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A model for fd phage penetration and assembly

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Below 15°C, chloroform causes fd phage to contract to I-forms, which are compact structures about 1/3 as long as the original phage. Above 15°C, chloroform causes I-forms to contract to even more compact spheroidal S-forms. Here we show that the coat protein structure in I-forms is the same as the protein structure in the phage and the protein structure in S-forms is the same as the protein structure in bilayers. The conversions from fd→I-forms→S-forms are therefore suggested to mimic steps in fd penetration. The same conversions, in reverse order, are suggested to mimic steps in fd assembly.

Filamentous phage assembly

1. INTRODUCTION

The fd phage has circular single-stranded DNA encased in a filametous capsid constructed of α-helical subunits arranged in a helicoid bundle [1–3]. The major coat protein of fds, pVIII, has just 50 amino acids [4] and is almost all helix [5–9], with a hydrophobic core and oppositely-charged ends [10]. The capsid also contains 4 additional minor proteins, 2 at each end [2]. The mass of these minor proteins totals only about 2% of the mass of pVIII [1–3].

Phage penetration involves insertion of the pVIII protein into the inner membrane of the host as the DNA enters the cell [11,12]. The orientation of the phage relative to the host membrane is the same for penetration and subsequent assembly [13,14], which occurs without cell lysis [2]. A substantial fraction of pVIII from infecting phage is re-used and appears in progeny [11,12,14]. Evidently, penetration and assembly involve the same steps, but in reverse order [3,15].

Both penetration and assembly involve host factors [2]. Assembly requires, in addition, several phage proteins [1,2] and, perhaps, the cell membrane potential [16]. Finally, the phage must cross both the inner and outer membrane, but this seems to be accomplished in a single step by means of adhesion zones [17]. In this communication we ignore these complicating factors and the outer membrane as has been done by others [3,18,19]. Based on the properties of contracted forms of fd phage produced in vitro by exposure to chloroform [20–22], we propose a model for fd penetration and assembly.

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2. MATERIALS AND METHODS

2.1. *Preparation of fd, oxidized fd, I-forms, S-forms and SDS–pVIII complexes*

Filamentous phage was grown on *E. coli* JM101 kindly provided by Dr. Gerald Hazelbauer, and purified by polyethylene glycol precipitation followed by banding on KBr density gradients. *N*-Bromosuccinimide (NBS) oxidation was accomplished by incremental additions of 0.56 mM NBS to solutions of fd phage at 0.38 mg/ml to give 1.2 mol NBS/mol pVIII or 3.4 mol NBS/mol pVIII. The reaction mixture was gently stirred in the dark at room temperature for 30 min before appropriate dilution for CD spectroscopy or before centrifugation into pellets for Raman spectroscopy. The fd phage was contracted to I-forms, and I-forms were converted to S-forms, as described by others [20–22]. SDS-pVIII complexes were made by dissociating fd in 1% SDS. The fd DNA was not removed from these complexes because previous work showed that the same CD spectra were obtained with or without the DNA [23].

2.2. *Circular dichroism (CD) and Raman spectroscopies*

CD spectra were collected using a JASCO model J-40A spectropolarimeter calibrated with 10-camphorsulfonic acid recrystallized from benzene. For Raman spectroscopy, the samples were illuminated with the 5145 Å line from a Spectra-Physics model 164 argon ion laser using about 100 mW at the sample. Raman scattering was collected at 90° relative to the incident laser beam using a JY Ramanor HG-2S double grating equipped with a Spex Digital Photometer.

3. RESULTS

3.1. *CD and Raman spectra*

The primary effect of NBS is to oxidize the single tryptophan of the phage capsid. This conclusion is reached from the loss of the tryptophan-specific Raman peaks, one of which is shown in Fig. 1, with only the slightest change in the tyrosine-specific peaks (Fig. 1,

