INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

Name of the Student	:	Pooja Rani Kuri
Roll Number	:	176106028
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Name of Thesis Supervisor(s)	:	Prof. Pranab Goswami
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SHORT ABSTRACT

Rotavirus, a primary contributor to severe cases of infantile gastroenteritis on a global scale, causes significant morbidity and mortality in the under-five population, particularly in middle to low-income countries, including India. Effective management of rotavirus disease encompasses both suitable diagnostic strategies and effective therapeutic interventions. Implementation of WHO-approved live-attenuated vaccines in certain regions of the world brought about a significant reduction in rotavirus-led gastroenteritis hospitalizations. However, they are linked to susceptibility to intussusception and exhibit low efficacy, primarily attributed to the high genetic diversity of rotavirus, varying over time and across different geographic regions. Herein, molecular data on Indian rotavirus A (RVA) has been reviewed through phylogenetic analysis, revealing G1P[8] to be the prevalent strain of RVA in India. The conserved sequences of rotavirus capsid proteins VP7, VP4 and VP6 across G1P[8] strain were used to examine helper T lymphocyte, cytotoxic T lymphocyte and linear B cell epitopes. Twenty epitopes were identified after evaluation of factors such as antigenicity, non-allergenicity, non-toxicity, and stability. These epitopes were then interconnected using suitable linkers and an N-terminal beta defensin adjuvant. The in silico designed vaccine exhibited structural stability and interactions with integrins (αvβ3 and αIIbβ3) and toll-like receptors (TLR2 and TLR4) indicated by docking and normal mode analyses. The immune simulation profile of the designed RVA multiepitope vaccine exhibited its potential to trigger humoral as well as cell-mediated immunity, indicating that it is a promising immunogen. These computational findings indicate potential efficacy of the designed vaccine against rotavirus infection.

Among the capsid proteins, the inner capsid protein, VP6, has received much attention as a promising candidate for next-generation vaccines against rotaviruses owing to its abundance in virion particles and high conservation. However, the formation of inclusion bodies during prokaryotic VP6 expression poses a significant hurdle to its research and application. Here, we employed experimental and computational approaches to investigate its inclusion body formation

and aggregation-prone regions (APRs). Heterologous recombinant VP6 expression in *E. coli* BL21(DE3) cells resulted in inclusion body formation, confirmed by transmission electron microscopy revealing amorphous aggregates. Thioflavin T assay demonstrated incubation temperature-dependent aggregation of VP6 inclusion bodies. Computational predictions of APRs in RVA VP6 protein were performed using sequence-based tools (TANGO, AGGRESCAN, Zyggregator, Waltz, FoldAmyloid, ANuPP, Camsol intrinsic) and structure-based tools (SolubiS, CamSol structurally corrected, Aggrescan3D). Out of a total of 24 consensus APRs identified, 21 were found to be surface-exposed in the VP6 structure. All identified APRs exhibit a predominance of hydrophobic amino acids, with proportions ranging from 33% to 100%. Computational identification of these APRs corroborates our experimental observation of VP6 inclusion body or aggregate formation. Characterization of VP6's aggregation propensity facilitates understanding of its behaviour during prokaryotic expression and opens avenues for protein engineering of soluble variants, advancing research on rotavirus VP6 in pathology, therapy, and diagnostics.

The rotavirus-led fatal infantile gastroenteritis globally demands a portable, specific, and low-cost diagnostic tool for its timely detection and effective surveillance in a mass population. Consequently, the design and development of an advanced biosensing technique for its detection is of paramount importance. A highly conserved 23-nucleotide sequence, 5' GCTAGGGATAAGATTGTTGAAGG 3', was identified through analysis of human RVA VP6 gene sequences and designated as the target. A molecular beacon of 33 nucleotides was designed with the sequence 5'[Fluorescein] ATAGTCCTTCAACAATCTTATCCCTAGCACTAT[Dabcyl]3', incorporating stem and loop regions. Secondary and tertiary structure characterization confirmed the desired stem-loop structure without internal secondary structures. The thermal stability of the molecular beacon-target complex was investigated using a temperature vs. Gibbs free energy change plot, melting curve analysis based on absorbance vs. temperature, and an experimental fluorescence resonance energy transfer melting assay. The melting temperature of the molecular beacon-target complex was experimentally determined to be 62°C. Spectral analysis showed fluorescence restoration in the presence of the synthetic VP6 target. Assay conditions were optimized with an excitation wavelength of 470 nm and a 10-minute incubation time. The assay demonstrated a linear correlation between fluorescence intensity restoration and target concentration, with a limit of detection of 18.8 nM. Interference studies with single mismatch, double mismatch, and scrambled targets revealed that the molecular beacon has strong specificity for the VP6 target, effectively discriminating against non-target sequences. This piece of work demonstrates the molecular beacon's potential as a sensitive and specific tool for detecting RVA VP6 gene, with promising applications in diagnostic assays for the rotavirus disease management.

This research enhances rotavirus disease management by leveraging the potential of capsid proteins for both therapeutic and diagnostic applications. The development of an RVA multiepitope vaccine, the probing of APRs in RVA VP6, and a sensitive molecular beacon-based genosensor for RVA detection offer promising advancements in disease prevention and detection, addressing key gaps in current strategies.