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The delta domain of the HK97 major capsid protein is essential for assembly

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ABSTRACT

The 102 residue N-terminal extension of the HK97 major capsid protein, the delta domain, is normally present during the assembly of immature HK97 procapsids, but it is removed during maturation like well-known internal scaffolding proteins of other tailed phages and herpesviruses. The delta domain also shares other unusual properties usually found in other viral and phage scaffolding proteins, including its location on the inside of the capsid, a high predicted and measured α-helical content, and an additional prediction for the ability to form parallel coiled-coils. Viral scaffolding proteins are essential for capsid assembly and phage viability, so we tested whether the HK97 delta domain was essential for capsid assembly. We studied the effects of deleting all or parts of the delta domain on capsid assembly and on complementation of capsid-protein-defective phage, and our results demonstrate that the delta domain is required for HK97 capsid assembly.

Introduction

The assembly of many viral capsids requires the participation of proteins which are not present in the mature capsid (King and Casjens, 1974; Showe and Black, 1973). These are called scaffolding proteins and occur in two varieties, internal and external (Dokland, 1999; Fane and Prevelige, 2003; Prevelige and Fane, 2012). The tailed dsDNA phages (Casjens and Hendrix, 1988) and herpesviruses (Homa and Brown, 1997) utilize internal scaffolding proteins that are present during initial assembly, but they are removed before or during DNA packaging and final maturation of the capsid. In the absence of a functional scaffolding protein, major capsid proteins fail to assemble correctly, often making irregular structures called monsters or spirals (Cerritelli and Studier, 1996; Earnshaw and King, 1978; Hagen et al., 1976), tubes of capsid protein called polyheads (Howatson and Kemp, 1975; Kellenberger, 1980), or less frequently, smaller-than-normal complete capsid shells (Choi et al., 2006; Earnshaw and King, 1978; Thuman-Commike et al., 1998). Internal scaffolding proteins, as their name implies, occupy a significant space within procapsids and therefore must be removed before DNA can be fully packaged. This is often accomplished through the action of a capsid maturation protease, but scaffolding proteins sometimes exit through specialized ports in the capsid that close during maturation, as occurs for phage P22 (Prasad et al., 1993). Scaffolding proteins have also been found to have secondary structures that are dominated by α-helices (Morais et al., 2003; Tuma et al., 1996).

HK97 is a temperate dsDNA bacteriophage with a $T=7$ icosahedral capsid that does not have a separate scaffolding protein for capsid assembly (Duda et al., 1995b). Instead, the major capsid protein has a delta domain, a 102-amino-acid N-terminal extension, which is removed by the phage-encoded protease before DNA packaging. The term delta domain applies to parts of a major capsid protein (mcp) that are absent from mature capsids, originally applied to phage T4 (Tsugita et al., 1975). A schematic of HK97 capsid assembly and maturation is shown in Fig. 1. Assembly begins with the formation of hexamers and pentamers of the major capsid protein (Xie and Hendrix, 1995). These, collectively called capsomers, along with the portal protein and phage encoded protease, assemble into the immature Prohead I, with the delta domains in the interior of the capsid (Conway et al., 1995). Once assembly is complete, the protease is activated and cleaves the delta domain into peptides, and then cleaves itself (Duda et al., 2013). The liberated peptides appear to escape through holes in the shell (Conway et al., 1995; Duda et al., 1995b), leaving an empty capsid, Prohead II, which is ready for DNA packaging through the portal (Duda et al., 1995a). DNA packaging causes the capsid to expand and crosslink into the mature form, Head II.

Like viral scaffolding proteins, the HK97 delta domain is present during initial assembly, but absent in the mature structure (Conway et al., 1995; Duda et al., 1995b), suggesting that it may play similar crucial roles during the early steps of assembly. In most tailed-phage genomes, the scaffolding gene lies between the

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portal gene and the major capsid protein gene (Casjens and Hendrix, 1988; Hendrix and Duda, 1998). In the case of HK97, the delta domain, though fused to the major capsid protein, holds the same position in the genome. For a number of systems, including P22 (Greene and King, 1996, 1999; Weigele et al., 2005), P2 (Cerritelli and Studier, 1996), and the herpesviruses (Kennard et al., 1995), the respective scaffolding proteins bind to capsid interiors via their C-terminal ends, just as the HK97 delta domain is tied to the rest of the mcp by its C-terminus. In the absence of a detailed structure, the HK97 delta domain is predicted to be composed of three α-helical regions (Conway et al., 1995) with a short β-sheet near its C-terminus. The highly α-helical nature of the HK97 delta domain has been confirmed by Raman spectroscopy (Benevides et al., 2004), showing that it truly shares this common property of scaffolding proteins. Additionally, the sequence of the first two predicted α-helices of the delta domain give high scores when algorithms that calculate the probability of forming coiled-coils are applied (Conway et al., 1995). In herpesviruses, P22, T4, λ, and ∅29, proper capsid assembly is dependent on the presence of the scaffolding protein (Earnshaw and King, 1978; Ray and Murialdo, 1975; Tatman et al., 1994; Thomsen et al., 1994; Keller et al., 1986, 1988; Hagen et al., 1976; King et al., 1980). The HK97 delta domain shares many properties with other scaffolding proteins; therefore, one could hypothesize that the delta domain is required for HK97 capsid assembly. To test this, we made deletions of all or parts of the delta-domain segment of the HK97 major capsid protein gene and tested the assembly phenotypes of the mutants using plasmid expression studies. Our results confirm that the HK97 delta domain is essential for assembly.

