Biophysical Studies of the Pf1 Coat Protein in the Filamentous Phage, in Detergent Micelles, and in a Membrane Environment†

I. Azpiazu,‡ J. C. Gomez-Fernandez,§ and D. Chapman*,‡

Department of Protein and Molecular Biology, Royal Free Hospital School of Medicine, London NW3, United Kingdom, and Departamento de Bioquimica, Facultad de Veterinaria, Universidad de Murcia, Murcia, Spain

Received March 30, 1993; Revised Manuscript Received June 21, 1993®

ABSTRACT: During the assembly of the Pf1 phage, the membrane-bound coat proteins convert into subunits of the filamentous phage. Fourier-transform infrared (FT-IR) transmission spectroscopy has been applied to a study of the secondary structure of these coat proteins when present (a) in the phage, (b) in detergent micelles, and (c) in a phospholipid membrane aqueous system. Suspensions of the Pf1 phage in H_2O and ^2H_2O show an amide I band at 1652 cm^{-1}, indicative of a high content of the α-helical structure present. Oriented films of the Pf1 phage studied by polarized FT-IR transmission spectroscopy indicate that the α-helical structures as well as the tyrosine residues (band at 1515 cm^{-1}) are both aligned along the axis of the phage. When the Pf1 coat protein is present in sodium dodecyl sulfate detergent micelles and in lipid membrane systems, the FT-IR spectra show an amide I band at 1657–1658 cm^{-1}, also indicative of a predominantly α-helical secondary structure. H/^{2}H amide proton exchange studies show that when present in a phospholipid membrane system some 50–60% of the Pf1 protein exchanges rapidly, while the rest undergoes slow exchange. This is consistent with a proportion of the protein being exposed to the solvent and the other being embedded in the lipid bilayer. The presence of a band at 1630–1640 cm^{-1} is indicative of the presence of random structures. Oriented lipid films containing the Pf1 coat protein studied by internal reflectance polarized FT-IR spectroscopy indicate that the average α-helical structures of the Pf1 coat protein are oriented perpendicular to the plane of the lipid membrane. To establish whether the amino-terminal region penetrates the lipid bilayer and perturbs the lipid chains, a fluorescence polarization study was carried out using the probe diphenylhexatriene. These studies and additional calorimetric studies of the gel–liquid-phase transition of the lipid in protein/lipid systems are consistent with a perturbation expected only from a single helix spanning the lipid bilayer.

Filamentous Pf1 phage is a nonlytic virus that infects bacteria of *Pseudomonas aeruginosa*, strain K. Under the electron microscope, the filamentous Pf1 phages appear as thin cylinders 6 nm thick and approximately 2 μm long (Marvin & Wachtel, 1975). Neutron and X-ray diffraction studies indicate that the viruses are hollow cylinders comprised of thousands of subunits of a 46 amino acid long coat protein, with the viral DNA packed inside (Marvin & Wachtel, 1975; Day et al., 1988). The coat protein subunits are arranged as helical rods, slightly tilted upon the filament axis and intercalated between their neighbors packing into left-handed spirals that ascend along the axis of the phage. During viral infection, these coat proteins are initially integral membrane proteins in the host membrane (Smilowitz et al., 1972; Webster & Cashman, 1973). The coat proteins leave the membrane during the assembly of the phage to become one of the thousands of subunits which form the new viral particles (Marvin & Wachtel, 1975).

The Pf1 phage has been previously studied using techniques such as circular dichroism (CD) spectroscopy. These studies indicate that the Pf1 phage contains almost 100% α-helical structure (Day et al., 1988). The tertiary structure of the protein in the dodecylphosphocholine (DPC) detergent micelles has been determined using 2-D ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectroscopy. The Pf1 coat protein in this detergent micelle adopts an arrangement of two stable α-helical segments (Shon et al., 1991; Schiksnis at al., 1987). With the assumption that this structure was conserved in lipid membranes, solid-state NMR spectroscopy of uniformly ^{15}N-labeled and site-specific ^{15}N-labeled Pf1 protein was used to deduce the orientation of the two helical stretches adopted by the protein within the lipid bilayer structure. These studies led Shon et al. (1991) to propose a model for the Pf1 protein in a phospholipid membrane where a long hydrophobic region (about 24 amino acids) forms a membrane-spanning α-helix while the amino-terminal region (1–14) is arranged as an amphipathic α-helix lying parallel to the lipid bilayer plane.

