Mimicking Initial Interactions of Bacteriophage M13 Coat Protein Disassembly in Model Membrane Systems

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ABSTRACT: The structure and changes in environment of the M13 major coat protein were studied in model systems, mimicking the initial molecular process of the phage disassembly. For this purpose we have systematically studied protein associations with various detergents and lipids in two different coat protein assemblies: phage particles and S-forms. It is remarkable that the major coat protein can change its conformation to accommodate three distinctly different environments: phage filament, S-form, and membrane-bound form. The structural and environmental changes during this protein transformations were studied by site-directed spin labeling, fluorescence labeling, and CD spectroscopy in different membrane model systems. The phage particles were disrupted only by strong ionic detergents [sodium dodecyl sulfate (SDS) and cetyltrimethylammonium bromide and (CTAB)] but were not affected by sodium cholate and sodium deoxycholate, nonionic detergents, and dilauroyl-L-α-phosphatidylcholine (DLPC) lipid bilayers. Conversion of the phage particles into S-forms by addition of chloroform rendered the coat protein accessible for the association with different ionic and nonionic detergents, as well as DLPC lipids. The disruption of the S-form by all detergents studied was instantaneous but was slower with DLPC vesicles. Only small unilamellar vesicles effectively solubilized the S-form. The data suggest that the viral protein coat is inherently unstable when the major coat protein is exposed to amphiphilic molecules. During conversion from the phage to the S-form, and subsequently to the membrane-bound form, the coat protein undergoes pronounced changes in environment, and in response the α-helix content decreases and the local protein structure changes dramatically. This adaptation of the protein conformation enables a stable association of the protein with the membrane.

Bacteriophage M13 coat is a filamentous phage composed of approximately 2800 copies of the major coat protein and a few copies of the minor coat proteins located at both ends of the phage (*1*). During phage disassembly the virus leaves its coat proteins in the *Escherichia coli* cytoplasmic membrane, while DNA is ejected in the cytoplasm (*2*). An intriguing question about phage disassembly is how the major coat protein is incorporated into the host membrane from a very tightly packed and stable phage coat.

M13 is a very stable virus, and it is hardly affected by lipid bilayers and detergents (*3, 4*). The only well-documented exception is SDS,[1] a strong anionic detergent, which easily disrupts the phage particle (*5, 6*). Also, the phage particle is stable at high temperature (up to 90 °C), and it is not sensitive to protease activity, high or low values of pH, and different salt concentrations (*3, 7*). The phage particle, however, is sensitive to the mechanic stress of ultrasonication, and it is highly sensitive for addition of chloroform, which in turn makes it susceptible for different detergents (*1*). The strong native architecture of the phage coat seems to be maintained primarily by hydrophobic interactions between the individual coat proteins (*7*). The coat proteins form a tube around the viral DNA, with a flexible amphiphatic N-terminus located at the outside of the coat and the basic C-terminus interacting with the DNA at the inside of the coat. The hydrophobic domain of the major coat protein is located in the central section of the protein sequence, and it interlocks the coat protein with its neighboring subunits. The packing of the coat protein subunits is very tight as can be seen from the X-ray structure (*7*) and solid-state NMR data (*8*).

Upon disassembly of the virus particle this tight coat structure, however, must be released. It has been speculated (*9*), that the major coat protein in the phage particle is stable only when surrounded by copies of other major coat proteins. It was also suggested that the stability of the virus particle can be compromised at the unprotected ends of the filament, where addition of solvent may affect the major coat protein conformation and its affinity for other proteins (*9*). This seems to be supported to some extent by the observation

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[1] Abbreviations: CD, circular dichroism; DLPC, dilauroyl-L-α-phosphatidylcholine; DOPC, dioleoyl-L-α-phosphatidylcholine; ESR, electron spin resonance; NMR, nuclear magnetic resonance; IAEDANS, *N*-[[(iodoacetyl)amino]ethyl]-1-sulfonaphthylamine; SDS, sodium dodecyl sulfate; CTAB, cetyltrimethylammonium bromide; MML, L-α-monomyristoyllecithin; DDM, *n*-dodecyl β-D-maltoside; cmc, critical micellar concentration; ANS, 1-anilinonaphthalene-8-sulfonate.

