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Bright fluorescence of a novel protein from *Vibrio vulnificus* depends on NADPH and the expression of this protein is regulated by a LysR-type regulatory gene

Chun Chin Chang,[a] Yin Ching Chuang,[b] Yu Chung Chen,[c] and Ming Chung Chang[a,*]

[a] *Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan, ROC*
[b] *Department of Medical Research, Chi Mei Medical Center, Tainan, Taiwan, ROC*
[c] *Department of Food Science, National Chung Hsing University, Taichung, Taiwan, ROC*

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Abstract

A blue fluorescent protein, BfgV, belonging to the short-chain dehydrogenase/reductase superfamily, was found in a non-bioluminescent pathogen *Vibrio vulnificus* CKM-1. This protein has fluorescence spectra with two excitation peaks at 283 and 352 nm, and one emission peak at 456 nm. BfgV fluoresces through effectively augmenting the intrinsic fluorescence of NADPH bound to it. *Escherichia coli* transformants expressing this protein can emit eye-detectable fluorescence. A LysR-type transcriptional regulator gene *bfgR* was found at the vicinal upstream region of *bfgV* in CKM-1 genome. The clues that products of *bfgR* can specifically bind to *bfgR–bfgV* intergenic promoter region and the deletion of *bfgR* significantly decreases the expression of *bfgV* reveal *bfgR* is a repressor gene of *bfgV* in *V. vulnificus* CKM-1.

Keywords: Fluorescence; NADPH; Short-chain dehydrogenase/reductase; *Vibrio vulnificus*

Luciferases are proteins that can catalyze bioluminescence reactions to produce light. These proteins exist in bioluminescent organisms which comprise a notable diversity of species [1,2]. The best known organisms capable of producing luciferase are bacteria that mostly belong to three genera: *Photobacterium*, *Vibrio*, and *Xenorhabdus* [2,3]. Each kind of luciferase in these bacteria usually comprises two different subunits of polypeptides, LuxA and LuxB [2,4]. The luminescent phenomenon producing by luciferase is due to the oxidation of long-chain aliphatic aldehyde to fatty acid [2,5].

Unlike luciferases, fluorescent proteins, such as green fluorescent protein (GFP) [6,7] and lumazine protein [8], cannot produce light by themselves. They simply transform short-wavelength light from other sources to another visible light. GFP fluoresces by virtue of a resonance structure which is post-translationally modified from an internal Ser-Tyr-Gly sequence [7,9], while lumazine protein fluoresces due to a highly fluorescent and non-covalently bound ligand, 6,7-dimethyl-8-D-ribityllumazine [8,10]. In addition, some NAD(P)H-dependent proteins can also fluoresce through bound fluorescent NAD(P)H molecules [11–13].

In our research of a pathogenic *Vibrio vulnificus* CKM-1, we accidentally found a fluorescent protein gene, *bfgV* (designated as *bfpvv* in the previous report), in this bacterium [14]. Sequence analysis revealed that BfgV belongs to the short-chain dehydrogenase/reductase (SDR) superfamily which contains more than 1000 members whose functions are known or unknown [15,16]. *Escherichia coli* transformants expressing *bfgV* could conspicuously fluoresce with pale blue light when irradiated with long-wavelength ultraviolet [14]. In this study, fluorescent BfgV was purified and its fluorescence spectrum was determined. Our experiments discovered the reason why BfgV fluoresce. In addition, the gene

* Corresponding author. Fax: +886-6-2754697.
E-mail address: mcchang@mail.ncku.edu.tw (M.C. Chang).

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which regulates the expression of *bfgV* was also identified in CKM-1 genome.

Materials and methods

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. Unless noted otherwise, bacteria were either grown in Luria–Bertani (LB) broth or on LB agar. Isopropylthio-β-D-galactoside (IPTG) was used as inducer at 1 mM in broth and 0.1 mM in agar plates.