In this paper, we use FT-IR spectroscopy to study the conformation of the Pf1 coat protein in the phage, in detergent micelles, and reconstituted in a lipid membrane environment. Infrared spectroscopy is a useful technique because the spectra report on the molecular vibrations of all the components (proteins, lipids, etc.) present in the system. Information about the secondary structure can be deduced by studying the amide I and II bands of proteins which originate from molecular vibrations of the peptide groups. The amide I band corresponds predominantly to the carbonyl stretching vibration and is sensitive to the hydrogen-bonding and hence to the secondary structure present (Jackson et al., 1989; Haris & Chapman, 1992). The amide II band originates from the bending vibrations of the amide NH groups. Valuable information about the stability and solvent exposure of protein and domains can be obtained by observing the reduction of the amide II′ band intensity upon H/^{2}H exchange.

† This work was financed by grants from the Welcome Trust (J.C.G.-F. and D.C.) and by a studentship (I.A.) from the Royal Free Hospital School of Medicine.

‡ Royal Free Hospital School of Medicine.

§ Universidad de Murcia.

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

0006-2960/93/0432-10720$04.00/0

Additionally, internal reflectance FT-IR spectroscopy is used to study the orientation of the secondary structure of the Pf1 protein within the lipid bilayer. Other biophysical techniques such as polarization of the fluorescent probe diphenylhexatriene and differential scanning calorimetry are used to examine the degree of lipid chain perturbation caused by the Pf1 coat protein embedded within the lipid bilayer.

MATERIALS AND METHODS

Reagents were obtained from Sigma, except L-α-dimyristoylphosphorylcholine (DMPC) which was purchased from Fluka and -α-dipalmitoylphosphorylcholine (DPPC) which was supplied by Lipid Products. Host cells *Pseudomonas aeruginosa* strain K and bacteriophage Pf1 samples were kindly provided by Dr. R. N. Perham (Cambridge, U.K.). One liter of TY medium culture of the host cells grown to early log phase (OD_{600} = 0.25) was infected with Pf1 phage at a multiplicity of infection of 15 (Schiksnis et al., 1987). About 12–18 h later, bacteria were removed by low-speed centrifugtion. Solid NaCl was added to the supernatant up to a concentration of 0.5 M, which after 6–10 h was clarified by centrifugation. Addition of poly(ethylene glycol) 8000 (PEG 8000) to a final concentration of 5% precipitated the phage which was then recovered by low-speed centrifugation. Virus purification was accomplished by equilibrium sedimentation of 0.4 g/mL CsCl in a Beckmann SW15 at 35 000 rpm for 18 h. The viscous layer of virus was separated dropwise and dialyzed 3 times against Tris/EDTA buffer, pH 8. The concentration of Pf1 phage was calculated by UV spectroscopy using an extinction coefficient of 2.07 mg·cm^{-1} at 270 nm (Day & Wiseman, 1976). Yields of Pf1 phage were usually about 100 mg.

Preparation of Coat Protein in Detergent Micelles and Lipid Vesicles. Phage samples were prepared by precipitation with 5% PEG/0.5 M NaCl and then resuspended in the appropriate volume. For studies in ^2H_2O, several precipitations and resuspension steps were performed in ^2H_2O solutions until H_2O was removed. This was checked by the absence of H–O–D infrared bands at 3400 cm^{-1} in the transmission spectrum of the solution.

For studies in detergent micelles, Pf1 phage samples (2 mg/mL) were solubilized in 20 mg/mL sodium dodecyl sulfate (SDS) for 60 min at 37 °C. For studies of the Pf1 coat protein in lipid membranes, DMPC was first dissolved in organic solvent, dried, and dispersed in buffer by strong vortexing at 30 °C. To reconstitute the Pf1 coat protein into lipid vesicles, two methods were used: (i) Phage samples (1 mg/mL) and aqueous suspensions of 2.5–5 mg/mL DMPC were sonicated in a pear-shaped flask intermittently in a MTE sonication bath at a temperature of 30–33 °C until the solution was translucent; (ii) 1 mg/mL phage and 2.5–5 mg/mL DMPC were solubilized separately in sodium deoxycholate solution (5 and 15 mg/mL, respectively) for 60 min at 37 °C; then the two solutions were mixed, and the detergent was removed by dialysis against Tris buffer at room temperature. Incorporation of the coat protein in the lipid vesicles was checked by centrifugation on a sucrose gradient. All samples of the Pf1 coat protein in detergent micelles and in phospholipid suspensions were freeze-dried for concentration.

