Structural Changes Accompanying Chloroform-Induced Contraction of the Filamentous Phage fd†

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Received April 26, 1993; Revised Manuscript Received July 7, 1993®

ABSTRACT: Contact with a chloroform/water interface at 2 °C induces contraction of fd filamentous phage into rodlike I-forms; this contraction is accompanied by a decrease in the magnitude of circular dichroism spectral intensity near 222 nm and an increase near 210 nm. Comparisons with circular dichroism spectra of 100% helical poly-L-lysine and *N*-bromosuccinimide-oxidized fd phage indicate that the spectral change accompanying the fd to I-forms transition is due primarily to a change in the contributions from the single tryptophan (W26) of the major coat protein, with probably no significant change in the α-helix content. Further contraction of the rodlike I-forms to spherical S-forms at 25 °C is accompanied by a substantial general decrease in the magnitude of the ellipticity throughout the 230–210-nm region, which is indicative of a decrease in the α-helix content of the major coat protein. The similarity of the circular dichroism spectrum of S-forms with that of coat protein in detergents suggests that the S-form coat protein resembles the coat protein in lipid bilayers. The intrinsic fluorescence of W26 is quenched without red-shift (but perhaps a barely detectable blue-shift) following fd contraction to I-forms and S-forms. The accessibility of W26 to aqueous quenchers does not change significantly upon contraction. However, interaction with hydrophobic quenchers is dramatically altered in the contracted forms in a manner suggesting that the environment surrounding the tryptophan changes from native-protein-like in the fd filament to molten globule-like in the I-form rods and S-form spheroids. As discussed herein, certain features of these data support previous suggestions that chloroform-induced filamentous phage contraction may provide information about phage penetration and assembly *in vivo*.

The class I filamentous bacteriophages (fd, f1, M13) (Marvin & Hohn, 1969; Frank & Day, 1970) contain about 2700 copies of the major coat protein (pVIII), several copies of four different minor coat proteins (pIII, pVI, pVII, pIX), and a molecule of closed, circular single-stranded deoxyribonucleic acid (DNA)[1] [reviewed in Makowski (1984); Rasched andd Oberer (1986); Day et al.(1988); and Model and Russel (1988)]. The almost entirely α-helical major coat protein (Day, 1966, 1969; Marvin et al., 1974; Williams & Dunker, 1981; Thomas et al., 1983; Grygnon et al., 1988; Clack & Gray, 1989), which comprises about 98% of the protein in the capsid (Marvin & Hohn, 1969; Nozaki et al., 1976), forms a complex bundle with axes almost parallel to the long axis of the phage and with a hole inside for the DNA (Marvin, 1966, 1989, 1990; Marvin et al., 1974; Makowski & Caspar, 1978; Glucksman et al., 1992). The four minor coat proteins cap the two ends, with pIII and pVI at one end and pVII and pIX at the other (Grant et al., 1981; Grant & Webster, 1984; Goldsmith & Konigsburg, 1977; Woolford et al., 1977; Lin et al., 1980; Simons et al., 1981).

Filamentous phage penetration and assembly are intimately associated with the host cell membrane, which accounts for the central hydrophobic core evident in the amino acid sequence of pVIII (Asbeck et al., 1969; Nakashima & Konigsberg, 1974). Upon infection, pVIII is deposited in the inner membrane of the host cell (Smilowitz et al., 1972; Smilowitz, 1974) and is later reused and combined with newly made pVIII during assembly of progeny phage (Trenkner et al., 1967). Assembly of the phage takes place at or in the membrane as a phage DNA binding protein, pV (Salstrom & Pratt, 1971), is displaced from the newly synthesized DNA (Mazur & Zinder, 1975) by both infecting and newly synthesized membrane-bound pVIII. Extrusion of the phage through the membrane during assembly does not cause lysis; the cells grow and divide while continuing to produce phage (Marvin & Hohn, 1969). The phage assembly process also requires noncapsid phage proteins pI and pIV [Horabin and Webster (1988), Brissette and Russel (1990), and Guy-Caffey et al. (1992), reviewed in Model and Russel (1988)] and the host protein thioredoxin [Russel and Model (1983, 1985) and reviewed in Russel (1991)].

The pVIII molecule has been shown to exist in three very different conformations. In the intact phage, the protein is at least 90% or more α-helical (Day, 1969; Marvin et al., 1974a; Williams & Dunker, 1981; Thomas et al., 1983; Grygnon et al., 1988; Clack & Gray, 1989; Glucksman et al., 1992). In many detergents and in lipids containing unsaturated double bonds or certain head groups and at high amphiphile to protein ratios, the α-helix content decreases to 50–60% (Ikehara et al., 1975; Cavalieri et al., 1976; Nozaki

† This work was initiated with support from NIH Grants GM 25937 and 25097 awarded to A.K.D. and Molecular Kinetics, Inc., and was completed in partial fulfillment of the Ph.D. degree by L.M.R.

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® Abstract published in *Advance ACS Abstracts,* October 1, 1993.

[1] Abbreviations: deoxyribonucleic acid, DNA; circular dichroism, CD; DPPC, dipalmatoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; 2,2,2-trichloroethanol, TCE; sodium dodecyl sulfate, SDS; nuclear magnetic resonance, NMR; tryptophan, W; phenylalanine, F; human growth hormone, HGH; adrenocorticotropic hormone, ACTH; bovine serum albumin, BSA; tryptamine, TAM; ultraviolet, UV.

0006-2960/93/0432-10479$04.00/0

et al., 1976, 1978; Dunker et al., 1982; Spruijt et al., 1989). At low lipid to protein ratios, after removal of detergent by dialysis, or in certain saturated lipids such as DPPC or DMPC, the fd coat protein assumes a structure containing 50–75% β-sheet with little or no α-helix (Makino et al., 1975; Nozaki et al., 1978; Dunker et al., 1982; Spruijt et al., 1989).

The ability of pVIII to take on different conformations may reflect the different environments encountered during the phage life cycle. Reversal of the β-sheet structure back to a helical form for membrane-associated pVIII protein has not yet been accomplished (Nozaki et al., 1978; Dunker et al., 1982; Spruijt et al., 1989), but such reversal has been accomplished by dissolving lipid-associated β-form pVIII in detergents (Dunker et al., 1982). It is generally assumed that the *in vivo* membrane-associated conformation of pVIII is the 50–60% α-helix form (Nozaki et al., 1976, 1978), especially given the similarity in structure to that of the proposed membrane-associated form of the filamentous phage Pf1 coat protein (Shiksnis et al., 1987, 1988; Nambudripad et al., 1991; Schon et al., 1991). However, a biological role for the β-sheet structure seems possible, especially given that removal of detergent or lipid *in vitro* favors the β-form and given that phage assembly requires a lipid-depletion step as the protein moves from the membrane environment to the phage environment.

Regardless of whether or not pVIII exhibits a β-form structure transiently during assembly, phage assembly almost certainly includes a substantial conformational change of pVIII, from 50–60% α to >90% α (Nozaki et al., 1976), and phage penetration probably depends on a similar structural change in reverse (Dunker et al., 1991a,b).

Filamentous phages undergo large changes in shape upon exposure to a chloroform/water interface (Griffith et al., 1981; Manning et al., 1981; Lopez & Webster, 1982). This is a temperature dependent process: rods (I-forms) are formed at 2 °C while spherical structures (S-forms) are formed at 25 °C. S-forms can be obtained directly from phage or by reexposing I-forms to the chloroform/water interface at 25 °C. Neither I-forms nor S-forms exhibit any measurable infectivity (Griffith et al., 1981; Manning et al., 1981). The contracted forms are more labile to detergents than is the intact phage, implying that contraction might activate the phage for membrane penetration (Griffith et al., 1981; Manning et al., 1981). Further support for this proposal comes from observations suggesting that the I-forms can spontaneously fuse with preformed, fluid-phase lipid vesicles (Manning et al., 1982). Of special note are findings that I-forms share properties with condensed protein structures known as molten globules (Dunker et al., 1991b), especially because these findings support proposals that the molten globule state might be involved in the translocation of proteins across membranes (Bychkova et al., 1988) and in the insertion of proteins into membranes (Dunker et al., 1991b; van der Goot et al., 1991).