Purification of BfgV. The BL21(DE3)/pFP21 was raised in LBA (LB broth containing 50 μg/ml ampicillin) broth. Isopropylthio-β-D-galactoside (IPTG) was added to induce BfgV overproduction. Cells were harvested by centrifugation, washed three times with 20 mM phosphate buffer (pH 7.0), and then disrupted by ultrasonication. Cell debris was removed by centrifugation and filtration by 0.22 μm filters. Clear cell lysate was applied to SOURCE 15S (Pharmacia; 1.6×10 cm) column, and then isocratic elution with the same buffer was performed. The brightest fractions were concentrated by ultrafiltration membrane (Amicon; 30,000 NMWL), then applied to Superdex 200 HR column (Pharmacia; 1×30 cm) equipped in the Pharmacia FPLC system, and eluted with TG (40 mM Tris, 1 mM EDTA, 0.1 M NaCl, 0.2 mM DTT, and 5% glycerol; pH 8.0) buffer. All above-mentioned purification steps were performed at 4 °C. The purest BfgV fraction (>91% homogeneity in SDS–PAGE) was used for the following studies.

Determination of the fluorescence spectra of BfgV. The fluorescence spectra of BfgV were measured with a Perkin–Elmer LS50B luminescence spectrometer at 25 °C. To determine emission peaks, an arbitrary short-wavelength UV (e.g., 290 nm) was used to excite BfgV and the emission spectrum was scanned from 350 to 600 nm. After the emission peak was determined, excitation scanning was performed to identify the excitation peaks of BfgV. Finally, excitation wavelengths were confirmed by checking against previously found emission maxima.

Reverse-phase HPLC analysis of the alkaline extract of purified BfgV. The alkaline extraction and high-performance liquid chromatography (HPLC) method for detection of NAD(P)H described by Stocchi [19] was used to treat the purified BfgV solution. The LiChrospher 100 RP-18e column (250×4 mm i.d.) (Merck) was used for separation. The mobile phase was 0.1 M KH_2PO_4 (pH 6.0) containing 10% (v/v) of CH_3OH and the flow rate was 1 ml/min. The standard solutions of NADH and NADPH were prepared in TG buffer (pH 8.0). Molar absorption coefficients of 6220 and 6290 $M^{-1} cm^{-1}$ at 340 nm were used to determine the concentration of NADPH and NADH, respectively.

Determination of DNA sequence around bfgV. The DNA fragments in the vicinity of *bfgV* gene in the CKM-1 genome were amplified by the method described by Min and Powell [20]. For upstream fragment, two primes GW345R (5′-CAC CGC TTG CAT ACC ATT TAG C-3′) and GW610R (5′-CAC CCA TAT CCA CTT TCC ATG C-3′) based on the *bfgV* sequence were used for amplification of upstream DNA fragments. The GW610R was first used to extend and enrich ssDNA of the target sequence by PCR. The ssDNA products were purified and tailed with poly(dC) by terminal deoxyribonucleotidyltransferase (New England BioLab). The semi-nested PCR was then performed, using GW345R and oligo$(dG)_{14}$ as primer. All semi-nested PCR products were loaded onto a 0.7% agarose gel for separation. The major DNA bands were sliced off, purified from the gels, and used as templates for sequencing.

Preparation of BfgR fusion protein and promoter binding studies. BfgR-HisTaq fusion protein was produced by BL21(DE3)/pREG906 transformants by IPTG induction. Cells were grown and then harvested by centrifugation, washed with PBS, resuspended in PSI buffer (0.02 M Na_2HPO_4, 0.5 M NaCl, and 10 mM imidazole, pH 7.4), and ultrasonicated. The supernatant was filtered through a 0.22 μm membrane and then purified by HisTrp chelating column (Pharmacia). The purified BfgR fusion protein with >95% homogeneity was dialyzed

