Pathway and Thermodynamic Analysis of HIV-1 Immature Particle Assembly

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1. Abstract

We have studied HIV-1 particle assembly in vitro, using Gag protein purified from E.coli. This protein lacks myristate at its N-terminus and the p6 domain at its C-terminus, and has an 84-residue deletion within the MA domain. The purified protein is soluble, but in the presence of cofactors it assembles into virus-like particles (VLPs) closely resembling authentic immature HIV-1. Cofactors include inositol hexakisphosphate (IP6) and nucleic acids.

We assess assembly in vitro by differential scanning fluorimetry, obtaining the thermostability of the respective species and both the Kd and the cooperativity of the IP6 response. Particles assembled upon addition of IP6 are denatured at a temperature ~ 10 □C higher than unassembled protein, reflecting the stabilization of Gag conformation due to Gag-Gag and Gag-IP6 contacts within VLPs.

Studies with Gag mutants yield further information on the mechanism of IP6 action. At physiological ionic strength, the highly charged polyanion IP6 binds to many sites on Gag (Datta et al., JMB 2007). This interaction is salt-sensitive, indicating that it is largely electrostatic in nature. We find that IP6-induced assembly is absolutely dependent upon the basic character of the nucleocapsid (NC) domain, even though in assembled VLPs, IP6 is localized in the capsid (CA) domain. Cooperativity of the IP6 response is drastically enhanced if the NC domain is replaced with a trimerizing isoleucine-zipper domain, but not with a dimerizing leucine-zipper domain. Taken together with other results, the results suggest that IP6-induced assembly is always initiated by the juxtaposition of Gag molecules at their C-termini, either by clustering of basic NC domains around IP6 or by zipper-mediated trimerization.

We have also characterized the IP6 response of Gag mutants which alter residues in CA that interact with IP6 in wild-type VLPs. In addition, recent data suggest that the pathway of assembly in response to RNA is quite different from that induced by IP6. This is significant since both RNA and IP6 participate in assembly of authentic particles in vivo.