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# LysGH15B, the SH3b Domain of Staphylococcal Phage Endolysin LysGH15, Retains High Affinity to Staphylococci

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**Abstract** LysGH15, a phage endolysin, exhibits a particularly broad lytic spectrum against *Staphylococcus aureus*, especially methicillin-resistant *S. aureus* (MRSA). Sequence analysis reveals that this endolysin contains a C-terminal cell wall binding domain (SH3b), which causes the endolysin to bind to host strains. In this study, the substrate binding affinity of the SH3b domain (LysGH15B) was evaluated. A fusion protein of LysGH15B and green fluorescent protein (LysGH15B–GFP) were cloned and expressed in *Escherichia coli*. Laser scanning confocal microscopy was used to detect the fluorescence of the treated cells irradiated at different excitation wavelengths and to determine the binding activity of LysGH15B–GFP and GFP. We found that LysGH15B–GFP not only generated green fluorescence, but, more importantly, also displayed specific affinity to staphylococcal isolates, especially MRSA. In contrast, the single GFP did not display any binding activity. The high affinity was attributed to the portion of LysGH15B and the binding activity of the fusion protein was specific to staphylococci. This study provides an insight into the SH3b domain of LysGH15. The specific binding activity may cause LysGH15B to serve as an anchoring device, and offer an alternative approach for cell surface attachment onto staphylococci.

## Introduction

Endolysins (also known as lysins) from bacteriophages are cell wall hydrolases synthesized at the end of the phage lytic life cycle that are involved in cell lysis and the release of the progeny particles from host cells [16, 22]. One common feature of lysin that infects gram-positive bacterial species is a modular structure, consisting of at least two separate functional domains: an N-terminal catalytic domain and a C-terminal cell wall binding domain [12].

We recently reported a novel staphylococcal phage lysin, LysGH15, which displayed a particularly broad host range and strong lytic activity against *Staphylococcus aureus*, especially methicillin-resistant *S. aureus* (MRSA) [6, 7]. Analysis of its amino acid sequence revealed that LysGH15 has a modular structure similar to those of LysK, phi11, MV-L, and LysH5, with two catalytic domains, specifically a CHAP (amidohydrolase/peptidase) domain and a central amidase-2 (*N*-acetylmuramoyl-L-alanine amidase) domain, as well as a C-terminal SH3b cell-binding domain (CBD) [14–16, 18].

Detailed studies of the CHAP domain of LysK have been conducted previously, and found that this domain sustains lytic activity against staphylococci [10]. However, to the best of our knowledge, there was only one cell wall-targeting (CWT) domain of staphylococcal phage lysin (ClyS) which has been used for binding to staphylococci via fluorescence tag [3, 17]. In this study, to evaluate the substrate binding affinity of the LysGH15B (the SH3b domain of LysGH15), we generated the fusion protein LysGH15B–GFP and determined its specific affinity to staphylococci.

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## Materials and Methods

### Construction of Expression Vectors

LysGH15B (Fig. 1a) corresponds to the SH3b portion of LysGH15 (GenBank accession no. HM015284). To amplify the *lysGH15B* and *GFP* genes for cloning and plasmid constructions, the full genome of the phage GH15 and the pEGFP vector were, respectively, used as templates. The *lysGH15B* gene was PCR-amplified using SH3b-*Sal*I (ACGCGTCGACAGGTGGAACTCCCTGACAAG) and SH3b-*Eco*RI (CGGAATTCTGGAAAAAGAACCAATACG) primers (enzyme sites are underlined), whereas the *GFP* gene was amplified using GFP-*Sal*I (ACGCGTCGAC**GGGGG TT CT**GTGAGCAAGGGCGAGGAG) and GFP-*Hin*dIII (CCCAAGCTTGTACAGCTCGTCCA) primers (the bold type indicate the linkers). The PCR products were digested with the corresponding restriction enzymes and cloned into the *Eco*RI/*Sal*I (*lysGH15B*) and *Sal*I/*Hin*dIII (*GFP*) sites of a pET-28a (+) vector, generating the plasmid pET28a-Lys-GH15B-GFP. The *LysGH15B–GFP* gene was amplified using SH3b-*Xho*I (CCGCTCGAGTGGAAAAAGAACCAATACG) and GFP-*Bam*HI (CGGGATCCCTACTTGTACAGCTCGTCCA) primers. This fragment was cloned into the *Xho*I/*Bam*HI sites of the vector pET15b, generating the expression vector pET15b-LysGH15B-GFP. The *GFP* gene was also inserted into the same sites of pET15b using GFP-*Xho*I (CCGCTCGAG**GGGGGTTCT**GTGAGCAAGGGCGAGGAG) and GFP-*Bam*HI primers.

