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Generation of deletion mutants of Goat Vitronectin spanning the residues from 340 to 355 by inverse PCR based mutagenesis

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Abstract

Vitronectin is one of the two major extracellular matrix proteins having multiple roles in cell physiology of human beings and animals. The protein structure has many domains including a heparin binding domain. The heparin binding domain of Human vitronectin plays an important role in angiogenesis, hemostasis, wound healing and is also implicated in tumor growth and metastasis, binding of microbes. The role of heparin binding domain of goat vitronectin has not been studied in detail. The present study involved generation of two deletion mutants of goat vitronectin spanning the residues 341 to 355 of the proposed region, by PCR based sited directed mutagenesis combined with homologous recombination. The mutants could be generated successfully without any undesired alterations. Small scale induction of these mutant clones was successful which could be used for production of recombinant protein. Functional studies of the protein like cell and microbial binding can be done in future using these mutant proteins.

Keywords: Vitronectin, Goat, Heparin binding region, Mutagenesis

1. Introduction

Vitronectin is a multifunctional glycoprotein present in the extracellular matrix and platelets, produced by liver predominantly. It is also known as S protein, serum spreading factor and epibolin. Vitronectin is found predominantly as a monomer in blood and as an oligomer in the extracellular matrix and platelet releasates (Schvartz *et al.*, 1993) [1]. Human vitronectin is known to have domain organization containing Somatomedin B domain, Connecting region, Hemopexin like domains and the Heparin binding domain (Suzuki *et al.*, 1984) [2]. Among the species, bovine, goat and trout vitronectin are unique in having two RGD motifs (Mahawar *et al.*, 2008) [3]. VTN plays diverse roles in wound healing, metastasis, regulation of membrane attack complex (MAC) formation angiogenesis, hemostasis, cell adhesion and spreading, cell migration, tissue repair, etc (Chillakuri *et al.*, 2010) [4]. Vitronectin promotes neutrophil infiltration and enhances inflammatory process during infections. It also protects the bacterial surface from MAC mediated lysis and enhances adhesion of bacterium. Vitronectin play an important role in bacterial pathogenesis by conferring bacterial serum resistance and in internalization of pathogens (Singh *et al.*, 2010) [5]. Malignant cells are also reported to use VTN for migration and blocking this interaction may be a therapeutic strategy in cancer treatment (Smith *et al.*, 2010) [6]. VTN binds to many biological substances like thrombin-antithrombin III (Jenne *et al.*, 1985) [7], the complement C5b-7 complex (Podack *et al.*, 1977) [8] heparin (Hayman *et al.*, 1983) [9]. These interactions are responsible for its multifunctional physiological roles like blood coagulation, complement modulation, cell attachment and spreading etc.

Goat vitronectin heparin binding domain has been characterized recently and it indicated that putative binding sites for ligand binding are available similar to human vitronectin (Murugavel *et al.*, 2025) [10]. These putative residues of goat VTN HBD is implicated in multimerisation of

the protein and is not involved in Vero cell binding. The present study was aimed to create precise deletion mutants of the heparin binding region spanning the residues 340 to 355 by PCR based site directed mutagenesis for further characterization and functional studies.

2. Materials and Methods

Materials

Restriction enzyme DpnI and a 1 kb DNA ladder were obtained from New England Biolabs (USA). Pfu DNA polymerase was procured from Fermentas Life Sciences (USA). The prokaryotic expression vector pPROExHT system (Life Technologies, USA) and plasmid pVnFL encoding recombinant full-length goat vitronectin was obtained from the laboratory repository. The PrepEase™ gel extraction kit was purchased from USB (USA). Agarose and ethidium bromide were purchased from Bangalore Genei (India). Deoxynucleotide triphosphates (dNTPs) were obtained from Biogene (USA). Oligonucleotide primers were synthesized by GCC Biotech Pvt Ltd (Kolkata, India). All other reagents used were of analytical grade and high purity.

Methods

Deletion Mutagenesis and PCR

Recombinant full-length goat vitronectin plasmid obtained from the laboratory repository was revived and isolated using the alkaline lysis method. The amino acid region spanning residues 340-355 of goat vitronectin was compared with consensus sequences of known heparin-binding proteins and was found to contain overlapping putative heparin-binding motifs. Based on this analysis, two deletion mutants were generated: one lacking residues 341-348 (R3D; Δ 341-348) and another lacking residues 349-355 (R4D; Δ 349-355). Deletion mutagenesis of the targeted region was performed using inverse PCR based homologous recombination (Pathak *et al*, 2019)^[11], with minor modifications.