Infrared Spectroscopy. Samples were rehydrated to give a final concentration of Pf1 protein of 3–4% in H_2O and 2–2.5% in ^2H_2O buffer. Typically, about 50 μL of the sample was placed between two CaF_2 windows separated by a 6–12-μm tin for measurements in H_2O or a 50-μm Teflon spacer in ^2H_2O solution. The cell was mounted on a thermostated vertical cell holder with the temperature set at 30 °C. The chamber was purged continuously with dried air. For each sample, 400 scans in H_2O or 100 scans in ^2H_2O solution were recorded at 4-cm^{-1} resolution on a Perkin-Elmer 1750 Fourier-transform infrared spectrometer. Buffer and sample spectra were recorded separately and subtracted, to obtain the difference spectra of the protein. Traces of water vapor were subtracted with a water vapor spectrum until a flat line was obtained in the frequency region of 1800–1700 cm^{-1}.

For transmission polarization studies, phage aliquots of 100 μL (2–4%) were oriented on a rectangular CaF_2 window by manually stroking with a sharp needle, while the liquid was being simultaneously dried with a current of nitrogen gas, as described by Fritzsche et al. (1987) in their studies of the fd phage. The film was enclosed in a transmission cell using a Teflon spacer of 1 mm and a CaF_2 window, mounted on a fixed vertical cell holder. A KRS-5 grid polarizer was used to polarize the infrared radiation, and the parallel beam was made to coincide with the direction of stroking.

Deconvolution routines were used to generate band-narrowed spectra from the original difference spectra of the protein. In all cases, an estimated natural half-bandwidth at half-height (HWHH) of 12 cm^{-1} was used with a resolution enhancement factor (K) of 2. Components in the amide I band were assigned in agreement with Jackson et al. (1989) and Byler and Susi (1986). The degree of H/^{2}H exchange was estimated by measuring the amide I′ band to amide II′ band intensity ratio (Rath et al., 1991; Lee et al., 1987).

Attenuated Total Internal Reflectance. Thin films were formed by spreading suspensions of lipid vesicles onto a germanium crystal (angle of incidence of 45°) and drying with a current of N_2 gas. The dichroic character of the infrared bands of the terminal methyl chain of the lipid acyl chains at 2874 and 1200 cm^{-1} served as an indicator of the degree of perpendicular orientation of the lipid molecules in the film (Fringeli & Gunthard, 1981).

The germanium crystal was installed on a vertical ATR accessory. Some 500–2000 spectra were accumulated on a Mattson FT-IR spectrometer series 4060, purged continuously with dried air, and equipped with a TGS detector. Spectra of the germanium crystal with and without film were recorded separately as single-beam spectra, ratioed to give transmission spectra, and then plotted as absorbance = $2\log(R_i/R_o)$, where R_i and R_o correspond to final and initial reflectance, respectively.

Differential Scanning Calorimetry. Calorimetric data were recorded using a Perkin-Elmer DSC-7 differential calorimeter following the procedure described by Gomez-Fernandez et al. (1980). Scanning rates of 4 °C/min and sensitivites of 1 mcal/s were used. Samples were prepared by the detergent dialysis method and followed with two cycles of centrifugation and resuspension to ensure elimination of traces of the detergent. Samples were hermetically sealed in Perkin-Elmer aluminum "volatile" sample pans. Satisfactory performance of the instrument was checked using pure DMPC and DPPC aqueous suspensions.

The enthalpies were determined by integrating the areas associated with the phase transition. After the measurements, the phospholipid contents of the pans were determined by solubilizing the pan contents in 6% SDS at 70 °C and analyzing the phosphate content as described by Bartlett (1959). The Pf1 protein content was measured by UV spectroscopy at 270 nm by comparison with solutions of Pf1 phage solubilized in 6% SDS.

