

Title: Biophysical and Structural characterization of Hepatitis A Virus (HAV) capsid protein VP1 generated in a heterologous expression system

Abstract

Hepatitis A Virus (HAV) is a quasi-enveloped picornavirus, causes acute hepatitis in humans and infects approximately 1.4 million individuals annually, which does not include the asymptotically infected population. HAV, from being endemic in the Indian subcontinent and the far east, is slowly becoming a threat to public health. This is because improved socioeconomic conditions in specific pockets has drastically reduced the population of resistant individuals and increased the probability of outbreaks, with increased possibility of liver failure. The available vaccines against HAV are based on generation of infectious particles in culture, and are therefore too expensive for universal vaccination efforts. HAV is very slow growing in culture and procuring large quantities of purified virus drives up the cost of generating inactivated or live attenuated vaccines. Under the given circumstances, generating cheaper subunit vaccines against HAV is a priority. Recombinantly generated HAV structural proteins may also be utilized for other usages, like development of effective diagnostic tests.

We attempted several strategies for recombinant production of one of the major capsid proteins, VP1, from HAV in *E. coli*. While several efforts resulted in the formation of soluble aggregates or co-elution of VP1 with the bacterial chaperone GroEL, correctly folded VP1 was eventually generated in a discrete oligomeric form upon purification of the protein from inclusion bodies and refolding. The oligomers resemble pentamers of VP1 from other picornaviruses and appear to have the correct secondary and antigenic surface structure. These complexes can be utilized for

understanding the molecular pathway of HAV capsid assembly and can also have potential biomedical usages in prevention and diagnostics of HAV infections.