

Construction, Expression, and Purification of ZNF191 (243-368) Zinc Finger Deletion Mutants

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Abstract

The C-terminal region of the zinc finger protein ZNF191(243-368) contains a putative DNA-binding domain containing four Cys₂His₂ zinc finger motifs. To understand the properties and functions of the zinc finger motifs, a deletion gene of ZNF191(243-368) was inserted into pGEX-4T-2. The recombinant vector was transformed into Escherichia coli BL21, and a glutathione S-transferase (GST) fusion protein was expressed and purified using glutathione agarose affinity resin. The results show that this expression system can be used to express and purify zinc finger deletion mutants of ZNF191(243-368), providing a basis for further investigation of this protein.

Keywords: Affinity purification; Deletion mutant; GST fusion protein; Zinc finger protein; Recombinant vector.



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1. Introduction

The zinc finger protein 191 (ZNF191) gene encodes a 368 amino acid Krüppel-type (C₂H₂) zinc finger protein, which includes a putative DNA-binding domain with four Cys₂His₂ zinc finger motifs at its C-terminus. ZNF191(243-368), the zinc finger region of ZNF191, presumably acts as the main functional region of ZNF191. However, the actual function of ZNF191(243-368) remains unclear [1, 2]. To study the properties, structure, and functions of ZNF191 and ZNF191(243-368), their in vitro expression and purification is crucial.

Although exogenous proteins have been expressed using various expression systems, inclusion body formation, exogenous protein toxicity, and side-chain modification can influence expression and complicate purification [3, 4]. Therefore, it has been difficult to obtain proteins for in vitro study. In our previous studies, the expression and purification of ZNF191(243-368) were proved very difficult because of the formation of inclusion bodies. Fusion expression systems can facilitate protein expression and simplify protein purification [5]. However, each expression system has unique characteristics that affect how they can be used. For example, we attempted to use the His-tag expression system to promote the expression and purification of exogenous ZNF191(243-368); however, the His-tag affected the properties and structure of ZNF191(243-368). In addition, the glutathione S-transferase (GST) expression system has also been used to generate GST-ZNF191 (243-368) [6].

Although GST-ZNF191 (243-368) is not stable, its degradation can be controlled, and the zinc finger protein can be cleaved from the GST fusion protein at a thrombin digestion site between the proteins [7]. To fully understand the function of ZNF191 (243-368), especially the properties, structures, and functions of each zinc finger motif, various zinc finger deletion mutants of ZNF191 (243-368) must be expressed and purified. The GST fusion expression system is a simple and quick method that exploits the specific affinity of GST for glutathione agarose affinity resin.

This study aimed to express and purify the GST fusion protein of ZNF191 (243-368) deletion mutants, providing the basis for further investigation of the properties and functions of ZNF191 (243-368) and ZNF191.

2. Materials and Methods

Table-1. Primers Used to Generate the Zinc Finger Deletion Mutant

Mutants	Forward Primer	Reverse Primer
GST-dF4	5'- CGCGGATCCAGAAATCCCTCT CGAAAGAAACAA-3'	5'- TCCCCGGGTTAAGGTTTCTCCCCA GTATG-3'
GST-dF3F4	5'- CGCGGATCCAGAAATCCCTCT CGAAAGAAACAA-3'	5'- TCCCCGGGTTAAGGTTTTTCTCCA GTGTGG-3'
GST- dF2F3F4	5'- CGCGGATCCAGAAATCCCTCT CGAAAGAAACAA-3'	5'- TCCCCGGGTTAAGGTTTCTCCCCA CTGTGAAT-3'
GST-dF1	5'-	5'-

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	CGCGGATCCATACCTACACTT CTCACACCCTTTCGT-3'	TCCCCGGGGTTAAACTTCCACAAC ATTCAGAAG-3'
GST-dF1F2	5'- CGCGGATCCATGTTTACAGTTC TTACACCCTTTCGG-3'	5'- TCCCCGGGGTTAAACTTCCACAAC ATTCAGAAG-3'
GST-dF1F2F3	5'- CGCGGATCCATACTTACGCAA GTCACACCCAAAAGC-3'	5'- TCCCCGGGGTTAAACTTCCACAAC ATTCAGAAG-3'
GST-dF1F4	5'- CGCGGATCCATACCTACACTT CTCACACCCTTTCGT-3'	5'- TCCCCGGGGTTAAGGTTTCTCCCCA GTATG-3'
GST-dF1F3F4	5'- CGCGGATCCATACCTACACTT CTCACACCCTTTCGT-3'	5'- TCCCCGGGGTTAAGGTTTTTCTCCA GTGTGG-3'
GST-dF1F2F4	5'- CGCGGATCCATGTTTACAGTTC TTACACCCTTTCGG-3'	5'- TCCCCGGGGTTAAGGTTTCTCCCCA GTATG-3'

* dF denotes the zinc finger was deleted.