Briefly, Mutagenesis PCR was carried in a volume of 25 μ L reaction mix in eppendorf gradient thermal cycler using standard conditions. The annealing temperature was fixed at 71.5°C and 71°C for R3D and R4D respectively. After PCR amplification, 1 μ L of DpnI (20 U) was added to the reaction mixture, which was then incubated at 37 °C for 16-18 hours to selectively digest the parental methylated plasmid DNA. After a recovery period of 1 hour in 1 mL of antibiotic-free LB broth, the transformed cells were plated onto LB agar containing ampicillin (100 μ g/mL) and incubated at 37°C overnight. Individual colonies were selected and cultured overnight in 5 mL LB broth supplemented with ampicillin (100 μ g/mL).

Mutant Detection PCR

Selected bacterial colonies were screened for the presence of deletions using mutant detection PCR, which distinguishes wild-type and mutant constructs based on differences in amplicon size. Deletion mutants yielded shorter PCR products compared with the wild-type construct upon agarose gel electrophoresis.

PCR amplification was carried out using goat wild-type vitronectin-specific primers, as described above, for 29 cycles with an annealing temperature of 61 °C for 1 minute. Plasmid DNA isolated from selected colonies served as the template, while recombinant wild-type goat vitronectin plasmid was used as the template control.

PCR products were resolved on 1.0% agarose gels prepared in 1× Tris-acetate-EDTA (TAE) buffer and visualized under

ultraviolet illumination using a gel documentation system. Clones yielding reduced-size amplicons in the mutant detection PCR were selected and submitted for DNA sequencing (GCC Biotech Pvt Ltd, Kolkata, India) to verify and confirm the targeted deletions. The sequence of the mutagenic primers used were given in table 1.

Table 1: Sequence of the mutagenic primers

HBD R3D Forward	5' TCC CCC CGG GCC CAT CGC AAA CGT TAC CGC TCG 3'
HBD R3D Reverse	5' ACG TTT GCG ATG GGC CCG GGG GGA GCT GGG AGC 3'
HBD R4D Forward	5' TCT GCG CGG CGC CTC CGA AGC CGT GGC CGA GGC 3'
HBD R4D Reverse	5' ACG GCT TCG GAG GCG CCG CGC AGA CTT AGT CAT 3'

Small-Scale Expression Analysis of Recombinant Clones

Positive recombinant clones were evaluated for protein expression under small-scale induction conditions. Individual colonies were inoculated into Luria-Bertani (LB) medium supplemented with ampicillin and incubated overnight at 37°C with continuous shaking. The overnight culture was subsequently diluted 1:100 into fresh LB broth containing ampicillin and allowed to grow at 37°C until the optical density at 600 nm reached approximately 0.6, indicating mid-logarithmic phase.

Prior to induction, 1 mL of the culture was collected and designated as the uninduced control. The remaining culture was induced by adding isopropyl β -D-1-thiogalactopyranoside (IPTG) to a final concentration of 1 mM. Both induced and uninduced cultures were further incubated at 37°C with vigorous agitation for 3 to 5 hours.

Following incubation, bacterial cells were harvested by centrifugation, and the resulting pellets were stored at -20°C until further analysis. For protein extraction, each pellet derived from 1 mL of culture was suspended in 20 μ L of sterile normal saline, followed by the addition of 30 μ L of 5× SDS sample loading buffer. The mixture was incubated at room temperature for 5 minutes to ensure complete denaturation of proteins. Protein expression was subsequently analysed by electrophoresis on a 5%-15% linear gradient sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE).

3. Results

Generation and Validation of Deletion Mutants

Site-directed mutagenesis using an inverse PCR strategy was performed to generate targeted deletions within the proposed functional region of the gene. Successful amplification of the modified constructs was confirmed by mutant detection PCR, which demonstrated the expected reduction in amplicon size compared with the wild-type template of 1.3kb, consistent with the designed deletions.

Sequence analysis of the amplified mutant constructs verified the precise removal of the intended nucleotide segments without the introduction of unintended mutations or frame shifts. Two deletion variants were successfully generated, corresponding to amino acid residues 341-348 and 349-355, respectively. Sequencing chromatograms confirmed the integrity of the remaining coding sequence in both constructs.

Small-Scale Expression of Deletion Mutants

The recombinant deletion mutants were evaluated for inducible protein expression under small-scale conditions. SDS-PAGE analysis of induced and uninduced cultures revealed a prominent protein band at approximately 60 kDa in

IPTG-induced samples. This band was absent or faint in the corresponding uninduced controls, confirming efficient induction of mutant protein expression. The expression was optimal between 2-3 hours post IPTG induction for both the mutants. Both deletion constructs exhibited comparable expression levels, indicating that removal of residues 341-348 and 349-355 did not significantly impair protein stability or inducible expression under the tested conditions.

