Purification and Characterization of Nipah Virus Nucleocapsid Protein Produced in Insect Cells

ABSTRACT

The nucleocapsid (N)protein of Nipah virus (NiV) is a major constituent of the viral proteins which play a role in encapsidation, regulating the transcription and replication of the viral genome. To investigate the use of a fusion system to aid the purification of the recombinant N protein for structural studies and potential use a diagnostic reagent, the NiV n gene was cloned into the pFastBacHT vector and and his tagged fusion protian was expressed in Sf9 insect cells by recombinant baculovirus. Western blot analysis of the recombinant fusion protein with anti-Niv antibodies produced a band of approximately 62 kDa. At time course study showed that the highest level of expression was achived after 3 days of incubation. Electron microscopic analysis of the NiV recombinant N fusion protein purified on a nickel-nitrilotriacetic asid resin column revealed different types of structures, including spherical, ring-like, particles. The light-scattering measurements of the recombinant N protein also confirmed the polydispersity of the sample with hydrodynamic radii of small and large types. The optical density spectra of the purified recombinant fusion protein reavealed a high A 260 / A 280 ratio, indicating the presence of nucleic acids. Western blotting and enzyme-linked immunosorbent assay result showed that the recombinant N protein exhibited the antigenic sites and conformation necessary for specific antigen-antibody recognition.