### Expression and Purification of LysGH15B–GFP and GFP

Exponentially growing cultures of *Escherichia coli* BL21 containing pET15b-LysGH15B-GFP or pET15b-GFP were induced by incubation with 1 mmol $l^{-1}$ isopropyl-$\beta$-D-thiogalacto-pyranoside (IPTG) for 6 h at 25°C with shaking. Subsequently, bacterial cells were washed with 20 mmol $l^{-1}$ sterile PBS and disrupted with an ultrasonic disintegrator. The resultant clear lysates were centrifuged at 10,000 rpm for 15 min at 4°C. For protein purification, the supernatant was dialyzed against PBS, added to (Ni–NTA, nickel matrix) His·Bind slurry, and eluted according to the manufacturer's instructions (Merck-Novagen). The protein file was analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

### Binding of LysGH15B–GFP to *S. aureus*

1-ml aliquot of stationary phase *S. aureus* culture (YB57, MRSA strain) was collected and washed twice using PBS. The cells were dyed with 20 μmol $l^{-1}$ Hoechst No. 33342 fluorescent dyes for 10 min at 37°C, washed five times with PBS, and then resuspended in 100 μl of PBS. The cells were then incubated with either GFP or the LysGH15B–GFP protein for 15 min at 37°C. After incubation, the cells were collected by centrifugation at 8000 rpm for 5 min, washed five times with PBS, and resuspended in 100 μl of PBS. Laser scanning confocal microscopy (LSCM) was used to detect the fluorescence of the treated cells that were irradiated at different excitation wavelengths and to determine the binding activity of LysGH15B–GFP and GFP.

### The Effectiveness of LysGH15B–GFP to Inhibit the Lytic Activity of LysGH15

Mid-log-phase cells of the YB57 strain were washed and resuspended in sterile PBS, and the cell concentration was modified to an $OD_{600}$ of 1.2. The YB57 substrate was pre-incubated with LysGH15B–GFP (final concentration, 40 μg $ml^{-1}$) for 15 min at 37°C, and the excess fusion was washed out with sterile PBS. The phage lysin LysGH15 (40 μg $ml^{-1}$) was added to the bacterial suspension and incubated for 30 min at 37°C. The $OD_{600}$ was again determined. As controls, the washed YB57 was treated with 40 μg $ml^{-1}$ LysGH15, 40 μg $ml^{-1}$ LysGH15B–GFP, or elution buffer alone.

### Binding Specificity of LysGH15B–GFP

To determine the binding range of LysGH15B–GFP, 53 *S. aureus* strains [6] and 6 *Staphylococcus epidermidis* were treated with Hoechst No. 33342 fluorescent dye and LysGH15B–GFP, as described above. LSCM was used to

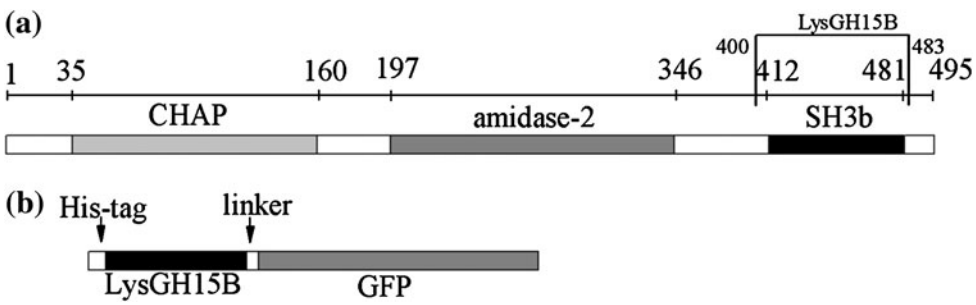


**Fig. 1** **a** Schematic of LysGH15 and its three domains. LysGH15 contains CHAP, amidase-2, and SH3b domains. LysGH15B (residues 400–483) is part of the fusion protein LysGH15B–GFP. **b** Schematic of LysGH15B–GFP