We have been studying the contracted forms in order to understand the relationship between these large shape changes and the structural changes of the coat protein. Here, we report circular dichroism and fluorescence spectra and fluorescence quenching studies demonstrating that the fd to I-form conversion occurs without a substantial change in the helix content of the coat protein but with a significant change in the environment of tryptophan 26. The I-form to S-form conversion occurs with a substantial decrease in the helix content of the coat protein leading to a conformation that is very similar to that found for the coat protein in a membrane environment. These results, along with previous work (Griffith et al., 1981; Manning et al., 1981; Lopez & Webster, 1982; Dunker et al., 1991b), suggest that I-forms and S-forms may provide insight about intermediate states of pVIII in fd penetration and assembly. A model for pVIII conformational changes during fd penetration and assembly based on these findings has been published in an earlier, preliminary report (Dunker et al., 1991a).

MATERIALS AND METHODS

Materials. The acrylamide was obtained from Eastman-Kodak, CsCl from Sigma, and TCE from Aldrich. Other chemicals such as sodium chloride, borate, and so on, were reagent grade.

Phage Growth and Purification. Methods for growth and purification of fd phage followed the long standing methods of centrifugation to remove the host cells, polyethylene glycol phase separation, differential centrifugation, and equilibrium centrifugation on density gradients (Wiseman et al., 1976; Arnold et al., 1992a).

Preparations of I-Forms and S-Forms. I-forms were made by stirring an aliquot of phage (usually 0.14 mg/mL in 0.01 M borate, pH 8.2) with an equal volume of chloroform at 11 400 rpm for 5 s at 2 °C using a Dremel tool equipped with an oval-shaped, Teflon-coated tip for stirring. Following stirring, the phases were separated by centrifugation in a table-top centrifuge for 1 min. The supernatant (containing I-forms) was withdrawn and in some cases diluted with 0.25 M NaCl to give a final solution of I-forms in 0.1 M NaCl, 0.01 M borate, pH 8.2. I-forms were made and centrifuged in a 4 °C cold room and kept below 10 °C during all subsequent steps. S-forms were prepared from I-forms by stirring an aliquot of I-forms with an equal volume of chloroform at 25 °C and diluted in the same manner as described above for I-forms.

Previous workers showed that I-form and S-form preparations yield homogeneous particles as judged by gradient centrifugation (Griffith et al., 1981; Manning et al., 1981; Lopez & Webster, 1982). The homogeneity of the I-form and S-form preparations studied herein was confirmed both by gradient centrifugation and by agarose gel electrophoresis in the absence of denaturants (Roberts, 1990). Furthermore, UV spectral analysis suggests that the samples retain their full complement of DNA (Roberts, 1990), which is in agreement with previous work showing that the DNA remains associated with the I-form and S-form particles (Griffith et al., 1981; Manning et al., 1981; Lopez & Webster, 1982).

For fluorescence studies, residual chloroform remaining in the aqueous phase was removed by blowing a stream of water-saturated nitrogen across the top of the samples. Chloroform removal was monitored by increases in tryptophan fluorescence resulting from loss of chloroform quenching; typically, several minutes of exposure to the nitrogen stream was required for the tryptophan fluorescence to stop increasing.

CD Spectra. I-form spectra were collected at 7 °C; S-form spectra were collected at 25 °C. Spectra of intact phage were collected at both temperatures. The temperature was controlled by use of a Lauda circulating water bath connected to a jacketed CD cell. Samples were prepared on three separate occasions, and one or two spectra were collected for each sample. Each CD spectrum was digitized with a Jandel Scientific 2200/2100 digitizer and buffer-subtracted; the spectra collected for samples prepared in an identical manner but at different times were averaged together.

A serious problem for CD spectroscopy of large particles is that differential light scattering can alter the spectra (Gitter-Amir et al., 1976; Mao & Wallace, 1984). Due to the shape

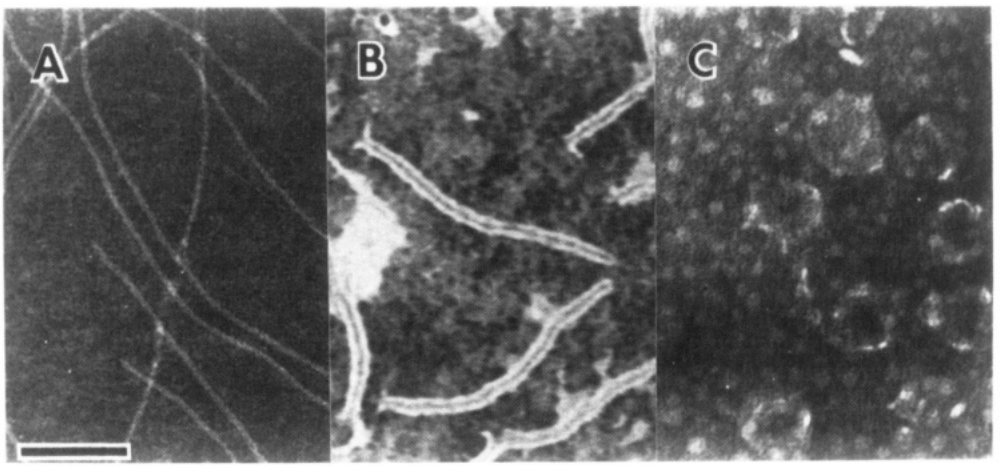

FIGURE 1: Electron micrographs of fd, I-forms and S-forms. I-forms were made from fd at 2 °C, and S-forms were made from I-forms at 25 °C by interaction with a chloroform/water interface as described in the Materials and Methods. Bar equals 0.2 μm. a: fd. b: I-forms. c: S-forms.

changes in the filament to I-forms to S-forms conversions, which are accompanied by light scattering increases in the order of S-forms > I-forms > filaments (Roberts, 1990), spectral distortions from differential light scattering are a potential problem. To test for light scattering artifacts, the detector acceptance half-angle was varied for each sample from about 30° to about 3° by moving the sample relative to the detector. Essentially no changes were observed in the ellipticities near 220 and 210 nm as a function of detector acceptance half-angle, demonstrating that differential light scattering did not make significant contributions to the CD spectra of any of the samples.

A second serious problem in determining accurate ellipticities is accurate determination of the protein concentration. The fd concentration was determined by using the known extinction coefficient of 3.84 cm^2/mg at 269 nm (Berkowitz & Day, 1976). Because exposure of the phage to the chloroform/water interface results in some loss of protein with apparently little or no loss of DNA and the conversion of fd to I-forms and S-forms might involve hypo- or hyperchromism in protein and/or DNA chromophores, the concentration of the contracted forms had to be determined by other than simple UV absorbance measurements.

I-form and S-form protein concentrations were determined by first diluting samples into 0.1 M NaOH to dissociate the protein complexes and eliminate sample differences, followed by use of the Coomassie Brilliant Blue dye binding assay (Bradford, 1976). NaOH-treated fd was used to generate the standard curve (Roberts, 1990).

The decrease in absorbance at 280 nm for I-forms and S-forms in SDS as compared to fd in SDS was also used for determining protein concentration. Here, the assumption is that there is no loss of DNA during S-form and I-form preparation. In this case, the differences between the absorbance spectra of fd, I-forms, and S-forms dissolved in SDS would be due solely to protein losses that occur during the preparation of I-forms and S-forms. The ellipticities at 222 nm calculated using the SDS and Bradford methods to determine protein concentration differed by less than 10%.

Fluorescence. Fluorescence emission spectra were collected on an SLM-4800 (SLM Instruments, Urbana, IL) equipped with a DME-2 monochromator controller and interfaced with an IBM-PC compatible computer from Greenleaf, Inc. Spectra were collected using an excitation wavelength of 295 nm, an excitation bandwidth of 4 nm, and an emission bandwidth of 8 nm. Excitation and emission polarizers were set at 0 and 55°, respectively. Typically, three spectra were collected for each sample at 2 and 6 °C, and the results were averaged together, as very little difference was observed for data collected at these two temperatures.

Fluorescence quenching experiments were performed by adding equal aliquots of fd, I-forms, or S-forms to solutions containing various amounts of quencher. Preparing the samples in this manner avoids the problem of high local concentration of quencher that would occur upon addition of concentrated quencher to the samples. It is necessary to avoid these high local quencher concentrations because the contracted forms lose their DNA in the presence of high concentrations of salt (Griffith et al., 1981; Manning et al., 1981) and may suffer less obvious structural changes in the presence of high concentrations of salt or of nonionic quenchers.

