Virology 506 (2017) 84–91

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

On the catalytic mechanism of bacteriophage HK97 capsid crosslinking

DanJu Tso[1], Craig L. Peebles, Joshua B. Maurer, Robert L. Duda, Roger W. Hendrix*

Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260, USA

ARTICLE INFO

Keywords:
Bacteriophage assembly
Virus capsids
Virus maturation
Capsid stabilization
Covalent cross-linking
Genetic selection

ABSTRACT

During maturation of the phage HK97 capsid, each of the 415 capsid subunits forms covalent bonds to neighboring subunits, stabilizing the capsid. Crosslinking is catalyzed not by a separate enzyme but by subunits of the assembled capsid in response to conformational rearrangements during maturation. This report investigates the catalytic mechanism. Earlier work established that the crosslinks are isopeptide (amide) bonds between side chains of a lysine on one subunit and an asparagine on another subunit, aided by a catalytic glutamate on a third subunit. The mature capsid structure suggests that the reaction may be facilitated by the arrival of a valine with the lysine to complete a hydrophobic pocket surrounding the glutamate, lysine and asparagine. We show that this valine has an essential role for efficient crosslinking, and that any of six other amino acids can successfully substitute for valine. Evidently none of the remaining 13 amino acids will work.

1. Introduction

The protein capsid (head) of bacteriophage HK97 is assembled initially into a structure known as Prohead I, which has 415 copies of the major capsid protein (MCP) arranged in T=7 icosahedral symmetry, 12 copies of the portal protein, and about 50 copies of the maturation protease (Conway et al., 2007; Duda et al., 1995a, 1995b). Maturation of Prohead I to the superficially similar Prohead II entails proteolysis, removing the N-terminal 102 amino acids from each copy of the MCP inside the closed shell, and autoproteolysis of the protease.

The resulting peptides probably diffuse through small holes in the Prohead lattice. Expansion converts Prohead II to mature Head, the final structure. Expansion is triggered by DNA packaging *in vivo* or by solvent changes *in vitro*. It involves extensive conformational changes within and between copies of the MCP, resulting in a capsid diameter increase (Conway et al., 1995; Duda et al., 1995a). Perhaps the most striking feature of expansion is that ≈400 covalent crosslinks form between pairs of MCP subunits (Popa et al., 1991), with the result that MCP monomers are linked into covalently continuous rings of 6 or 5 subunits (Duda, 1998; Duda et al., 1995a). The rings are mutually interlinked topologically at the 3-fold positions of the capsid lattice (Helgstrand et al., 2003; Wikoff et al., 2000), forming the robust structure we have called viral chainmail. Efficient crosslinking requires that the subunits first assemble into capsids (Duda et al., 1995a, 1995b; Hendrix and Duda, 1998), and capsid expansion is required for crosslinking (Duda et al., 1995a; Gan et al., 2004). Efficient crosslinking in turn is essential to produce viable HK97 phage (Ross et al., 2005).

1.1. Mechanism of crosslinking

We describe here experiments to elucidate the autocatalytic chemical mechanism of crosslinking. The crosslink is an isopeptide (amide) bond between the side chains of lysine 169 (K169) of one subunit and asparagine 356 (N356) of an adjacent subunit (Duda et al., 1995a). All of the crosslinks are clearly visible in the high-resolution structure of mature Head (Helgstrand et al., 2003; Wikoff et al., 2000). The Head structure also shows that K169 is part of a β-hairpin, termed the E-loop, that extends partway over the surface of the adjacent subunit, bringing K169 near the location of N356 of the adjacent subunit to which it will crosslink (Fig. 1). Structural and biochemical experiments show that the E-loops in Prohead I or Prohead II are poorly ordered and extend away from the outer surface of the capsid shell; the E-loops dock on an adjacent subunit and become ordered as part of the conformational changes that underlie capsid expansion (Conway et al., 2001; Gan et al., 2004, 2006; Lee et al., 2008; Li et al., 2005; Wikoff et al., 2006). The K169 and N356 residues that are destined to react are ~35 Å apart in Prohead II, but once the E-loop docks on the adjacent subunit, K169 projects into a cleft on the adjacent subunit to reach its N356 partner (Gan et al., 2006; Gertsman et al., 2009; Wikoff et al., 2006, 2000).

