LANDSCAPE PHAGE LIBRARIES AS A SOURCE OF BIOSELECTIVE NANOMATERIALS

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ABSTRACT

New generations of targeted diagnostic, therapeutic and imaging nanodevices require robust molecular recognition interfaces that bind target receptors with high specificity and selectivity. Landscape phages are nanoparticles displaying on their surface thousands of specific ligands. We constructed a novel landscape library, in which random 9-mer peptides are presented on the phage surface in 4000 copies. The new library and previously constructed 8-mer library have been used to select phages that bind proteins (streptavidin, TEM1 β-lactamase) and pathogenic bacteria *S. typhimurium*. Phage-borne peptides selected from two libraries mostly have no overlapping or common motifs indicating that the libraries have distinct repertoires of binding phages. Selected phages were used as recognition interfaces in phage-based microarrays and biosensors. Constructed landscape phage libraries can serve as an universal source for novel stable bioselective nanomaterials with many applications in medicine and technology.

Keywords: filamentous phage, landscape libraries, nanoparticles

1 INTRODUCTION

Ff class of filamentous phage (f1, M13 and fd) are long thread-like flexible particles. Their tubular capsid consists of 2,700 subunits of the 50-residue major coat protein pVIII arranged in a helical array. Each pVIII subunit is largely α-helical; half of its 50 amino acids are exposed to the solvent, the other half is buried in the capsid. One tip of the phage virion is capped with five copies of proteins pVII and pIX, another tip - with five copies of pIII and pVI [1, 2]. Viral DNA of different size, including recombinant genomes with foreign DNA inserts, can be accommodated in the phage capsid whose length is altered to match the size of the enclosed DNA by adding fewer or more pVIII subunits during the phage assembly.

In phage peptide libraries a foreign coding sequence is spliced in-frame into a phage coat protein gene, so the "guest" peptide encoded by that sequence is fused to a coat protein and displayed on the virion surface. When a foreign coding sequence is spliced into the major coat protein gene gpVIII, the guest peptide is displayed on every pVIII subunit. Such phages are known as "landscape phages" [3-4]. It was demonstrated that phages selected from landscape phage libraries can bind organic ligands, proteins and antibodies, resist stress factors such as chloroform or high temperature [3-4]. Phage-derived probes inherit the extreme robustness of wild-type phage [5] and allow fabrication of bioselective materials by self-assemblage of phage or its composites on metal, mineral or plastic surfaces. Therefore landscape libraries could be considered as a universal source of new selectable nanomaterials.

Although the landscape phages could retain their ability to infect *E. coli* and form phage progeny, it is not surprisingly that these chimeras are defective in some degree. It was observed, that even in the highly diverse libraries different guest peptides are presented unequally. The disproportionate representation of different phage clones and their depletion during phage reproduction may affect efficiency of selection of clones with the optimal fitness. Therefore, there is a need to develop new phage libraries with extended and different repertoires of displayed landscapes. In this work we describe construction of 9-mer landscape library (f8/9), based on no-phage-producing vector f8-6, and compare its properties with the 8-mer landscape library (f8/8) constructed earlier. We found, that although these libraries have similar size and diversity, phages selected from both libraries in parallel selection and with common selectors (streptavidin, TEM1 β-lactamase and of *Salmonella typhimurium* cells), mostly have no overlapping or common motifs of displayed peptides and therefore represent different phage families. These results justify development of separate landscape libraries covering different areas of a virtual "sequence space".

