MODULATING AFFINITY OF LANDSCAPE PHAGE NANOPARTICLES BY MUTAGENESIS OF THE MAJOR COAT PROTEIN

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ABSTRACT

Phage peptide libraries are invaluable source of the ligands specifically binding numerous analytes. In landscape libraries guest peptides are fused to each copy of the major coat protein pVIII and whole phage particle operates as a bioselective nanodevice. To explore how amino acids neighboring a guest peptide on phage surface influence a binding ability of the phage nanoparticle, we constructed collection of mutant phages carrying ß-galactosidase binding peptide ADTFAKSMQ and random mutations in the middle part of the pVIII protein. We found that such mutations can improve or completely destroy binding ability of the phage. These features correlate with the common charge formed by mutated amino acids. Influence of the guest peptide on the diversity of mutations in the neighboring area of pVIII was also revealed. The concept of "separate" landscape libraries as a new source of phage nanoparticles with modulated diversity of displayed peptides is formulated and discussed.

Keywords: landscape phage, mutations, nanoparticles

1 INTRODUCTION

Landscape phage display libraries proved to be invaluable source of the ligands specifically binding numerous analytes [1-7]. Diversity and repertoire of the libraries are critical for the successful selection of ligands. In landscape libraries guest peptides are fused to the N-terminal part of each of 4000 copies of the major coat protein pVIII and form unique binding "landscapes" on the phage surface, so that the whole phage particle operates as a bioselective nanodevice with genetically determined regular structure. In spite of the high potential value of the landscape phages as a novel class of bioselective nanomaterials, their detailed structures and functioning remain unsolved. Furthermore, even the principle question on a status of the foreign peptides on the phage body as conformationally independent domains or phage-constrained units remains unaddressed. According to some data peptides responsible for the binding abilities of the selected landscape phage could not bind appropriate targets in a form of free peptides [1, 4]. Another set of data contradicts to this observation and imply that foreign peptides in the landscape phages exist as independent domains with their own intrinsic conformations, in the same way as they operate in a synthetic form or in the type 3 phage vectors [1, 5]. For example, when landscape phage libraries were used for epitope-mapping, phage displayed peptides affinity selected against mono- or polyclonal antibodies were found to be identical or highly homologous to continuous epitopes of the corresponding antigens [1, 4, 6].

In this study we demonstrated that mutations of amino acids surrounding the displayed peptide fused to the major coat protein pVIII can dramatically change binding proficiency of the peptide towards its target receptor, increase it or completely destroy. The influence of the peptide on the spectrum of such mutations of pVIII in viable phages was also observed. Based on the data found in this study we developed a concept of the new "split" libraries with increased repertoire of the peptides with structural and functional diversity.

2 COLLECTION OF MUTANT PHAGES AND MUTANT PROPERTIES

The exposed moiety of the fusion pVIII protein in a landscape phage can be divided conventionally into four areas shown in Fig. 1 by different colors: a guest peptide (white), amino acids 5-11 (grey), amino acids 12-19 (yellow) and amino acids 20-24 (red). According to the model shown in Fig. 1, a guest peptide on the phage surface is surrounded by four areas belonging to the neighboring subunits (shown as yellow and red) and one area of the same subunit (shown as grey).

A model phage 1G40 carrying peptide ADTFAKSMQ was selected previously as a strong binder of ß-galactosidase (ß-gal) *E. coli* (Kd = 30 nM) from the f8/8 library. Peptide itself did not bind of ß-galactosidase [4]. We extensively randomized the area adjacent to the displayed β-Gal-binding peptide (amino acids 12-19) by cloning a synthetic oligonucleotide duplex, which encodes the peptide DTFAKSMQ, into the previously designed f8α-library (Fig.1) that presents a multimillion-clone collection of phages with random amino acids 12-19 [7]. A composition of RF DNA's isolated from bacterial cells, which were infected with

~10^8 unique phage clones of the f8α-library, was cleaved by *Pst*I and *Bam*HI endonucleases

