Characterization of the nucleic acid binding activity of inner core protein VP6 of African horsesickness virus.

de Waal P.J., and Huismans H.

Department of Genetics, University of Pretoria, Pretoria, South Africa, 0002

Minor structural protein VP6 is the putative helicase of African horsesickness virus (AHSV), an orbivirus in the Reoviridae family. As a first step towards characterizing of the enzyme activity of VP6, we investigated how the protein interacts with double-stranded (ds) RNA and other nucleic acids. The AHSV VP6 gene was expressed using a baculovirus and a bacterial expression system. Nucleic acid binding was assayed using an electrophoretic migration retardation assay and a nucleic acid overlay protein blot assay. VP6 bound double and single stranded RNA and DNA in a NaCl concentration sensitive reaction. The isoelectric point (pI) of truncated VP6 peptides was found to be important in determining the ability to bind to dsRNA. Of six truncated peptides investigated, two partially overlapping peptides bound dsRNA at pH 7.0, while other peptides with the same overlap did not. The distinction between the peptides appeared to be the pI, which was basic in the case of the peptides that did bind and acidic with respect to the peptides that did not bind. If the pH of the binding buffer was raised above the pI of those that bind (pH 10), no binding was observed and if it was lowered to less that of the lowest pI (pH 6.0), binding could be induced. The results suggest that dsRNA binding in the blot assay is very strongly affected by the net charge on the peptide.