Fluorescence quenching data were fit to the following equation (from Lakowicz, 1983)

$$\frac{F_o}{F} = (1 + K_{SV}[Q])e^{V[Q]} \quad (1)$$

where F_o is the fluorescence in the absence of quencher, F is the fluorescence in the presence of quencher, K_{SV} is the (Stern–Volmer) quenching constant, V is the static quenching term, and [Q] is the concentration of quencher. The curve fitting to obtain K_S and V was accomplished by a least-squares procedure using the KaleidaGraph software package (Synergy Software, 2457 Perkiomen Ave., Mt. Penn, PA 19606) implemented on a Macintosh SE. For the curve fitting to eq 1, the KaleidaGraph software package requires initial guesses for the K_S and V values. To check the validity of the resulting values, simulations were carried out using data derived from eq 1 with input K_S and V values and with the inclusion of appropriate-sized random errors. In these simulations, the KaleidaGraph software package retrieved essentially the exact input K_S and V values from the derived data, regardless of the initial guesses used for K_S and V (the program reached the final solutions much faster if good guesses were supplied initially).

Electron Microscopy. Samples were stained with 2% PTA, placed on carbon-coated copper grids, and viewed with a Hitachi H-300 electron microscope operating at 75 KV.

RESULTS

Electron Microscopy of fd, I-Forms, and S-Forms. Electron micrographs of fd and its contracted forms are

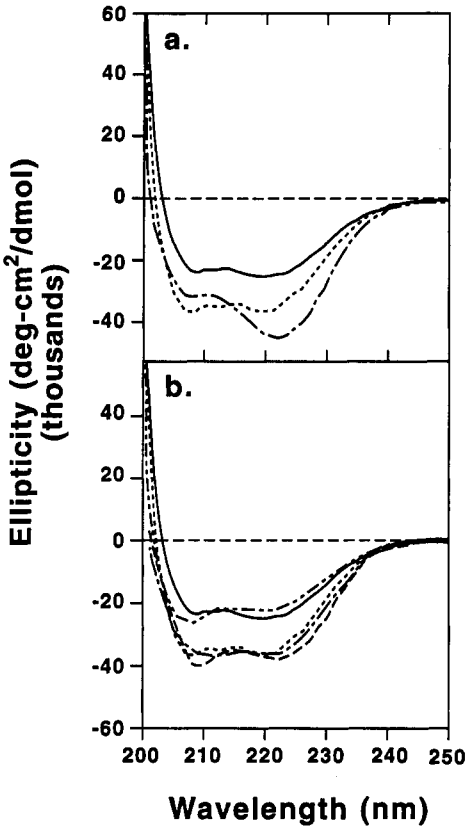

FIGURE 2: CD spectra of fd, I-forms, and S-forms. CD spectra were collected at 6 °C for fd, I-forms, and S-forms at about 84 μg/mL in 0.01 M borate, pH 8.2. The low temperature was important to prevent spontaneous conversion of I-forms to S-forms. In a, the spectra, from most to the least negative ellipticity at 222 nm, are fd (— - —), I-forms (- - -), and S-forms (—). In b, the CD spectra of I-forms and S-forms of a are compared with the CD spectra of NBS-treated fd by Arnold et al. (1992a), 100% helical poly-L-lysine (Chen et al., 1972), and SDS/pVIII complexes. The SDS/pVIII complexes were prepared by dissociating fd in 1% SDS in 0.01 M borate, 0.1 M NaCl without removal of the DNA. The spectra in b, from most to least negative ellipticity at 230 nm, are from poly-L-lysine (– –) , NBS-treated fd (— - —), I-forms (- - -); S-forms (—), and SDS/pVIII complexes (- - - - -).

presented in Figure 1. These photographs show the large changes in shape that occur upon contraction of the intact filament to rod-shaped I-forms and thence to spheroidal S-forms.

CD Spectra of Phage, I-Forms, and S-Forms. The CD spectra of I-forms and S-forms have significantly different shapes or magnitudes compared to the spectrum of fd. Conversion from phage to I-forms involves a decrease in the magnitude of the ellipticity at 222 nm and an increase at 208 nm (Figure 2a). Conversion from I-forms to S-forms leads to a general decrease in magnitude throughout the 210–230 nm region (Figure 2a).

The intensity of the phage CD spectrum has a magnitude consistent with the high helix content of pVIII, but the shape of the CD curve is anomalous (Ikehara et al., 1975; Nozaki et al., 1976; Williams & Dunker, 1977; Day & Wiseman, 1978). This anomalous shape has been attributed to unexpectedly large contributions from the single tryptophan, W26 (Day and Wiseman, 1978; Arnold et al., 1992a). Oxidation by *N*-bromosuccinimide (NBS) removes the tryptophan contributions, rendering a normal-shaped CD spectrum (Arnold et al., 1992a). Comparison of the I-form spectrum with the NBS-treated fd spectrum, in which the anomalous tryptophan contributions become removed, and the spectrum for 100% α-helical poly-L-lysine shows the striking similarities among all three spectra (Figure 2b).

The large change in the CD spectrum of S-forms compared to fd (Figure 2a) yields a CD spectrum with a magnitude very similar to that of the fd coat protein mixed with SDS (e.g., pVIII/SDS complexes), except that the I-form CD spectrum is apparently red-shifted compared to that from the SDS/pVIII complexes (Figure 2b).

Tryptophan Fluorescence. CD spectral shape changes suggest a marked alteration in the environment of tryptophan as the phage contracts from fd to I-forms and S-forms. Such a change in the tryptophan environment should lead to a corresponding change in the fluorescence of the contracted forms compared to intact phage.

Initial preparations of I-forms and S-forms yielded highly variable fluorescence intensities. This variability turned out to be the result of variable residual chloroform, which was thereby discovered to be an extraordinarily potent inhibitor of tryptophan fluorescence in the I-form and S-form particles. To obtain the intrinsic fluorescence spectra without chloroform quenching, chloroform was removed by blowing a stream of humidified nitrogen over the samples. For comparative purposes, fd phage was diluted into chloroform saturated buffer, which does not cause contraction and which causes only a small decrease in fluorescence. Chloroform removal was monitored by the fluorescence intensity, which increased initially and reached a stable plateau for each sample after a few minutes. It was found to require much more time to remove chloroform from I-form and S-form as compared to the fd samples.

The extraordinary chloroform quenching noted above is illustrated by comparing the fluorescence spectra of fd, SDS/pVIII complexes, I-forms, and S-forms after equivalent dilutions back into chloroform saturated buffers so that they all have comparable levels of chloroform (Figure 3a) and following removal of chloroform, but without adding it back (Figure 3b). Note that chloroform is a far more effective quencher of the tryptophan in I-forms and S-forms than in fd phage or pVIII/SDS complexes as evidenced by comparing the spectra in figure 3a with those in Figure 3b.

With regard to the fluorescence in the absence of chloroform, the fd and pVIII/SDS complexes exhibit similar intensity levels and similar λ_{max} values very near 343 nm (Figure 3b). The fluorescence intensity of both I-forms and S-forms is markedly decreased compared to intact fd or SDS/pVIII complexes, but with very little change in the emission maxima: λ_{max} (I-forms) ≈ 342 nm and λ_{max} (S-forms) ≈ 341 nm.

Fluorescence Quenching. To further investigate the tryptophan environments, Stern–Volmer plots (Figure 4) were constructed for chloroform, trichloroethanol (TCE), acrylamide, Cs^+, and I^-. The dynamic and static quenching constants, K_{SV} and V, respectively, were determined from these data (Table I). The quenching constants for acrylamide, Cs^+, and I^- show little change as the phage is converted to I-forms and S-forms or as pVIII/SDS complexes are formed and are consistent in every case with the tryptophans being substantially protected from the solvent. In contrast, the quenching by chloroform and TCE show large changes as the phage converts to I-forms and S-forms (Table I), providing additional evidence for changes in the tryptophan environment.

The pVIII/SDS complexes exhibit remarkable quenching behavior in TCE solutions, with an abrupt increase in quenching near 0.15 M TCE (Figure 4b). This abrupt change is to be contrasted with the steady upward bend observed for

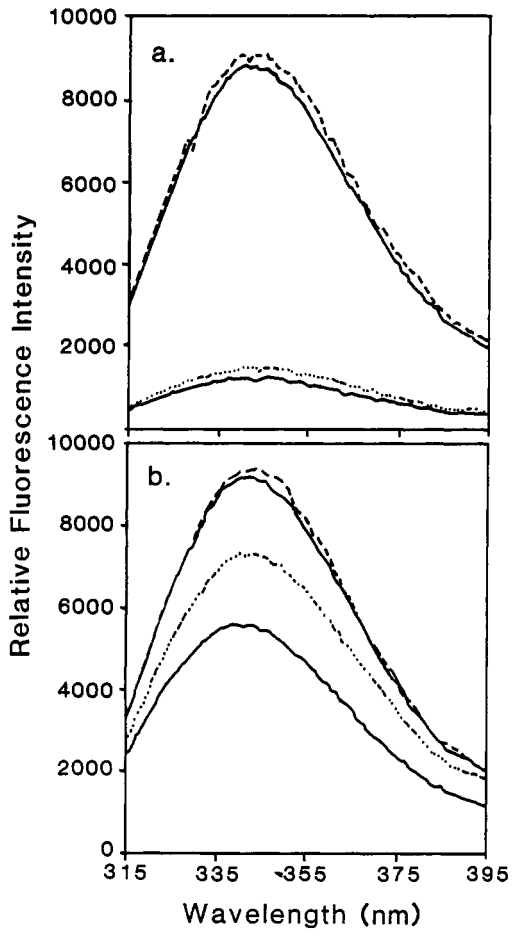

FIGURE 3: Fluorescence spectra of SDS/pVIII complexes, fd, I-forms, and S-forms. SDS/pVIII complexes were prepared as described in the legend of Figure 2. Fluorescence spectra were taken (a) without chloroform removal (b) following chloroform removal. I-form and S-form spectra were collected at 6 °C, spectra of SDS/pVIII complexes at 22 °C, and fd spectra at both temperatures. The spectra, from highest to lowest, are SDS/pVIII complexes (- - -), fd (—), I-forms (···—), and S-forms (—).