observe the fluorescence of these strains after treatment with the fusion protein. *Streptococcus*, *Bacillus subtilis*, *Salmonella enteritidis*, *Klebsiella pneumoniae*, and *E. coli* were also included in experiments to determine the specificity of the binding activity of LysGH15B–GFP.

## Results and Discussion

### The Expression and Purification of LysGH15B–GFP

The fusion protein contained the His-tag, LysGH15B, linker, and GFP components in order from the N-terminal to the C-terminal (Fig. 1b). A His-tag was also located in the N-terminal region of single GFP. Full expression vectors, pET15b-GFP and pET15b-LysGH15B-GFP, were identified by PCR and sequence detection, and were introduced into competent *E. coli* BL21 cells. SDS-PAGE analysis revealed protein bands at about 28 and 38 kDa in the *E. coli* BL21 cells that contained pET15b-GFP and pET15b-LysGH15B-GFP (Fig. 2), respectively, after being induced by IPTG. The homogeneous band also emerged in the sample containing the purified His-tagged protein. The bands accorded with the predicted sizes of GFP and LysGH15B–GFP.

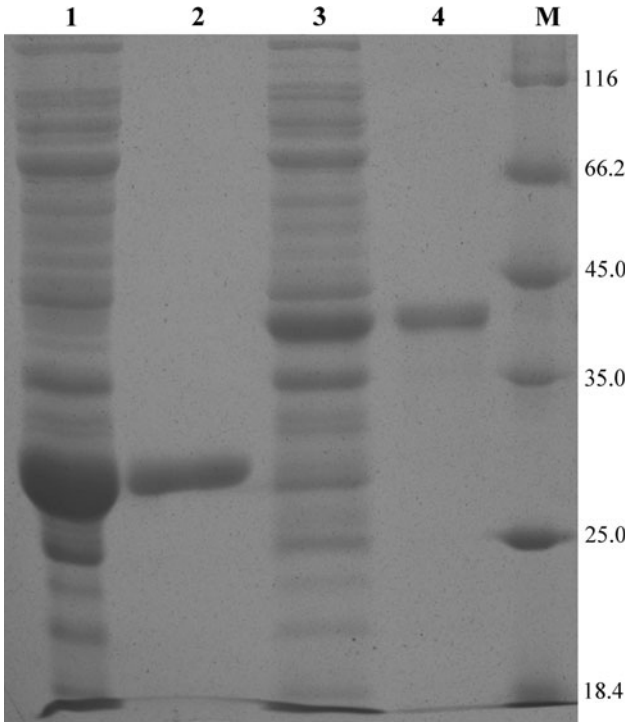


**Fig. 2** Protein profiles of N-terminal His·6-tagged LysGH15B–GFP and GFP extracts from expression bacterial strains, respectively. Every lane was added with 15 µl total volume. *Lane 1 E. coli* BL21 (Coden Plus) cells containing a pET15b-GFP construct induced with IPTG. *Lane 2* the purified GFP fraction (about 28 kDa). *Lane 3* pET15b-LysGH15B-GFP-containing *E. coli* BL21 (Coden Plus) cells induced with IPTG. *Lane 4* the purified LysGH15B–GFP fraction (about 38 kDa). *Lane M* the molecular mass marker (kDa)

### LysGH15B–GFP Binds to *S. aureus* Isolate

Blue fluorescence emitted by Hoechst No. 33342 fluorescent dyes was found near the center of the cells of the YB57 strain when examined at 405 nm wavelength, and green fluorescence emitted by GFP was observed around the LysGH15B–GFP-treated YB57 cells at 488 nm wavelength (Fig. 3). However, no green fluorescence was present around YB57 cells treated with GFP under the same conditions. These results demonstrate that LysGH15B–GFP binds to YB57 cells, and that the binding activity and green fluorescence of LysGH15B–GFP were due to the LysGH15B and GFP components, respectively.