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that phage particles with an amber mutation in the minor coat protein gp6 and gp3 at the host entering end of the phage particle are less stable (*10*, *11*). However, it is very difficult to monitor disassembly *in vivo*. The first events in phage infection have been mimicked in vitro by Griffith and coworkers (*3*, *4*) using a chloroform−water interface. They found that upon addition of chloroform, the long filamentous structure contracted in an ordered way. This is a temperature-dependent process; at 2 °C rods (I-forms) are formed, while at 25 °C spherical structures (S-forms) are formed. In this paper only the S-form will be used. The S-form structure contains all coat proteins with approximately two-thirds of the DNA ejected outside of the S-form. It was also suggested (*4*), on the basis of sucrose density gradient centrifugation, that the S-form interacts with preformed phospholipid vesicles. Since the major coat protein from the S-form associated with the lipids, they suggested that the S-form might be involved in the phage disassembly process as an intermediate step. Formation of the S-form may provide a mechanism for a release of the viral DNA and coat protein association with the lipid bilayers. However, there is no evidence that chloroform or chloroform-like molecules participate in the phage disassembly, as well as there is no evidence of S-form formation in vivo (*12*, *13*).

Despite a disagreement about the significant role of the S-form during the infection process, it is very interesting to note that the major coat protein has the ability to change its conformation, which allows the protein to exist in distinctly different environments: phage filament, S-form, and membrane-bound form. During conversion from one form to another, the coat protein undergoes an extreme change in environment, and in response its structure is almost certain to change.

Since, ultimately the major coat protein−lipid interactions in the host membrane are responsible for the solubilization of the protein coat and concomitant DNA release, we mimicked possible changes of the protein structure and changes in its local environment during the initial interactions of the phage disassembly in membrane model systems. Because hydrophobic interactions are expected to play an important role in this process, a V31C major coat protein mutant, located in the center of the hydrophobic part of the protein, was produced. This mutant was labeled with a nitroxide spin label for studies with ESR spectroscopy and a fluorescence label for fluorescence spectroscopy. Associations of different detergents and phospholipids with the coat protein were systematically studied with two well-defined protein associations: phage particles and S-forms. Changes in the overall secondary structure of the major coat protein upon phage disruption were followed by CD spectroscopy. The local structural changes in the hydrophobic part of the protein, responsible for the coat stabilization, were determined by site specific spin labeling of the V31C major coat protein mutant followed by ESR spectroscopy. The polarity of the protein in different environments during phage disruption was followed by site-specific fluorescence AEDANS labeling of the V31C major coat protein and exposure to the polar quencher acrylamide. The information obtained allowed us to determine structural and environmental changes of the coat protein during conversion from filamentous form to S-form and subsequent association with the membrane.

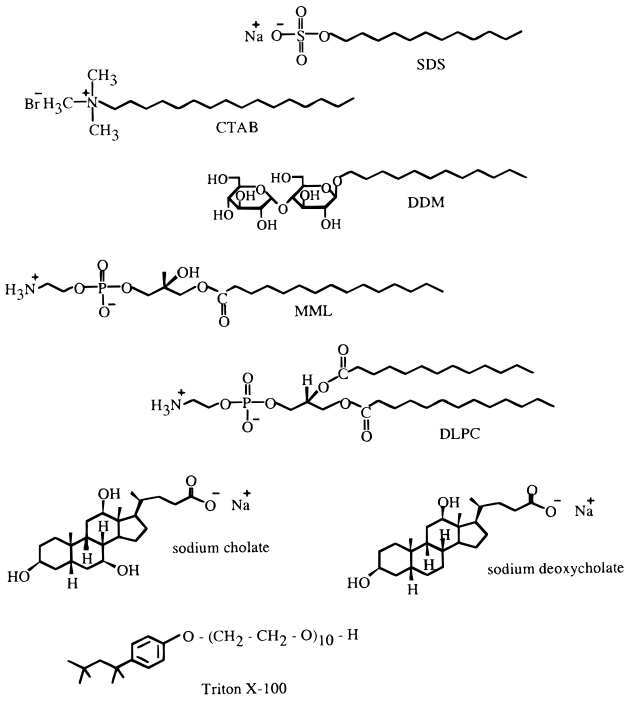

FIGURE 1: Structural formulas of sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB), dodecyl β-D-maltoside (DDM), monomyristoyllecithin (MML), dilauroyl-L-α-phosphatidylcholine (DLPC), sodium cholate, sodium deoxycholate, and Triton X-100.