Table 1
Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristic(s)	Reference or source
Bacterial strains		
V. vulnificus		
CKM-1	Clinical isolate; Sm^r	[14]
URD101	CKM-1 *bfgR*::pSVI236; Km^r	This study
E. coli		
XL1 blue	[F′ Tn10 *pro*AB *lac*^{q} ZΔM15] *rec*A1 *end*A1 *gyr*A96 *thi*-1 *hsd*R17 *sup*E44 *rel*A1	Stratagene
BL21(DE3)	*gal hsd*S (λ c*Its*857 *ind*1 *Sam*7 *nin*5 *lac*UV5-T7 gene1); host carrying the T7 RNA polymerase gene	[18]
S17-1	*rec*A *pro hsd*R RP4-2-Tc::Mu-Km::Tn7; conjugal donor; Sm^r	[21]
Plasmids		
pET21b	Cloning vector; *lac* operator-controlled T7 promoter, optional C-terminal HisTag, T7 terminator, *lacI* coding sequence, multiple cloning sites, Ap^r	Novagen
pFP21	pET21b with 751-bp *Xba*I–*Xho*I insertion, including complete 720-bp ORF of *bfgV* and 31-bp upstream non-coding sequence, generated by PCR from chromosome of CKM-1	This study
pREP21	pET21b with 1056-bp *Xba*I–*Xho*I insert, including complete 918-bp ORF of *bfgR* and 137-bp of *bfgR–bfgV* intergenic promoter region	This study
pREG906	pET21b with a *Nde*I–*Xho*I insertion, including a complete ORF of *bfgR* fusing with $(His)_6$-*Taq* sequence at C-terminal, Ap^r	This study
pCVD442	Positive selection suicide vector, *sacB*; *R6K γ ori*, *mob* of RP4, multiple cloning site, Ap^r	[33]
pSVI001	pCVD442 with *bla* gene replaced by *kan* gene, Km^r	This study
pSVI236	pSVI001 with 236-bp *Sal*I–*Sph*I fragment encoding internal coding sequence (the 28th to 263th bp) of *bfgR*, Km^r	This study
pPGST322	pBR322 with an *Nhe*I–*Hin*dIII insert which containing a complete 137-bp *bfgR–bfgV* promoter fragment fused with *Schistosoma japonicum*-born *gst*, Ap^r	This study

against TP buffer (20 mM Tris, 100 mM KCl, 1 mM DTT, 0.2 mM EDTA, and 10% glycerol; pH 7.9). As to the promoter binding study, the 137 bp of *bfgR–bfgV* intergenic promoter fragments was generated by PCR and labeled with DIG-11-ddUTP at 3′ ends using terminal transferase. The binding reaction mixture contained approximately 1 ng of labeled fragments, 15–40 ng of purified BfgR protein, and 1 μg of poly(dI–dC). Competitor (pREP21) or non-competitor (pET21b) DNA was added at 200 ng where noted. All binding reactions were carried out in a total volume of 12 μl PB buffer (20 mM Tris, 1 mM DTT, 0.2 mM EDTA, 100 μg/ml BSA, and 10% glycerol; pH 7.9) at 25 °C for 30 min. Electrophoresis was carried out in 5% polyacrylamide gel in 0.5× TBE buffer. The blotting and chemiluminescent detection were performed according to the protocols in the kit.

Construction of bfgR insertional mutant. To construct *bfgR* mutant, conjugation between S17-1/pSVI236 and CKM-1 was carried out by the methods described by Simon et al. [21]. Briefly, the donor strain, S17-1/pSVI236, and the recipient strain, CKM-1, were grown overnight in LB broth separately and then cells were recovered by centrifugation. Equal amounts of the CKM-1 and S17-1/pSVI236 transformants were mixed, then spotted on LB agar, and incubated at 37 °C for 3–8 h. Then, the mixed cells were spread on thiosulfate–citrate–bile–sucrose (TCBS) agar (to select against the *Escherichia coli* donor) [22] supplemented with 80 μg/ml kanamycin (to screen for *bfgR* mutant). The suspect mutants were selected and streaked again for pure culture. One of the insertional mutants, named URD101, was confirmed to carry the correct insert in its genome by Southern blot and primer walking mathod described above.

Western blot analysis. Cells were first grown in marine broth (Difco). Equal amounts of total cell proteins from different samples (CKM-1, URD101, CKM-1/pPGST322, and URD101/pPGST322) were separated in 12% SDS–PAGE and transferred to Hybond-p membrane (Pharmacia). The membrane was blocked with PBS-TM (phosphate-buffered saline containing 0.02% (w/v) Tween 20 and 5% (w/v) skim milk) and reacted with 1:4000 diluted anti-GST antisera (Pharmacia) or rabbit polyclonal antisera against BfgV protein for 1.5 h at room temperature. The detection was performed using 1:20,000 diluted anti-goat IgG (for anti-GST) or anti-rabbit IgG (for anti-BfgV) conjugated with peroxidase and ECL detection kit (Pharmacia).