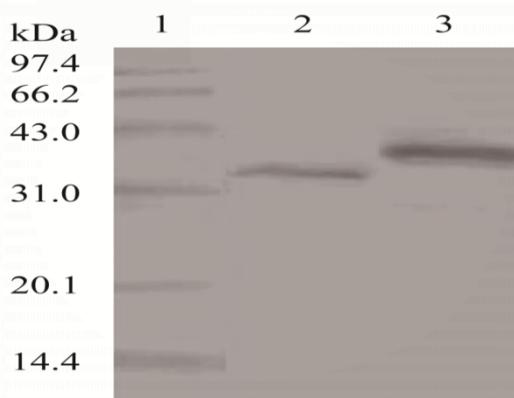
A cDNA fragment encoding the zinc finger region of ZNF191 was subcloned by PCR to create a GST fusion protein. The oligonucleotide primers (Union Genetic Company, Shanghai, China) used in the polymerase chain reaction (PCR) are shown in Table 1. The PCR template was the pTSA-ZF vector [8]. The zinc finger gene was amplified using a forward primer (which introduced a BamHI site) and a reverse primer (which introduced a SmaI site). The PCR-amplified fragment and the pGEX-4T-2 (Amersham Pharmacia) vector were each incubated with BamHI and SmaI, gel purified, and ligated together using T4 ligase (BioLabs). The ligation product was transformed into Escherichia coli BL21 cells. The bacterial clone was verified by DNA sequencing.

Lysogeny broth (LB; 50 mL) containing 100 µg/mL ampicillin was inoculated with a single, freshly picked colony containing the expression plasmid, and then incubated overnight at 37°C. The overnight culture was diluted 100 fold in 500 mL of 2YT medium and then grown at 37°C to an optical density at 600 nm (OD₆₀₀) of 0.6. Isopropyl-β-D-1-thiogalactopyranoside was then added to a final concentration of 0.5 mM, and cells were induced for 3 h at 30°C. The cells were harvested by centrifugation at 6,000 rpm and 4°C, resuspended in 100 mL ice cold phosphate-buffered saline (PBS; pH 7.4) with 10 mM β-mercaptoethanol, and then lysed with lysozyme at 4°C for approximately 30 min. The mixture was supplemented with 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, and 5 U/mL DNase I (Sango Company, Shanghai, China) with stirring for 30 min at 4°C to aid protein solubilization, and then centrifuged at 15,000 rpm for 30 min at 4°C. The supernatant was mixed with 10 mL of Glutathione Sepharose 4B slurry (Amersham Pharmacia Biotech) and then shaken for 30 min. The affinity matrix was extensively washed with PBS until it reached an OD₂₈₀ < 0.02, and the GST fusion proteins were eluted in elution buffer (50 mM Tris and 100 mM reduced glutathione, pH 8.0). The eluted solution was concentrated using an Amicon YM-10 centrifugal filter unit (Millipore) and passed through a Sephadex G75 column.

The purified proteins were mixed with 2 × SDS loading buffer and boiled for 10 min to prepare the samples. The samples were detected by 15% Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

3. Results and Discussion

Figure-1. SDS-PAGE of GST-dF1F4 and GST-ZNF191(243-368)



Lane 1: protein marker, Lane 2: GST-dF1F4, Lane 3: GST-ZNF191 (243-368).

The DNA sequence of the recombinant plasmid demonstrated that the N-terminus of the zinc finger gene was linked with the C-terminus of the GST gene. This plasmid expressed a fusion protein that contains C2H2-type tandem zinc finger motifs at the C-terminus of the fusion protein. Bacterially expressed GST fusion protein was purified by Glutathione Sepharose 4B and Sephadex G75 column in accordance with a previously described method.

The proteins were expressed under optimum conditions: the bacteria grown to OD600 = 0.6 under 30 °C, then induced by 0.5 mM IPTG for 3 h. The protein purified by column was detected by SDS-PAGE.

SDS-PAGE analysis of the purified proteins shows a prominent band of the expected size of the fusion protein, approximately 40 kDa of GST-ZNF191 (243-368), and 35 kDa of GST-dF1F4 (Figure 1). The corresponding proteins showed a single band, indicating that the protein purity was higher than 90% and the position of the electrophoretic band was consistent with the expected molecular weight of the proteins. This indicated that target proteins were obtained in vitro, and GST expression systems are suitable for expressing and purifying the zinc finger deletion mutants of ZNF191(243-368).

4. Conclusion

The zinc finger deletion mutants of ZNF191 (243-368) can be obtained from GST expression system, which is simple for expressing and purifying of zinc finger proteins. And purified zinc finger can be used to further examine the function of ZNF191.

Acknowledgements

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