MATERIALS AND METHODS

Phage Labeling. The major coat protein mutant V31C was grown and purified as described previously (*14*, *15*). The concentrated phage solution was spin-labeled with 3-maleimidoproxyl at a spin label-to-phage ratio of 5/1 (mol/mol) in a 150 mM NaCl and 10 mM Tris-HCl buffer, pH 8.0, at 37 °C for 2 h. The labeling reaction was stopped by adding an excess of cysteine to the reaction mixture. Free spin label was removed by dialysis at room temperature against a 100-fold excess of 150 mM NaCl and 10 mM Tris-HCl buffer, pH 8.0. The dialysis buffer was changed four times every 12 h. The major coat protein mutant V31C was also labeled with the IAEDANS fluorescence label as described above for the spin-labeled phage, but labeling and dialysis were carried out in the dark to prevent photodegradation of the AEDANS.

Phage Disruption in Detergents and Lipids. Spin-labeled or fluorescence-labeled phage solution (8−16 mg/mL) was mixed with an equal volume of concentrated detergent solution to obtain the final detergent concentration well above the critical micellar concentration (cmc). The detergents used in this paper are shown in Figure 1. Final detergent concentrations were 30 mM SDS, 100 mM sodium cholate, 100 mM sodium deoxycholate, 20 mM cetyltrimethylammonium bromide (CTAB), 100 mM dodecyl β-D-maltoside (DDM), 100 mM monomyristoyllecithin (MML), and 3% Triton X-100. The detergent solutions were prepared in 150 mM NaCl and 10 mM Tris-HCl buffer, pH 8.0. The phage−detergent mixtures were vigorously vortexed before application to either ESR, fluorescence, or CD experiments. The samples for the CD measurements were diluted in 50 mM phosphate buffer, pH 8.0. Small unilamellar vesicles of DLPC and DOPC were prepared as follows. The lipids were

dissolved in chloroform, evaporated onto the surface of the glass tube by nitrogen gas, and dried under vacuum for at least 2 h to remove traces of chloroform. The lipids were resuspended in 150 mM NaCl and 10 mM Tris-HCl buffer, pH 8.0, and sonicated on a Branson B15 cell disrupter for 20 min, until a clear opalescent solution was obtained. Large lipid vesicles were removed by centrifugation at 12 000 rpm for 30 min in a Sigma-220MC centrifuge. Small unilamellar vesicles were incubated with phage (20 mg of lipids/mg of phage) at 37 °C for 2 h with occasional mixing.

S-Form Preparation. The S-form was prepared by gently vortexing a mixture of equal volumes of filamentous phage and chloroform for 5 s/min at least five times at room temperature as described by Griffith et al. (*3*). After phase separation the aqueous supernatant containing the S-form was collected and the remaining chloroform was removed under a flow of nitrogen. The filamentous phages were transformed completely to uniform spherical morphology (S-form) as checked by electron microscopy (data not shown). Since S-form is not stable over a prolonged period of time, fresh S-form was prepared for each set of experiments.

S-Form Disruption in Detergents and Lipids. The S-form was disrupted in 30 mM SDS, 100 mM sodium cholate, 100 mM sodium deoxycholate, 20 mM CTAB, 100 mM DDM, 100 mM MML, and 3% Triton X-100 final concentration. The detergent solutions were prepared in 150 mM NaCl and 10 mM Tris-HCl buffer, pH 8.0. The S-form was incubated with small unilamellar DLPC or DOPC vesicles as described above for the disruption of phage particles.

ESR Spectroscopy. ESR spectra were recorded on a Bruker ESP 300E spectrometer equipped with a 108TMH/9103 microwave cavity at room temperature as described previously (*14*).

Fluorescence Spectroscopy. Fluorescence spectroscopy was performed with a Perkin-Elmer LS-5 luminescence spectrophotometer at room temperature. The emission spectra of AEDANS-labeled major coat protein mixed with different detergents were collected with an excitation wavelength of 340 nm and an excitation and emission bandwidth of 5 nm. The emission spectra were recorded from 400 to 550 nm. The background intensity from the labeled wild-type phage in the same buffer was recorded under the same experimental conditions and was subtracted from the corresponding spectral intensity. Steady-state quenching studies were performed by addition of acrylamide as described by Spruijt et al. (*15*).