Nucleotide sequence accession number. The GenBank accession number for the sequence presented in this paper is AF080431.

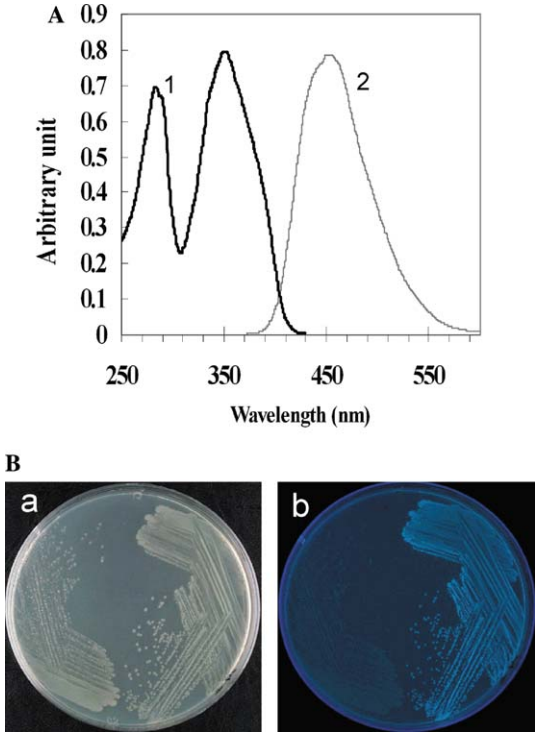

Fig. 1. Fluorescence spectra of BfgV and photographs of fluorescent *E. coli* colonies expressing BfgV protein. (A) Excitation spectrum (curve 1) of BfgV measured by fixing emission at 456 nm shows major and minor peaks at 352 and 283 nm, respectively. Emission spectrum (curve 2) of BfgV activated at 352 nm has a major peak at 456 nm. (B) *E. coli* BL21 and its transformants harboring pFP21 were cultured on LBA agar for 16 h. IPTG was added in agar as inducer. (a) A culture plate under white light: left part BL21, right part BL21/pFP21 and (b) same plate under 365 nm UV light.

Results

The fluorescence spectra of BfgV

The purified BfgV in TG buffer was put into a fluorometer for spectra determination. Curve-1 and curve-2 in Fig. 1A represent the excitation and emission spectra, respectively. Two excitation peaks were observed: one weak peak at 283 nm and the other at 352 nm. Only one major emission peak appeared at 456 nm (Fig. 1A). This emission wavelength corresponds to visible pale-blue color and coincides with the fluorescence emitting from the BL21(DE3)/pFP21 transformants which overexpressed *bfgV* (Fig. 1B).

HPLC analysis of the alkaline extract of BfgV

Because the fluorescent spectra of BfgV are similar to those of NAD(P)H-dependent oxidoreductase [11,12]. We therefore speculated that BfgV may contain NAD(P)H and its fluorescence may be related to these cofactors. Thus, an alkaline extraction followed by reverse-phase HPLC analysis was adopted to determine whether the purified BfgV contained NAD(P)H or not. As shown in Fig. 2, the standard solutions of NADPH and NADH separated by this method had a retention time of 3.36 and 5.78 min, respectively. The alkaline extract of purified BfgV had a major peak retained at 3.36 min, a value identical to that of NADPH. This result suggested NADPH linked with BfgV (Fig. 2). If NADPH were required for BfgV to fluoresce, elimination of NADPH should diminish the fluorescence intensity. Therefore, NADPH-dependent L-glutamic dehydrogenase (GLDH; EC. 1.4.1.3.) was used to confirm the role of NADPH in the fluorescence phenomenon of BfgV. When GLDH and its substrates, α-ketoglutarate and ammonium chloride, were added into purified BfgV solution, the fluorescence intensity of BfgV immediately and significantly decayed within 40 s (data not shown). These results showed that not only does NADPH bind to BfgV, but also NADPH plays a key role in the fluorescent phenomenon of the BfgV.

