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Mechanism of Coliphage M13 Contraction: Intermediate Structures Trapped at Low Temperatures

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The filamentous coliphage M13 can be transformed into a spherical particle (termed spheroid) by exposure to an interface of water and a slightly polar but hydrophobic solvent such as chloroform at 24°C. We report here that exposure of M13 filaments to a chloroform-water interface at 2°C trapped the phage particles in forms morphologically intermediate to filaments and spheroids. These structures were rods 250 nm long and 15 nm wide, and each had a closed, slightly pointed end, an open flaired end, and a hollow central channel. The final contraction of these intermediates (termed I-forms) into spheroids was dependent upon both temperature and the presence of the solvent-water interface but was apparently independent of both the minor phage coat proteins and the virion DNA. Although stable in an aqueous environment, I-forms, in contrast to filaments, were readily disrupted by detergents, suggesting that the phage structure had been altered to a form more easily solubilized by membrane lipids. These solvent-induced changes might be related to the initial steps of phage penetration in vivo.

The filamentous coliphage M13 (fd, f1) offers an attractive model system for studying the membrane association of virus infection and the general effect of membranes on the structure of nucleoprotein complexes. The virus capsid is constructed of viral B protein subunits helically arranged to form a long rigid tube around the circular single-stranded DNA (11, 14-16). The phage are resistant to heating to 80°C and are also resistant to exposure to 1% Sarkosyl and 8 M urea (12). Furthermore, when phage are sheared into fragments, the DNA remains tightly bound inside the broken filaments. The coat protein, however, is released from the DNA when M13 penetrates and infects its host cell and is found dissolved in the bacterial inner membrane (12, 20, 21). The mechanisms involved in this transition from a highly stable protein filament to lipid soluble subunits have been elusive. An understanding of these events may provide general insights into the interaction of complex protein assemblies with cell membranes.

We recently discovered that exposure of M13 filaments to a chloroform-water interface initiates a highly ordered contraction of the filament into spherical particles that we termed spheroids (9). Two-thirds of the viral DNA was found to emerge from a hole in the spheroid protein shell, whereas the DNA spanning the origin of replication remains bound inside. In this new form, the phage coat protein is easily dispersed in detergents and is susceptible to protease attack. Although we suggested that the chloroform-water interface mimics the in vivo activation of M13 filaments, the details of this process were obscure.

In this report, we describe the isolation and characterization of stable virion particles which appear to be intermediates in the transition of filaments to spheroids. Contraction of the filament through these intermediate morphologies was dependent upon the temperature of the solvent-water interface. Furthermore, the contraction appeared to involve non-cooperative, localized changes in the filament structure and was independent of both the minor coat proteins and virion DNA. We believe the interface mimics the in vivo activation of the M13 filament. Possible mechanisms of virion penetration are discussed below.

MATERIALS AND METHODS

Phage and bacterial strains. The M13 phage used in the experiments described here were from a stock grown on *Escherichia coli* H560 purified by Triton X-100 and Sarkosyl washes and banded in CsCl (8). The stock contained 20% miniphage. fd phage were grown on *E. coli* K37 cells and purified as described above. Mini-free f1 were a gift from R. Webster.

I-form and spheroid preparation. Purified phage stocks were diluted in TE buffer (0.01 M Tris, 0.001 M

EDTA [pH 7.5]) to a DNA concentration of 50 μg/ml or less. Equal volumes of phage and chloroform (or another solvent) were brought to the desired temperature in separate tubes before being mixed. We prepared I-forms by gently mixing ice-cold phage and chloroform 3 to 4 times (5 s each) over a 60- to 90-s period with a Vortex mixer. The phases were separated by brief centrifugation at 2°C, and the aqueous layer was collected. Spheroids were prepared by an identical procedure but at room temperature. Samples were sedimented in 5 to 20% sucrose gradients in TE buffer for 2 h at 20°C (spheroids) or 2°C (I-forms) in an SW41 rotor (39,000 rpm). Samples were banded in CsCl as described previously (9).

