ABSTRACT

Rotaviruses cause severe diarrhea in humans and animals around the globe. One of its nonstructural proteins, NSP4, has been identified as the viral enterotoxin. This ER-localized glycoprotein is known to play crucial roles in the viral morphogenesis as well. Its cytoplasmic tail domain performs a myriad of functions in various stages of the life cycle of the virus. We have taken up structural investigations of this domain in order to obtain insights into the mechanism of virulence, species-dependent variation of the extent of infectivity, calcium binding properties and the interaction of the protein with double layered particles, capsid proteins of the virus and other cellular proteins. We crystallized the segment encompassing the virulence determining and the interspecies variable regions of NSP4 from different strains of the virus with variations in virulence properties and sequence. We were successful in obtaining the crystals of the recombinant forms of NSP4 fragments from some of the strains. While previously reported NSP4 protein fragments were shown to be tetrameric $\alpha$-helical coiled coils, NSP4\textsubscript{95-146} from an asymptomatic human strain ST3 and a symptomatic bovine strain MF66 have been found to form an unexpected and hitherto unknown pentameric coiled-coil structure which is presented in the thesis. The appearance of a new oligomeric state of the protein prompted us to explore the conditions relating to different oligomeric states of the protein. Results of structure analysis combined with gel chromatography experiments revealed that pH, presence of $\text{Ca}^{2+}$ ions and interaction with membranes dictate the oligomerization of this particular domain.

The molecular replacement method was employed to determine the structure of the first protein, ST3:NSP4\textsubscript{95-146}. Since the only known model available was that of a tetramer or a dimer of dimers, extensive attempts were carried out using these models to search for tetrameric structures but with no success. A more systematic approach with manual interventions at intermediate stages of molecular replacement yielded a solution which clearly showed the pentameric nature of the molecule. Refinement of the structure was complicated by severe anisotropy of the data and the presence of twinning and rotational pseudosymmetry in the crystal with its axis very
close to the twin operator axis. A clear molecular replacement solution could be obtained for MF66:NSP4_{95-146} using the refined structure of the pentameric ST3:NSP4_{95-146} as the search model.

Two significant structural features of the proteins presented here are that (i) the Ca^{2+} ion found at the core of the tetrameric structures coordinating with residues from the four chains is absent in ST3:NSP4_{95-146}, and (ii) extra electron density appears inside the core of the pentameric structure of MF66:NSP4_{95-146} which is elongated and spans about 20 residues on the N-terminal side of the helix which is largely hydrophobic in nature and is likely to correspond to a lipid/membrane molecule that interacts with the protein. Even though the existence of interaction between NSP4 and membranes was clearly established earlier, this is the first visualization of the interaction which also clearly indicates that, only in the pentameric structure, a lipid could be accommodated. Both these results are new and have implications in the functioning of NSP4 in many stages of the virus life cycle related to its association with Ca^{2+} and the membranes. Three other pentameric structures of dimensions similar to those reported here are available. A comparison of all these structures revealed membrane association, structural plasticity and interaction with hydrophobic ligands in the core as their common features.

Through the investigations presented here we demonstrated the structural plasticity of the rotaviral NSP4 protein and its ability to exist in different oligomeric states probably as a functional requirement to regulate and/or mobilize Ca^{2+} ions and to interact with membranes or due to the influence of a combination of these factors at various compartments of the cell with varying pH during the virus life cycle. Though it is difficult to predict at this point what the implications of these observations are on the structure of the entire C-terminal domain of NSP4, these results should give a new direction to the investigations involving NSP4 and its functions.