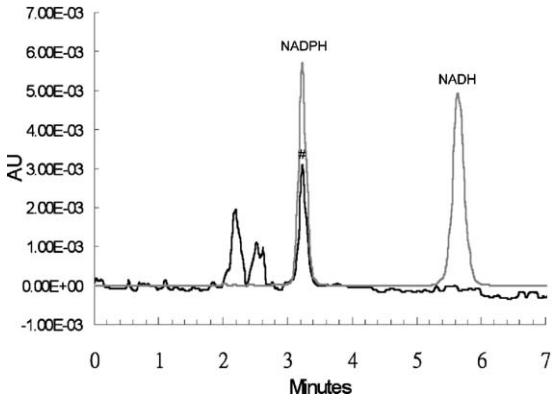

Fig. 2. Separation of NADPH from extracts of purified fluorescent BfgV by reverse-phase HPLC. A 20 μl mixture of 6.7 μM NADPH and 9.3 μM NADH was subjected to HPLC separation to generate a standard chromatogram of these two compounds (gray curve). The retention time (RT) of NADPH and NADH was 3.36 and 5.78 min, respectively. A 200 μl of potassium hydroxide extract of purified BfgV, was analyzed under the same conditions (black curve). A major peak of the sample curve, denoted by "#" symbol, had the same RT as NADPH in the standard chromatogram. The concentrations of these two compounds were traced as absorbance units (AU) at 340 nm.

Efficient augmentation of the intrinsic fluorescence of NADPH by BfgV protein

Since NADPH plays an important role in the BfgV fluorescence, the fluorescence intensity of purified BfgV was compared to that of free NADPH with the same concentration determined from this protein. The result showed the ratio of relative fluorescence intensity (RFI) of BfgV–NADPH complex to NADPH alone at 456 nm is about 10:1 as the excitation wavelength was set at 340 nm (optimum for free form NADPH) (Fig. 3). If the excitation were shifted to the optimum for BfgV–NADPH complex at 352 nm, the RFI ratio at 456 nm would slightly increase to 11:1 (data not shown). Therefore, the apo-BfgV protein has the ability to positively influence the intrinsic fluorescence of NADPH molecules bound to it.

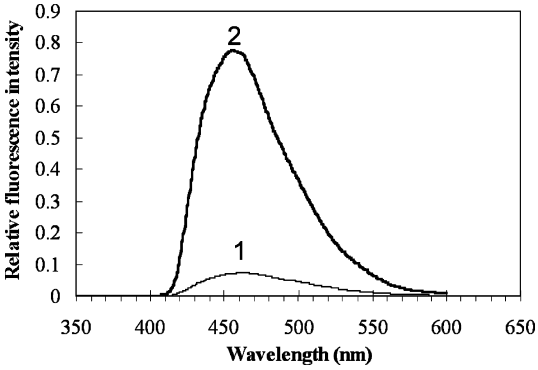

Fig. 3. Comparison of fluorescence intensity of BfgV and free NADPH. NADPH was prepared at the same concentration as the alkaline extract of purified BfgV. NADPH (curve 1) and BfgV (curve 2) were excited at 340 nm in TG buffer.

Localization of a transcriptional regulator gene in the vicinal upstream region of bfgV

DNA sequence around *bfgV* revealed that there was no structure gene clustered with *bfgV* to form an operon. However, an open reading frame (designated as *bfgR*) of 915 bp located in the vicinal upstream region was noted. The orientation of this gene is reverse to that of *bfgV* (Fig. 4). Comparison of the deduced amino acid sequence of the BfgR with other sequences in the GenBank database revealed BfgR has significant similarity to LysR-type transcriptional regulators such as CrgA [23] with 65% similarity and MetR [24] with 57% similarity. The N-terminal region of BfgR possesses "helix–turn–helix" (HTH) motif which is a signature of LysR-type regulator and get involved in DNA recognition and binding (Fig. 4) [25].