Electron microscopy. Thin carbon support films were partially floated from small pieces of mica directly onto 100-μl droplets of phage samples. The mica was withdrawn, and the carbon was floated onto 1% uranyl acetate droplets. Electron microscopy (EM) grids were placed on the carbon and then picked up with forceps, and the excess stain was drained from the grids with filter paper. Samples were normally fixed in formaldehyde and glutaraldehyde before being stained (7).

RESULTS

Isolation and characterization of M13 I-forms. In our previous study, we showed that exposure of an M13 filament to a chloroform-water interface at room temperature induces a precise change into a hollow spherical particle that we termed a spheroid. More recently, we found that if the chloroform treatment was carried out at 2°C, structures very different from M13 filaments or spheroids were visualized by direct mounting of the material onto carbon supports and shadow-casting with tungsten (Fig. 1). These particles were tubes which were uniformly shorter and thicker than the phage filaments. Measurement of their length revealed that the filaments had contracted 3.5-fold (from 850 to 250 nm) (Fig. 2). When they were visualized by negative staining with uranyl acetate, rods of the same length (250 nm) were observed, and their width was determined to be 15 nm, as contrasted to the 5.5-mm width of the phage filaments. Furthermore, these rods always exhibited one closed, slightly pointed end, one open flaired end, and a hollow central channel (2 nm) into which the stain penetrated (Fig. 3). Many of the rods displayed structures at one end that resembled the knoblike adsorption complex of the filament previously visualized by Gray et al. (6). Ice-cold chloroform treatment of M13 miniphage (extreme deletion mutants 15 to 50% of the length of wild-type filaments [8]) and f1/pBR322 chimera (1.7 times the normal phage length [2]) produced homogeneous populations of particles identical to those from M13 wild-type phage except for being proportionately shorter or longer. Because the results described below suggest that these particles are intermediates in the transition between filaments and spheroids, they will hereafter be termed I-forms.

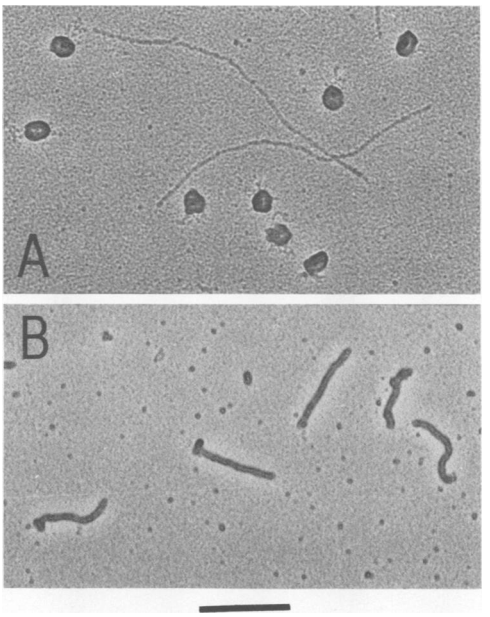

FIG. 1. *Visualization of the effect of chloroform treatment of M13 filaments at different temperatures. Treatment at room temperature produced a mixture of spheroids and filaments (A), whereas treatment at 2°C produced I-form particles (B). Samples were mounted on carbon films and were rotary tungsten shadowed. Bar equals 0.25 μm.*

When ice-cold M13 filaments were blended with a Vortex mixer with chloroform for 60 s and sedimented in a 5 to 20% neutral sucrose gradient, they moved somewhat more slowly than spheroids run in a parallel gradient (Fig. 4). When similarly prepared I-forms were centrifuged to equilibrium in CsCl, bands of two distinct densities were seen, one at a slightly heavier density than untreated filaments and one at a significantly lighter density (Fig. 5). The denser band corresponded exactly to the density of spheroids run in a parallel gradient (1.31 g/cm^3), whereas the lighter band corresponded to the density of empty spheroid shells (lacking DNA). The ratio of the absorbance at 260 and 280 nm was decreased in the lighter band, and, when [^{3}H]thymidine-labeled I-forms were banded in CsCl, the label was detected only in the more dense peak. No change in the appearance of the I-forms was observed by EM after sucrose sedimentation or CsCl banding. Therefore, like spheroids, I-form morphology appeared to remain stable in 5 M CsCl, even though the DNA had been lost from some of them.