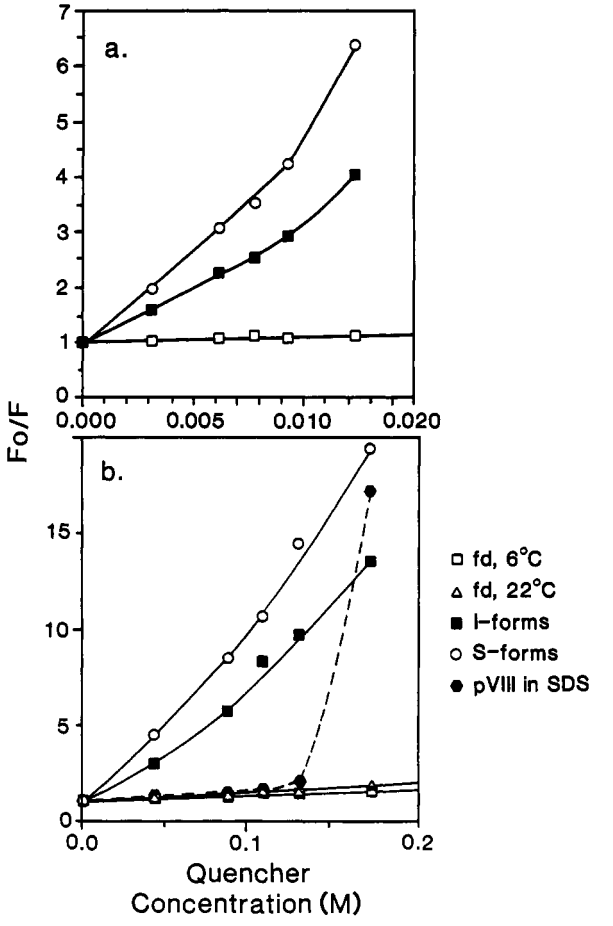

FIGURE 4: Stern–Volmer plots for fd, I-forms, S-forms, and pVIII/SDS complexes. The fd phage, I-forms, and S-forms at the same total protein concentrations were diluted equivalently into borate buffer containing various concetrations of chloroform (a) or TCE (b). The resulting fluorescence of each sample was measured at 342 nm at 6 °C. The resulting data yielded the Stern–Volmer quenching curves for fd (□–□–□), I-forms (■–■–■), and S-forms (○–○–○). For the pVIII/SDS complexes, which were prepared as described in the legend to Figure 5, the experiments were carried out at 22 °C to avoid precipitation of SDS. For comparative purposes, quenching of fd phage was also studied at 22 °C. These curves are shown by: fd phage at 22 °C (△–△–△) and pVIII/SDS complexes at 22 °C (●–●–●). Note that the curves for chloroform quenching of fd phage at 6 °C, fd phage at 22 °C, and pVIII/SDS complexes were all essentially coincident when viewed at the scale used in a, so just one curve is shown.

I-forms and S-forms. Such abrupt increases in TCE quenching have precedent and were interpreted in terms of a TCE-induced conformational change of the protein under study, leading to large increases in TCE binding and quenching (Eftink et al., 1977). Note that, after the conformational change, the TCE quenching of the tryptophans in pVIII/SDS complexes, in the I-forms and in the S-forms, are all very similar, suggesting that the environments are similar in all three cases.

DISCUSSION

CD Spectra. The CD spectra of large particles are potentially affected both by differential light scattering and by absorptive flattening (Gitter-Amir et al., 1976; Mao & Wallace, 1984). Changing the detector acceptance angle is a simple test for differential light scattering (Mao & Wallace, 1984); such tests indicate that differential light scattering did not contribute substantially to the CD spectra presented herein. There is no such simple test for absorptive flattening, which arises from the shielding of some chromophores by others in large aggregates [see Gitter-Amir et al. (1976) and Mao and Wallace (1984) and references cited therein]. However, absorptive flattening leads to a decrease in the ellipticities as the extinction coefficient increases; for helices this results in a reduction of the amplitudes of the 208- and 190-nm bands compared to that of the 222 nm band (Gitter-Amir et al., 1976), with a spectral shape over the 260–200-nm region vaguely resembling that of the filament samples, not those of the I-form and S-form samples. Yet for the filaments, both calculation (Day & Hoppenstadt, 1971) and experiment (Arnold et al., 1992a) strongly suggest that such optical effects do not distort the CD spectrum obtained from a sample of this material. Thus, overall, it is unlikely that the CD spectra from filaments, I-forms, and S-forms suffer major distortions from the above mentioned optical effects. However, small distortions from these effects cannot be ruled out, especially for S-forms, which would be most subject to such optical distortions due to its spherical shape (see below).

The major coat protein, pVIII, constitutes 98% of the protein in the phage (Marvin & Hohn, 1969; Nozaki et al., 1976; Goldsmith & Konigsberg, 1977). In addition, the CD signal from the phage DNA is small because of the small amount of DNA in the phage, about 12% by weight (Hoffmann-Berling et al., 1963; Berkowitz & Day, 1976), and because of the low magnitude of the CD spectrum of DNA in the 200–240 nm region [see Bush (1974); Wells & Yang (1974); Hanlon et al. (1975)]. For these reasons, the changes in the CD signals

Table I: Fluorescence Quenching of fd, I-Forms, S-Forms, and SDS/pVIII Complexes

quencher		fd	I-forms	S-forms	SDS/pVIII complexes
acrylamide	K_{SV}	3.1 ± 0.4	3.6 ± 0.4	2.6 ± 0.4	
	V	~0	~0	~0	
	K_{SV}	4.6 ± 0.3[b]			5.0 ± 0.2[b]
	V	~0			~0
Cs^+	K_{SV}	<1	<1	<1	
	V	~0	~0	~0	
I^-	K_{SV}	<1	<1	<1	
	V	~0	~0	~0	
chloroform	K_{SV}	8.3 ± 5.4	160 ± 50	280 ± 60	
	V	~0	10 ± 3	45 ± 6	
	K_{SV}	7.9 ± 2.9[b]			6.6 ± 1.7[b]
	V	~0			~0
trichloroethanol	K_{SV}	3.0 ± 0.4	27 ± 6	100 ± 22	
	V	~0	5 ± 2	2 ± 0.5	
	K_{SV}	4.7 ± 1.0[b]			5.5 ± 2.0[b]
	V	~0			~0

[a] The dynamic and static quenching constants, K_{SV} and V, respectively, were estimated by fitting the equation $F_0/F = (1 + K_{SV}[Q])e^{V[Q]}$ to the fluorescence data, where F_0/F is the ratio the fluorescence intensity of the sample without the quencher, F_0, divided by the fluorescence intensity in the presence of the quencher, F, and [Q] is the molar concentration of the quencher. Static quenching constant values between 0 and 1 M^{-1} were rounded down to ~0 since such small values give essentially straight lines in the Stern–Volmer plots over the concentrations used. [b] The measurements for the pVIII/SDS complexes and for a set of fd controls were carried out at 22 °C; all other measurements were performed at 6 °C. SDS/pVIII complexes were not studied at the low temperature due to SDS precipitation; I-forms were not studied at the higher temperature due to temperature sensitivity. fd was studied at both temperatures to provide control experiments.

from fd, I-forms, and S-forms primarily reflect the structural changes in pVIII.

