Effect of Mutation on Aggregation-Prone Protein

Stabilization of the von Willebrand Factor, a domain found in the honeybee egg yolk protein Vitellogenin, by inducing mutations in the expressing gene

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BACKGROUND

Vitellogenin (Vg) is an ancient, multifunctional protein and the main yolk precursor for eggs in honeybee and all egg-laying animals. Honeybee Vg acts, among other functions, as lipid transporter and an antioxidant. Its titer positively correlates with the honeybee's oxidative stress tolerance[1], and an elevated Vg titer is linked to prolonged life span in honeybees[2]. The von Willebrand Factor (vWF) is a conserved structural element of Vg and likely plays an important role in interactions with lipid or other proteins, contributing to the important functions of the protein. The domain doesn't behave well in bacterial expression system and undergo forms of aggregation and fragmentation. We here modify an expression system of the vWF with the aim of producing a workable NMR sample for exploring structure-function relationships of this part of Vg. The project involves introducing mutations in the WT gene, express protein and analyze the protein structure to validate the prediction by Aggrescan.

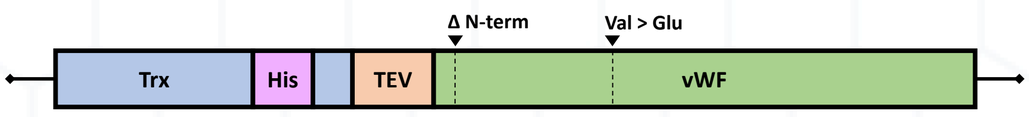


Fig 3: The insert elements in pET32a-Trx-vWF construct. vWF expressing gene inserted in pET32a plasmid. The location of mutations we aim to cause is determined from the prediction from Aggrescan, shown in Fig 2.

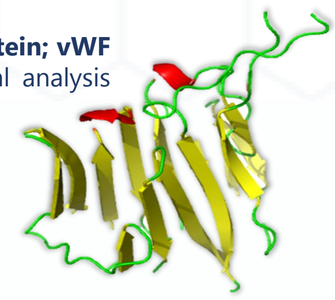
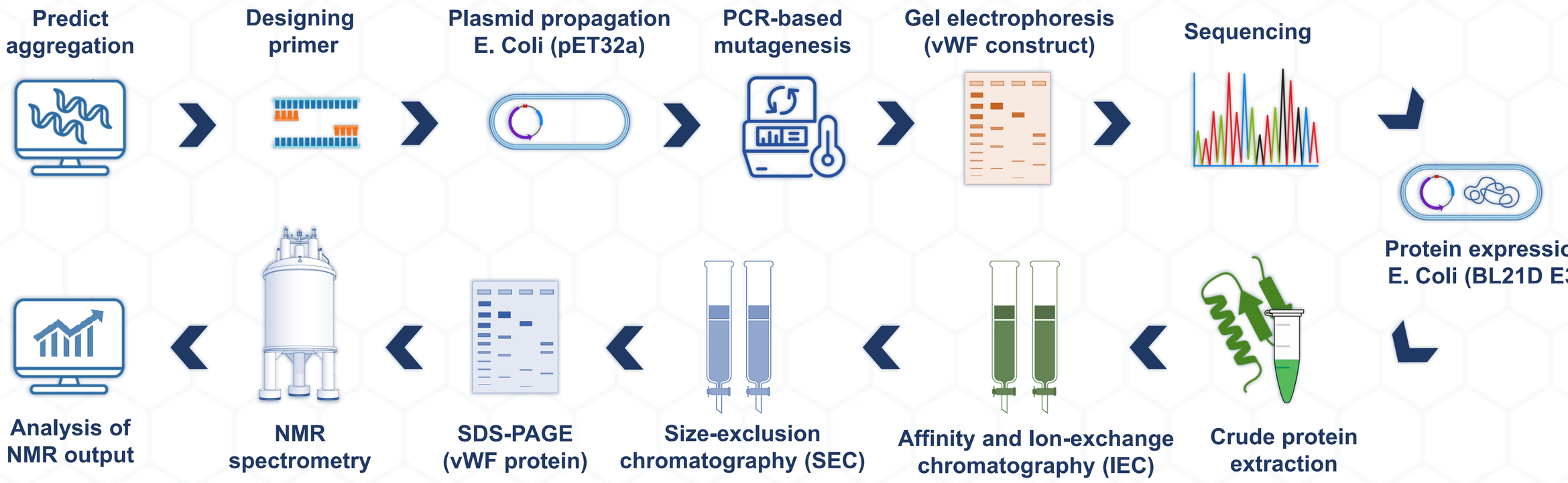