M13 filaments are extremely resistant to treat-

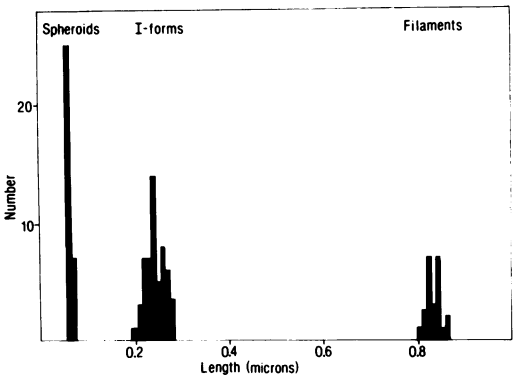

FIG. 2. *Comparison of the lengths of the M13 filaments and intermediates with the diameters of the spheroids. Measurements were obtained from samples stained with uranyl formate.*

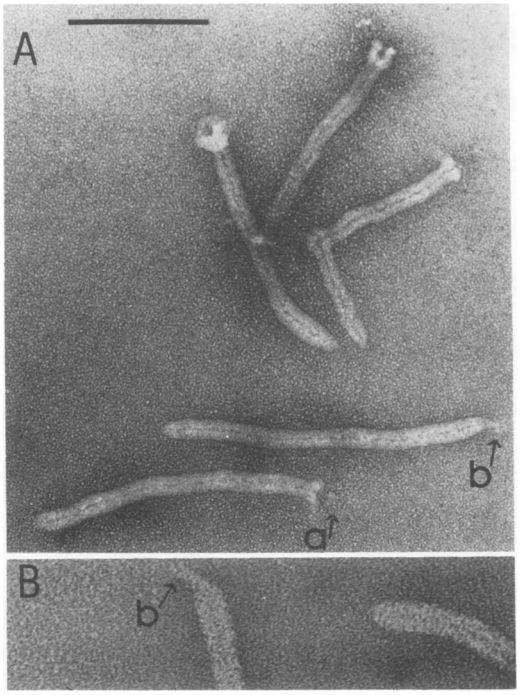

FIG. 3. *Visualization of I-form particles stained with uranyl acetate and showing the stained central channel (A). Knob and stem structures resembling the virion adsorption complex can be seen before (b) and after (a) the end of the particle flaired open. Magnified view (B) shows the knob and stem complex (arrow) on one I-form and the opposite end (lacking the complex) of a second I-form. Bar equals 0.1 μm.*

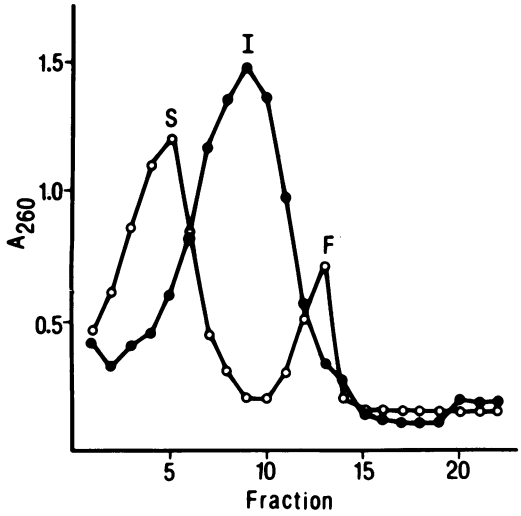

FIG. 4. *Comparison of the sedimentation behaviors of M13 filaments after exposure to a chloroform-water interface on ice (●) or at room temperature (○). Samples were run in parallel gradients, and the profiles were superimposed. Visualization of the particles in each peak by negative staining revealed spheroids (S), I-forms (I), and filaments (F). Sedimentation was from right to left. A_{260}, Absorbance at 260 nm.*

ment with a variety of agents, including heat, detergents, proteases, and urea. In sharp contrast, spheroids are much more labile (9). The sensitivity of I-forms was tested by using negative staining and EM. I-forms were as easily disrupted as spheroids under conditions in which filaments remained intact (Table 1).