Data from ORD, UV, NMR, CD, and Raman spectroscopy all suggest that pVIII in the phage is at least 90% helical (Day, 1966, 1969; Thomas et al., 1983; Marvin et al., 1974; Opella et al., 1987; Grygnon et al., 1988), with one study estimating 98% α-helix (Clack & Gray, 1989). Recent models derived from X-ray diffraction confirm a very high helix content for the fd/M13 type filamentous phage, with only a few residues at the carboxy terminus being nonhelix (Glucksman et al., 1992). Thus, an overall estimate of about 90% helix is most consistent with current data.

The intensity of the fd phage CD spectrum (Figure 2a) has long been known to have a magnitude consistent with the high helix content of pVIII, but the shape of the curve is anomalous, with an unusually deep minimum at 222 nm and a shoulder, not a minimum, at 208 nm (Ikehara et al., 1975; Nozaki et al., 1976; Williams & Dunker, 1977; Day & Wiseman, 1978). This anomalous shape has been attributed to unexpectedly large contributions from the single tryptophan, W26 (Day & Wiseman, 1978; Arnold et al., 1992a). Oxidation of the phage by *N*-bromosuccinimide has been shown to remove the tryptophan contributions, leaving a spectrum appropriate for mostly helical proteins (Arnold et al., 1992a).

Conversion from phage to I-forms involves a decrease in the magnitude of the ellipticity at 222 nm and an increase at 208 nm (Figure 2a). These changes are similar to those observed by NBS oxidation, which removes the tryptophan contributions. Indeed, comparison of the I-form spectrum with the NBS-treated fd spectrum and the 100% α-helical poly-L-lysine spectrum show striking similarities among all three spectra (Figure 2b). These similarities demonstrate that the contraction from fd to I-forms involves essentially no change in the pVIII secondary structure, which is nearly all helix both before and after the remarkable morphological change. Rather, the CD spectral change accompanying the fd to I-forms contraction is evidently due primarily, if not entirely, to a change in the environment of W26.

The W26 contribution to the CD spectrum of fd has unexpectedly large ellipticities of about −520 000 ± 110 000 deg-cm^2/dmol at 224 nm and +490 000 ± 80 000 deg-cm^2/dmol at 208 nm. These extraordinarily large ellipticities have been tentatively attributed to oscillator coupling between the indole of W26 and the benzene ring of a phenylalanine (F45) from a neighboring subunit in the phage capsid. The extraordinarily large magnitude of the ellipticities from the putative coupled oscillators suggests that the two chromophores must be very close together, probably in van der Waals contact (Arnold et al., 1992a).

There are three primary side-by-side contacts between the helical, rod-shaped pVIII subunits in the fd capsid. For one of these subunit contacts, the ends of one of the rod-shaped subunits are shifted by about 16 Å relative to the neighbor. The second contact involves a shift of about 32 Å. This subunit contact is suggested to include the putative W26/F45 interaction leading to the unusually large tryptophan contribution. The third contact involves a shift of about 48 Å. The diagrams or models in Marvin (1989), Glucksman et al. (1992), or Arnold et al. (1992a) can be consulted for more details. Given these primary subunit contacts, contraction to ~1/3 the length as in the filament to I-forms conversion means that pairs of contacting proteins would have to move relative to each other by ~11 Å (e.g., 16 − 16/~3 ≈ 11), ~22 Å (e.g., 32 − 32/~3 ≈ 22) and ~32 Å (e.g., 48 − 48/~3 ≈ 32). These displacements are far more than enough to separate the W26/F45 pair and thereby eliminate this possible contributor to the abnormally large tryptophan CD spectrum in the filament.

The similarity of NBS-treated fd to helical poly-L-lysine (Figure 2b) supports the previous use of the latter in the CD reference set for fd (Clack & Gray, 1989). The CD spectra of helices are length dependent (Woody & Tinoco, 1967). Thus, the extraordinary length of the fd helices, which are ~40 residues long (Glucksman et al., 1992), compared to those of water-soluble proteins typically used for referencing CD spectra, which range from ~4 to <20 in length with an average near 10–12 residues (Arnold et al., 1992b), probably accounts for the need to use helical poly-L-lysine as a CD reference.

Conversion from I-forms to S-forms leads to a general decrease in the magnitude of the ellipticity throughout the 210–230-nm region (Figure 2a), yielding a CD spectrum with a shape and magnitude very similar to that of the pVIII/SDS complexes, except for an apparent slight red-shift of the I-forms spectrum (Figure 2b). The apparent red-shift corresponds to a reduced intensity of the I-forms spectrum at shorter wavelengths. Although the I-forms CD spectrum is not exactly the same shape as reported previously for spectra distorted by absorptive flattening (Gitter-Amir et al., 1976; Mao & Wallace, 1984), a small absorptive flattening of the I-form CD spectrum compared to that of the dispersed pVIII/SDS complexes could be a contributing factor to the apparent red-shift.

When compared to helical poly-L-lysine, the pVIII/SDS CD spectrum is consistent with about 60% helix, a value which is further supported by previous estimates of secondary structure made by Raman spectroscopy (Williams et al., 1980), also with helical poly-L-lysine in the reference set. Recent

NMR studies on pVIII/SDS complexes suggest both helical and nonhelical regions (Henry & Sykes, 1992). Unpublished extensions of this analysis are consistent with two helical segments, one from residues 7–15 and the other from residues 24–47, for a total of 33 helical residues, yielding an overall helix content of 66% (G. D. Henry, personal communication).

Fluorescence Spectra. The tryptophan fluorescence emission λ_{max} of fd reported here to be 343 nm is substantially red-shifted compared to values near 330 nm that have been reported previously (Day et al., 1979; Arnold et al., 1992a). This red-shift is due to the use of polarizers in the present study compared to the lack of polarizers in the previous studies [see discussion in Lakowicz (1983)].

It is commonly found that decreases in fluorescence intensity are accompanied by red-shifts due to increasing polarity of the environment (Lakowicz, 1983). In contrast to the usual case, the decrease in intensity as the fd filaments convert to I-forms and S-forms is accompanied by a very slight blue-shift: 343 nm (fd), 342 (I-forms), and 341 (S-forms). The similarity of the λ_{max} values suggest that the tryptophan environments are of similar polarity in all of these structures.

Initial preparations of I-forms and S-forms yielded highly variable fluorescence intensities which turned out to be due to variable residual chloroform in the samples; in this way it was discovered that chloroform is an extraordinarily potent quencher of tryptophan fluorescence in the I-form and S-form particles as shown by the marked reduction of the fluorescence emission in these particles (Figure 3a). In contrast, little change in the λ_{max} or fluorescence intensity is observed for fd or pVIII/SDS complexes when diluted into chloroform saturated buffers (Figure 3a and b).

Systematic fluorescence quenching studies provide a second method to investigate changes in the tryptophan environment as fd contracts into I-forms and S-forms. The resulting Stern–Volmer plots for chloroform, TCE, acrylamide, Cs^+, and I^- (Figure 4) and the resulting quenching constants (Table I) do indeed provide further insight about the nature of the tryptophan environments in the phage, I-forms, S-forms, and pVIII/SDS protein complexes.

The quenching by acrylamide, Cs^+, and I^- show little change as fd is contracted to I-forms and S-forms or as the coat protein is dissolved in SDS. This lack of change in quenching by polar quenchers is consistent with the lack of change in the λ_{max} value of the emission spectra, and both suggest that the tryptophan is substantially protected from the solvent in all the different forms. Given the considerable degree of protection from the solvent for all the forms, the decreased fluorescence intensity for I-forms and S-forms as compared to fd is probably due to altered intramolecular quenching, resulting from changes in the environment of the indole ring. Identification of the component or components responsible for this increased quenching would lead to additional insight regarding the structure of the I-form and S-form structures.

In contrast to the results with the more polar quenchers, the quenching by the less polar chloroform and TCE shows large changes as the phage converts to I-forms and S-forms (Figure 4 and Table I). The static quenching constants, K_{SV}, range from 3 to 8 M^{-1} for chloroform and TCE quenching of fd (Table I). Such values are within the range previously observed for single tryptophan globular proteins (Table II). Likewise, K_{SV} values for chloroform and TCE quenching of pVIII/SDS complexes fall within this range as well (at least at low quencher concentrations, see below). On the other hand, the K_{SV} values for chloroform and TCE quenching of I-forms and S-forms are unusually large, ranging from 27 to 280 M^{-1}! Such large magnitudes for K_{SV} are atypical, exceeding even the magnitudes observed for completely unprotected indole groups in water (e.g., compare these magnitudes to the quenching constants for the unprotected indole of tryptamine, TAM, in Table II).

