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COAT PROTEIN CONFORMATION IN M13 FILAMENTS, I-FORMS AND SPHEROIDS

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Circular dichroism studies of the filamentous coliphage M13 were carried out to determine conformational changes in the major capsid protein (the B protein) that occur during contraction of the filaments to I-forms and spheroids. The α-helicity of the B protein is somewhat lower in the I-forms than in filaments and much lower in spheroids. This conformational change may explain the increased detergent and lipid solubility of both I forms and spheroids relative to filaments.

Filamentous coliphages (M13, fd, f1) penetrate male E. coli cells by inserting the major capsid protein (the B protein) into the bacterial inner membrane (1-3). A variety of studies have shown that the B protein is nearly 100% α-helical in the virion, but only 50% α-helical when incorporated into lipid bilayers (4-10). The mechanism by which this change is accomplished has been difficult to deduce, especially because the virus capsid is extremely stable in the presence of lipids and even most detergents.

We recently demonstrated that exposure of M13 filaments to a hydrophobic but slightly polar interface induces a highly ordered, morphological change in the virus capsid and makes it detergent and apparently lipid soluble (11,12).

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When an aqueous suspension of M13 filaments is gently mixed with an equal volume of chloroform at room temperature, the 900nm x 5nm filaments contract into stable hollow spherical particles 40nm in diameter (spheroids). Two-thirds of the viral DNA is extruded from a hole in the spheroid shell and the third spanning the origin of viral DNA replication remains bound inside (11). Our evidence indicates that the solvent-water

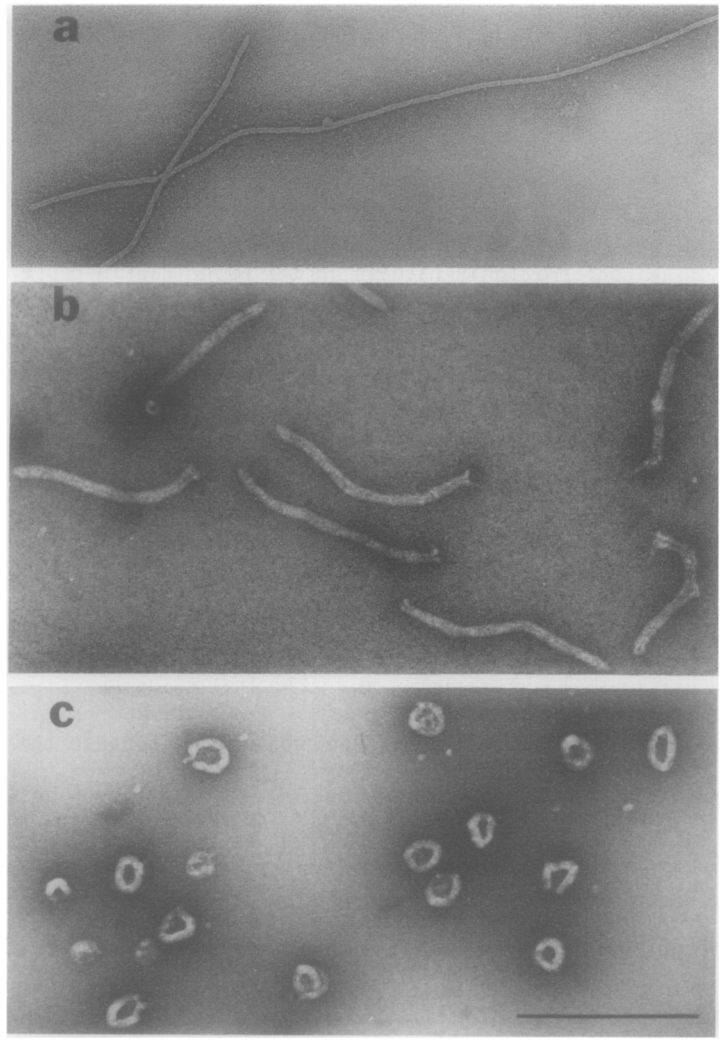

Figure 1. Visualization of the chloroform induced contraction of coliphage M13. Normal virus filaments (a) briefly shaken with ice-cold chloroform contract into I-forms, (b) treatment of filaments or I-forms at room temperature produces spheroids, (c) samples were stained with uranyl acetate. Bar equals 0.2 μm.

interface is required and that many hydrophobic but slightly polar solvents induce nearly identical changes. Filaments exposed to a chloroform-water interface at 2°C contract into particles that appear to be intermediates (I-forms) in the filament-to-spheroid contraction process. These I-form particles are stable in an aqueous environment at temperatures up to 37°C, but contract into spheroids if reexposed to a solvent interface at temperatures above 15°C (figure 1). Contraction appears to involve noncooperative, localized changes in the filament structure and causes the capsid protein to become susceptible to treatment with detergents and proteases (11). Our recent evidence indicates that I-forms and spheroids, in contrast to filaments, can apparently fuse with small unilamellar lipid vesicles at temperatures above the phase transition temperature of the lipid (13). We have postulated that the changes induced by the chloroform:water interface may be similar to changes that occur *in vivo* during virus penetration, converting the capsid to a form more readily dispersed in a phospholipid bilayer. It was not know if the contraction of the filament- and the concommitant changes in capsid properties - involved only a rearrangement of the B proteins with respect to each other, or an actual change in the conformation of the B protein. In this report, we describe circular dichroism (CD) studies that indicate that the conformation of the B protein is different in filaments, I-forms, and spheroids.