The high-resolution structure of mature Head shows an unexpected feature that may provide insights into the catalytic mechanism of

* Correspondence to: University of Pittsburgh, Department of Biological Sciences, A323 Langley Hall, 4249 Fifth Ave, Pittsburgh, PA 15260, USA.
E-mail address: rhx@pitt.edu (R.W. Hendrix).
[1] Current address: Department of Surgery, Robert Wood Johnson Medical School, Rutgers University, New Brunswick, NJ 08901, USA.

http://dx.doi.org/10.1016/j.virol.2017.03.011
Received 15 December 2016; Received in revised form 22 March 2017; Accepted 23 March 2017
Available online 27 March 2017
0042-6822/

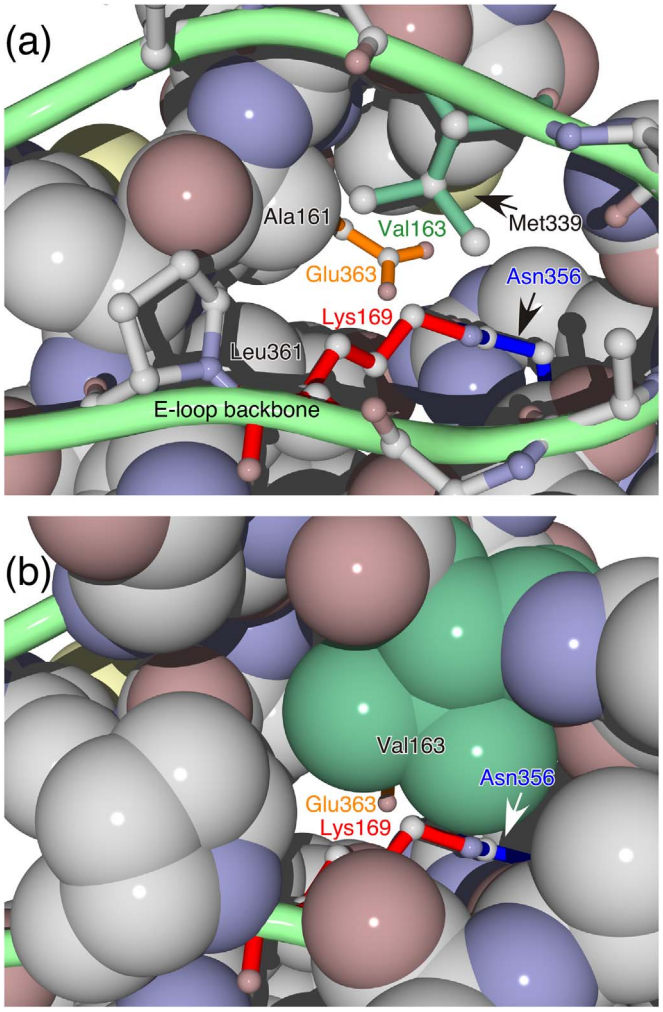

Fig. 1. Structure of the crosslinking reaction site. The view is from the outside of the capsid and represents the structure of the mature head in which the crosslink has formed**. Top panel:** The green loop represents the E-loop of the subunit that supplies the reactive lysine Lys 169). The side chain of Lys 169 is shown as red sticks and is attached via the crosslink to Asn 356 (blue sticks) from the adjacent subunit. The side chain of Val 163 is shown as green sticks, and the side chain of catalytic residue Glu 363, which is part of a third subunit, is shown as orange sticks. Most other side chains are represented as space-filling models, and the side chains of Met 339 and Leu 361 are indicated. **Bottom panel:** Same as top panel, except that Val 163 and the other E-Loop residues are shown in space-filling form.

crosslinking: the carboxylate of glutamate 363 (E363) from a third subunit is located ~2 Å from the crosslink (Helgstrand et al., 2003; Wikoff et al., 2000). E363 is in a comparable position in Prohead II, where the crosslink has not yet formed (Gertsman et al., 2009). We proposed that E363 may play a catalytic role in crosslinking (Helgstrand et al., 2003; Wikoff et al., 2000). Subsequent experiments support this hypothesis (Dierkes et al., 2009) and further suggest that an important property of E363 for performing this catalytic role is its ability to accept a proton from K169—that is, to act as a general base.