Hybrid forms demonstrate non-cooperative contraction and gradual DNA release. (i) Non-cooperative contraction of the filament. To probe the very first changes in the filaments induced by exposure to the chloroform-water interface, we mixed a suspension of filaments with an equal volume of chloroform at 2°C for 3 s with a Vortex mixer. As soon as the phases had separated (ca. 10 s), a sample of the aqueous supernatant was withdrawn and immediately diluted into five volumes of TE buffer with 1% formaldehyde. The samples were then further fixed with 0.5% glutaraldehyde. Hybrid particles in which only part of the filament had thickened to the width characteristic of I-forms were seen by EM (Fig. 6B). These I-form–filament particles constituted only a few percent of the total population. The remaining particles were complete I-forms and apparently unchanged filaments. Similar results were obtained without the fixation steps, and the hybrid particles were stable for several hours on ice or at room temperature.

To determine whether the I-forms would change to spheroids upon being warmed, we incubated suspensions of I-forms (centrifuged free of chloroform) at temperatures between −4 and 37°C for various amounts of time. As deter-

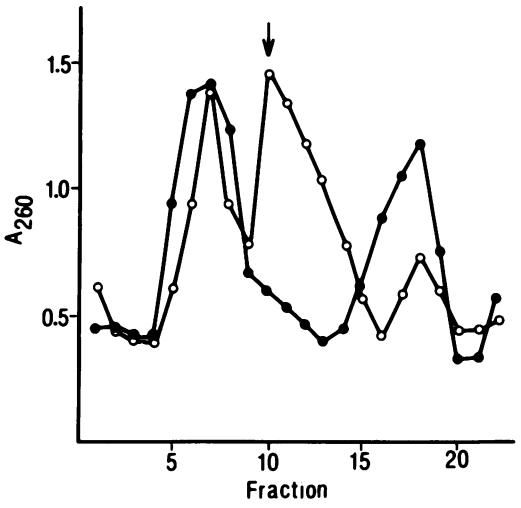

FIG. 5. *Equilibrium density banding of M13 filaments after treatment with chloroform on ice (●) or at room temperature (○). Suspensions of M13 filaments were gently mixed with an equal volume of chloroform for 60 s at each temperature with a Vortex mixer. After removal of the chloroform, samples were mixed with solid CsCl and buffer and banded to equilibrium. Arrow indicates the peak position of untreated M13 filaments run in a third gradient. A_{260}, Absorbance at 260 nm.*

mined by EM, the morphology of the I-forms was stable at all temperatures for several hours. However, with prolonged incubation at higher temperatures (22 to 37°C), the I-forms appeared disordered into a variety of heterogeneous structures, none of which was spheroids. The characteristic morphology of I-forms was also maintained after 12 h of exposure to ice-cold chloroform. However, when a preparation of I-forms was treated with chloroform for a second time at temperatures above 15°C, 100% of the I-forms were converted to spheroids after less than 20 s of exposure to the solvent. Reexposure to chloroform at temperatures slightly below 15°C resulted in the conversion of some I-forms to spheroids and produced particles with a morphology intermediate to I-forms and spheroids (Fig. 6D).

In these studies, we also observed that other hydrophobic but slightly polar solvents, notably acetyl acetone, isobutanol, and ethyl acetate, could trigger the contraction of filaments into spheroids at room temperature. When these solvents were tested for their ability to cause the conversion of I-forms into spheroids, two solvents, ethyl acetate and isobutanol, were found by EM to induce rapid and complete contraction of the I-forms into spheroids. The third solvent, acetyl acetate, disrupted many of the I-forms and caused aggregation of those remaining.

TABLE 1. *Sensitivity of filaments, I-forms, and spheroids to various treatments*[a]

Treatment	Disruption of particle		
	Filaments	I-forms	Spheroids
1% Triton X-100	−	+	+
1% Sarkosyl	−	+	+
Urea (M)			
0.5	−	−	−
1.0	−	−	−
2.0	−	−	−
5.0	−	+	+
Pronase (500 μg/ml)[b]	−	+	+
Subtilisin (500 μg/ml)[b]	−	+	+
Heating (°C)			
37	−	−	−
45	−	+	+
75	−	+	+
90	−	+	+
40% Formamide	−	+	+
1 M NaCl	−	+	+
5 M CsCl[c]	−	+	+

[a] Filaments, freshly prepared I-forms, and spheroids were treated and examined by EM. Complete disruption (+) or stability (−) of the particles was noted for each treatment. Dissolution of the spheroids by the detergents was verified by sucrose velocity sedimentation.