Results and discussion

Expression of the HK97 major capsid protein without the delta domain

The HK97 maturation protease is not necessary for the full-length major capsid protein to assemble into Prohead I (Duda et al., 1995a, 1995b) and appears to be incorporated into proheads by binding to the delta domain (Conway et al., 1995; Duda et al., 2013). This suggests that the protease would not be needed for assembly in the absence of the delta domain. Therefore, we deleted the protease and the entire delta domain from our standard expression plasmid to make a delta-less variant called pNoDelta1. In this plasmid, the Met start codon of HK97 major capsid protein, gp5, was moved from its normal position to just upstream of codon 104, which encodes the first residue of the mature HK97 major capsid protein. This plasmid failed to express any detectable protein (not shown), so we added the ribosome binding site and the first five codons from the efficiently expressed bacteriophage T7 major capsid protein (Olins et al., 1988; Olins and Rangwala, 1989) to make plasmid pNoDelta2. This plasmid produced ample delta-less HK97 major capsid protein (Fig. 2). The proteins from pNoDelta2 and a control plasmid were expressed under radiolabelling conditions (Fig. 2A) and samples were collected 1, 5, and 45 min after label addition, along with the remaining soluble and pellet fractions of the culture. The wild-type control produced copious full-length 42 kDa mcp, which was also cleaved to the mature 31 kDa size during the experiment (Fig. 2A, left) indicating normal assembly and conversion to Prohead II. Plasmid pNoDelta2 expressed a protein that migrated similarly to the mature mcp at all time-points and at levels comparable to that of the wild-type protein (Fig. 2A, right). However, most of the protein was in the pellet fraction at the end of the experiment, suggesting that it is insoluble.

In order to determine whether the truncated protein was capable of assembling into recognizable structures such as capsomers or proheads, we repeated the expression experiments using our high-level expression protocol and analyzed the pellet, supernatant, and PEG (polyethylene glycol precipitate) fractions (Fig. 2B and C). In this experiment, the control plasmid was a protease knockout plasmid (protease-) that expresses only the full-length HK97 major capsid protein. This plasmid produced a 42 kDa band as expected for uncleaved major capsid protein (lanes 1–3, Fig. 2B) in the SDS gel. The pNoDelta2 plasmid produced a band that migrated as expected for its size (indicated by a dot in lane 4 in Fig. 2B), but this band only appeared in the pellet fraction,

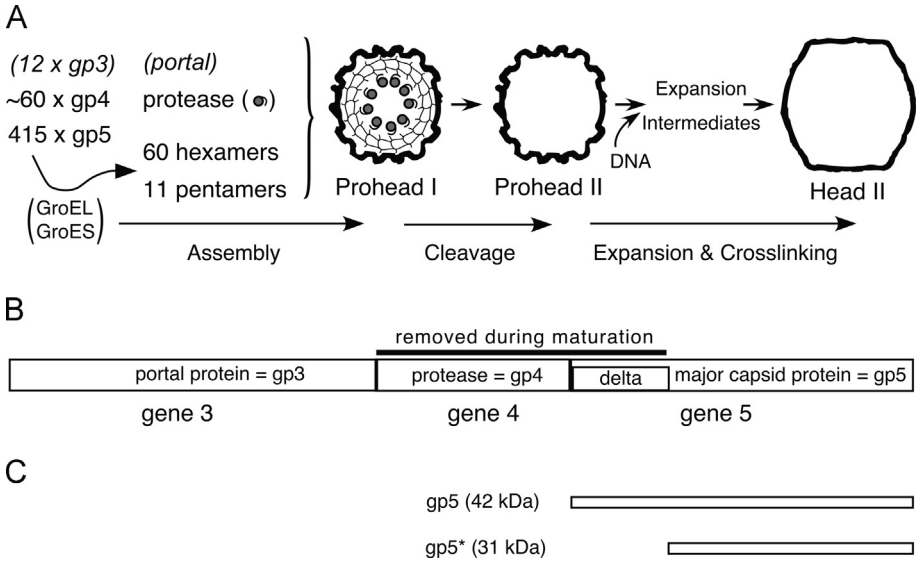

Fig. 1. HK97 Capsid Assembly Pathway. (A) The *E. coli* chaperonin proteins GroEL and GroES assist in the folding and assembly of the mcp subunit into hexamers and pentamers. These, collectively called capsomers, assemble, along with the portal and the protease, into Prohead I. The active protease, present inside the capsid, cleaves the delta domains, then cleaves itself. The resulting peptides escape from the capsid, leaving an empty Prohead II shell. DNA is packaged through the portal and induces the capsid to transform, via a series of expansion intermediates, into the mature Head II. Head II is stabilized by covalent crosslinks and is ready to attach a tail to form a viable phage. (B) HK97 capsid assembly genes with protein names. The HK97 protease substrates, including the delta domain are identified. (C) The major forms of the HK97 major capsid protein.