CD Spectroscopy. CD measurements were performed at room temperature on a Jasco J-715 spectrometer in the wavelength range 190−290 nm with a 10 mm path length. The CD settings were 100 s scan time, 1 nm bandwidth, 0.1 nm resolution, and 125 ms response time. Up to 20 spectra were accumulated to improve the signal-to-noise ratio. Background spectra, consisting of the same buffer, were recorded under the same experimental conditions. Difference spectra were generated by subtracting the background spectra from the corresponding spectra. For CD spectroscopy both wild-type and labeled phage particles were used.

RESULTS

The CD spectra of M13 phage filament, S-form, and cholate-associated coat protein are given in Figure 2, both

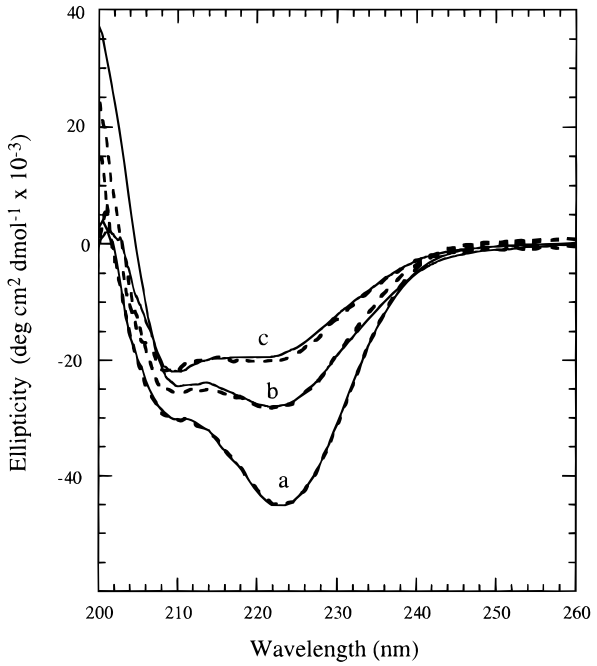

FIGURE 2: CD spectra at room temperature of the major coat protein (a) in filamentous phage, (b) in intact S-form, and (c) solubilized from the S-form in sodium cholate. The solid lines are for wild-type major protein; the dashed lines are for spin-labeled V31C major coat protein.

for the wild-type major coat protein and spin-labeled V31C major coat protein. As can be seen on comparing the spectra of both types of protein, there is no difference in the characteristic minima at 208 and 222 nm, indicating that protein modification does not alter the secondary structure. The CD spectra of the S-form and cholate-associated coat protein have significantly different line shapes and spectral intensities as compared to the filamentous phage particles. The intensity of the CD spectrum of the phage filaments is consistent with a high α-helix content of the major coat protein, although the line shape is anomalous around 222 nm (*16*). Conversion of the filamentous phage to the S-form dramatically decreases the ellipticity at 222 nm and to a smaller extent at 208 nm. Association of the S-form with sodium cholate further decreases the ellipticity throughout the 208−240 nm region. The CD spectra of the major coat protein associated with other detergents (data not shown) are similar to those of the coat protein in sodium cholate and are consistent with previous reports in the literature (*6*, *13*, *17*). The higher CD intensity of the coat protein in the S-form indicates that the α-helical content is higher as compared to the coat protein associated with detergents.

The wavelengths of the fluorescence emission maximum of the AEDANS-labeled V31C coat protein in filamentous phage and in the S-form in the presence of different detergents are given in Table 1. The wavelength of the fluorescence emission maximum of the coat protein in the filamentous phage in the absence of amphiphiles was 491 nm and was not changed significantly upon addition of the detergents sodium cholate, sodium deoxycholate, DDM, MML, and Triton X-100, respectively. However, in the presence of SDS and CTAB, the fluorescence emission maximum increased to 500 and 475 nm, respectively. Conversion of phage filaments to the S-form shifted the wavelength of the fluorescence emission maximum to 462

