doi:10.1016/j.jmb.2011.05.006 *J. Mol. Biol.* (2011) **410**, 226–240

Contents lists available at www.sciencedirect.com

Journal of Molecular Biology

journal homepage: http://ees.elsevier.com.jmb

Conformational Changes in Bacteriophage P22 Scaffolding Protein Induced by Interaction with Coat Protein

G. Pauline Padilla-Meier[1] and Carolyn M. Teschke[1,2]*

[1]*Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06269, USA*
[2]*Department of Chemistry, University of Connecticut, Storrs, CT 06269, USA*

Received 4 March 2011; received in revised form 28 April 2011; accepted 5 May 2011
Available online 14 May 2011

Edited by J. Karn

Keywords:
cross-linking;
fluorescence energy transfer;
virus assembly;
protein folding;
procapsids

Many prokaryotic and eukaryotic double-stranded DNA viruses use a scaffolding protein to assemble their capsid. Assembly of the double-stranded DNA bacteriophage P22 procapsids requires the interaction of 415 molecules of coat protein and 60–300 molecules of scaffolding protein. Although the 303-amino-acid scaffolding protein is essential for proper assembly of procapsids, little is known about its structure beyond an NMR structure of the extreme C-terminus, which is known to interact with coat protein. Deletion mutagenesis indicates that other regions of scaffolding protein are involved in interactions with coat protein and other capsid proteins. Single-cysteine and double-cysteine variants of scaffolding protein were generated for use in fluorescence resonance energy transfer and cross-linking experiments designed to probe the conformation of scaffolding protein in solution and within procapsids. We showed that the N-terminus and the C-terminus are proximate in solution, and that the middle of the protein is near the N-terminus but not accessible to the C-terminus. In procapsids, the N-terminus was no longer accessible to the C-terminus, indicating that there is a conformational change in scaffolding protein upon assembly. In addition, our data are consistent with a model where scaffolding protein dimers are positioned parallel with one another with the associated C-termini.

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Introduction

Scaffolding proteins are required by numerous double-stranded DNA (dsDNA) icosahedral viruses to catalyze the proper assembly of their transient precursor capsid, known as procapsid. These viruses use either an internal scaffolding protein, an external scaffolding protein, or both to assemble their procapsids. Some viruses recycle the scaffolding proteins for further rounds of assembly, while others enzymatically degrade them upon DNA packaging.[1–3] It is clear from studies of many viruses that scaffolding proteins are indispensable for proper assembly, and yet there is no clear structural understanding of their fundamental function.

Bacteriophage P22 provides a paradigm for the assembly of dsDNA viruses. The *in vivo* morphogenic pathway of the $T=7$ *Salmonella* bacteriophage P22 involves the coassembly of 415 molecules of monomeric coat protein with 60–300 molecules of an internal scaffolding protein, as well as some minor injection proteins and the portal protein complex (which occupies one of the 5-fold vertices), to form a procapsid.[4,5] P22 scaffolding protein is known to direct procapsid assembly. Without scaffolding

**Corresponding author.* Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06269, USA. E-mail address: teschke@uconn.edu.

Abbreviations used: dsDNA, double-stranded DNA; FRET, fluorescence resonance energy transfer; WT, wild type; NEM, *N*-ethyl maleimide.

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protein, high concentrations of coat protein will assemble into aberrant forms: $T=4$ empty procapsids and spiral structures. The spiral structures appear to have their 5-fold and 6-fold vertices located inappropriately, so that closed procapsid structures do not form.[6,7] Scaffolding protein is also responsible for the incorporation of ejection proteins and the portal complex.[8] dsDNA is actively packaged into procapsids through the unique portal vertex.[8] Concomitant with DNA packaging, scaffolding protein exits from immature capsids to take part in additional rounds of assembly, and the capsids expand to form mature virions.[9,10] In the processes of the folding and assembly of P22 procapsid proteins, none is covalently modified or proteolyzed. Nevertheless, the structure of the procapsid examined by cryo electron microscopy reveals that coat protein subunits are found in seven quasi-equivalent conformations: six in hexons and one in pentons.[10,11] Thus, during assembly, coat protein monomers must be 'switched' into the necessary conformations to produce a closed icosahedral procapsid. Scaffolding protein has been suggested to control the proper switching of capsid proteins.[12] How conformational switching occurs and is controlled by scaffolding proteins during assembly for any icosahedral virus is not understood. *In vitro*, 420 copies of coat protein and ~300 copies of scaffolding protein assemble into a procapsid-like particle that has the same size and general morphology of *in vivo* assembled procapsids, although without the portal complex or minor proteins.[4,13,14]