[b] Samples were incubated for 5 min at 37°C.

[c] DNA was released from a fraction of the I-forms and spheroids.

(ii) DNA extrusion. By using hybrid particles, we could determine when the DNA was first extruded from the virion. To visualize DNA emerging from I-forms, we incubated *E. coli* single-stranded binding protein (SSB [19]) with I-forms, and the resulting complexes were fixed, mounted on carbon support films, and rotary tungsten shadowed as previously described (7, 9). The DNA remained inside full I-forms, but as further contraction occurred, the DNA was ejected from the rounded end of the particles (Fig. 7).

These changes are independent of DNA structure and the minor coat proteins. Recent studies in this laboratory have shown that as long as the DNA remained within the I-forms or spheroids, it was in a duplex-like structure, but it opened into a single-stranded loop upon extrusion from the spheroids (J. D. Griffith, S. El Saidy, and S. Hester, J. Mol. Biol., in press). To determine whether the ability of a portion of

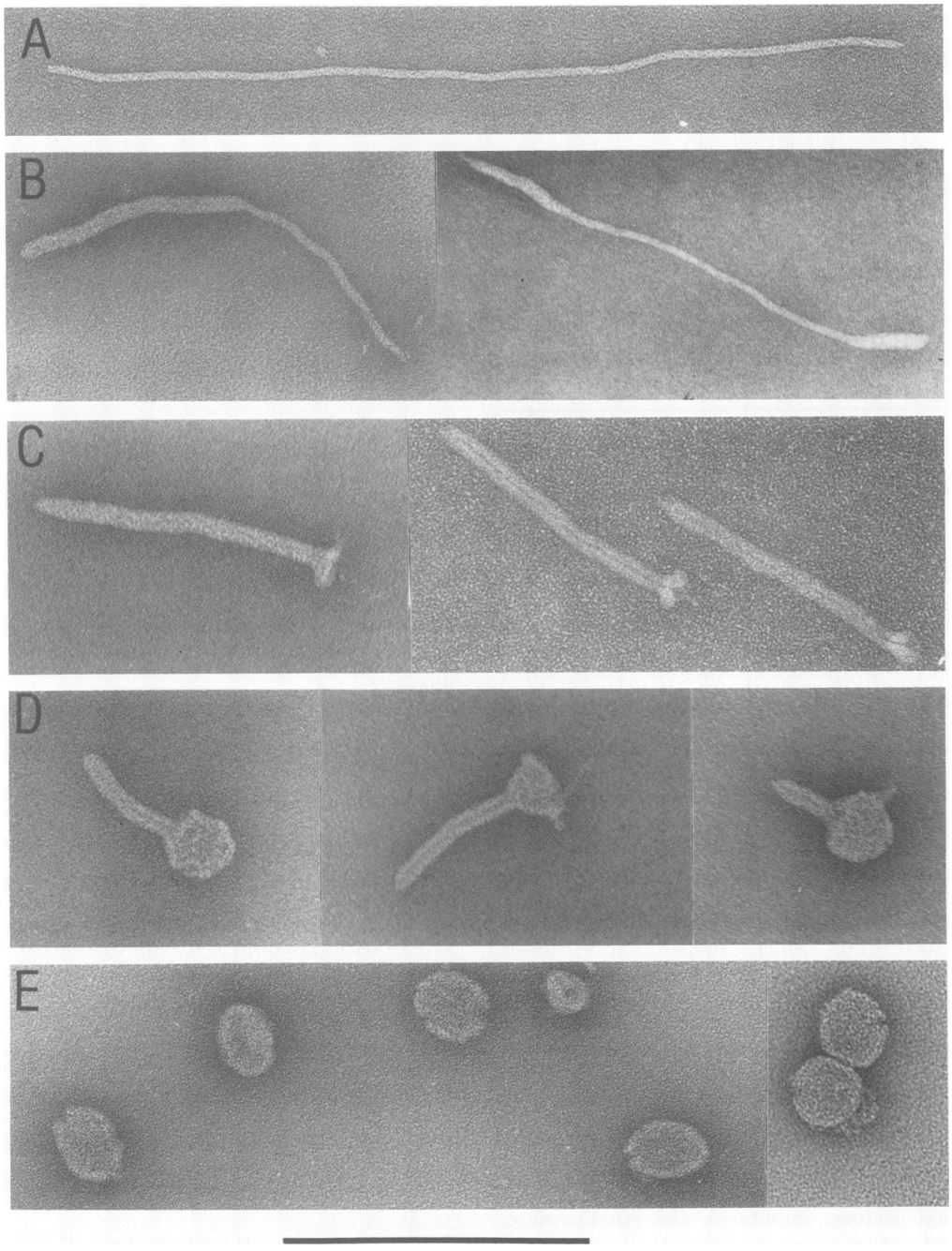

FIG. 6. *The ordered sequence of events in the contraction of M13 filaments. M13 filaments (A), exposed to a chloroform-water interface on ice for a brief time, produced hybrid I-form–filament particles (B) which convert to I-forms upon further exposure (C). Conversion of the I-forms to I-form–spheroid (D) and finally spheroid (E) particles occurred as the temperature of the interface was raised above 15°C. Samples were stained with uranyl acetate. Bar equals 0.25 μm.*