2 LANDSCAPE LIBRARIES AND THEIR CHARACTERISTICS

For construction of the f8/9 library we used vector f8-6 (Fig.1) to ensure that there was no contamination of the library with residual wild-type phage that could overgrow the recombinant clones during successive amplification. Double stranded randomized fragments were ligated into cleaved vector DNA followed by electroporation of resulting recombinant DNA molecules into *E. coli* cells. A portion of transformed bacterial clones (named here the

"pre-library") was grown on an indicator plate with tetracycline, while the major part of transfected bacteria were cultured in a liquid media with tetracycline. The size of the pre-library (2×10^9 clones) was determined using a proportion of the clones grown on the indicator plate. The phage library isolated from the liquid culture containing 4.4×10^{13} phage virions was named the *"primary library"*. It was observed that about half of the transfected bacteria in the pre-library produced phage particles, in agreement with our previous observations. It was determined by growing in liquid cultures individual pre-library clones, separation of bulk cells by centrifugation and titering the phage remaining in supernatants. *The complexity (the size)* of f8/9 library (1.2×10^9 clones) – the number of primary clones able to produce phage – was determined as a portion of the primary clones producing phage particles (61%) compared to the total number of primary clones (2×10^9). The rest 39% of the pre-library clones did not produce phage because they contained a stop codon TAG in different positions of the gene encoding major coat protein pVIII, as was demonstrated by PCR amplification of the corresponding segment of viral DNA and its sequencing. Thus, we found that complexities of f8/8 and f8/9 libraries were similar and differ from their theoretical complexities (1.28×10^{11} for f8/9 library encoded by DNAs Gnk(nnk)$_8$ where n = G, A, T, or C and k = G or T; and 4.16×10^9 for f8/8 library encoded by DNA's Gnk(nnk)$_6$nnG).

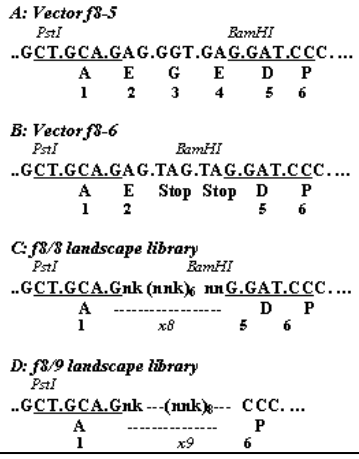

Fig.1. Vectors and libraries. Nucleotide sequences correspond to the N'-terminal part of gpVIII. Randomized structures shown as nnk, where **n** =A, T, G, C, and **k** =G, T. N'-terminal amino acid structures of pVIII shown in capital letters; randomized amino acids – as *x8* in f8/8 and *x9* in f8/9 library). Numbers are corresponding to the number of amino acids of pVIII

The quality of a phage display library can be characterized by its *completeness* or *diversity* - the proportion of all possible sequences actually present in the library. *Population diversity* of the libraries was estimated from the sequences of a limited number of the members of the libraries (73 for f8/8 and 61 for f8/9) using the statistical program RELIC POPDIV [8]. Calculated population diversities of f8/8 and f8/9 primary libraries (0.0091 +/- 0.0045 and 0.0029+/-0.0016 respectively), were 37 and 3 time lower than the estimated completeness of these

	Selectors		
	β-lactamase	**Streptavidin**	***S.typhimurium***
f8/8	EPKPTPAA[2] DPKPTAAA[4] DPRPESAP SDTSSPGQ VNTSSPGQ[6] DPPKRPDV[5] DPSSRQTP VTGSPPST[2] VSPPSHST VPPQSNSM ERPAPQLQ VTPSPTPQ DRVQPAMQ[3] DNASAPRS EHPQPPTP APVHQESS	VPEGAFSS[7] VPEGAFTS[3] VPEGAFGS VPEGAFST VPEGAFSQ VPESAFAQ VPDGAFST VPDSAFNT VPDGAFSQ[2]	VTPPQSSS VTPPTSPQ VTPSSPHS VTPQGSHP VSTQSTHP TPGQPSHP VPPPSPQS[2] VPPPSPHS[3] VPPPSASS VPPPSQSQ[2] VPPPSNPS VPPPGQHQ VPPSSSSP VPQQDKAQ
	Selectors		
	β-lactamase	**Streptavidin**	***S.typhimurium***
f8/9	ARSVAMSDS ASSVAMSDS VLSSDHNED VSSSDHNED[2] DHLNVASSD ERTQDGSSD DQRGDRDDT DFGYAKEDT VPSGDVSME VQGYGPSMD ENTGTSIPE DQSGAVGMG ENLSMVTTT DSGTALSMA VSSGTGPDG VSMEVAPDA GMGPEYGGD VTAPSTAED AIETTVGDD ASSPGIGSE EPQTLYGTQ GHTGGLEED VHNGNLRLD GDSGTGDSH EMDTGKDGN ATFSVPEAD	VPVGAYSDT AALGHPAMD[20] VDEGHPAME[2] ASMSGDSGD DHGNTVNTA DNQWQDTSS DNQYGGTFD DPVSASRDD DSSGSVGMD DSTGGVDEN DTSYLNVTE DYARDDGTN EKNSDDGMG GDPSGDAES GGFTSDTNG GGPLMQAHD GPHGNEESD GRSDWQGAN GSVVGDEGS VGGGERSTD VGMGLTVDD VPGGTVPED VSSIQVNNE VYNGAATGD VYVDGPPEN	ELPLAFGND[2] ELPLDPGLD GSYSDMVDN[2] GVYSDISGD VNYDDMTST[2] VPYADMSES[2] DAFSQSATD[2] VAEPVDLPA AGMTYDLPD[2]

