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Phage Display of Catalytically Active Staphylococcal Nuclease

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Abstract—Staphylococcal nuclease (SNase), a 14 kD enzyme that catalyzes the hydrolysis of single- and double-stranded nucleic acid, was fused to the N-terminus of the gene III (pIII) protein of filamentous phage fdtet. The SNase-pIII protein is infective and the catalyzes DNA hydrolysis, demonstrating that functional SNase can be displayed on the phage surface.

Introduction

Phage display has been used as a powerful tool in biological screening. It has been shown that peptides,[1–3] antibodies,[4,5] hormones[6] and enzymes[7] can be fused to the coat protein of filamentous phage. The resulting fusion phage can be screened based on either binding affinity of biological activity. We now report the fusion of the enzyme SNase on the N-terminus of pIII protein, a minor coat protein of the filamentous phage fdtet required for infectivity. SNase is a relatively nonspecific enzyme that accelerates phosphodiester bond hydrolysis in DNA and RNA approximately 10^{16}-fold over the uncatalyzed rate.[8] The SNase – pIII fusion protein folds properly, as determined by the infectivity and the catalytic activity of the fusion phage. This system may allow the generation of mutant SNases with novel specificities and activities.

Results and Discussion

Cloning of SNase into fAFF

The fAFF1 vector[3] was used to express SNase on the phage surface. This vector contains two non-complementary BstX I sites ($^{5'}$CCATTCTACTGG$^{3'}$ and $^{5'}$CCAGACAACTGG$^{3'}$) within the leader sequence of pIII protein. The first BstX I site introduces a mutation in the leader sequence. In order to restore the correct leader sequence and clone the SNase gene, polymerase chain reaction (PCR) was used to introduce two BstX I sites at both ends of the SNase structure gene encoded in the plasmid pKJSN1. The BstX I digested SNase gene fragment was ligated with a synthetic DNA fragment made of two complementary oligonucleotides, ON 71 ($^{5'}$CTCTCACTCGGCCAGGGTG$^{3'}$) and ON72 ($^{5'}$CTGGCCAGAG TGAGAGTAGA$^{3'}$). This synthetic DNA fragment restores the correct leader sequence and serves as a linker between the fAFF1 vector and the SNase gene (Fig. 1). The ligated fragment was then cloned into the fAFF1 vector, which had been previously digested with BstX I, to yield plasmid fdSN. The correct sequence of fdSN was confirmed by dideoxy sequencing.

Properties of the phage

The bacteriophage vector fAFF1 produced non-infectious phage particles as determined by the absence of colony formation. This is consistent with the fact that vector fAFF1 contains a frameshift mutation in the pIII gene. In contrast, vector fdSN typically gave ~10^9 cfu/mL of phage particles from overnight culture. This shows that in the fusion phage, pIII proteins were properly assembled on the surface of the phage particles. The chromogenic plate assay clearly showed the presence of a pink halo for ~10^9 cfu of fdSN phage particles. The size of the pink halo was comparable to that produced by 200 pg of SNase protein. The fAFF1 phage, on the other hand, showed no detectable pink halo. Western blot of the whole phage with anti-SNase antibodies showed that the SNase co-migrated with the phage particles. This demonstrated that we have indeed

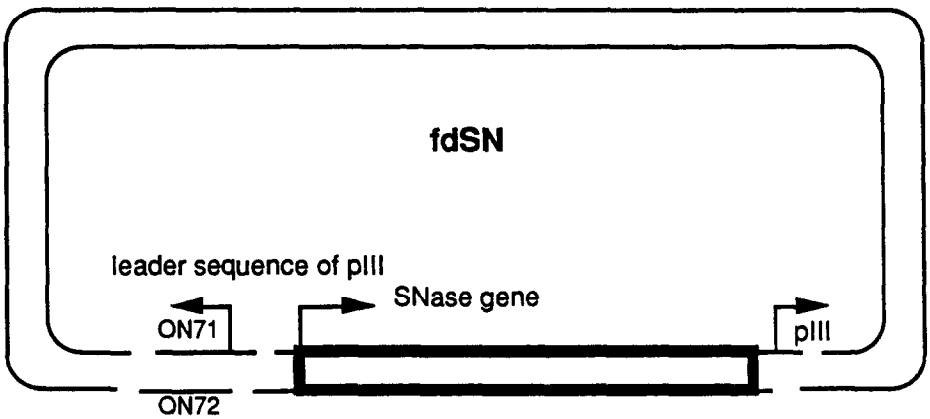

Figure 1. Construction of the fusion phage vector.

constructed active SNase on phage surface. The K_m^{DNA} for the fusion phage was determined to be 38.6±6.5 μg/mL, compared with 55.9±3.2 μg/mL for wild type SNase under the assay conditions. This system should make it possible to generate mutant SNases with novel activities and specificities by screening fusion phage libraries for their ability to bind novel nucleotides, metal ions or synthetic substrates.