Regulation of bfgV by bfgR in V. vulnificus CKM-1

To test whether *bfgR* is a regulator gene of *bfgV* or not, DNA mobility shift assay was first performed to examine the binding of purified BfgR protein to the 137-bp *bfgR–bfgV* intergenic promoter fragment. For DNA mobility shift assay, the PCR-generated promoter fragments were labeled and incubated with various amounts of purified BfgR fusion protein. As shown in Fig. 5, BfgV retarded the labeled DNA fragments and caused a band-shift in a dose-dependent manner (Fig. 5, lanes 2–4). Furthermore, unlabeled plasmid DNA (pREP21) containing the intact *bfgR–bfgV* intergenic promoter region showed distinct competition for BfgR away from the labeled fragments (Fig. 5, lane 5), while the same amount of the vector DNA (pET21b) did not (Fig. 5, lane 6). The results revealed that BfgR could specifically bind to this promoter fragment and suggested this protein might be a transcriptional regulator for *bfgV*.

To study whether BfgR controlled the expression of *bfgV* in *V. vulnificus* CKM-1, a *bfgR* insertional mutant, URD101, was constructed. Then, the expression of *bfgV* in both wild-type CKM-1 and mutant URD101 as a function of growth was examined by Western blot. The result indicated no BfgV could be detected in CKM-1 strain during log phase and only a low level of BfgV was discovered as the cells reached the stationary phase (Fig. 6A, lanes indicating 2–8 h growth of CKM-1). This is in contrast to the situation observed for URD101 mutant, in which the BfgV could be detected at all growth phases (Fig. 6A, lanes indicating 2–8 h growth of URD101). Moreover, we also fused the 137-bp promoter fragment to a promoterless *gst* reporter gene to become a promoter probe, pPGST322. This probe was introduced