Table II: Comparison of λ_{max} and K_{SV} Values

protein/sample[a]	K_{SV} (acrylamide)	K_{SV} (TCE)	λ_{max} (nm)	ref
azurin	~0		308	
RNase T	1.1	1.0	324	*e,f*
HGH	3.0		342	*e,f*
human serum albumin	3.1	20[b]	342	*e,f*
strep. nuclease	5.2	8.0	334	*e,f*
monellin	5.2	4.1	342	*e,f*
glucagon	10.5	10.0	352	*e,f*
ACTH	13.0		352	*e,f*
TAM	33	22	355	*g*
TAM/SDS	10.5	42[b]	338	*g*
BSA	3.7	230[b]	340	*e,f*
fd	3.1	3.0	332,[c] 343[d]	*h* and this paper
I-forms	3.6	27[b]	nd, 342[d]	this paper
S-forms	2.6	100[b]	nd, 341[d]	this paper

[a] The proteins listed above contain single tryptophans; the abbreviations are as follows: HGH = human growth hormone; ACTH = adrenocorticotropic hormone. Nonprotein samples and a protein (BSA) with more than one tryptophan are given for the comparison; abbreviations are as follows: TAM = tryptamine; SDS = sodium dodecyl sulfate; BSA = bovine serum albumin. [b] The F_o/F plots for these proteins and the SDS/indole sample exhibit measurable upward curvature, indicating non-zero values for V, the static quenching term (see Table I). [c] The λ_{max} for fd was determined in *h* to be 332 nm with an excitation wavelength of 295 nm when polarizers were not used; an earlier paper (*i*) reported 330 nm for λ_{max} with an excitation of 280 nm, and again polarizers were not used. nd = not determined. [d] The λ_{max} values for fd, I-forms, and S-forms determined in this study with polarizers in place. [e] Eftink, M. R., & Ghiron, C. (1976) *Biochemistry 15*, 672–680. [f] Eftink, M. R., Zajicek, L. J., & Ghiron, C. (1977) *Biochem. Biophys. Acta 491*, 473–481. [g] Eftink, M. R., & Ghiron, C. (1976) *J. Phys. Chem. 80*, 486–493. [h] Arnold, G. E., Day, L. A., & Dunker, A. K. (1992) *Biochemistry 31*, 7948–7956. [i] Day, L. A., Wiseman, R. L., & Marzec, C. J. (1979) *Nucleic Acids Res. 7*, 1393–1403.

Stern–Volmer plots exhibit upward curvature, corresponding to non-zero values for the static quenching constant, V, when there is an interaction between the fluorescent moiety and the quencher molecule or when the quencher molecule accumulates in the region surrounding the fluorescent moiety (Lakowicz, 1983). Thus, the upward curving plots (Figure 4) and the associated non-zero V values for the quenching of I-forms and S-forms by chloroform and TCE (Table I) suggest that I-forms and S-forms, but not fd, bind the chloroform and TCE molecules. This could explain the longer times required to remove the residual chloroform from I-form and S-form samples as compared to the fd samples as noted in the results.

Additional support for the binding of hydrophobic molecules by I-forms and S-forms comes from observations on proteins with hydrophobic binding pockets, e.g. the bovine and human serum albumins, and from observations on indole groups associated with SDS micelles. The two albumins and SDS micelles would all be expected to bind hydrophobic groups and all show quenching behavior similar to that of I-forms and S-forms, namely, protection from hydrophilic quenchers with typical K_{SV} values and zero V values in contrast to very large K_{SV} values and non-zero V values for hydrophobic quenchers (Table II).

The pVIII/SDS complexes exhibit remarkable quenching behavior in TCE solutions, with an abrupt increase in quenching near 0.15 M TCE (Figure 4b). Such abrupt

increases in TCE quenching have precedent and were interpreted in terms of a TCE-induced conformational change in the protein under study, leading to a large increase in TCE binding (Eftink et al., 1977). Here, we suggest that the higher concentrations of TCE likewise induce a conformational change for the protein component in the pVIII/SDS complexes.

A more detailed consideration of the pVIII/SDS complexes provides additional insight regarding the quenching by TCE. The pVIII molecule exists as a dimer in the pVIII/SDS complexes (Henry & Sykes, 1990b; Henry & Sykes, 1992). The tryptophan indole ring and one other side chain exhibit unusual resonances (Henry & Sykes, 1992). Our results show that tryptophans appear to be in native protein-like environments from which the TCE (at lower concentrations) and chloroform are both excluded. One possible explanation for both the unusual NMR resonance (G. D. Henry, personal communication) and for the protection from TCE and chloroform is that the tryptophans are located at the protein/protein interface in the SDS-associated dimer. However, at higher TCE concentrations, above 0.15 M, the tryptophan environment changes abruptly, probably as a result of a TCE-induced protein conformational change, which would be expected to expose the trytophan to the SDS micelle environment. The resulting degree of quenching becomes very similar to that in I-forms and S-forms (see Figure 4b), suggesting that the tryptophans in I-forms and S-forms are in SDS micelle-like environments.

What is the difference between the hydrophobic environment inside an SDS micelle and the hydrophobic environment inside a native protein? The former is relatively dynamic whereas the latter is relatively static. The dynamic, nonrigidly packed hydrophobic tails of the SDS micelles readily dissolve hydrophobic molecules, thus accounting for the atypical quenching of an SDS-associated indole by TCE (Eftink & Ghiron, 1977), whereas the more rigidly packed hydrophobic interiors of native proteins are better able to exclude hydrophobic molecules, thus accounting for the typical quenching by TCE for the several single tryptophan proteins listed in Table II.

The quenching data for TCE and chloroform therefore suggest that the difference in the tryptophan environment in fd as compared to I-forms and S-forms is that the hydrophobic groups in fd are rigidly packed, like those in native proteins, whereas the hydrophobic groups in I-forms and S-forms are nonrigidly packed. Such a change could also contribute to the large change in the tryptophan CD spectral contribution as well. When the proteins convert from rigid to nonrigid sidechain packing, tryptophan CD contributions are lost (Ohgushi & Wada, 1983) because the asymmetric, rigid environment leading to the CD signal in the former becomes lost due to motional loss of asymmetry in the nonrigid interactions of the latter.

Are I-Forms and S-Forms Assemblies with Molten Globule-like Properties? Proteins that are perturbed so as to have nonrigidly packed side chains are called molten globules (Ohgushi & Wada, 1983). According to several studies, molten globules are characterized by having native-like secondary structure (as do I-forms and S-forms) with a compact shape (as do I-forms and S-forms), but are distinguished from native proteins by having nonrigid side-chain packing and nonfixed tertiary structure (Dolgikh et al., 1981; Ohgushi & Wada, 1983; Goto & Fink, 1989; Kataoka et al., 1993; Handel et al., 1993; Barrick & Baldwin, 1993). By all of these criteria, the conversion from fd to I-forms and S-forms involves conversion from a native-like to a molten globule-like form.

The fluorescent molecule 1-anilinonaphthalene-8-sulfonate (ANS) has been used to probe the molten globule state (Semisotnov et al., 1991; Morii et al., 1991; Handel et al., 1993). Classical studies show that, as the ANS probe moves from water into the heme binding pocket in apomyoglobin, there is an increase in intensity of about 245-fold and a blue-shift from 515 nm in water to 454 nm when in the binding pocket (Stryer, 1965). When this probe combines with various molten globular protein forms, smaller intensity increases, typically 10- to 150-fold, and smaller blue-shifts, typically to the 460–490 nm range, are observed. The blue-shifts and intensity increases are evidently the result of ANS becoming associated with the nonpolar, nonrigidly packed regions of the molten globular forms (Semisotnov et al., 1991; Morii et al., 1991; Handel et al., 1993).

We previously showed that association of ANS with fd phage leads to only very slight increases in fluorescence intensity, but association with I-forms or S-forms leads to massive, greater than 50-fold, increases in fluorescence intensity (Dunker et al., 1991b). The λ_{max} values from these published spectra, 475 nm for I-forms and 483 nm for S-forms, fall exactly within the range reported for ANS associated with typical molten globules. Thus, ANS binding provides further evidence that the fd phage to I-forms and S-form conversions resemble typical conversions from native-like to molten globule-like forms.

The large size of ANS compared to chloroform and TCE is strong evidence that the nonrigidly packed regions are probably fairly large and not merely confined to small pockets in the vicinity of the indole side chains. In addition, our data suggest a new test for the conversion to the molten globule-like state, namely, atypically potent fluorescence quenching by TCE and chloroform.

