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Combinatorial search for the ligands that specifically recognize the streptococcal collagen-like proteins Scl1 and Scl2

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Abstract. Phage-encoded peptide libraries are often used to study biomolecular interactions. We employed this combinatorial approach to identify ligands specific for the streptococcal collagen-like proteins, Scl1 and Scl2, which are expressed on the cell surface by group A *Streptococcus*. Several sequence motifs displayed by phages were selected for their binding ability to different Scl variants.

Keywords: Group A *Streptococcus*; Scl ligands; Phage display; PCR from plaques

1. Introduction

The streptococcal collagen-like proteins, Scl1 and Scl2, are cell surface proteins of the human pathogenic bacterium group A *Streptococcus* (GAS). The extracellular portion of mature Scl proteins is composed of the arbitrarily designated N-terminal noncollagenous variable (V) region and the adjacent collagen-like (CL) region. In addition, Scl1 proteins harbor the so-called linker (L) region containing different numbers of direct repeats. Scls form conserved collagen-like triple-helices and exhibit two-domain lollipop-like structural

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organization [1]. This structural organization seems particularly suited for a ligand binding where the stalk-forming CL region projects the globular V region away from the bacterial surface, facilitating interactions with potential targets. At the same time, both Scl proteins produced by different GAS strains vary significantly in their primary sequence and show different ligand-binding specificities [2]. Here, we used phage display approach to identify ligands specific for different Scl variants.

2. Materials and methods

Several recombinant rScl proteins, such as P144 and P163 derived from different Scl1 or Scl2 variants, were produced using the *Strep*-tag II expression and purification system [1], and were used as "bait proteins" in search for specific ligands. All rScl constructs containing a C-terminal *strep*-tag II (WSHPQFEK) were immobilized onto *Strep*-Tactin-coated microplate wells (IBA-GmbH). An M13 (gpIII) phage-display 7-mer random-peptide library was used to select phages that bound to the rScl constructs absorbed onto microplate wells (PhD-7; New England BioLabs). rScl proteins (100 μg/ml) were immobilized at room temperature for 1 h and then blocked with TBST/2% BSA. 100 μl containing 10^{10} phages displaying random peptides was added to each well and incubated at room temperature for 1 h. The unbound phages were washed away while the bound phages were eluted with 0.2 M glycine–HCl pH 2.2 and amplified in the *Escherichia coli* ER2387 [3]. Phages recovered following three or four rounds of panning were plated for single plaques by mixing with the *E. coli* ER2387 (OD = 0.5) and 3 ml of 0.7% soft agar onto LB-agar containing IPTG–Xgal–Tet (50 μg/ml–20 μg/ml–20 μg/ml).

Single plaques were touched with toothpicks, immersed in a microplate wells containing PCR cocktail, and then dipped into microplate wells containing phage-storage buffer. Two forward primers F1 (5′-ATGATTGACCGTCTGCGC) and F2 (TGTCGGCGCAACTATCG) were designed and tested using a reverse-96 sequencing primer and control ssDNA supplied with the kit (Fig. 1A). PCR was carried out in a 96-well microplate format with Deep Vent DNA Polymerase (New England BioLabs) using DNA Engine Dyad thermocycler (Bio-Rad). The PCR products were cleaned with QIAquick 96 PCR Purification system (QIAGEN) and analyzed by cycle DNA sequencing using Big Dye Terminator mixes and an ABI 3100 automated capillary DNA sequencing instrument.

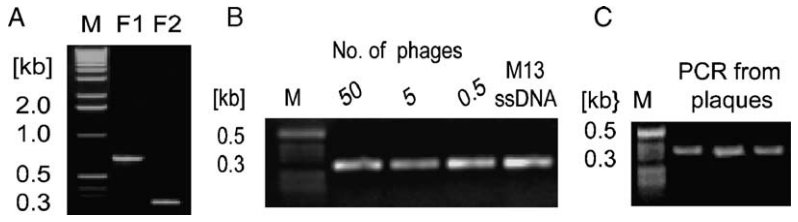

Fig. 1. DNA-template amplification for sequence analysis. (A) Primer design and testing with control phage ssDNA. (B) Sensitivity of PCR detection using purified phage particles. (C) DNA amplification directly from phage plaques. All PCR samples (A–C) were analyzed in 1.3% agarose gel. M; 1-kb DNA size marker (Invitrogen).