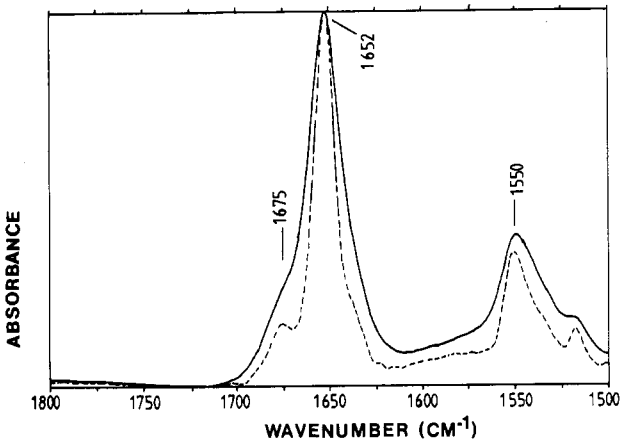

FIGURE 1: Transmission FT-IR spectrum of the coat protein in 20 mg/mL phage in H_2O (solid trace) and its deconvolved spectrum (dashed trace) obtained with a resolution enhancement factor of 2 and a bandwidth of 12 cm^{-1}.

Fluorescence Polarization Studies. Fluorescence polarization measurements were made using a Perkin-Elmer MPF-4B fluorometer. The temperature of the sample was thermostatically controlled and set at 37 °C. The fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH) (an aliquot of a 10 mM solution in tetrahydrofuran) and lipid were added to the organic solvent solution, before drying, to give a probe: lipid molar ratio of 1:500. The use of DPH as a fluorescent probe for the study of hydrophobic regions of lipid bilayers has been discussed in detail by various workers (Shinitzky et al., 1971).

In the fluorescence polarization experiment, the sample was excited with a polarized beam, and the components of the emission parallel or perpendicular to the direction of the excitation beam were measured. The polarization of the emitted fluorescence, P, was determined as

$$P = (I_{par} - I_{perp}G)/(I_{par} + I_{perp}G)$$

where I_{par} and I_{prep} were the intensities of the parallel and perpendicular components of the fluorescence, respectively, and G is a correction factor for the instrumental response of the two components of the emission (Lakowicz, 1983).

To ensure that polarization due to light scattering was not occurring, polarization, P, was measured before and after diluting the sample. In cases where dilution gave an increase in P, the samples were diluted until the value of P remained constant.

According to Hoffman et al. (1981), the values of polarization approach an exponential curve of the type $P = 1 - e^{-MX}$ as the concentration of intrinsic proteins increases. M is an empirical quantity of the approximate number of lipid molecules or probes which fit around the circumference of the intrinsic molecule embedded in the membrane. To derive M, the polarization values for each sample had to be transformed. The values were normalized relative to a minimum value of 0 for the polarization value of the pure lipid ($P_o = 0.25$) and a maximum value of 1 for the estimated maximum. It was estimated that 95% of maximum polarization was reached at $P = 0.375$. For the calculation of M, curves of the form $P = 1 - e^{-MX}$ for several values of M were fitted to the experimental values.

RESULTS

Figure 1 show the FT-IR transmission spectrum of Pf1 phage when present in H_2O solution. The coat protein represents 95% of the phage mass and 99% of the total protein of Pf1 phage, while only some 5% of the phage mass corresponds to the DNA (Day et al., 1988). The infrared spectrum is hence dominated by the contribution of the coat protein subunits in the filamentous phage. The amide I band (width at half-height of 24 cm^{-1}) is positioned at 1652 cm^{-1}, which corresponds to α-helical structure (Jackson et al., 1989). The deconvolved spectrum reveals a small component at 1675 cm^{-1}, which is attributed to β-turn structures (Krim & Bandekar, 1986; Jackson et al., 1989). Quantitative analysis of the secondary structure by factor analysis of the amide I band (Lee et al., 1990) indicates an α-helical content of approximately 90–100% of the protein in the Pf1 filamentous phage, in agreement with other reports (Day et al., 1988). In ^2H_2O solution (not shown), some reduction of the amide II′ band intensity occurs over a period of 2 h, implying that part of the structure of the coat protein in the phage is available for exchange to occur.