Fig. 1: AlphaFold 3 predicted model of the expressed protein; vWF domain. The AF3 output is input for Aggrescan's structural analysis and aggregation prediction shown in Fig 2.

Fig 2: Aggrescan4D plot of predicted aggregation propensity of vWF. The x-axis is the amino acids; the y-axis is the aggregation propensity score. Residues with score above 0.0 are considered part of a structural aggregation-prone region; higher scores indicate a greater propensity for aggregation. The plot shows the positions of the mutations introduced in the expressed protein; N-term deletion and Val to Glu (V→E).

METHODS



RESULTS

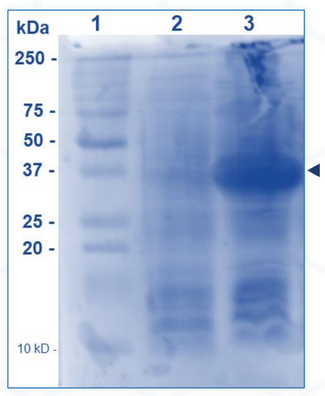


Fig 4: Expression of mutated Trx-vWF in BL21D E3 cells. The cells were treated with ITPG, inducing the gene expression of the target protein. Lane 2: Bacteria lysates from un-induced culture; Lane 3: Bacteria lysates after induction with 1 mM ITPG. The gel show a distinct band after induction at ~ 40 kDa, as expected, suggesting successful expression.

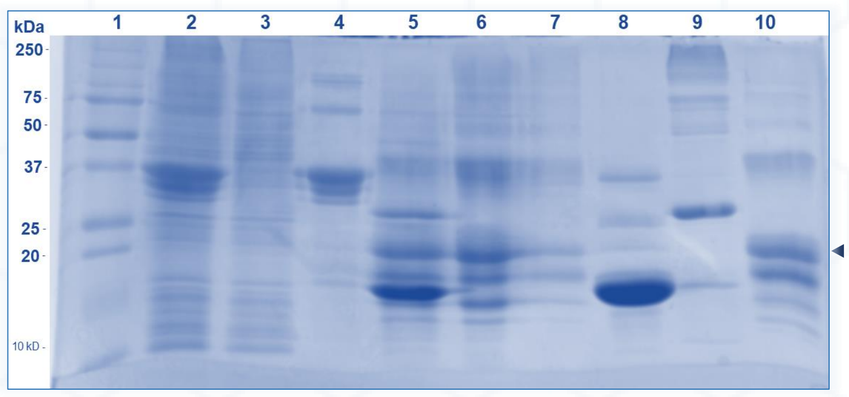


Fig 5: Purification of mutated vWF domain. After lysis of the cells expressing recombinant protein, a series of separations techniques based on affinity and size were applied, as shown in, lane 2; cell lysate, lane 3; flow-through of first His-trap, lane 4; His-trap bound, lane 5: TEV digestion, lane 6; flow-through of second His-trap, lane 7, 8, 9; His-bound after TEV digestion, lane 10; vWF after size-exclusion chromatography. The gel shows a band at approx. 22 kDa as expected, but many other bands are also present.