Table 1: Fluorescence Emission Wavelength Maximum of the AEDANS-Labeled V31C Mutant Coat Protein in Filamentous Phage and in the S-Form after Solubilization in Different Detergents[a]

treatment	λ_{max} for filaments (nm)	F/F_0	λ_{max} for S-form (nm)	F/F_0	cmc
	491	0.60	462	0.90	
DLPC	491	0.60	471	0.93	<0.01
sodium cholate	489	0.64	463	0.81	15.00
sodium deoxycholate	491	0.63	465	0.79	6.00
CTAB	475	0.75	473	0.74	0.92
Triton X-100	488	0.64	480	0.68	0.24
DDM	490	0.63	481	0.72	0.60
MML	490	0.62	486	0.71	0.01
SDS	500	0.55	502	0.54	0.90

[a] The final detergent concentrations were 30 mM SDS, 20 mM CTAB, 3% Triton X-100, 100 mM sodium cholate, 100 mM sodium deoxycholate, 100 mM DDM, and 100 mM MML. The AEDANS-labeled coat protein in filamentous phage and solubilized from S-form in different detergents was quenched by a 125 mM final concentration of acrylamide. The maximum decrease in fluorescence intensity is given as F/F_0, where F_0 is the fluorescence intensity of the nonquenched sample. Tbe cmc values of the detergents were obtained from refs *18* and *28*, taking into account the sample salt concentration in the case of ionic detergents.

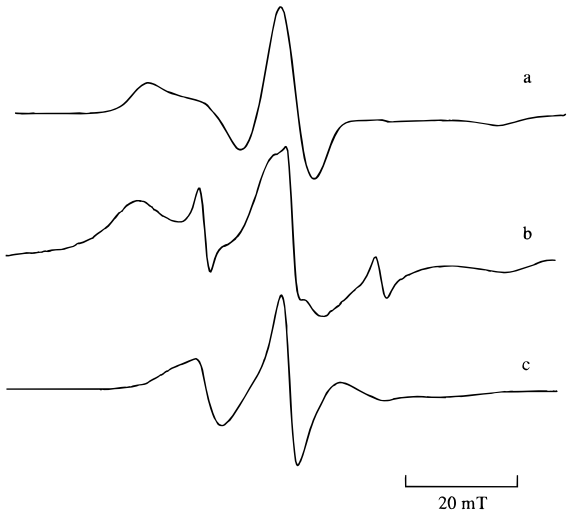

FIGURE 3: ESR spectra of spin-labeled V31C major coat protein (a) in filamentous phage, (b) in intact S-form, and (c) solubilized from the S-form in CTAB. Spectra were recorded at room temperature and were normalized to the same central line height.

nm in the absence of amphiphiles. When the S-form was mixed with detergents, the fluorescence emission wavelength maximum increased again. The influence of acrylamide, a water-soluble quencher, on the fluorescence intensity of the AEDANS-labeled V31C major coat protein is given in Table 1. The fluorescence of detergent-associated fluorescence-labeled coat proteins with a higher emission wavelength maximum was quenched more efficiently than detergent-associated coat proteins with a lower fluorescence emission wavelength maximum.

The ESR spectra of spin-labeled V31C coat protein in the filamentous phage, converted to S-form, and mixed with CTAB at room temperature are given in Figure 3. The spectrum of the spin-labeled filamentous phage is characteristic for a strongly immobilized spin label with an outer splitting $2A_{max}$ of 6.44 mT, as given in Table 2. The spectrum of the filamentous phage was not affected by addition of 100 mM sodium cholate, 100 mM sodium deoxycholate, 100 mM DDM, 100 mM MML, or 3% Triton X-100. The mobility of the spin probe, however, dramatically increased upon mixing with 30 mM SDS and 20 mM CTAB, giving values for $2A_{max}$ of 4.85 and 5.16 mT, respectively. Conversion of the spin-labeled filamentous phage to the S-form in the absence of amphiphiles resulted in an immobilization of the spin label, giving a value of $2A_{max}$ of 6.68 mT, and a substantial line broadening of the spectrum. An additional mobile component appeared in the spectrum. As shown in Figure 3, association of the spin-labeled S-form with CTAB detergent significantly reduced the outer hyperfine splitting. This ESR spectrum is characteristic for an intermediate motion with an outer splitting $2A_{max}$ of 5.16 mT. The spectrum was indistinguishable from that of the spin-labeled filamentous phage after mixing with CTAB (data not shown). The outer hyperfine splitting after mixing spin-labeled S-form with sodium cholate, sodium deoxycholate, Triton X-100, DDM, MML, and SDS is given in Table 2. The outer hyperfine splitting of spin-labeled S-form decreased significantly with all detergent studied.

