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# Expression, purification, crystallization and structure determination of the N terminal domain of Fhb, a factor H binding protein from *Streptococcus suis*

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ABSTRACT

Fhb is a surface virulence protein from *Streptococcus suis*, which could aid bacterial evasion of host innate immune defense by recruiting complement regulator factor H to inactivate C3b deposited on bacterial surface in blood. Here we successfully expressed and purified the N terminal domain of Fhb (N-Fhb) and obtained crystals of the N-Fhb by sitting-drop vapor diffusion method with a resolution of 1.50 Å. The crystals belong to space group C2 with unit cell parameters $a = 127.1$ Å, $b = 77.3$ Å, $c = 131.6$ Å, $\alpha = 90°$, $\beta = 115.9°$, $\gamma = 90°$. The structure of N-Fhb was determined by SAD method and the core structure of N-Fhb is a β sandwich. We speculated that binding of Fhb to human factor H may be mainly mediated by surface amino acids with negative charges.



## 1. Introduction

*Streptococcus suis*, a gram-positive facultative anaerobe bacterial, is an important zoonotic pathogen associated with a wide range of diseases in pigs including meningitis, septicemia, pneumonia, endocarditis and arthritis [1,2]. *S. suis* could be transmitted to humans by close contact with pigs or pork and infections mainly occur in slaughterhouse workers and those who handle infected pork [3,4]. Among the identified 35 serotypes, *S. suis* serotype 2 is the major cause of the associated diseases in pigs and humans [5–7]. Before 1998, cases of *S. suis* infection have been reported sporadically worldwide [8,9]. However, two large-scale outbreaks of *S. suis* characterized by streptococcal toxic shock like syndrome emerged in China (One in Jiangsu Province in 1998 and the other in Sichuan Province in 2005). These incidents have drawn scientific community's attention to this pathogen [10].

To better survive in blood of the host, *S. suis* has evolved many sophisticated means to evade host innate immune defense, particularly the complement system attacks, and to maintain a high level of bacteremia [11–14]. Similar to HIV and the serum-resistant *Borrelia* [15,16], *S. suis* is also able to recruit complement regulator factor H from the serum [14]. When bound to bacterial cell surface, factor H could negatively regulate the host immune response by inactivating the complement component C3b deposited on bacterial cell surface [14,17]. Several studies have proven that bacterial cell surface proteins play important roles in recruiting complement regulator factor H, such as BbCRASP for *Borrelia burgdorferi*, fhbB for *Treponema denticola* and Fhbp for *Neisseria meningitis* [18–20]. In terms of *S. suis*, Fhb could recruit complement regulator factor H to bacterial cell surface and contribute to evasion of complement mediated attack and bacterial survival in blood [14]. Fhb was initially identified by immune proteomics and was proven to be a promising vaccine candidate for prevention and control of *S. suis* infection [21,22].

Although many factor H binding proteins of several pathogens have been characterized, molecular structures have been determined for only 3 bacterial produced factor H binding proteins such as BbCRASP for *B. burgdorferi*, fhbB for *T. denticola* and Fhbp for *N. meningitis* [23–25]. The sequence identity between these proteins is low (Figure S1) and the three determined structures do not share

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structure homologue (Figure S2). The major sequence motifs or structural signatures for factor H recognition remain unknown. Determination of additional factor H binding protein structures and further elucidation of bacterial factor H recognition mechanism are still important for infectious diseases prevention and treatment. Fhb, a 77 KD cell surface anchor protein, is composed of four parts including a signal peptide with 44 amino acid residues, an N-terminal factor H binding domain N-Fhb, a C-terminal domain and an LPXTG cell wall anchor motif. The N-Fhb protein is rich in β strand. In this study, we reported the expression of N-Fhb in *E. coli* and its purification and crystallization. Furthermore, we also determined the crystal structure of N-Fhb using Se-SAD method.

## 2. Materials and methods

### 2.1. Materials

The pfu polymerase and T4 DNA ligase were purchased from NEB, while the two restriction enzymes Nde I and Xho I were purchased from Takara. Chromatographic columns including a His Trap 5-ml column and a Superdex-200 column were purchased from GE Healthcare. The proteinase cocktail inhibitor was purchased from Roche and the commercial crystal screening kits were all purchased from Hampton Research.

