

# Evolutionary Selection of Bone Mineral Hydroxyapatite Binding Peptides Using Landscape Phage Library

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## ABSTRACT

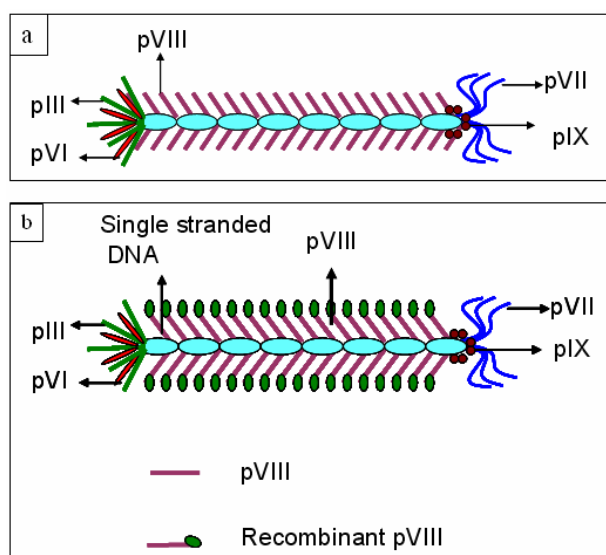
The goal of this work is to identify a peptide that can specifically bind bone mineral hydroxyapatite  $[(Ca_5(PO_4)_3OH)_2]$  from a phage-displayed random peptide library. Instead of using pIII library where random peptides are displayed at the tip of filamentous phage, we used landscape phage libraries, in which random octa-peptides or nona-peptides are displayed on all the N-termini of the pVIII (major coat protein) of the tetracycline resistant phage (fd-tet). The crystalline hydroxyapatite (HA) powder was used as a target during biopanning. We selected clones that bind with high affinity to HA. Three 8-mer and two 9-mer peptides were found to bind to HA with high affinity. The motif alignments showed that the selected binding peptides share common motifs with proteins found in bone including collagen, biglycan, osteocalcin, and osteonectin. Our results indicate that both collagen and non-collagenous proteins can contribute to HA binding during bone formation.

**Keywords:** bone, phage, phage display, binding peptide, hydroxyapatite, biominerals

## 1. INTRODUCTION

M13, fd, and f1 are filamentous bacteriophages which have single stranded genomic DNA of ~6,000-6,500 nucleotides. The DNA is surrounded by an outer coat which is composed of thousands of alpha-helical major coat proteins named pVIII. Several copies of minor coat proteins including pIII, pVI, pVII, and pIX are present at the tips of the phage (Fig. 1a) [1]. Phage-displayed random peptide libraries were created by genetically modifying the filamentous phages. During this course of library creation, degenerate oligonucleotides were spliced in frame into one of the coat proteins, which upon expression would give rise to a peptide fused to the coat protein towards the exposed surface of the phage. Thus each phage particle would display multiple copies of a particular peptide (Fig. 1b) [2]. There are two types of phage-displayed random peptide

libraries. One is pIII library, where a foreign peptide is fused to 5 copies of pIII at the tip of the phage. Another is pVIII library, where a foreign peptide is fused to each of the several thousand copies of pVIII that constitute the side wall of phage. The library used in this work is a pVIII library called landscape phage library. We generated and used a landscape phage library in which the peptides were displayed on all the 3,946 copies of the major coat protein of Fd-tet bacteriophages. The use of landscape phages as alternatives for antibodies are due to several robust features of the phage such as resistance to heat [3] and organic solvents [4]. During bone biomineralization, the bone proteins interact with HA precursor to nucleate HA and organize nucleated HA into ordered arrays along collagen fibers. However, so far, there is still debate on whether collagen or non-collagenous proteins nucleate and interact with HA during bone formation. Therefore, using phage display technology to identify a HA-binding peptide will shed light into the mechanism of bone biochemistry.