Table 2: Outer Splitting of the ESR Spectra of Phage Filaments and the S-Form Solubilized with Different Detergents and Lipids[a]

treatment	$2A_{max}$ (mT) filaments	$2A_{max}$ (mT) S-form
	6.44	6.68
DLPC	6.44	5.89
sodium cholate	6.44	5.71
sodium deoxycholate	6.44	5.73
CTAB	5.16	5.16
Triton X-100	6.44	5.94
DDM	6.44	5.80
MML	6.44	5.35
SDS	4.85	4.85

[a] The final detergent concentrations are the same as in Table 1.

To study the type of association of DLPC and DOPC with the S-form, small unilamellar vesicles were incubated with either phage particles or S-forms. There was no interaction between DLPC or DOPC bilayers and phage filaments. The S-form, however, was disrupted when incubated with DLPC bilayers. The disruption was much slower than with detergents and was possible only with small unilamellar vesicles. The fluorescence emission wavelength maximum of the AEDANS-labeled coat protein associated with DLPC bilayers was 471 nm. This is somewhat higher than found for the S-form, and the fluorescence intensity was only slightly quenched by acrylamide (see Table 1). The CD spectrum of the major coat protein associated with lipids was similar to the spectra obtained with detergent-associated forms of the coat protein, indicative for an α-helix secondary structure. The ESR spectrum of the spin-labeled coat protein associated with lipids was characteristic for an immobilized spin label with an outer splitting of $2A_{max}$ of 5.89 mT. Small unilamelar DOPC vesicles could also disrupt the S-form; however, the results were not well reproducible. Furthermore, small unilamellar DOPC vesicles were not stable during incubation and tended to form larger vesicles (data not shown).

DISCUSSION

Bacteriophage M13 major proteins form a very stable protective coat around the viral DNA, which is not easily disrupted by various chaotropic reagents (*3*). The stability

of the coat, however, must be compromised during uncoating of the phage particle, when DNA enters into the host cell and coat proteins are incorporated into the host cytoplasmic membrane. The data in this paper clearly indicate that it is possible to disrupt the protein coat with different detergents and lipids. The latter, however, is a special case, and we will first discuss the more general case of phage disruption by detergents.

Phage Filament. The large values of the outer splitting in the ESR spectrum of the spin-labeled V31C mutant are consistent with a tight protein subunit packing in the phage particle, as proposed by Marvin et al. (*7*). According to this model, residue 31 is probably located at the bottom of a fairly deep cleft. Amino acid residue 31 of the mutant protein is located in the middle of the protein hydrophobic domain. In the phage structure, however, this residue appears to be accessible to both the spin-label and the more bulky IAEDANS label, which has a relatively high fluorescence emission maximum (491 nm) and a good exposure for acrylamide quenchers (see Table 1). This indicates a quite well exposed position in the phage. Although located in a more hydrophilic environment, the spin label is considerably immobilized by surrounding amino acids in the filamentous phage structure, as can be inferred from the large values of the outer splitting in the ESR spectrum.

To solubilize the phage coat, the detergent has to bind to the protein aggregate and penetrate into the hydrophobic interior (*18*, *19*). As deduced from the spin-label ESR studies, fluorescence labeling experiments, CD spectroscopy, and electron microscopy experiments, the filamentous phage particle after mixing with detergents was disrupted only with the detergents SDS and CTAB. All other detergents and lipids used did not change ESR, CD, or fluorescence spectral parameters (see Tables 1 and 2). This is consistent with an undisrupted filament structure, as seen by electron microscopy (data not shown). There was no influence of the labeling of the mutant on the disruption properties of the phage particle, as deduced from CD experiments of the wild-type and fluorescence- or spin-labeled protein. This shows that the labeled V31C mutant coat protein is a good reporter for the behavior of the native coat protein.