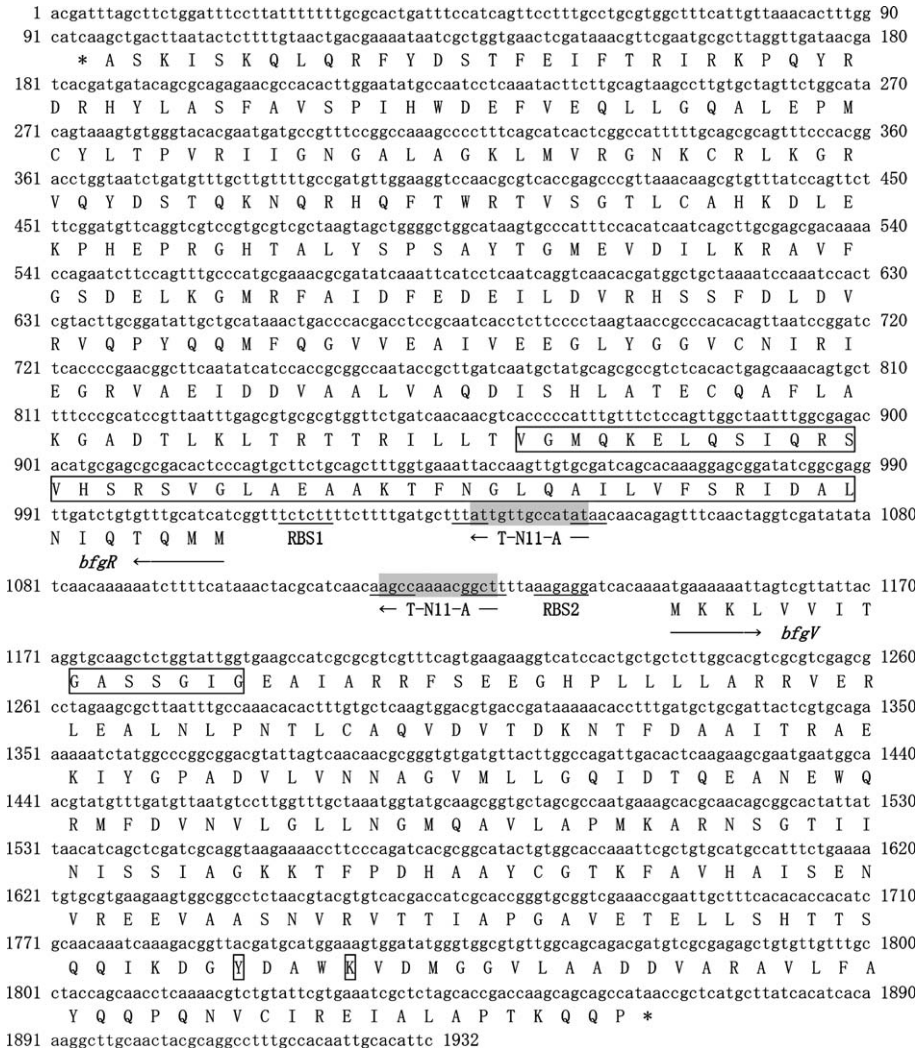

Fig. 4. Nucleotide and amino acid sequences of the *bfgR* and *bfgV* genes. The nucleotide sequences of the non-transcribed strand of *bfgR* and the transcribed strand of *bfgV* are shown. Their deduced amino acid sequences are also present. Two putative LysR-type recognition sequences, T-N11-A, in intergenic region are shadowed and the inverted repeats are underlined. Possible ribosome-binding sites for both *bfgR* and *bfgV* are underlined and denoted as RBS1 and RBS2, respectively. The "helix–turn–helix" sequence in *bfgR* and the SDR-superfamily conserved motif and residues in *bfgV* are boxed. The asterisk symbol in this figure indicates stop codon site.

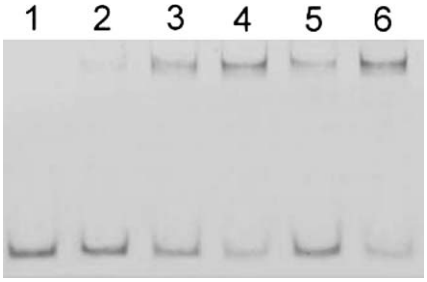

Fig. 5. Electrophoretic mobility shift assay of the 137-bp *bfgR–bfgV* intergenic fragments with purified BfgR. Lanes: 1, no BfgR; 2, 3, and 4, BfgR at 15, 25, and 40 ng, respectively; 5, same as lane 4 but with unlabeled competitor DNA (i.e., pREP21 at 200 ng); and 6, same as lane 4 but with unlabeled pET21b as non-competitor DNA at 200 ng.

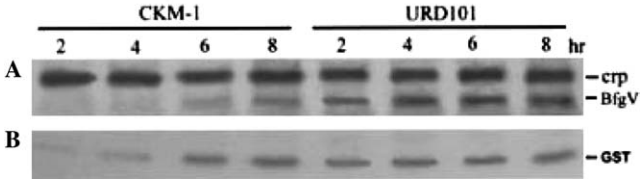

Fig. 6. Western blot analysis of BfgV expression. (A) Wild-type CKM-1 and mutant URD101 or (B) wild-type and mutant transformants containing pPGST322 were grown in marine broth and sampled every 2 h. Equal amounts of total proteins of each sample were resolved by SDS–PAGE and then Western blot was performed with (A) anti-BfgV antibody or (B) anti-GST antibody. Two–four hours, exponential (or log) phase; 6–8 h, stationary phase. crp, cross-reacting protein.

[26] to CKM-1 and URD101, and then the protein level of glutathione *S*-transferase (GST) in both strain was compared. The results of Western blot showed the GST expression pattern was similar to that of BfgV in both CKM-1 and URD101. Therefore, the expression of *bfgV* is repressed, especially during log phase, and this repression requires a wild-type *bfgR* gene.

Discussion

The *bfgV* was the first fluorescent protein gene found in *V. vulnificus*. Its product BfgV matches the characteristics of SDR superfamily proteins, including the size of ≈250 residues, with only 15–30% residue identity between members in this superfamily, functionally conserved residues such as Tyr145 and Lys149 in the catalytic site, a conserved GlyXXXGlyXGly motif in the N-terminal part and their using NADP(H) or NAD(H) as cofactor [15,16]. HPLC analysis and NADPH-dependent GLDH reaction demonstrated that NADPH is responsible for the fluorescent phenomenon of BfgV. Because NAD(P)H binding proteins are ubiquitous in living cells, we had ever tried to know if there was any other fluorescent protein like BfgV in CKM-1. The GenBank of CKM-1 was screened again, and all recombinant plasmids in blue fluorescent transformants were isolated and sequenced. We found their inserts all contained intact *bfgV*. We also screened some bacteria GenBanks at hand to look for any other gene with similar fluorescent trait like *bfgV*, but no one could be found. Besides, there was still no report about NAD(P)H binding protein giving living cells naked-eye-detectable fluorescence except BfgV. Therefore, this protein is rather unique.