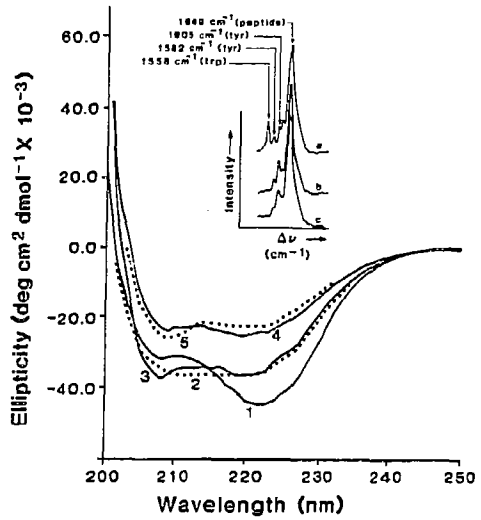

Fig. 1. CD Spectra of fd, I-forms, S-forms and SDS–pVIII complexes. The CD spectra of fd, I-forms, and S-forms were collected at 6°C; the CD spectrum of SDS–pVIII complexes was collected at room temperature due to precipitation of SDS at low temperatures. Protein concentrations in all cases were about 50 μg/ml. The CD spectra represent: (curve 1, solid) fd; (curve 2, dotted) NBS-oxidized fd, 3.4:1; (curve 3, solid) I-forms; (curve 4, solid) S-forms and (curve 5, dotted) SDS–pVIII complexes. Inset: the Raman spectra of fd and NBS-oxidized fd were collected and digitally water-subtracted as described previously [7]. The curves are: (a) fd; (b) NBS-oxidized fd, 1.2:1; and (c) NBS-oxidized fd, 3.4:1.

inset). The oxidation of the tryptophan is essentially complete by an NBS/pVIII ratio of 3.4/1, as evidenced by a complete loss of the tryptophan specific peak (Fig. 1, inset) and as corroborated by essentially complete loss of intrinsic tryptophan fluorescence [24].

NBS-oxidized fd, I-forms, and fd phage all contain the coat protein in a nearly 100% helical form. This conclusion is based on the data of Fig. 2. The position of the amide I band for fd and NBS-oxidized fd and the intensities of CD spectra for I-forms and NBS-treated fd are all very similar to the corresponding spectra for 100% α-helical poly-L-lysine [6,25], showing that pVIII has high helix content in all 3 particles. Overall these data show that the fd to I-form conversion occurs with little or no change in the structure of pVIII.

The CD spectrum of S-forms is like that of pVIII complexed with SDS (Fig. 1). The CD spectrum of SDS–pVIII complexes has previously been shown to be essentially the same as the CD spectrum of pVIII in lipid bilayers [23,26–28]. These CD spectra are consistent with about 50–60% helix [26–28], which agrees with a Raman estimate of 55% helix using the amide I band [6]. The Raman studies indicated that the SDS–pVIII complexes contain a mixture of helix, turns and random, with very little sheet [6], suggesting a transmembrane helix and nonhelical ends for the membrane-bound form. Recent NMR studies of SDS–pVIII complexes support such a structure [29].

Solid-state NMR studies on the coat protein of a related phage, Pf1, also support the concept of a transmembrane helix motif for the membrane-bound form of filamentous phage coat proteins; however, the ends of the Pf1 coat protein [30] are not the same as proposed here for pVIII.

4. DISCUSSION

4.1. *A proposed model for fd penetration and assembly*

I-forms are morphologically intermediate between the fd phage and the membrane-bound structures [20–22]. I-forms are trapped by low temperatures, which is appropriate for a role as an assembly intermediate. Being spheroidal [20–22], S-forms have the morphology of membranes.

Here we shown that I-forms contain pVIII with the same structure as in the phage, and that S-forms contain pVIII with the same structure as when in bilayers. Thus, I-forms and S-forms have the expected properties of intermediates, both structurally and morphologically. Essentially no extrapolation is required to propose a pathway for pVIII during penetration consisting of the following: fd→I-forms→S-forms→bilayers, and the reverse pathway for assembly (Fig. 2).

Additional support for an I-form-like structure in the penetration pathway (e.g. Fig. 2, Region 2) comes from the recent isolation and initial characterization of chloroform-resistant mutants that we have called fd^{cr}. Following chloroform exposure, several of the fd^{cr} mutants apparently exhibit infectious I-forms and infectious particles having internal segments with I-form-like morphologies, which suggests partial conversion to I-forms (Dunker and Ensign, work in progress). These mutants suggest that I-forms are involved in penetration, and, by inference, also in assembly.