4. Discussion

The successful generation of precise deletion mutants through inverse PCR demonstrates the reliability of this approach for targeted modification of the gene of interest. The absence of off-target mutations upon sequencing further validates the specificity of the mutagenesis strategy.

Importantly, both deletion variants retained robust expression at the expected molecular weight of approximately 60 kDa, suggesting that the deleted regions are not essential for protein expression or stability in the bacterial system. These findings indicate that the constructs are suitable for subsequent large-scale expression and purification.

The availability of these deletion mutants provides valuable tools for downstream functional analyses, including cell-binding assays, multimerization studies, and bacterial interaction assays. Such investigations will help delineate the functional significance of residues 341-348 and 349-355 in the biological activity of the protein.

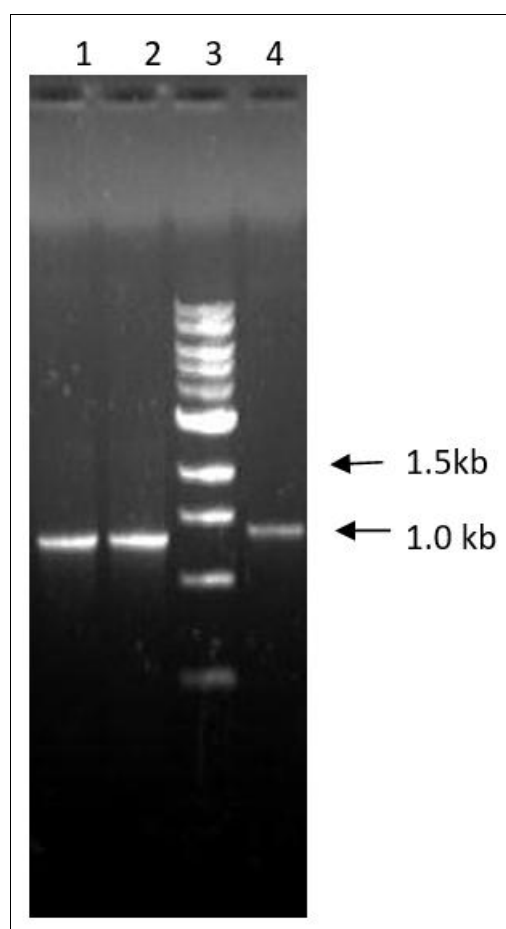


Fig 1: Mutant detection PCR

Lane 1- R3D amplicon Lane 2- R4D amplicon Lane 3- molecular weight markers
Lane 4 - Wild Vitronectin amplicon.
Note the difference in size of amplicons.

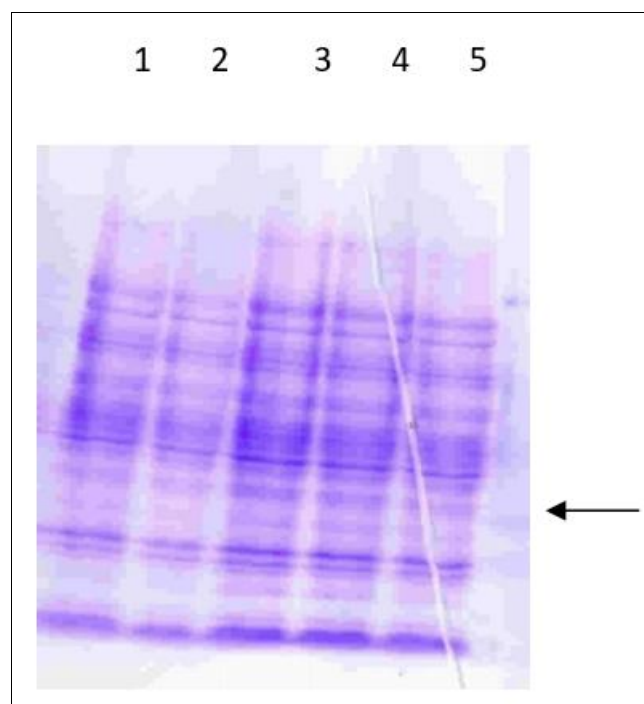


Fig 2: Small scale induction R3D clone

Lane 1- Uninduced clone Lane 2-5: 1 hour, 2 hour 3 hour 4 hour post induction respectively.
Induced protein expression shown in arrow.