### 2.2. Cloning, expression and purification of N-Fhb

The recombinant N-Fhb fragment is from 139 to 343aa. The gene for N-Fhb was amplified by PCR from the genomic of *S. suis* strain 05ZYH33. The primers are Fhb-F: 5′ CGCCATATGAAGCAACAGTCGCCATTAATTC 3′ and Fhb-R: 5′ CCGCTCGAGCTAAGTTTTTTCTTTCTCAAGGG 3′. The PCR product was digested with Nde I and Xho I, ligated into the plasmid pET28a using T4 DNA ligase and transformed to *E. coli* strain DH5α. Positive clones were selected by agar plates containing 100 μg/ml kanamycin and verified by DNA sequencing. Finally the recombinant expression vector was transformed into *E. coli* strain BL21 (DE3). The transformed BL21 cells were cultured in LB broth containing 100 μg/ml kanamycin at 37 °C to $OD_{600}$ = 0.8–1.0 and then induced by 0.5 mM IPTG at 30 °C for 4 h. Bacterial pellets were harvested by centrifugation at 5000 rpm for 10 min at 4 °C and re-suspended with buffer A (10 mM Hepes pH 7.5 and 500 mM NaCl) containing proteinase inhibitor cocktail. The pellets were lysed by high pressure cell cracker and the cell debris was removed by centrifugation of the lysates at 13,000 rpm for an hour at 4 °C. The supernatant was filtered with 0.22 μm filter and loaded onto Ni affinity chromatography column equilibrated with buffer A. The contaminated protein was washed with buffer containing 10 mM Hepes pH 7.5, 500 mM NaCl and 60 mM imidazole. The target protein was eluted with 10 mM Hepes pH 7.5, 500 mM NaCl and 250 mM imidazole. Further purification was achieved by gel-filtration chromatography on Superdex 200 equilibrated with buffer A. The purified protein was desalted with crystallization buffer (10 mM Hepes pH 7.5 and 200 mM NaCl) and concentrated to about 50 mg/ml. Se labeled N-Fhb protein was expressed and purified as follows. The recombinant expression vector was transformed into B834 competent cells and the cells were cultured in medium containing Se labeled methionine at 37 °C for 6 h and were induced with 0.5 mM IPTG at 30 °C for 6 h. The purification procedures were the same as the native protein. The purified Se labeled protein was concentrated to about 30 mg/ml.

### 2.3. Crystallization

The protein sample was centrifuged at 12,000 rpm for 10 min at 4 °C to clarify the solution before crystallization. Crystal screening was carried out at 18 °C with crystal screen agent kits I and II, PEG Ion Screen, Natrix and Index using the sitting drop vapor diffusion method. Drops containing 1 μl protein solution and 1 μl reservoir solution were equilibrated with 100 μl reservoir solution. Crystal optimization was performed by refining the initial condition with adjustment of protein concentration and precipitant concentration.

**Table 1**
Data collection and refinement statistics of N-Fhb.

| Parameter | N-Fhb |
|---|---|
| Data collection | |
| Wavelength (Å) | 0.9793 |
| Resolution (Å) | 50–1.5 |
| Space group | C2 |
| Cell parameters (Å) | |
| a(Å) | 127.1 |
| b(Å) | 77.3 |
| c(Å) | 131.6 |
| α, β, γ (°) | 90, 115.9, 90 |
| Completeness (%)[a] | 97.4 (95.6) |
| Redundancy[a] | 7.4 (7.2) |
| I/Sig I[a] | 40.6 (2.9) |
| Total reflections | 352,817 |
| Unique reflections | 169,683 |
| $R_{merge}$ (%)[b] | 6.3 (95) |
| Refinement | |
| Resolution (Å) | 1.50 |
| $R_{work}$ (%)[c] | 0.161 |
| $R_{free}$ (%)[d] | 0.189 |
| Protein atoms | 8006 |
| Water molecules | 1078 |
| *RMS deviations* | |
| Bond lengths (Å) | 0.022 |
| Bond angles (°) | 1.932 |
| B-value ($Å^2$) | 20.7 |
| *Ramachandran plot (%)* | |
| Favored regions (%) | 97 |
| Allowed regions (%) | 3 |
| Disallowed regions (%) | 0 |
| PDB code | 5BOB |

[a] Values in parentheses are for the highest-resolution shell (1.55–1.50 Å for Fhb 2.75–2.7 Å for Fhb bound to Gb2).
[b] $R_{merge} = \Sigma|(I_{hkl})-<I>|/\Sigma(I_{hkl})$, where $I_{hkl}$ is the integrated intensity of a given reflection and <I> is the mean intensity of symmetry equivalents.
[c] $R_{cryst} = \Sigma||F_{obs}|-|F_{calc}||/|F_{obs}|$, where $F_{obs}$ and $F_{calc}$ are the observed and calculated structure factors, respectively.
[d] $R_{free}$ is the R-factor calculated using a randomly selected 5% of reflection data withheld from the refinement.

### 2.4. Data collection and processing and structure determination

Data was collected at the X Ray Beam line BL17U at Shanghai

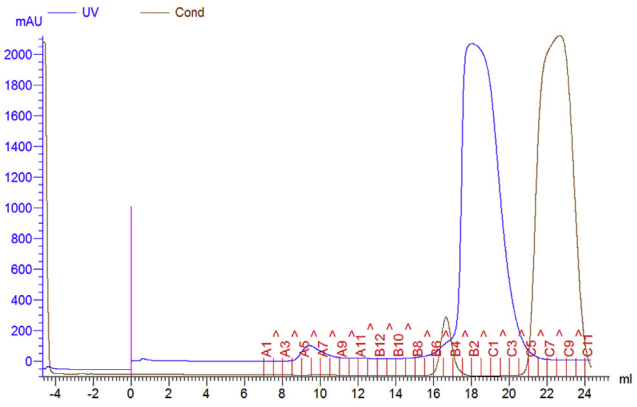


**Fig. 1.** The purification profile of N-Fhb on Superdex 200.