The two detergents that disrupt filamentous particles, the anionic SDS and the cationic CTAB, have in common a flexible monoacyl chain 12 or 16 carbons long, attached to a charged headgroup. From geometry consideration there are two possible reasons for the ability of these two detergents to disrupt the phage particle. First, they have a single charge, and second, they both have a flexible hydrophobic tail. It has been suggested by Roberts and Dunker (*13*) that the nonrigidly packed hydrophobic tails of the SDS molecules readily penetrate and dissolve hydrophobic protein aggregates. This also applies for CTAB. In contrast, the rigid and flat negatively charged sodium cholate and sodium deoxycholate detergents are unable to disrupt the phage particle. This indicates that tightly packed protein coat does not allow the penetration of rigid and relatively bulky molecules to the hydrophobic interior of the protein coat. Although a flexible hydrophobic tail is clearly important in phage disruption, its presence alone is not sufficient. For example, DDM, Triton X-100, and MML all have a comparable single flexible hydrophobic chain, but they are unable to disrupt the phage particle. DDM and Triton X-100 are nonionic detergents, which suggests that charge is important in phage disruption. It is, however, interesting to note also that the zwitterionic MML is not able to disrupt the phage particle. This would indicate that in addition to a single flexible acyl chain a net detergent charge is needed for phage disruption.

S-Form Phage. Conversion of the filamentous phage particle to the S-form is accompanied by a significant structural and environmental change. The fluorescence blue shift of the AEDANS-labeled V31C major coat protein (see Table 1), and a decreased accessibility to acrylamide quencher, indicate a more hydrophobic environment of the fluorescence label in the S-form (*15*). In addition, the conversion to the S-form removes the anomalous high negative ellipticity at 222 nm in the CD spectrum of the phage (see Figure 2). This anomaly in the CD spectrum has been tentatively attributed to a strong absorption of chromophore oscillator coupling between Trp26 and Phe45 of the two neighboring proteins in the phage particle labeled as 0 and 11 in the index notation used by Marvin et al. (*7*). This coupled oscillator breaks down during conversion into the S-form, whereby neighboring proteins slide along their length relative to each other (*13*). The conversion to the S-form also significantly decreases the side-chain mobility of the spin-labeled part of the protein (see Figure 3). This is in good agreement with the spin-labeled Y24C−V31A mutant used to study the transition from the filamentous phage to the S-form by Khan et al. (*29*). This reduced side-chain mobility of the spin-labeled V31C contradicts the idea introduced by Dunker et al. (*20*) that the S-form resembles molten globules. The principal feature of molten globules is the nonrigid side-chain packing (*21*). In addition, Dunker et al. (*20*) showed that the S-form but not filamentous phage binds ANS (1-anilinonaphthalene-8-sulfonate). ANS binds either to the exposed hydrophobic groups on the protein or to the nonrigid side chains packed in the protein aggregate (*20*). An appearance of the sharp ESR component upon conversion to the S-form suggests that a small fraction of the spin labels (5−10%) has a nonrigid side-chain packing, which may explain at least part of the ANS binding to the S-form. On the basis of the observed changes it must be concluded that the initial protein−protein interactions in the filamentous phage are broken during the conversion to the S-form.

It is interesting to note that chloroform, which is hydrophobic, significantly changes the hydrophobic protein−protein interactions of the filamentous phage particle, allowing protein sliding and a change of morphology. However, chloroform is essentially unable to solubilize the major coat protein. On the other hand, all amphiphilic molecules studied were able to disrupt the S-form. The data also show that the spatial constraints preventing phage solubilization are no longer present in the S-form, because the bulky detergents sodium cholate, sodium deoxycholate, and Triton X-100 are able to disrupt the spherical particles. Inspection of electron microscopy pictures of our S-forms and already published S-forms clearly indicates the presence of at least one relatively large aperture in the spheroid structure at the place where DNA is emerging (*3*, *12*, *13*, *20*, *22*). The appearance of the additional small mobile component in the ESR spectrum upon conversion to the S-form is consistent with part of the labels being in a different

environment, presumably provided by this aperture. The apertures in the S-form structure will permit an enhanced accessibility of the major coat proteins in the S-form for interaction with detergents. Once the detergent has a direct access to the hydrophobic part of the major coat protein, the disruption proceeds very rapidly toward a complete solubilization of the protein aggregate. The solubilization of the S-form upon addition of the detergents was practically instant on the time scale of our experiments.