**Figure 1.** Structure of wild-type phage (a) and display of peptide on major coat (b).

## 2. EXPERIMENTAL

We constructed and used two types of landscape phage libraries [5] termed f8/8 ( $2 \times 10^{11}$  cfu/ml) and f8/9 ( $1.4 \times 10^{11}$  cfu/ml) landscape phage libraries, respectively. Eight and nine amino acids were displayed randomly on the major coat protein of vector f8-1 (fd-tet derivative) in f8/8 and f8/9 library, respectively. We adopted a biopanning approach by modifying the protocol in reference [6] and performed a step gradient chromatography where the virions were adsorbed on the HA powder sitting on the filter disc of an HA column in 10 mM PBS (10 mM  $\text{NaH}_2\text{PO}_4$ , and 0.15 mM NaCl, pH adjusted to 7.0 with NaOH). The phage libraries (f8/8 and f8/9) were first diluted to  $10^9$  virions/ml by diluting 25  $\mu\text{l}$  of phage library in 275  $\mu\text{l}$  of TBS (the total volume is 300  $\mu\text{l}$ ). The selected HA-specific phages were amplified and propagated. Finally, the specific part of the phage genome was subjected to PCR amplification and sequenced to obtain the sequences of the HA-binding peptides, followed by a binding assay to determine the best HA-binding peptide.

## 3. RESULTS & DISCUSSION

About one hundred clones were randomly picked up from the plates and analyzed. The clones were cultured and a segment of the genomic DNA that encoded the displayed 8-mer and 9-mer peptides was sequenced. We then subjected both the 8-mer and 9-mer sequences to motif analysis using Relic program which searches for continuous motifs within the peptide population. This program also aligns segments of specified length that occur more than once in a peptide population [7]. Three 8-mer peptides (DNNSTLSR, GEEASGSS and EPSGPTVQ) from f8/8 and two 9-mer peptides (EGAGRESSS and GTPLMSDEG) from f8/9 library showed greatest binding even at stringent conditions. All these three sequences showed preferential binding to HA when compared to the rest of the selected clones. All the selected peptides were analyzed to see if there is any similarity between the motifs found in the selected peptides and in the bone proteins. The analysis showed that there are obvious similarities between the selected 8-mer and 9-mer peptides and the bone proteins. The motif alignments showed that the selected binding peptides share common motifs with bone proteins such as collagen, bone silaprotein, biglycan, osteocalcin, and osteonectin (Figs. 2 and 3).

### COLLAGEN TYPE I

786 G P S G P A G P T G A 795  
E P S G P T V Q  
E P S G P T V Q

### BONE SIALOPRETEIN

100 E A E N T T L S A T T L G Y G E D A T P G T G Y T G 126  
S Q S V T T L S E Q P G E E A S G S S  
D N N T L S R

### BIGLYCAN

25 W D F I L D D G P F M M N D E E A S G A D 45  
A S S L D T G P G E E A S G S S

### OSTEONECTIN

84 L D E N N T P M C V 93  
D N N S P P N G  
D N N S T L S R

**Figure 2.** Motif alignments between selected 8-mer peptides and proteins found in bone. The first line in every set shows the bone protein sequence (flanked with amino acid numbers) that matches with the insert peptides. The following lines show the selected peptide sequences. Amino acid consensus sequences are shown highlighted. The amino acid sequences of bone proteins were taken from NCBI web site.

### COLLAGEN TYPE I

1012 A C P P G E S G R E G A P G A E G S P G R E G S P G A K G D R G E T G P A G P P G A P G A 1050  
A Q L E T S S R E E G A G R S S S A D K S E T I S A  
E G A G R E S S S  
E S G R M G E M D

### BONE SIALOPROTEIN

263 E Y I G V N E Y D N G 273  
G S G V N E D D

### BIGLYCAN

30 D D G P F M M N D E E A S G A D T S G V L 50  
G P T L M S D E G A S V S G G L  
D F T S G V S  
E S G S G V M

### OSTEONECTIN

33 T V A E V I E V S V G A N P V Q V E 50  
D E S I A V G A  
D S R D A N E V Q

### OSTEOCALCIN

24 K P S G A E S S K G A A 35  
A G V E S V S S T  
E G A G R E S S S

**Figure 3.** Motif alignments between selected 9-mer peptides and proteins found in bone. The first line in every set shows the bone protein sequence (flanked with amino acid numbers) that matches with the insert peptides. The following lines show the selected peptide sequences. Amino acid consensus sequences are shown highlighted.

#### 4. CONCLUSION

Landscape phage display has been successfully applied to identify bone mineral specific peptides. Three 8-mer and two 9-mer peptides were found to show high binding affinity. Some selected peptides share motifs with some proteins found in bone. Our results indicate that both collagen and non-collagenous proteins in bone can contribute to the binding of bone minerals.

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