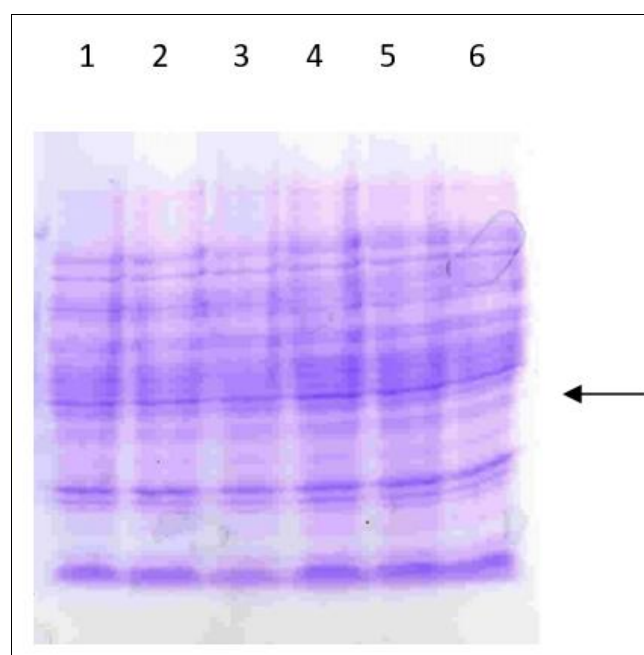


Fig 3: Small scale induction R4D clone

Lane 1- Uninduced clone Lane 2-6: 1 hour, 2 hour, 3 hour, 4 hour, 5 hour post induction respectively.
Induced protein expression shown in arrow.

5. Conclusion

Inverse PCR based mutagenesis of the proposed region was successful as evidenced by mutant detection PCR. The mutants showed desired deletions with no undesirable changes upon sequencing of mutants. Two deletion mutants spanning the regions 341-348 and 349-355 were created successfully. Small scale induction of the mutants were successful indicating protein induction at 60 kDa regions

when compared with the uninduced controls. These could be used for bulk production of the desired mutants in future which could be used for functional studies like cell binding, multimerisation studies and bacterial binding studies.

Conflict of Interest

Not available

Financial Support

Not available

References

1. Schwartz I, Seger D, Shaltiel S. Vitronectin. *Int J Biochem Cell Biol.* 1999;31(5):539-544.
2. Suzuki S, Pierschbacher MD, Hayman EG, Nguyen K, Ohgren Y, Ruoslahti E. Domain structure of vitronectin: alignment of active sites. *J Biol Chem.* 1984;259(24):15307-15314.
3. Mahawar M, Joshi P. Goat vitronectin: characterization and binding to *Staphylococcus aureus*. *Comp Biochem Physiol B Biochem Mol Biol.* 2008;149(3):410-418.
4. Chillakuri CR, Jones C, Mardon HJ. Heparin binding domain *in vitronectin* is required for oligomerization and thus enhances integrin mediated cell adhesion and spreading. *FEBS Lett.* 2010;584(15):3287-3291.
5. Singh B, Su YC, Riesbeck K. Vitronectin in bacterial pathogenesis: a host protein used in complement escape and cellular invasion. *Mol Microbiol.* 2010;78(3):545-560.
6. Smith HW, Marshall CJ. Regulation of cell signalling by uPAR. *Nat Rev Mol Cell Biol.* 2010;11(1):23-36.
7. Jenne D, Stanley KK. Molecular cloning of S-protein, a link between complement, coagulation and cell-substrate adhesion. *EMBO J.* 1985;4(12):3153-3157.
8. Podack ER, Kolb WP, Müller-Eberhard HJ. The SC5b-7 complex: formation, isolation, properties and subunit composition. *J Immunol.* 1977;119(6):2024-2029.
9. Hayman EG, Pierschbacher MD, Ohgren Y, Ruoslahti E. Serum spreading factor (vitronectin) is present at the cell surface and in tissues. *Proc Natl Acad Sci U S A.* 1983;80(13):4003-4007.
10. Murugavel S, Joshi P. Elucidation of heparin binding domain of goat vitronectin by deletion mutagenesis. *Indian Vet J.* 2025;102(3):1-4.
11. Pathak H, Sokkalingam M, Prasanth L, Devi K, Joshi P. *Staphylococcus aureus* autolysins interact with caprine vitronectin without involving the heparin binding domain and the second arginine-glycine-aspartic acid motif of the host protein. *Arch Microbiol.* 2019;201(5):639-647.

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