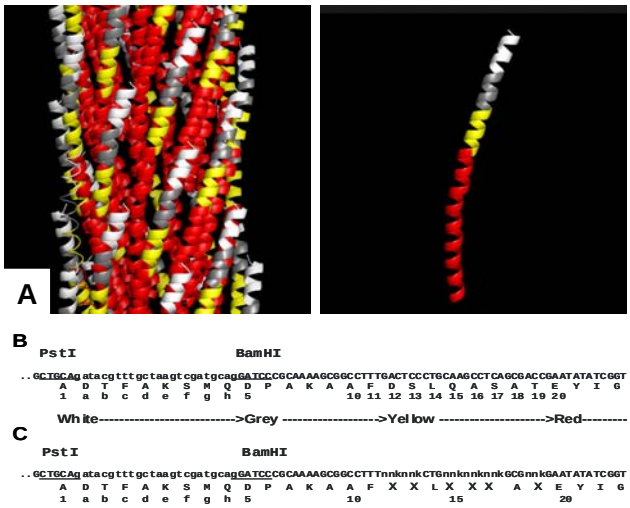

Figure 1: A. Three dimensional structure of the β-Gal binding phage capsid (left) composed of subunits of the major coat protein pVIII (right). White areas correspond to the position of the guest peptides, grey – to the 5 -11 amino acids, yellow - to the area of 12-19 amino acids, and red – to 20 - 24 amino acids exposed at the phage surface. Area corresponding to 24 -50 amino acids are buried inside the capsid.
B, C: Fragment of nucleotide sequence of the pVIII gene and amino acid sequence of the mature pVIII protein in β-Gal binding phage (B) and in mutants (C). Numbers correspond to amino acids; guest amino acids are indicated as a-h. In mutants, random oligonucleotide insert designated as nnk (where n =A, G, C, o T; k= G, T) with X denoting a corresponding random amino acid.

and ligated with the synthetic duplex. The ligated RF DNA's were electroporated into *E. coli* cells yielding 1.2×10^6 transformed primary clones. The yield of transformed clones was unexpectedly low considering the high competence of the cells (3.3×10^8 CFU per 1 μg of RF DNA) and amount of DNA (4 μg) used for transformation, that could hint on a poor viability of the major portion of the transformed bacterial clones. (Our previous attempts to obtain collection of mutants by randomizing area of 12-19 amino acids in pVIII of phage 1G40 using oligonucleotide-directed mutagenesis of single stranded DNA of 1G40 phage was not very successful, the number of obtained clones were significantly lower then in described here strategy). Sequence analysis shown that only 23% of the obtained phage collection carried β-Gal-binding peptide and mutations, the rest were presented by original clones from f8α-library without incorporated peptide. To enrich collection for the clones of interest we performed 3 rounds of affinity selection against immobilized β-Gal and clones from original and obtained sets (85 clones altogether) were analyzed for binding activity in direct ELISA, where immobilized phages bound β-Gal from solution (data are not shown). To exclude a possibility that ELISA signals vary because of different adsorption of assayed landscape phages to the plastic, most of the clones were tested by competitive ELISA, where at first phage 1G40 was immobilized on the wells and the pre-incubated mixture of the phage of interest and β-Gal were added and analyzed for ELISA signal. The results of the competition ELISA were in agreement with the direct ELISA: the phages that bound ß-Gal strongly when they were immobilized on plastic surface demonstrated the strong binding with enzyme in solution, and *vise versa*—week binders demonstrated almost no binding capacities in both tests.

Constants of dissociation (kD) were determined for the most interesting clones (Fig.2).

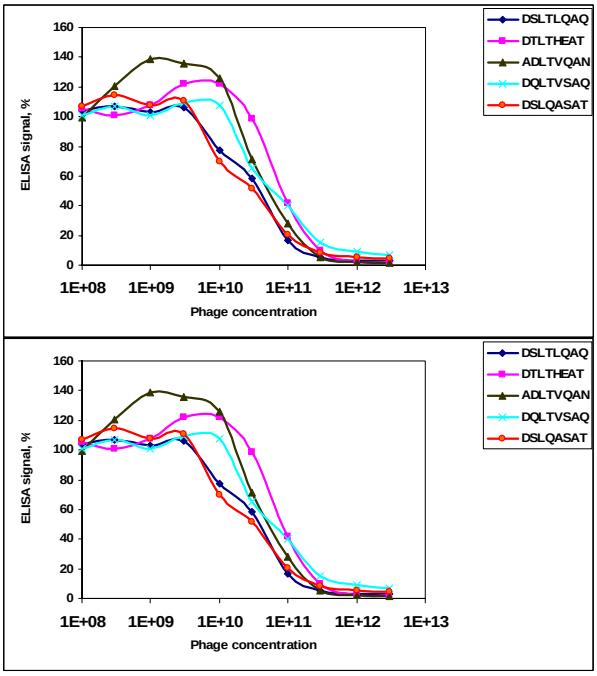

Figure 2: Binding of different mutants with β-Gal as measured by inhibition ELISA.
Phages displaying indicated peptides were pre-incubated at graded concentrations with β-Gal and then added to the wells of ELISA plate coated by detection phage 1G40, original β-Gal-binding phage carrying ADTFAKSMQ peptide. ELISA signals are presented as percentage of signal from the sample without phage, which was considered as 100%.