### Effectiveness of GH15B–GFP in Inhibiting the Lytic Activity of LysGH15

LysGH15 displayed a strong lytic activity toward the MRSA strain YB57; the $OD_{600}$ of YB57 cultures decreased about 65% after exposure to LysGH15 (Fig. 4). However, the YB57 cultures treated with LysGH15B–GFP were insensitive to LysGH15; the decrease of $OD_{600}$ only reached about 30%. As a control, we determined that LysGH15B–GFP alone had no effect on the $OD_{600}$ of YB57 cultures, similar to buffer treatment. Hence, LysGH15B–GFP displayed competitive inhibition to the lytic activity of LysGH15, and indirectly indicated the binding activity of LysGH15B–GFP to YB57 cells.

### Binding Specificity

The LysGH15B–GFP protein sufficiently bound to 43 *S. aureus* strains and 4 *S. epidermidis* strains. More importantly, the fusion protein bound to all 24 MRSA strains examined. In contrast, no green fluorescence was detected around *Streptococcus*, *B. subtilis*, *S. enteritidis*, *K. pneumoniae*, or *E. coli* strains after incubation with LysGH15B–GFP (data not shown). Therefore, the affinity of LysGH15B–GFP was apparently specific to staphylococci, at least based on these limited studies.

This study revealed that LysGH15B–GFP, which contains the SH3b domain of LysGH15, displayed a broad binding host range that is essentially consistent with the lytic spectra of LysGH15. Since, we have determined that the specific affinity of LysGH15B–GFP to staphylococci is attributed to its LysGH15B portion, it might be concluded that the lytic range of lysin is also decided by the SH3b portion [4, 5]. The ability of binding is closely related to