Although all detergents studied solubilize the S-form, the state of the protein after association with various detergents was not the same (see Tables 1 and 2). Since protein solubilization is an equilibrium between protein−protein and protein-amphiphile interactions, it is not surprising that protein association with the different detergents is not the same. For instance, sodium cholate is a weak detergent with a high cmc. It has a relatively low aggregation tendency, and it has a weak interaction with the protein, because it can be easy removed by dialysis. It has been shown previously that sodium cholate is unable to completely disrupt protein−protein interactions (*17*). This is consistent with our results, whereby disruption of the fluorescence-labeled S-form in sodium cholate preserves the hydrophobic environment to a great extent. In addition, a relatively low mobility of the spin-labeled protein in small sodium cholate micelles also suggests protein aggregation. The detergent SDS represents the other extreme. SDS is a strong anionic detergent with a low cmc. It is relatively insoluble in an aqueous environment, it has a high aggregation tendency, and it has a strong interaction with the protein. In addition, it has a strong ability to disrupt noncovalent protein−protein interactions. During disruption of the S-form in SDS, the polarity of the fluorescence-labeled part of the protein is changed dramatically from the situation in the S-form, and the mobility of the spin-labeled part of the protein was increased significantly. This is in agreement with a monomeric state of the protein in SDS (*5*). The fluorescence and spin-label characteristics of the labeled coat protein associated with other detergents used in this study are intermediate to the states of the protein described for sodium cholate and SDS.

S-Form Interaction with Phospholipids. Manning and Griffith (*4*) suggested that the major coat protein from the S-form was able to interact with lipids; however, they could not exclude nonspecific aggregation of the protein in the presence of lipids. In our experiments, the CD spectra of the S-form associated with DLPC lipid bilayers showed no sign of β-sheet secondary structure. It was shown by other groups that nonspecific protein aggregation of the major coat protein causes an irreversible β-sheet polymeric state (*23*−*25*). The CD results furthermore indicate that the secondary structure of the lipid-associated protein is comparable with a very well-characterized protein secondary structure in SDS (*5*). It is also interesting to note that the α-helix content of the coat protein decreases during disruption of the S-form (see Figure 2), which could indicate that the coat protein has an extended helical conformation in the S-form, in contrast to an L-shaped conformation suggested in lipid bilayers (*5*, *26*). The decrease in α-helical content is consistent with the formation of a loop connecting the two helices in L-form and absence of secondary structure in the C-terminus after the protein has dissociated from the viral DNA in the S-form.

In addition, the fluorescence data strongly suggest that the hydrophobic part of the protein is incorporated in the hydrophobic core of the lipid, because of the low fluorescence emission maximum and almost complete exclusion from acrylamide quencher. Finally, it must be emphasized that all the experimental data for the S-form major coat protein associated with lipids compare very well with the data of the major coat protein reconstituted into lipids from sodium cholate by dialysis (*14*, *15*). Although the two mechanisms of protein reconstitution into lipid bilayers, S-form solubilization and reconstitution from the cholate, differ markedly, the end results agree well. In both cases the major coat protein is reconstituted into the membrane.

Effective solubilization of the S-form was only achieved when small unilamellar DLPC vesicles were used. Since the lipid monomer concentration in the solution is low, and the majority of the lipids form vesicles, it is unlikely that saturation of the S-form and subsequent solubilization can be achieved. The demand for small vesicles, on the other hand, suggests that vesicles must be able to come in close proximity to the hydrophobic part of the protein in order to obtain protein solubilization. This is only possible near the aperture in the S-form, where the DNA is emerging. When the dimensions of the aperture, estimated to be 5−15 nm, are taken into account, this is possible with small but not with large vesicles. This may also explain why disruption with DOPC vesicles was not well reproducible. In the case of DOPC it was difficult to maintain small vesicles during relatively long incubation (2 h) with S-forms. The instability of small DOPC vesicles is in agreement with the finding that small highly curved DOPC vesicles are thermodynamically unstable (*27*). Since the requirement of small vesicles for S-form disruption also implies a stressed bilayer situation, it is conceivable that solubilization of the protein decreases the curvature and consequently the internal lipid stress. A similar situation may occur during phage disassembly in the cytoplasmic membrane, where locally around the disassembly site the lipids are expected to be in a stressed state.

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