We identified mutant phages with different affinity toward β-Gal: from mutants binding stronger then original phage 1G40 (phages DSLHGQAM, DQLNATAL, AELTTRAE with Kd 15.0, 17.8 and 23.4 nM respectively), mutants which bound β-Gal at the same range (DSLTLQAQ, ADLTVQAN, kD 37.5

and 46.9 nM), significantly lower (EDLTQRAL, kD=253.1), to the mutants which completely lost binding abilities (Table).

To confirm that characterized phages bear the intact β-Gal-binding peptide ADTFAKSMQ at the N-terminus of the fused pVIII protein, we used N-terminal amino acid sequence analysis for phage 1G40; phage DSLHGQAM with enhanced affinity towards β-Gal and three phages, which completely lost β-Gal-binding activity: DELTVAAN, QELSIQAE, and EDLSAMAG. Analysis confirmed that all five phage carried designated ß-Gal binding peptides. The conclusion was also proved by mass spectrometry of fused pVIII proteins from the same phages. Hence, the loss of binding activity in analyzed phages were due to the conformational changes in displayed peptide but not an alternative processing of fused pVIII leading to the destruction of this peptide. No common amino acid motifs in mutated area of pVIII protein for different groups of phages were identified. Analysis of the electrostatic charges of the mutant area of pVIII proteins revealed that the best binding ability of the phages corresponded to charge -0.9 - -1.0, whereas the low charge -2,3- -3.0 corresponds to mutants which lost their ability to bind β-Gal completely.

Strong binders of ß-Gal		Non binders of ß-Gal	
Phage clone	Net charge	Phage clone	Net charge
DSLQASAT (parent)	-1.09	DDLTASAI	-2.09
DSLHGQAM	-0.924	DELITEAH	-2.919
DQLNATAL	-1.09	DELTVAAN	-2.088
ADLTVQAN	-1.09	DLLTTQAE	-2.088
AELTTRAE	-1.086	DNLEMMAQ	-2.088
DQLTVSAQ	-1.09	DNLITMAD	-2.09
DSLTLQAQ	-1.09	DSLNEQAV	-2.088
EDLTQRAL	-1.088	DTLTENAV	-2.088
DTLTHEAT	-1.921	EDLNAQAL	-2.088
SNLEMMAT	-1.088	EDLSAMAG	-2.088
		EELESIAN	-3.083
		EELNGQAM	-2.086
		EELNQQAN	-2.086
		EELSNSAT	-2.086
		EELSQQAN	-2.086
		EELSVQAT	-2.086
		EELTLEAH	-2.917
		EELTNSAQ	-2.086

Table: Net charge in the area of amino acids 12-19 of pVIII of selected phages characterized by their affinity towards β-Galactosidase

We also compared the population diversity of the mutated amino acids in the obtained collection with the diversity of amino acids in the original f8α-library. It was observed that diversity of amino acid in the library of phages fused to the foreign β-Gal-binding peptide significantly decreased; we concluded that only small portion of the mutation in the middle of the pVIII protein could co-exist with peptide DTFAKSMQ in viable phages.

3 NOVEL "SEPARATE" LIBRARIES WITH INCREASED DIVERSITY

We demonstrated that the affinity of displayed peptids can be modulated by mutating the adjacent amino acids. This interference could be caused as intermolecular interaction of the different areas of pVIII protein as well as intramolecular interaction through forming of unique landscapes by neighboring pVIII subunits on the phage surface. These data allowed us suggesting a new concept of "separate" libraries. Indeed, if one functional peptide could co-exist only with determined set of the mutations, and *visa versa,* then new libraries could be constructed based on vectors with different mutations of 12-10 amino acids of the pVIII protein. The set of viable phages and accordingly, displayed peptides in each libraries may vary considerably since a well known censoring of landscape libraries will be different in each case. By this way, a considerable complexity of the landscape libraries will be obtained not just by simple increasing of the number of independent clones in a library, but also by using in parallel different libraries with their characteristic repertoire and functional and structural diversity. The concept of the "separate" landscape libraries may be advantageous in the search for the new phage nanoparticles with different binding propensities.

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