The fluorescence spectra of BfgV are very similar but not totally identical to two NADPH-dependent proteins: human estradiol 17β-dehydrogenase-NADPH (17β-HSD–NADPH) complex [12] and malic enzyme–NADPH complex [11]. The interaction between these two apoproteins and NADPH can cause obvious blue shift in emission light from about 460 nm (free form of NADPH) to 436 and 440 nm, respectively [11,12]. Unlike these two proteins, BfgV–NADPH complex only makes about 4 nm emission shift. In addition, we also found BfgV is capable of enhancing the intrinsic fluorescence intensity of bound-NADPH to approximately 10 times the value of NADPH alone. The increase of fluorescence intensity of bound-NAD(P)H was also found in the above-mentioned proteins [11,12,17]. However, their fluorescence augmenting ability seemed not so strong like BfgV. This phenomenon is not universal because there is at least one exception, glyceraldehyde 3-phosphate dehydrogenase, in which fluorescence intensity of bound-NADPH decreases [13]. Although the real mechanisms are not well understood, this phenomenon had been proposed to arise by conformational changes of the NAD(P)H after binding to the proteins [13]. Distinct binding manner may lead to different levels of conformational change and, subsequently, represent diverse fluorescence intensity of NAD(P)H.

After sequencing and analyzing the region around *bfgV* in CKM-1 genome, there were some reasons to predict an upstream *bfgR* gene might regulate the expression of *bfgV*: first, BfgR possesses important features shared by many LTTRs such as containing putative HTH structure (Leu8–Val50) and rich in basic amino acids at its N terminus. These structures were proposed to be responsible for DNA binding. Second, two putative binding motifs characterized by T-N11-A as the core of an inverted repeat for LTTRs binding [27] were found in the *bfgR–bfgV* intergenic region (Fig. 4); Third, the orientation of *bfgR* and *bfgV* is reverse, and the space between them is only 137 bp. The data of gel mobility shift assay, Western blot of BfgV expression in CKM-1 and URD101, and GST promoter probe assay in this studies indeed supported that *b fgR* is involved in the repression of *bfgV*, especially during log phase. Although *bfgR* was knocked out in URD101, this mutant was still non-fluorescent. One possible explanation is that the limited increase of BfgV is still insufficient to confer fluorescent phenotype. It is also much possible that there are some potential substrates which may further exhaust the fluorescent "BfgV–NADPH" into non-fluorescent "BfgV–NADP" complex. We therefore speculated that the fluorescence of BfgV might not be the main function like luciferase or GFP in its original host.

It had ever been reported the hydroxy group of a highly conserved Tyr residue (Tyr152 in the numbering system of the 3α/20β-hydroxysteroid dehydrogenase and Tyr145 in BfgV) in the catalytic sites of the members of SDR superfamily is crucial for enzymatic reaction [15,28]. If this Tyr residue was substituted by other residue, e.g., Phe, the enzymatic activity would be abolished [15]. Although BfgV belongs to the SDR superfamily, the potential enzymatic activity of this protein did not seem to correlate with its fluorescent property. In our previous study, a Tyr145Phe mutant still fluoresce and even brighter than wild-type BfgV [14]. This property favors this kind of BfgV mutant to become a reporter without influencing the metabolism in the host cells. In this study, we further proved NADPH is the key factor for BfgV to fluoresce and this protein has the ability to efficiently augment the intrinsic fluorescence of NADPH bound to it. Because NADPH is a general molecule in living cells, no matter they are aerobic or anaerobic, BfgV derived mutant molecule theoretically could be used as a reporter in both of these two cell types. As for reporter molecule, GFP is undoubtedly an admirable one used extensively. Some genetically modified GFPs, which significantly increase the efficiencies of protein folding, fluorophore formation, and (or) fluorescence intensity, had been constructed in the past [29–31]. However, GFP and its mutated molecules all require oxygen to form a special fluorophore for fluorescing. This requirement hinders their application under obligate anaerobic conditions [32]. Therefore, BfgV may be a good candidate to be applied in this field.

Acknowledgments

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