libraries ($1.4\times10^9/4.16\times10^9 = 0.34$ and $1.2\times10^9/1.28\times10^{11} =$ 0,0094 respectively) probably because of unequal presentation of different clones in the libraries and uneven positional distribution of amino acids in foreign peptides. It is interesting to note that population diversity of phage-producing clones in the pre-library f8/9 (0.0116+/- 0.0074) exceeds the diversity of the primary library f8/9 (0.0029+/- 0.0016) four times, indicating that population of the library changes during its growth in a liquid medium. Even more dramatic change in population diversity was observed when a portion of the primary library f8/8 was used for amplification in the presence of fresh *E.coli* K91BlueKan cells (0.0091 +/- 0.0045 and 0.0012+/-0.0006 for the primary and amplified libraries respectively). The decrease in population diversity of the library more than 7 times during its amplification can be attributed to differences in infectivity of individual clones in the library toward the host bacterium and differences of biosynthesis of distinct phage particles inside the bacterial cell.

We assumed that the diversities of f8/9 and f8/8 libraries were affected by *biological censoring* of phage producing clones during phage amplification. To check this hypothesis, amino acid profiles of the theoretical 8-mer and 9-mer libraries were compared with amino acid profiles of the real libraries Analysis of amino acid compositions within displayed peptides shown complete absence of cysteine, poor presentation of bulky hydrophobic (tryptophan and phenylalanine) and positively charged amino acids (lysine, arginine) but increased numbers of negatively charged amino acids (aspartic and glutamic acids) compare to theoretically randomized libraries of the same format. Comparison of the amino acid profiles for the f8/8 and f8/9 libraries with theoretically randomized libraries provides evidence of censoring of the real libraries.

3 AFFINITY SELECTION AND PHAGE CHARACTERISTICS

We hypothesized that using different landscape phage libraries may be advantageous in the search for specific ligands by affinity selection against different targets because the range of clones belonging to the same sequence family in each library may be different. Selection of both libraries with the same target should yield a greater variety of structurally non-overlapping clones. To prove this hypothesis, we surveyed both primary libraries in selection of binding phage against the same selectors: monomeric TEM-1 β-lactamase, tetrameric streptavidin and whole *Salmonella typhimurium* cells.

