonly used to block the plastic plates (R. Hirschfeld and C. J. Noren, unpublished work). Several peptides in the Ph.D.-7 library have demonstrated binding to streptavidin, with SLLAHPQ being the strongest consensus sequence (Refs. [1,15] and K. A. Noren and C. J. Noren, unpublished work). Peptides that bind other constituents of the materials employed in phage display have yet to be identified in the Ph.D.-7 library.

We have noted that the phage-displayed peptide HAIYPRH from the Ph.D.-7 library has been selected by several laboratories, including our own, using not only varied targets but also different screening systems (Table 1). HAIYPRH is not a target-unrelated peptide of the type described by Menendez and Scott because it never has been associated with binding a particular constituent of a phage display screening system [6]. However, it is unlikely that it would be a true binder of all the targets listed in Table 1. Here we present evidence that phage displaying the peptide HAIYPRH in the Ph.D.-7 library propagate at a dramatically faster rate than the majority of phage in the library, and we trace this phenomenon to a coincident point mutation elsewhere in the genome of HAIYPRH-bearing phage.

Materials and methods

Materials

The Ph.D.-7 Phage Display Peptide Library (lot 3), *Escherichia coli* ER2738 (F′*proA*$^{+}$*B*$^{+}$ *lacI*^{q} *Δ(lacZ)M15 zzf::Tn*10(Tet^{R})/*fhuA2 glnV Δ(lac-proAB) thi-1 Δ(hsdS-mcrB)5*), M13KE phage, the M13KE gIII cloning vector, the –96 gIII sequencing primer S1259S 5′d(CCCTC ATAGTTAGCGTAACG), the reverse *lacZα* sequencing primer S1233S 5′d(AGCGGATAACAATTTCACACAG GA), and all enzymes were supplied by New England Biolabs (Ipswich, MA, USA). Custom sequencing primers were synthesized at New England Biolabs. ChoiceCoat custom-coated samples of HisGrab (zinc chloride) and HisGrab (no metal) 96-well plate clear strips were a gift from Pierce Biotechnology (Rockford, IL, USA). Chelating Sepharose Fast Flow resin and horseradish peroxidase (HRP)-linked anti-M13 monoclonal antibody were obtained from Amersham Biosciences (Piscataway, NJ, USA). X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) and IPTG (isopropyl-β-D-thiogalactopyranoside) were obtained from American Bioanalytical (Natick, MA, USA). Polyethylene glycol (PEG) and ABTS (2,2′-azinobis[3-ethylbenzothiazoline-6-sulfonic acid], diammonium salt) were obtained from Sigma–Aldrich (St. Louis, MO, USA). All other materials and reagents were obtained from Fisher Scientific.

All methodology for the use of the Ph.D.-7 library, including media and solutions, ER2738 strain maintenance, phage amplification and titering, and purification of single-stranded M13 viral DNA, is described in the Ph.D.-7 Phage Display Peptide Library Kit manual [15] and in the literature [1]. DNA sequencing was performed by the New England Biolabs Sequencing Core Facility with an Applied Biosystems 3130xl Genetic Analyzer using a

[1] *Abbreviations used:* BSA, bovine serum albumin; HRP, horseradish peroxidase; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; IPTG, isopropyl-β-D-thiogalactopyranoside; PEG, polyethylene glycol; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), diammonium salt; LB, Luria–Bertani; Ph-HAIYPRH, M13 phage displaying the peptide sequence HAIYPRH; dNTP, deoxynucleoside triphosphate; RF, replicative form; qPCR, quantitative PCR; ELISA, enzyme-linked immunosorbent assay; MOI, multiplicity of infection; HAIYPRH, the peptide His-Ala-Ile-Tyr-Pro-Arg-His; SD, Shine–Dalgarno; CMV, cucumber mosaic virus; hTfR, human transferrin receptor; GFP, green fluorescent protein; CEF, chicken embryo fibroblast; HMGB1, high-mobility group protein 1; ASD, anti-Shine–Dalgarno; rRNA, ribosomal RNA; mRNA, messenger RNA.