Support for an S-form-like structure in the assembly pathway (e.g. Fig. 2, Region 3) comes from the previous work of Bayer and Bayer [31], who showed that the inner membrane of infected cells becomes resistant to cleavage by freeze fracture with the same time course as the accumulation of pVIII. An S-form-like region does not contain a cleavage plane. The accumulation of many such assembly sites [17] would be expected to render the inner membrane resistant to cleavage following infection and thus account for the previous observations [31]. Marvin [3] originally suggested that filamentous phage might assemble from lipid-free patches.

Recently a model has been proposed for the assembly of a related filamentous phage, Pf1 [19]. That model differs from the proposal in Fig. 2 in several respects. First and second, the model for Pf1 assembly lacks Regions 2 and 3. Third, the model for Pf1 assembly has the membrane-associated protein with a very different structure compared to pVIII in Region 4; specifically, the membrane-associated Pf1 coat protein was proposed to have transmembrane helix and a short surface-

Model For fd Penetration and Assembly

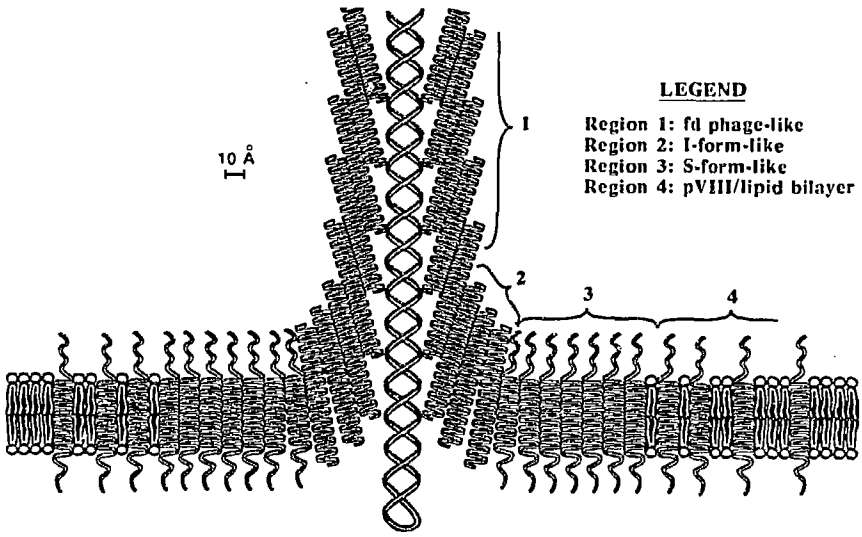

Fig. 2. Proposed pathway for pVIII during penetration and assembly. pVIII is proposed to pass through several sequential steps during phage penetration and assembly, including: (Region 1) a structure like fd phage; (Region 2) a structure like I-forms; this regions might comprise only a few annuli, with the fd→I-forms and the I-forms→S-forms conversions occurring one right after the other at the membrane surface; (Region 3) a structure like S-forms with pVIII having a transmembrane helix and non-helical ends; the loss of helix at the ends is proposed to be due to increased repulsion of like-charges as DNA- protein interactions are lost and as the highly charged ends come closer together following the contraction steps; (Region 4) the unusual lipid bilayer containing transmembrane pVIII.

bound helix with an unstructed, mobile loop connecting the two [19,30]. The 2 helical segements were proposed to join end-to-end, with the intervening loop remaining mobile and unstructured as the Pf1 phage assembly takes place.

In Fig. 2 the ends of the fd coat protein are proposed to convert from coil to helix during the process of assembly. The two structural pathways for the coat proteins of these 2 phages may both be correct and merely reflect differences in detail arising from amino acid sequence differences. The ends of pVIII are suggested to become non-helical in the membrane because of charge–charge repulsion that results from a loss of DNA-protein interactions and from decrease in separation between like-charges as the protein helices become aligned.

Another model for both fd and Pf1 penetration and assembly involves the coat protein becoming perpendicular to the phage axis in order to associate with adhesion zones that are proposed to join the inner and outer membranes with a particular morphology [3,15]. As far as we know, there is no compelling evidence to favor the parallel-type models of Fig. 2 and [19] over the perpendicular-type models of [3,15]. One indirect test would be to determine the orientation of the helices relative to the axis in I-form structures.

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