the DNA to change from a duplex to an open loop was necessary for spheroid formation, we photo-cross-linked the two opposing strands of DNA in situ in the I-forms before room-temperature chloroform treatment. This was accomplished by exposing I-forms to UV light (15 min at 4°C) in the presence of 2 μg of trimethyl psoralen per ml (Griffith et al., J. Mol. Biol., in

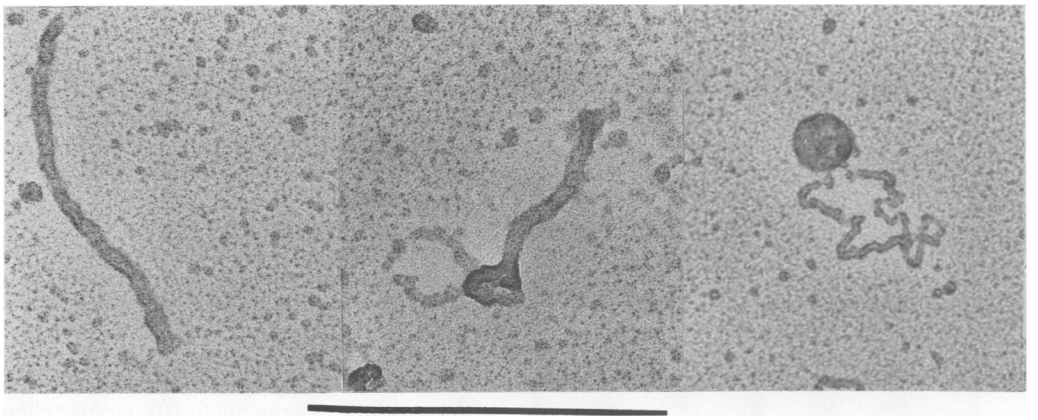

FIG. 7. *Visualization of DNA extrusion by using the E. coli single-strand DNA binding protein (SSB [9]). Addition of SSB to full-length I-forms (left panel) revealed no DNA exposed, but as further contraction to spheroids occurred (center and right panels), an amount of DNA appeared proportionate to the degree of contraction. Samples were prepared as described previously (9); the preparation included rotary shadow casting with tungsten. Bar equals 0.25 μm.*

press). Despite the cross-linked state of the DNA, I-form particles contracted into spheroids upon exposure to chloroform at 24°C, as revealed by EM. To examine this change in I-form particles lacking DNA, we banded I-forms in CsCl gradients as described above, and the empty virion shells from the less-dense peak (I-forms lacking DNA) were collected. When these empty capsids were treated with chloroform, they too contracted into spheroids (Fig. 8).