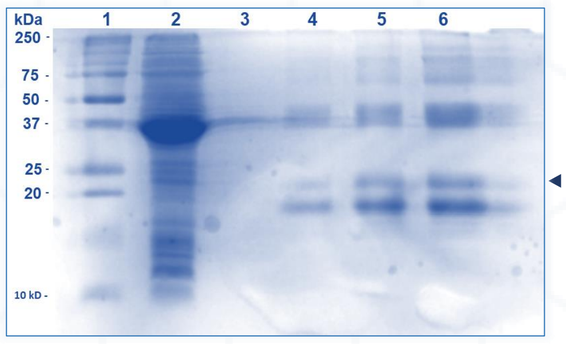


Fig 6: SDS-PAGE analysis of vWF domain after purification. Lane 4, 5, 6; vWF domain protein after ion exchange and size-exclusion chromatography. The gel show the vWF domain band at ~ 22 kDa, as expected (**insert same arrow***). Additional bands are visible, indicating possible degradation or contamination.

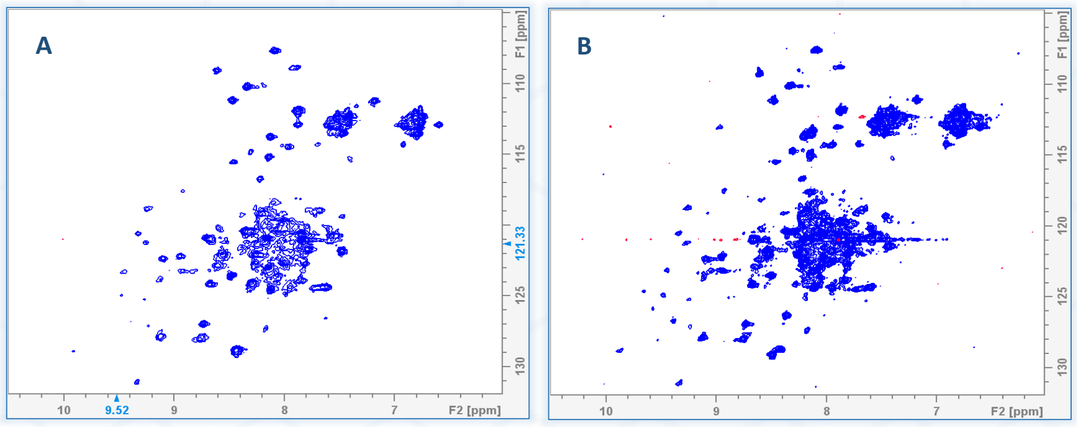


Fig 7: HSQC spectrum of ^{15}N labeled vWF domain protein recorded at (Bruker BioSpin) liquid-state high-field NMR spectrometer. Panel A represents 2D spectrum of vWF expressed protein with the mutations V→E and N-term deletion, acquired on a 600MHz instrument. Panel B represents vWF domain with the native sequence acquired at 850MHz. The result shows no difference between the WT and mutated vWF domain protein except for differences attributable to instrument fields strength.

CONCLUSION

The results of SDS-PAGE show the expected vWF band, but the presence of other bands indicates a possible degradation of the target protein, suggesting further optimization of the expression and purification is needed. The NMR results show that the introduced mutations (V→E) and N-term deletion have no reducing effect on the ability of the vWF domain of vitellogenin to form aggregations, as predicted in Aggrescan. Hence, different strategies like the use of large chaperones may be considered to improve the stability of the vWF Vitellogenin.

REFERENCES

1. **Salmela H, Harwood GP, Münch D, Elsik CG, Herrero-Galán E, Vartiainen MK, et al.** *Nuclear translocation of vitellogenin in the honey bee (Apis mellifera).* Apidologie. 2022;53(1):13.
2. **Havukainen, H., et al.,** *Vitellogenin Recognizes Cell Damage through Membrane Binding and Shields Living Cells from Reactive Oxygen Species *.* Journal of Biological Chemistry, 2013. 288(39): p. 28369-28381.

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