P22 scaffolding protein is composed of 303 residues, and its functional domains have been mapped through mutagenesis studies. The C-terminus has been identified as the coat-binding domain.[15–17] Residues 280–294 are the minimum residues required for coat binding and are highly negatively charged.[18] The N-terminus is postulated to be involved in the autoregulation of scaffolding protein gene expression through interaction with its own mRNA.[19–22] The N-terminus might also function as the signaling domain to control scaffolding protein exit during DNA packaging because N-terminal deletion mutants of scaffolding protein are unable to leave the procapsids upon initiation of DNA packaging.[18] Based on functional studies, P22 scaffolding protein is thought to be generally U-shaped, with the N-terminus and the C-terminus positioned in an anti-parallel fashion.[18] Biophysical studies of P22 scaffolding protein show that it is an elongated ellipsoid that is mainly composed of α-helices connected by unstructured regions[23,24] (22 Å in diameter × 247 Å in length).[25] It exists in a monomer–dimer–tetramer equilibrium in solution, but the monomers and dimers are proposed to be the species actively involved in procapsid assembly.[25] Internal scaffolding proteins from other viruses such as lambda, T4, and SPP1 are also elongated helical ellipsoids with flexible domains.[26–28]

Phi29 scaffolding protein, which is 100 residues in length, is a dimeric and elongated α-helical structure composed of a four-helical bundle with coiled coil tails at the N-terminus.[29] The NMR structure of the minimal coat-binding domain at the C-terminus of P22's scaffolding protein also contains a helix–turn–helix similar to phi29's N-terminus.[30–32] However, acquiring a high-resolution structure of the P22 scaffolding protein has remained a challenge, likely due to its inherent flexibility and multiple oligomeric states. The arrangement of the scaffolding protein inside procapsids is still ambiguous. Attempts to study scaffolding protein inside the procapsids of other dsDNA viruses such as T7 and phi29 have only shown possible network arrangements of inner scaffolding proteins.[29,33] Cryo electron microscopy studies on P22 that imposed icosahedral symmetry on the reconstructions have not been able to visualize the entire length of scaffolding protein, suggesting a nonicosahedral order inside the procapsids.[11] A 22-Å cryo electron microscopy difference map of procapsids with and without scaffolding protein revealed that the scaffolding protein C-terminus interacts with coat protein trimer tips and is arranged with a distance of 50 Å between adjacent scaffolding proteins.[34] A recent reconstruction of P22 procapsids with no symmetry imposed confirms the electrostatic interaction between the C-terminus of scaffolding protein and the N-terminal arm of coat protein. The reconstruction also indicates a second site for the interaction of the scaffolding protein C-terminus with the A-loop of the coat protein.[35] In total, this reconstruction indicated that each coat protein has an associated scaffolding protein.

In this study, we have investigated the general conformation and fold of scaffolding protein in solution, as well as inside procapsids, to increase our understanding of the ability of this protein to direct the assembly of procapsids through the use of fluorescence resonance energy transfer (FRET) and cross-linking. The orientation of the full-length scaffolding protein inside procapsids was also examined by cross-linking. We show that scaffolding protein has a significantly more complex fold than phi29's scaffolding protein and undergoes conformational changes when bound to the interior of procapsids. We also present evidence that scaffolding proteins are more closely associated inside procapsids than previously suggested.

Results

As noted above, the P22 scaffolding protein is one of the best-characterized scaffolding proteins of dsDNA bacteriophages and viruses. Nevertheless,

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