Sequences of peptides displayed on the surface of the selected landscape phages are shown in Table. Their binding to the target selectors was tested by ELISA. Most of the analyzed clones selected from both libraries against β-lactamase demonstrate the clustering into families, with a few "orphans" that did not belong to any identifiable family structure. All selected clones from the f8/8 library gave positive signals with the best binder DNASAPRS (the phages are designated by a sequence of a displayed peptide) bound at a level 25 times higher than the control phage f8-5. Most of phages selected from the f8/9 library (24/26) demonstrated binding in ELISA with the best binder, GHTGGLEED bound at a level 17 times higher than the negative control. So, phages selected against β lactamase from both libraries have different binding abilities and no common motifs. In affinity selection against streptavidin, we used f8/9 library and compare peptide structures with those obtained previously for f8/8 library [6]. Enriched 9-mer sublibrary from the third round of affinity selection did not contained the predominant family of the clones with common motif VPxGAY/FS/T as it was found for f8/8 library, but just one clone which belonged to that family. After additional two rounds of selection 50 analyzed clones turn out to be 6 clones with two predominant phages VPVGAYSDT (21 out of 50) and AALGPAMD (22 out of 50). All selected clones bound streptavidin with different affinity and the best binder from f8/9 library - VPVGAYSDT bound streptavidin with affinity 50 times higher then the best binder VPEGAFSS selected from f8/8 library (Fig.2). Affinity selection of f8/8 library against *S. typhimurium* cells revealed 14 clones belonging to 2 families and 2 unrelated clones while selection of f8/9 library detected 9 unique clones belonging to two families and 3 unrelated clones (50 clones have been analyzed in both cases, all clones bound *S. typhimurium* cells with different affinity). Best binders from f8/8 library were VPQQDKAD and VTPPQSSS and from f8/9 library – AGMTYDLPD and ELPLDPGLD. These experiments with three distinct selectors proved that phages selected from separate libraries have distinct structures of displayed peptides and no overlapping structural motives.

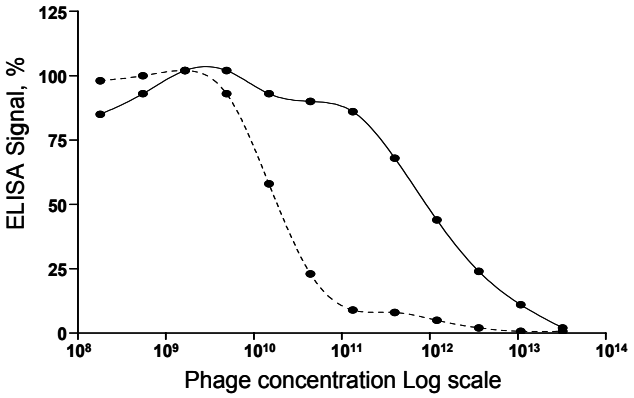

Fig.2. Competitive ELISA of two streptavidin binding phages: VPEGAFSS (dashed line) from the f8/8 library and VPVGAYSDT (solid line) from the f8/9 library.

4 ADVANTAGES OF USING SEPARATE LIBRARIES FOR AFFINITY SELECTION

The ultimate goal of the most phage display projects is identification of phage ligands binding strongly and specifically to their analytes. It may be assumed *a priori* that in every population of random peptides there are peptides with ideal fitness and highest affinity to the target, so called leading peptides. Theoretically, the leading peptide may be revealed by affinity selection. However, in practice, the leading peptide may be absent from the selected pool of phage clones because the theoretical complexity (total number of all possible peptide structures) of the library exceeds the actual observed complexity. We hypothesized that chances of revealing the leading ligands as well as clones with different binding properties may be considerably increased by using "separate libraries". These are libraries enriched by clones that belong to different families. To explore this hypothesis, we performed selection of phages from two distinct landscape libraries, f8/8 and f8/9, using the same selectors. Analysis of binding

phages selected from different libraries clearly showed that separate libraries create greater diversity in the repertoire of the clones represented in the library, provide a wider range of alternative clones and allow phage clones with maximal affinity to a particular target can be selected

One of the selected phage binding *S. typhimurium* cells with displayed peptide VTPPTQHQ has been successfully used as a specific interface in biosensors for Salmonella monitoring [7]. Streptavidin-binding phage VPVGAYSDT have been used in landscape phage based microarray. Hence constructed landscape phage libraries can serve as an universal source for novel stable bioselective nanomaterials with many potential applications in medicine and technology.

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