Implications for fd Assembly and Penetration. Because at least three host proteins (specified by the tol Q, tol R, and tol A genes) are required for phage penetration (Sun & Webster, 1986, 1987) and because two noncapsid viral proteins (pI and pIV) and at least one host protein (thioredoxin) are required for phage assembly [Horabin & Webster, 1988; Brissette & Russel, 1990; Guy-Caffey et al., 1992; Russel & Model, 1985; reviewed in Model and Russel (1988); Russel (1991)], it is unclear whether morphological changes of the isolated fd phage particles are relevent to penetration or assembly (Webster, personal communication).

On the other hand, I-forms and S-forms have several properties that seem to be appropriate for assembly or penetration intermediates. I-forms and S-forms resemble molten globules as discussed above. Recently, molten globules have been implicated in protein folding [reviewed in Ptitsyn (1987); Kuwajima, 1989], in the translocation across membranes (Bychkova et al., 1988), in the insertion of membrane proteins (Dunker et al., 1991b; van der Goot et al., 1991), and in the transfer of retinol from its binding protein into a membrane environment (Bychkova et al., 1992)—this last example is mentioned here because it involves radical changes in lipid/protein interactions. Given these examples, the molten globule-like nature of I-forms and S-forms is entirely appropriate for insertion into and exit from the host cell membrane.

S-forms have pVIII in a conformation resembling that found in membrane vesicles; indeed S-forms have certain features in common with membrane bilayers. That is, the morphology of S-forms resembles the shape of membrane bilayers, the charged ends of pVIII are hydrophlilic as are the phospholipid

head groups, and the nonpolar residues from sequence positions 19–40 are hydrophobic as are the fatty acid tail groups of bilayer-forming phospholipids.

I-forms have a morphology and coat protein structure that is intermediate between the intact phage and the membrane-like S-forms. The helical content of pVIII in I-forms is the same as that of pVIII in the phage; this is appropriate if I-form-like structures are on the pathway for the insertion of the protein into the membrane. Indeed, from the NMR results of Henry and Sykes (1990a,b, 1992, and personal communication), the conformational change as I-forms convert to S-forms most likely involves the interruption of a very long helix to form a short, amphipathic helix and a longer, more hydrophobic helix appropriate for spanning the membrane bilayer. Such a structural change is similar to, but not identical with, the one proposed for the Pf1 coat protein during assembly of this related filamentous phage (Nambudripad et al., 1991).

In addition to two helical forms (Ikehara et al., 1975; Cavalieri et al., 1976; Nozaki et al., 1976, 1978; Dunker et al., 1982; Spruijt et al., 1989), pVIII also exhibits a β-rich form when separated from lipids or detergents or when the protein/amphiphile ratio becomes too high (Makino et al., 1975; Nozaki et al., 1978; Dunker et al., 1982; Spruijt et al., 1989). Given that phage assembly necessarily involves concentration of pVIII even to the point of protein/lipid separation, it has remained unclear whether the β-rich form is involved transiently in the assembly process. Here, we report the existence of the two helical forms of pVIII in nonphage structures and yet in the complete absence of amphiphiles. It is unclear whether the helical structures of pVIII in I-forms and S-forms represent the thermodynamically most stable conformations or are kinetically-trapped metastable intermediates that eventually convert to the β-rich structures. However, even if these helical structures are only metastable in the absence of detergents or lipids, the lifetimes of the helices in I-forms and S-forms are obviously more than long enough for their participation in phage penetration and assembly.

Proteins whose function depends on multistep pathways can often be trapped into specific intermediates by lowering the temperature; thus, the trapping of I-forms by a lower temperature is also consistent with its intermediate status. The intermediate status of I-forms and the similarity of S-form coat protein to membrane-bound coat protein argue in favor of the relevance of phage contraction *in vitro* to phage penetration and assembly *in vivo*. Finally, it seems highly unlikely that a complicated bundle of helices such as the fd phage would have multiple pathways for telescoping into longer or shorter structures. Thus, we are in accord with previous workers who suggested that the changes in pVIII following conversion to I-forms and S-forms may mimic the conformational changes of pVIII during penetration and assembly (Griffith et al., 1981; Manning et al., 1981; Marvin 1978, 1989, 1990).

Implications for Other Multimeric Biomolecular Assemblies. The concept that a protein/nucleic acid complex can convert from a native-like form to a molten globule-like form that undergoes large shape changes has potential implications beyond the structure and assembly of filamentous phages.

ACKNOWLEDGMENT

We wish to thank Susan Johns of the Center for Visualization, Analysis and Design (VADMS) for the preparation of computer models that clarified the subunit interactions in the filaments and in possible subunit shifts leading to the contracted forms and also Greg Arnold for the CD spectrum of NBS-treated fd phage.

REFERENCES

Arnold, G. E., Day, L. A., & Dunker, A. K. (1992a) *Biochemistry 31*, 7948–7956.
Arnold, G. E., Dunker, A. K., Johns, S. J., & Douthart, R. J. (1992b) *Proteins: Struct., Funct., Genet. 12*, 382–399.
Asbeck, V. F., Beyreuther, K., Kohler, H., Von Wettstein, G., & Braunitzer, G. (1969) *Hoppe-Seyler's Z. Physiol. Chem. 350*, 1047–1066.
Aubrey, K. L., & Thomas, G. J. (1991) *Biophys. J. 60*, 1337–1349.
Axelsen, P. H., & Prendergast, F. G. (1989) *Biophys. J. 56*, 43–66.
Barrick, D. & Baldwin, R. L. (1993) *Protein Sci. 2*, 869–876.
Berkowitz, S. A. & Day, L. A. (1976) *J. Mol. Biol. 102*, 531–547.
Bychkova, V. E., Berni, R., Rossi, G. L., Kutyschenko, V. P., & Ptitsyn, O. B. *Biochemistry 31*, 7566–7571.
Bychovka, V. E., Pain, R. H., & Ptitsyn, O. B. (1988) *FEBS Lett. 238*, 231–234.
Bradford, M. M. (1976) *Anal. Biochem. 72*, 248–254.
Brissette, J. L., & Russel, M. (1990) *J. Mol. Biol. 211*, 565–580.
Bush, C. A. (1974) in *Basic Principles in Nucleic Acid Chemistry, II* (Ts'o, P. O. P., Ed.) pp 145–151, Academic Press, New York.
Cavalieri, S. J., Goldthwait, D. A., & Neet., K. E. (1976) *J. Mol. Biol. 102*, 713–722.
Chen, Y.-H., Yang, J. T., & Martinez, H. M. (1972) *Biochemistry 11*, 4120–4131.
Clack, B. A., & Gray, D. M. (1989) *Biopolymers 28*, 1861–1873.
Cooper, T. M. & Woody, R. W. (1990) *Biopolymers 30*, 657–676.
Day, L. A. (1966) *J. Mol Biol. 15*, 395–398.
Day, L. A. (1969) *J. Mol. Biol. 39*, 265–277.
Day, L. A., & Wiseman, R. L. (1978) in *The Single-Stranded DNA Phages* (Denhardt, D. T., Dressler, D. & Ray, D. S., Eds.) pp 605–625, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Day, L. A., Wiseman, R. L., & Marzec, C. J. (1979) *Nucleic Acids Res. 7*, 1393–1403.
Day, L. A., Marzec, C. J., Reisberg, S. A., & Casadevall, A. (1988) *Annu. Rev. Biophys. Chem. 17*, 509–539.
Dolgikh, D. A., Gilmanshin, R. I., Brazhnikov, E. V., Bychkova, V. E., Semisotov, G. V., Venyaminov, S. Y., & Ptitsyn, O. B. (1981) *FEBS Lett. 136*, 311–315.
Dunker, A. K., Fodor, S. P. A., & Williams, R. W. (1982). *Biophys. J. 37*, 201–203.
Dunker, A. K., Ensign, L. D., Arnold, G. E. & Roberts, L. M. (1991a) *FEBS Lett. 292*, 271–274.
Dunker, A. K., Ensign, L. D., Arnold, G. E. & Roberts, L. M. (1991b) *FEBS Lett. 292*, 275–278.
Eftink, M. R., & Ghiron, C. (1976a) *Biochemistry 15*, 672–680.
Eftink, M. R., & Ghiron, C. (1976b) *J. Phys. Chem. 80*, 486–493.
Eftink, M. R. Zajicek, J. L., & Ghiron, C. (1977) *Biochim. Biophys. Acta 491*, 473–481.
Fodor, S. P. A. (1981) M. S. Thesis, Washington State University.
Fodor, S. P. A., Dunker, A. K., Ng, Y. C., Carsten, D., Williams, R. W. (1981), in *Bacteriophage Assembly* (Dubow, M., Ed.) pp 441–447, Alan R. Liss, New York.
Frank, H., & Day, L. A. (1970) *Virology 42*, 144–154.
Gitter-Amir, A., Rosenheck, K., & Schneider, A. S. (1976) *Biochemistry 15*, 3131–3137.
Glucksman, M. J., Bhattacharjee, S. & Makowski, L. (1992) *J. Mol. Biol.* 226, 455–470.
Goldsmith, M. E., & Konigsberg, W. H. (1977) *Biochemistry 16*, 2686–2694.