Experimental

Reagents

Restriction endonucleases and T4 DNA ligase were obtained from New England Biolabs. [^{35}S]dATP was from Amersham. Single-stranded calf thymus DNA, Salmon testes DNA, SNase protein, sodium glycinate, toluidine blue O, polyethylene glycol (PEG) and cesium chloride (CsCl) were from Sigma. Agar was from DIFCO laboratories. Monoclonal anti-SNase antibodies were kindly provided by Dr D.C. Benjamin from University of Virginia.

Plasmids and E. coli *strains*

E. coli strain MC1061 (hsdR mcrB araD139 Δ(araABC-leu)7679 ΔlacX74 galK rpsL thi) and K91 were used. Filamentous bacteriophage vector fAFF1 was from Affymax Research Institute, Palo Alto, CA.[3] Plasmid pKJSN1 was obtained from K.Judice.[9] Bacteria were grown in either Luria-Bertani (LB) broth, containing tetracycline (20 μg/mL) or on solid LB agar plate, containing tetracycline (20 μg/mL).

Nucleic acid manipulations

Oligonucleotides were synthesized on a 391 DNA synthesizer and purified by denaturing polyacrylamide gel electrophoresis. Gel electrophoresis, phosphorylation, ligation, and transformations were performed as described.[10] Dideoxy sequencing was performed with kit from United States Biochemical, according to the manufacturer's protocol.

Phage purification

A 1 L culture of bacteria MC1061 cells bearing plasmid fAFF1 or fdSN were grown at 37 °C overnight in LB broth supplemented with 20 μg/mL tetracycline. All further steps were performed at 4 °C unless otherwise indicated. The cells were harvested by centrifugation at 5000 *g* for 15 min. To the supernatant 0.2 volume of 20 % PEG, 2.5 M NaCl was added. The solution was left on ice for 1 h and then the phage pellet was obtained by centrifugation at 7000 *g* for 20 min. The phage pellet was resuspended in TE buffer (10 mM Tris, pH 8, 1 mM EDTA) to a final volume of 16 mL. To the phage solution, 7.04 *g* of CsCl was added and the resulting solution was subjected to centrifugation at 200,000 *g* for 20 h. The phage band was collected and then dialyzed against 10 mM Tris, pH 8.

Determination of infectivity of the phage

Bacteria K91 cells (1 mL) were grown at 37 °C in LB broth to OD_{600} = ~0.8. The cells were infected with 10 μL of phage stock. After 40 min growth at 37 °C, the transfected bacteria were plated onto a LB agar plate containing 20 mg/mL of tetracycline. Colonies were fully developed after overnight incubation at 37 °C.

SNase assay

A chromogenic plate assay was used for quick determination of the activity of SNase fusion phage. In our experiments, 3 mL of the phage stock solution (1×10^{10} colony forming units (cfu)) or SNase (200 pg) were spotted on a plate containing 1 % agar, 1 % NaCl, 50 mM sodium glycinate (pH 9.9), 20 mM $CaCl_2$, single-stranded calf thymus DNA (100 μg/mL), and toluidine blue O (0.2 mg/mL). After incubation for 2 h at 37 °C, catalytic activity was determined by the presence of a pink halo against the blue background. A spectrophotometric assay was used for determining the K_m^{DNA} value of the SNase fusion phage. The K_m^{DNA} value was determined by varying the concentration of salmon testes DNA from 10 to 80 μg/mL in 40 mM sodium glycinate, pH 9.9, with 10 mM Ca^{2+}. Reactions were initiated by the addition of 10 μL of phage solution and the initial velocities were determined by monitoring the increase in absorbance at 260 nm.

Western blot of phage particles

The phage particles were loaded in duplicates onto a 1 % agarose gel with 25 mM Tris, pH 8.7, 250 mM glycine as the running buffer. After 16 h of electrophoresis at 7 mA constant current, the gel was soaked in transfer buffer (25 mM Tris, pH 8.7, 250 mM glycine, 2 % SDS, 20 % methanol) for two hours and then blotted onto immbolin membrane. Half of the membrane was stained with amido black and the other half was blotted with anti-SNase antibodies and developed as described.[10]

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