We proposed the model shown in Fig. 2 from the facts outlined above. We suggest that E363, the catalytic glutamate, accepts a proton from the ε-amino group of K169, converting the lysine ε-amino group into a good nucleophile that attacks the γ-carbon of N356 to form the observed isopeptide crosslink. The side chains of lysine and glutamate have very different pK_A's in aqueous solvent and thus a proton would not be expected to pass readily from lysine to glutamate. However, examination of the high-resolution structure of Head shows that the crosslink and the catalytic glutamate – which mark the presumed catalytic site – are enclosed within a hydrophobic environment

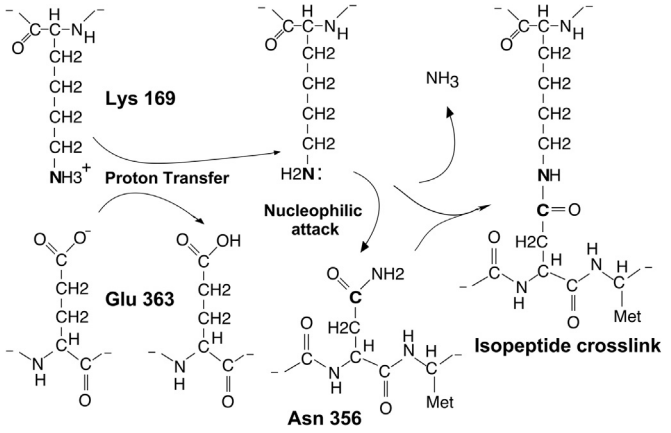

Fig. 2. Proposed reaction mechanism. The catalytic residue Glu 363 is proposed to accept a proton from the epsilon amino group of reactant Lys 169, rendering Lys 169 a good nucleophile that attacks the gamma carbon of the crosslinking partner, Asn 356. The proton transfer from Lys 169 to Glu 363 is proposed to be facilitated by a hydrophobic environment around the reaction site, of which a crucial component is supplied by Val 163.

bordered by the side chains of three other amino acids: Leucine 361, Methionine 339, and Valine 163 (Fig. 1). If the positive charge of K169 and the negative charge of E363 are indeed enclosed together in a hydrophobic pocket prior to crosslinking, the hypothesized transfer of a proton from K169 to E363 is expected to be energetically more favorable. Two of the three hydrophobic amino acid side chains that line the interior of the hydrophobic pocket occupy essentially the same positions in Prohead II as in Head. However the third hydrophobic residue, Valine 163 (V163), is part of the E-loop that also contains K169. The side chains of K169 and V163 extend from the same side of the E-loop with the consequence that when the E-loop docks on the neighboring subunit and positions K169 for crosslinking, it simultaneously positions V163 to complete the hydrophobic cage surrounding the crosslinking reaction site. The experiments reported here test various aspects of the reaction mechanism proposed in Fig. 2, particularly the role of V163.

2. Results

We wished to determine whether V163 plays an essential role in assembly and head maturation, and particularly whether V163 has a direct role in crosslinking. We changed the V163 codon (GTG) to an aspartate codon (GAT) in the context of plasmid pV0. This plasmid has HK97 genes *4* (protease) and *5* (MCP) under the control of a phage T7 promoter and an inducible T7 RNA polymerase (Duda et al., 1995b). This V163D mutant MCP is unable to support phage growth, as judged by its failure to complement an infecting phage bearing an amber mutation in gene *5*, the MCP gene (Fig. 3).

We expressed the V163D mutant MCP from the plasmid that also carries the protease to ask whether V163D can complete capsid assembly and maturation steps. With wild-type genes, this construct abundantly produces Prohead II that can crosslink efficiently in response to certain changes in solvent composition. The V163D mutant likewise produces Prohead II-like particles at the same high level seen for the wild type. We characterized these particles first by agarose gel electrophoresis (Fig. 4a). The mutant particles migrate as a band characteristic of Prohead II except faster migrating than wild-type Prohead II, a property we ascribe to the 420 extra negative charges on the outside of these Proheads due to the mutation. We also analyzed the V163D particles on an SDS polyacrylamide gel, which showed that all of the mutant MCP, like the wild type, had been processed to the 31 kDa cleaved form (Duda et al., 1995a, 1995b) (Fig. 4b). We conclude that mutant V163D MCP can fold, assemble, and undergo cleavage indistinguishably from wild-type MCP.