METHODS

Growth and purification of the phage have been previously described (11,14). I-forms were prepared by gently shaking a suspension of M13 filaments with an equal volume of ice-cold chloroform for 60 seconds, and collecting the aqueous phase. Spheroids were obtained by gently shaking the I-forms with an equal volume of chloroform at room temperature. Accurate measurements of the concentration of coat protein in samples for CD analysis were made spectrophotometrically using A_{260} = 3.78 for a 1 mg/ml solution of filaments, and assuming the protein constitutes 88.5% of the virion (5). Identical values were used for I-forms and spheroids since no appreciable amount of protein is lost during contraction (11). In addition, small amounts of virions labeled with ^{3}H-lysine were added to phage preparations prior to chloroform treatment, and specific activity relative to absorbance determined. The concentrations of coat protein in subsequent preparations of filaments, I-forms, or spheroids could then be determined by radioactive counts. A Cary 60 spectropolarimeter with a CD attachment was used for CD measurements and a mean residue molecular weight of 105 was

used to calculate molar elipticities. All measurements were made at 29°C. Each spectrum was repeated at least five times with several concentrations of virus.

RESULTS

As shown in figure 2, filaments, I-forms, and spheroids give rise to three different CD spectra. In each, elipticity minima are seen at 208nm and 222nm (wavelengths characteristic of α-helix). However, the intensity of the signal varies considerably among the three capsid structures. Filaments absorb more strongly at 222nm than at 208nm, producing a highly characteristic spectrum. The intensity of the minima at both wavelengths is substantially decreased in the I-form spectrum, and even more decreased in the spheroid spectrum. Although these spectra are of intact virus, not purified B protein, the changes we have noted can be attributed almost entirely to changes in the B protein signal since the contribution of DNA to the signal in the 200-250nm region is negligible and the minor coat proteins comprise less than 2% of the virion particles (5,6,15).

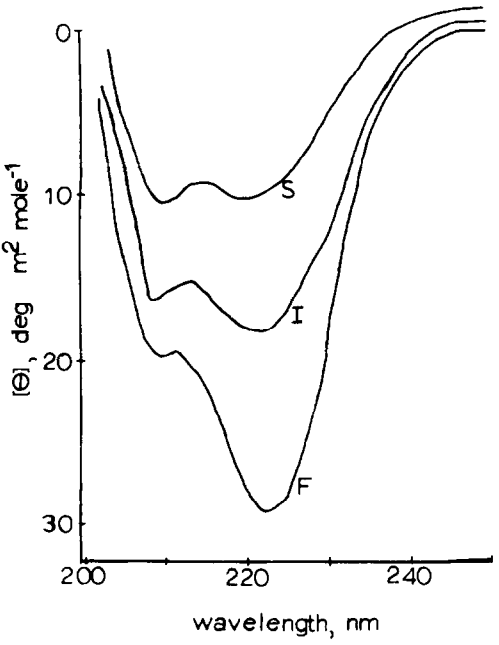

Figure 2. The CD spectra of filaments (F), I-forms (I), and spheroids (S). The molar elipticity minima of the filament spectrum differ slightly from previously reported valves (5,6) due to the inherent difficulties in determining the precise protein concentration in intect virus preparations.

DISCUSSION

CD, X-ray, ultraviolet, and laser Raman spectroscopy have been used to examine the conformation of the B protein in filaments (4,16-19). Each study has suggested that the percentage of α-helix is very high. For example, laser Raman data are most consistent with uniform α-helicity and less than 5% β structure (20). The CD data presented in this study suggest that the contraction of filaments to I-forms and spheroids results in a decrease in the α-helicity of the B protein. An approximation of the α-helicity of the B protein in the contracted phage can be obtained from the elipticity values at 208nm according to the equation of Greenfield and Fasman (21,22). By this criterion, the B protein is roughly 85% α-helical in I-forms and only about 50% α-helical in spheroids.

Although CD is not as refined a technique as laser Raman or X-ray analysis, it is well suited to the goals of this study. Circular dichroism is a good measure of percent α-helix when the α helicity of a peptide is high (21,22). Because the B protein is almost entirely α-helix, the altered conformation in I-forms and spheroids is readily detected.
In addition, CD data are obtained at relatively low concentrations (0.1 mg/ml) as compared to laser Raman (10-100 mg/ml), and X-ray (≈ 500 mg/ml) analysis. Thomas and Day have reported that intervirion interactions occur at high virus concentrations and low salt concentrations and result in changes in B protein conformation (20). Intervirion interaction is a potentially serious problem in the analysis of I-forms and spheroids which have a higher propensity to aggregate and disrupt at high concentration than do filaments. No detectable aggregation occurs at 0.1 mg/ml (11,12, and unpublished observations).

It has been suggested that the stable conformation of the B protein in membranes is not the α-helical conformation of filaments, but rather a form with decreased α-helix and up to 50% β structure (5-10). The CD spectrum

of spheroids is virtually identical to the spectrum of B protein incorporated into lipid bilayers, suggesting that the B protein conformation in spheroids is very similar to its conformation in membranes. This may explain the observed changes in detergent and lipid solubility that occur when filaments contract into I-forms and spheroids.

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