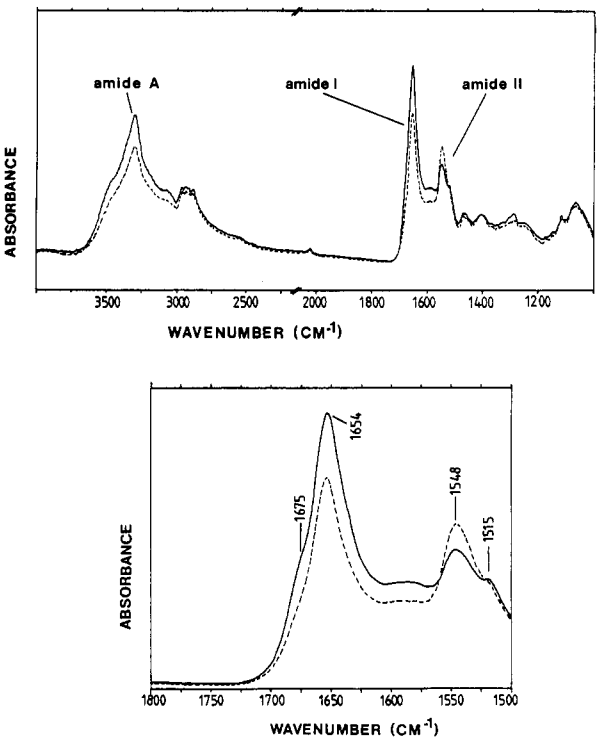

FIGURE 2: (Top) Transmission FT-IR spectra (4000–1000 cm^{-1}) of oriented films of Pf1 phage, obtained with a beam polarized parallel (solid trace) and perpendicular (dashed trace) to the direction of the axis of the phage. (Bottom) Infrared region (1800–1500 cm^{-1}) of transmission spectra of oriented films of Pf1 phage, obtained with beams polarized parallel (solid trace) and perpendicular (dashed trace) to the direction of the axis of the phage.

Figure 2, top panel, shows the transmission spectrum of oriented dried films of the Pf1 phage, recorded with polarized infrared radiation. Here the parallel polarized beam follows the direction of the axis of the Pf1 phage. With the parallel beam, there is an enhancement of various bands: the amide A band at 3200 cm^{-1} which originates mainly from N–H stretching vibrations (Susi, 1969) and the amide I band at 1654 cm^{-1}; a reduction is observed in the amide II band. In the spectra recorded with a perpendicularly polarized beam, the amide A and amide I bands are reduced, and only the amide II band is enhanced. The remaining bands exhibit no changes in intensity. This pattern in the infrared bands is consistent with the helices being aligned parallel to the axis of the phage. The amide I band region (Figure 2, bottom) reveals that the bands at 1675 and 1515 cm^{-1} show an increase in intensity with the parallel polarized beam. The latter band

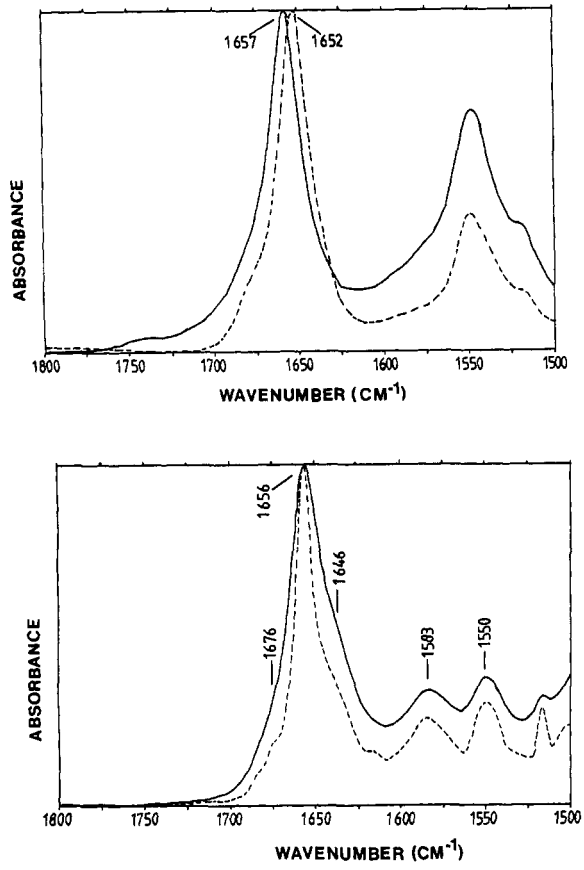

FIGURE 3: (Top) Transmission FT-IR spectra of the coat protein solubilized in SDS detergent micelles (solid trace) and 20 mg/mL phage in H_2O solution (dashed trace). (Bottom) Transmission FT-IR spectrum of the coat protein solubilized in SDS detergent micelles in ^2H_2O solution (solid trace) and its deconvolved spectrum (dashed trace).