M13 filament fragments obtained by French pressure cell shearing were used to determine whether the minor coat proteins were essential for these structural changes. Because the virion minor coat proteins are located at or near the ends of the filament (4, 5, 10, 18, 24), the fragments possessed, at most, the minor coat proteins from only one end. Those fragments from the center of the filament might have had no minor coat proteins. When the fragments were treated with chloroform for 60 s on ice and examined by EM, all filament fragments had converted into short fat tubes which resembled sections of I-forms (Fig. 9). No unchanged filament fragments were observed. Thus, it appears that these highly ordered changes in the virus capsid depended upon the structure or arrangement of the B protein and did not require either viral DNA or a full complement of minor coat proteins.

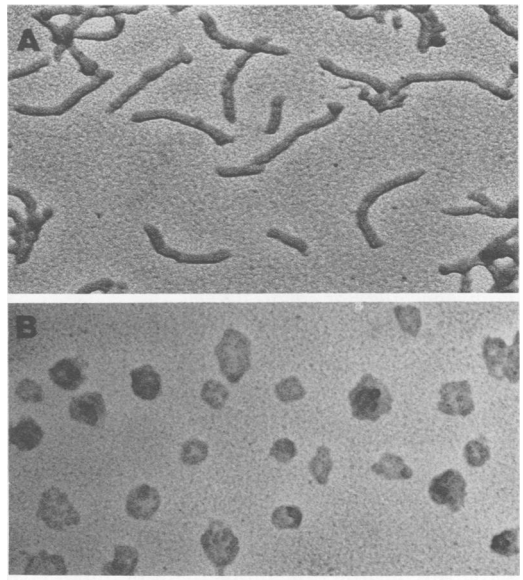

FIG. 8. *I-form particles lacking DNA contract into spheroids upon exposure to room-temperature chloroform. I-forms were banded to equilibrium in a CsCl gradient, and the upper peak (I-forms lacking DNA) was collected (A). When gently mixed with chloroform at 20°C with a Vortex mixer, these particles contracted into spheroids (B). Samples were mounted on carbon films and rotary tungsten shadowed. Bar equals 0.25 μm.*

DISCUSSION

In a previous report, we described the chloroform-induced contraction of M13 filaments into spheroids (9). The details of the contraction mechanism, however, remained to be elucidated. In this report, we described the isolation and characterization of particles that appeared to be intermediates in the transition of filaments to spheroids. By controlling the temperature during chloroform treatment, we documented an ordered sequence of events in the morphological transformation of the M13 virion (Fig. 6).

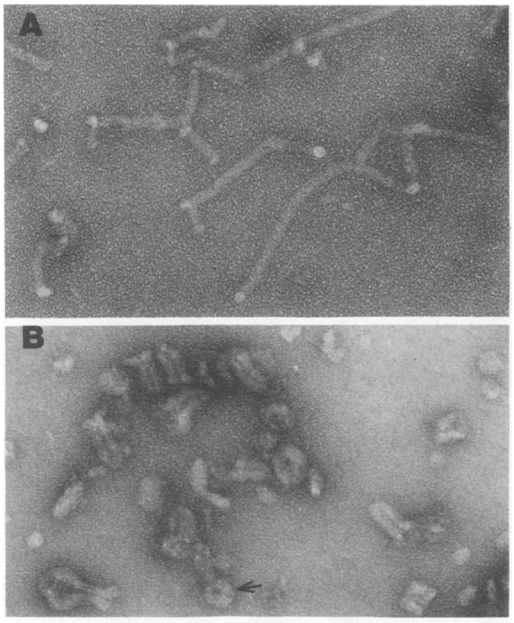

FIG. 9. *Visualization of the chloroform-induced contraction of fragmented M13. Phage filaments were sheared in a French pressure cell (A). Subsequent chloroform treatment produced short fat tubes resembling sections of I-forms (B). Small circular structures with a diameter similar to that of I-forms and showing a stained core (arrow) probably represent particles oriented perpendicularly to the plane of the grid (particles viewed end on). Samples were stained with uranyl acetate. Bar equals 0.1 μm.*

We showed that the contraction of the filaments was not necessarily cooperative. Hybrids possessing morphological characteristics of two structures (I-form–filament or I-form–spheroid structures) were isolated and remained stable in aqueous solution. Because these changes were observed in fragments of M13 filaments, apparently neither the presence of a complete virion structure nor the full complement of minor coat proteins was required for the occurrence of these events.