Goto, Y. & Fink, A. L. (1989) *Biochemistry 28*, 945–952.
Grant, R. A., Lin, T. C., Konigsburg, W., & Webster, R. E. (1981) *J. Biol. Chem.* 256, 539–546.
Grant, R. A., & Webster, R. E. (1984) *Virology 133*, 315–328.
Griffith, J., Manning, M., & Dunn, K. (1981) *Cell 23*, 747–753.
Grygnon, C. A., Perno, J. R., Fodor, S. P. A., & Spiro, T. G., (1988) *BioTechniques 6*, 50–55.
Guy-Caffey, J. K., Rapoza, M. P., Jolley, K. A., & Webster, R. E. (1992) *J. Bacteriol. 174*, 2460–2465.
Handel, T. M., Williams, S. A., & DeGrado, W. F. (1993) *Science 261*, 879–885.
Hanlon, S., Brudno, S., Wu., T. T., & Wolf, B. (1975) *Biochemistry 14*, 1648–1660.
Henry, G. D., & Sykes, B. D. (1990a) *Biochem. Cell Biol. 68*, 318–329.
Henry, G. D., & Sykes, B. D. (1990b) *J. Mol Biol. 212*, 11–14.
Henry, G. D., & Sykes, B. D. (1992) *Biochemistry 31*, 5284–5257.
Hoffmann-Berling, H., Marvin, D. A. & Durwald, H. (1963) *Z. Naturf. 18b*, 876–883.
Horabin, J. I. & Webster, R. G. (1986) *J. Mol. Biol. 188*, 403–413.
Ikehara, K., Utiyama, H., & Kurata, M. (1975) *Virology 66*, 306–315.
Kataoka, M., Hagihara, Y., Mihara, K., & Goto, Y. (1993) *J. Mol. Biol. 229*, 591–596.
Kuwajima, K. (1989) *Proteins: Struct., Funct., Genet. 6*, 87–103.
Lakowicz, J. (1983). *Principles of Fluorescence Spectroscopy*, Plenum Press, New York.
Lin, T. C., Webster, R. E., & Konigsberg, W. (1980) *J. Biol. Chem. 255*, 10331–10337.
Lopez, J., & Webster, R. E. (1982) *J. Virolog. 42*, 1099–1107.
Makino, S., Woolford, J. L., Tanford, C., & Webster, R. E. (1975) *J. Biol. Chem. 250*, 4327–4332.
Makowski, L., & Caspar, D. L. D. (1978) in *The Single-Stranded DNA Phages* (Denhardt, D. T., Dressler, D., & Ray, D. S., Eds.) pp 627–643, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Makowski, L., (1984) in *The Structures of Biological Macromolecules and Assemblies, Vol. 1: The Viruses* (McPherson, A., & Jurnak, F., Eds.) pp 203–253, Wiley, New York.
Manning, M., Chrysogelos, S., & Griffith, J. (1981) *J. Virol. 40*, 912–918.
Manning, M., Chrysogelos, S., & Griffith, J. (1982) *Biophys. J. 37*, 28–30.
Manning, M., Moore, M., Spremulli, L., & Griffith, J. (1983) *Biochem. Biophys. Res. Commun. 112*, 349–355.
Mao, D. & Wallace, B. A. (1984) *Biochemistry 23*, 2667–2673.
Marvin, D. A., (1966) *J. Mol. Biol. 15*, 8–17.
Marvin, D. A. (1978) in *The Single Stranded DNA Phages* (Denhardt, D. T., Dressler, D., & Ray, D. S., Eds.) pp 583–604, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Marvin, D. A. (1989) *Int. J. Biol. Macromol.11*, 159–164.
Marvin, D. A. (1990) *Int. J. Biol. Macromol. 12*, 15–138.
Marvin, D. A., & Hohn, B. (1969) *Bacteriol. Rev. 33*, 172–209.
Marvin, D. A., Pigram, W., Wiseman, R. L., Wachtel, E. J., & Marvin, F. J. (1974a) *J. Mol. Biol. 88*, 581–598.
Marvin, D. A., Wiseman, R. C., Wachtel, E. J. (1974b) *J. Mol. Biol. 82*, 121–138.
Mazur, B. J., & Zinder, N. D. (1975) *Virology 68*, 490–502.
Model, P., & Russel, M. (1988) in *The Bacteriophages* (Calendar, R., Ed.) Vol. 2, pp 375–456, Plenum Publishing Corp., New York.
Morii, H., Ichimura, K., and Uedaira, H. (1991) *Proteins: Struct., Funct. Genet. 11*, 133–141.
Nakashima, Y. & Konigsberg, W. (1974) *J. Mol. Biol. 88*, 598–600.
Nambudripad, R., Stark, W., Opella, S. J., & Makowski, L. (1991) *Science 252*, 1305–1308.
Nozaki, Y., Chamberlain, B., Webster, R., & Tanford, C. (1976) *Nature 259*, 355- 337.
Nozaki, Y., Reynolds, J., & Tanford, C. (1978) *Biochemistry 17*, 1239–1246.
Opella, S. J., Stewart, P. L., & Valentine, K. G. (1987) *Q. Rev. Biophys. 19*, 1–49.
Ohgushi, M., & Wada, A. (1983) *FEBS Lett. 164*, 21–24.
Rasched, I., & Oberer, E. (1986) *Microbiol. Rev. 50*, 401–427.
Roberts, L. M. (1990) Ph.D. Thesis, Washington State University.
Rossomando, E. F., & Zinder, N. D. (1968) *J. Mol. Biol. 36*, 387–399.
Russel, M., & Model, P. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 29–33.
Russel, M. (1991) *Mol. Microbiol. 5*, 1607–1613.
Salstrom, J. S., & Pratt, D. (1971) *J. Mol. Biol. 61*, 489–501.
Semisotnov, G. V., Rodionova, N. A., Razgulyaev, O. I., Uversky, V. N., Gripas', A. F., & Gilmanshin, R. I. (1991) *Biopolymers 31*, 119–128.
Schiksnis, R. A. , Bogusky, M. J., Tsang, P., & Opella, S. J. (1987) *Biochemistry 26*, 1373–1381.
Shon, K.-J., Kim, Y., Colnago, L. A., & Opella, S. J. (1991) *Science* 252, 1303–1305.
Simons, G. F. M., Konings, R. N. H., & Schoenmakers, J. G. G. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 4194–4198.
Smilowitz, H., Carson, J., & Robbins, W. P. (1972) *J. Supramolec. Struct. 1*, 8–18.
Smilowitz, H. (1974) *J. Virology 13*, 94–99.
Spruijt, R. B., Wolfs, C. J. A. M., & Hemminga, M. A. (1989) *Biochemistry 28*, 9158–9165.
Stryer, L.(1965) *J. Mol. Biol. 13*, 482–445.
Sun, T.-P., & Webster, R. E. (1986) *J. Bacteriol. 165*, 107–115.
Sun, T.-P., & Webster, R. E. (1987) *J. Bacteriol. 169*, 2667–2674.
Thomas, G. J., Jr., Prescott, B., & Day, L. A. (1983) *J. Mol. Biol. 165*, 321–356.
Trenkner, E., Bonhoeffer, F., & Gierer, A. (1967) *Biochem. Biophys. Res. Commun. 28*, 932–939.
van der Goot, F. G., Gonzalez-Mañaz, J. M., Lakey, J. H., & Pattus, F. (1991) *Nature 354*, 408–410.
Williams, R., & Dunker, A. K. (1977) *J. Biol. Chem. 252*, 6253–6255.
Williams, R., & Dunker, A. K. (1981) *J. Mol. Biol. 152*, 783–813.
Williams, R. W., Dunker, A. K., & Peticolas, W. C. (1980) *Biophys. J. 32*, 232–234.
Wells, B. D., & Yang, J. T., (1974) *Biochemistry 13*, 1317–1321.
Wiseman, R. L., Berkowitz, S. A., & Day, L. A. (1976) *J. Mol. Biol. 102*, 544.
Woody, R. W., & Tinoco, I., Jr. (1967) *J. Chem. Phys.* 46, 4927–4845.
Woolford, J. L., Steinman, H. M., & Webster, R. E. (1977) *Biochemistry 16*, 2694–2700.