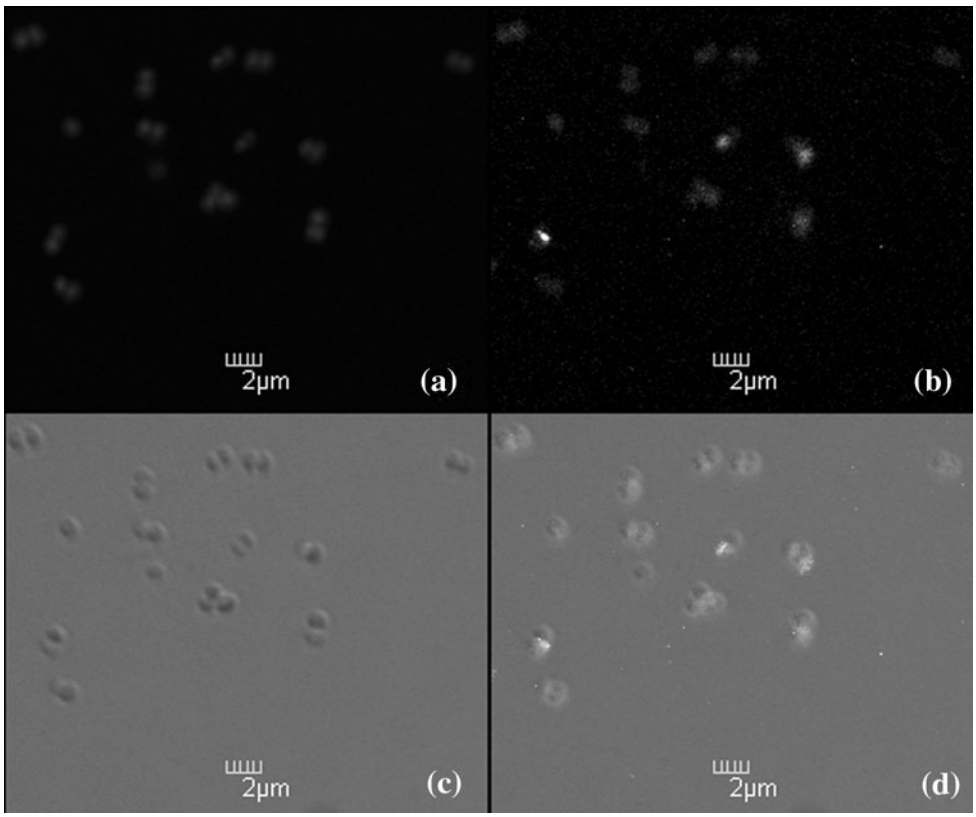


**Fig. 3** Localization of LysGH15B–GFP on YB57. YB57 was dyed with a 20 μmol $l^{-1}$ Hoechst No. 33342 fluorescent dye for 10 min at 37°C and incubated with LysGH15B–GFP for 15 min at 37°C. **a** Localization at 405 nm wavelength (blue fluorescence, emitted by Hoechst No. 33342 fluorescent dye). **b** Localization at 488 nm wavelength (green fluorescence, emitted by GFP). **c** Image of ordinary ray (normal light). **d** Overlay of the pictures shown in **a**, **b**, and **c**

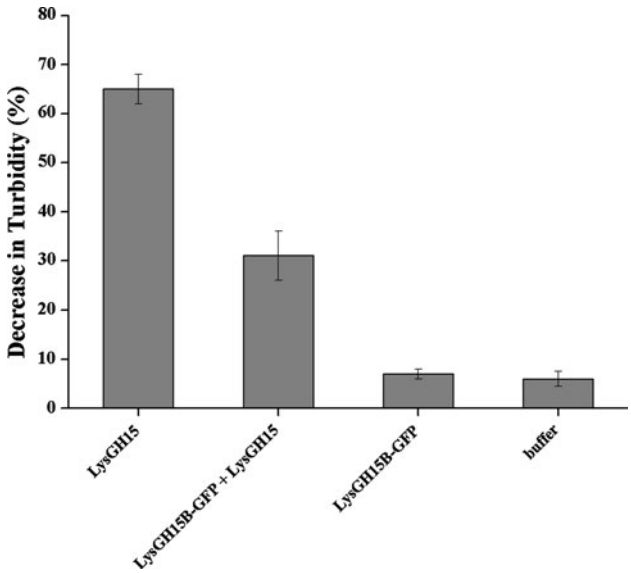


**Fig. 4** Competitive inhibition of LysGH15B–GFP on the lytic activity of LysGH15. YB57, which had been treated with 40 μg $ml^{-1}$ LysGH15B–GFP for 15 min, was washed by PBS and then exposed to 40 μg $ml^{-1}$ LysGH15 for 30 min. As controls, YB57 was treated with 40 μg $ml^{-1}$ LysGH15, 40 μg $ml^{-1}$ LysGH15B–GFP, or elution buffer alone for 30 min. Values represent mean ± SD ($n = 3$)

the lytic activity of lysin [19]. Hence, the host range and lytic activity of lysin may be changed by modifying the structure of the SH3b domain.

Phages have previously been used to detect corresponding bacterial strains [1, 8, 9]. However, there are some limitations to the development of fluorescence-tagged bacteriophages, such as the environmental contamination of phages. Phage lysins are not subjected to this limitation and, more importantly, they manifest a broader host range than the whole virus [2, 6]. Thus, compared with phages, the SH3b domain of lysin might be a better detection tool. LysGH15B–GFP not only generates green fluorescence but also maintains specific binding activity toward staphylococci. In addition, it is relatively inexpensive and convenient for mass production by industrial fermentation. Therefore, LysGH15B–GFP possesses great potential as a detection tool of staphylococci.

Because, LysGH15B has been demonstrated to possess the ability to target the heterologous protein GFP on the surface of staphylococci, and because there is no mutual effect between LysGH15B and GFP, it might be possible to use LysGH15B as an anchoring motif for the display of other proteins, such as antigens or functional enzymes, on the surface of staphylococci [13, 20, 21]. This strategy has practical applications for the development of oral vaccines, biocatalysts, whole-cell absorbents, and other purposes [11]. In conclusion, LysGH15B represents a new type of anchoring device and offers an alternative approach for cell surface attachment onto staphylococci.

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