3. Results

A PCR protocol was developed to quickly generate DNA-sequencing templates. Designed primer pairs yielded single DNA bands of expected sizes of ~0.7 kb and 335 bp with forward primers F1 and F2, respectively (Fig. 1A). DNA amplification was also successful when whole phage particles were used as either purified phages (Fig. 1B) or as crude material directly taken from the plaques (Fig. 1C).

The sequences of the phage-displayed peptides selected for binding to each rScl protein were aligned and analyzed for the presence of amino acid patterns. The consensus-binding sequences were deduced for each rScl variant, as exemplified by the consensus obtained for P144 (Fig. 2A). Probability of the occurrence of selected sequence motifs found within deduced consensuses, such as motifs SA_H and S_ _H, were further analyzed by combinatorial mathematics employing Bayes' formula [4] (Fig. 2B).

To substantiate the amino acid sequence analysis, 2 different phages (Ph144-3-1 and 3-2) selected on a P144 variant and displaying peptides with different but overlapping patterns of amino acids were amplified and used in ELISA-based binding assay [3]. Bound phages were detected with anti-gpVIII HRP-conjugated monoclonal antibody. Different levels of binding to P144 were recorded for both phages, while P163-immobilized wells were negative for the binding (Fig. 2C).

4. Discussion

In this work we provide our experience on the employment of the combinatorial approach to characterize the spectrum of Scl-protein targets and to identify ligands specific for different Scl variants.

DNA and protein sequences displayed by selected phages were obtained following a simple protocol in which sequencing templates were obtained by PCR directly from phage particles without prior DNA extraction and purification. This simple modification of the standard procedure significantly accelerated data analysis obtained by phage-display method. We were able to select phages harboring short sequence motifs that specifically bound to several rScl proteins and use this sequence data to deduce the

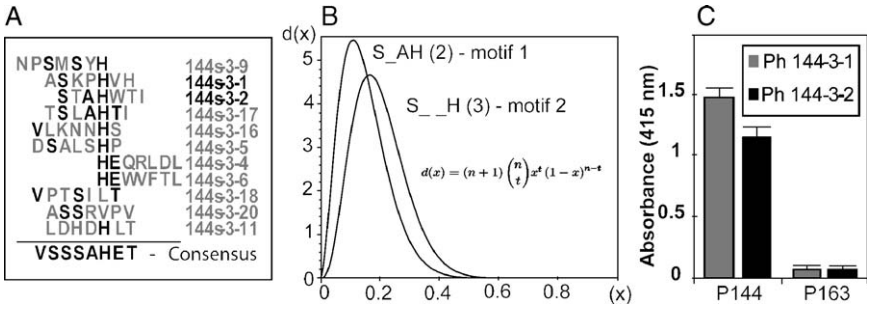

Fig. 2. Analysis of data obtained for P144. (A) Consensus binding motif was deduced from sequences displayed by phages selected on recombinant protein P144. (B) Density of conditional probability ($d(x)$) as a function of ratio (x) of the number of phages harboring shown sequence motifs to the number of all phages after final panning was calculated. (C) ELISA-based binding assay of selected phages to P144.

consensus-binding sequences for individual Scl variants. A BLAST search for short nearly exact matches performed using deduced consensus sequences identified potential Scl targets involved in signal transduction and cell communication, transport, and immune response.

In conclusion, our results show that phage display technology might be a powerful tool to identify natural and designed ligands that will specifically recognize cell surface Scl proteins produced by the human pathogenic bacterium, GAS.

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