We were unable to isolate I-forms after exposure of normal-length filaments to chloroform above 15°C and therefore cannot prove that contraction initiated above and below 15°C follows the same sequence of steps. However, two observations suggest that normal-length filaments simply contract from I-forms to spheroids too rapidly at room temperature to allow I-form visualization. First, I-forms of wild-type phage immediately convert to spheroids when exposed to room temperature chloroform, and second, filaments that do not form stable spheroids (i.e., miniphage and the pBR322 chimeric phage) contract into I-forms when treated with room-temperature chloroform. Amako and Yasunka (1) and Marvin (13) also visualized structures resembling I-forms after room-temperature ether treatment of Pf1 (a filamentous phage constructed somewhat differently than M13). Thus, contraction may be a common property of filamentous phage despite differences in length and capsid structure.

These changes in morphology may result from a conformational change in the coat protein subunits or a reorientation of the subunits with respect to one another. Optical (circular dichroism) studies of the major coat protein (B protein) showed that it changes from nearly 100% alpha-helicity in intact filaments to 50% alpha-helicity when solubilized in detergents or incorporated into lipid vesicles (3, 17, 23). It was proposed that a similar conformational change occurs when the B protein is inserted into the bacterial membrane (17). Work in this laboratory revealed very similar changes in circular dichroism spectra as filaments change to I-forms and then to spheroids (unpublished data).

Many aspects of the filamentous phage life cycle have been well characterized, but the mechanism by which the coat proteins are incorporated into the cell membrane and the DNA is extruded into the cell during penetration has remained elusive. The process has been especially puzzling because the native capsid does not disperse either in lipid bilayers or in detergents. Although we have not proven that the contracted phage forms have a counterpart in vivo, the characterization of I-forms and spheroids demonstrated that the capsid could exist in an alternative, more-soluble form and that the filament was constructed to allow preferential release of the DNA from the end of the virion that contains the knoblike adsorption complex. Furthermore, with our EM mapping showing which portion of the DNA was retained inside the spheroids, the experiments reported here lead us to suggest that the origin of DNA replication is the last segment of the phage genome to enter the cell. This conclusion is in agreement with the recent work of Webster et al. (R. E. Webster, R. A. Grant, and L. A. W. Hamilton, J. Mol. Biol., in press), who showed that in phage filaments, the origin of replication resides at the opposite end of the phage from the adsorption protein complex.

On the bases of these studies, we propose that in vivo, filamentous phage are "activated" by contact with a specific cell component(s), possibly the F pilus or the pilus basal structure. This activation initiates capsid changes necessary for insertion of the B protein into the membrane. The chloroform-water interface may mimic this

activation step, and, although the minor coat proteins were not necessary for this in vitro, they may well be required to initiate the process in vivo. From the temperature sensitivity of M13 contraction in vitro, we suspect that some aspects of M13 penetration in vivo also show temperature dependence. Indeed, Tzagoloff and Pratt showed that M13 penetration and uncoating (but not attachment) were blocked below 15°C (22).

Our studies with I-forms suggest that activation need not result in the rapid contraction of the filament and forcible ejection of the DNA. Rather they suggest that capsid rearrangement in vivo is a gradual stepwise rearrangement and that the portion of the capsid nearest the receptor (and nearest the membrane) is activated, dispersed into the membrane, and sequentially followed by the rest of the virion. As the B protein enters the membrane, it may transiently form a channel through which the viral DNA traverses the lipid bilayer. Completely contracted particles such as I-forms or spheroids may not exist in their entirety at the membrane surface, but they may appear in vitro because the coat protein cannot disperse into the solvent.

The remarkable 20-fold contraction of the M13 capsid in vitro suggests the provocative possibility that other protein filaments may undergo contraction in response to a hydrophobic environment and that changes in membrane interfaces may regulate the contraction of protein filaments such as those forming the eucaryotic cell cytoskeleton.

ACKNOWLEDGMENTS

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