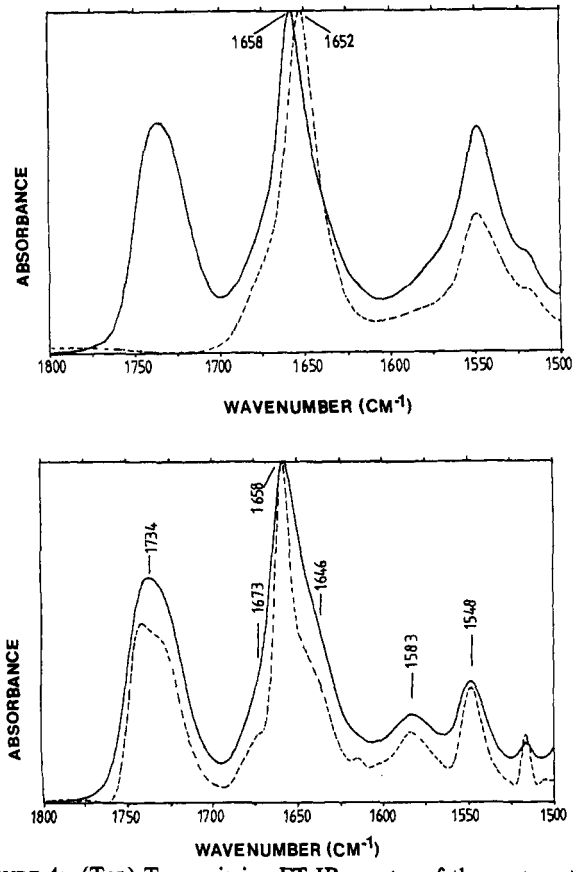

FIGURE 4: (Top) Transmission FT-IR spectra of the coat protein after reconstitution in DMPC vesicles (solid trace) and in 20 mg/mL phage in H_2O (dashed trace). (Bottom) Transmission FT-IR spectrum of the coat protein reconstituted in DMPC vesicles in ^2H_2O solution (solid trace) and its deconvolved spectrum (dashed trace).

at 1515 cm^{-1} originates from ring vibrations of tyrosine residues (Venyaminov & Kalnin, 1991).

Coat Protein in Detergent Micelles. The FT-IR spectrum of the Pf1 coat protein in SDS micelles in H_2O solution is shown in Figure 3, top panel, in comparison with the spectrum of the phage. It can be seen that the protein in detergent micelles gives rise to an amide I band with a similar bandshape as occurs with the phage. The frequency of the amide I band shifts from 1652 cm^{-1} in the phage to 1657 cm^{-1} in SDS micelles and DPC micelles (spectrum not shown). This higher amide I frequency is indicative of a weaker hydrogen-bonding occurring in the α-helical structures of the coat protein in the detergent micelle. Quantification of the secondary structure by factor analysis (Lee et al., 1990) indicates that the proteins in SDS and DPC micelles have a percentage of α-helix of approximately 90–100%. The ratio of the intensity of the amide I band to the amide II band is higher with the protein in the phage than that occurring with the protein in the detergent micelles.

In SDS micelles using ^2H_2O solution (Figure 3, bottom panel), the amide I′ band of the protein is broader and appears at 1656 cm^{-1}. In the deconvolved spectrum, a small band at 1630–1640 cm^{-1} is observed and appears to originate from a shift of band components present under the main amide I band at 1657 cm^{-1} with the protein in H_2O (Figure 3, top panel). These types of structures are assigned to random structures (Jackson et al., 1989). The intensity of the amide II′ band shows a partial decrease as a result of $H/^2H$ exchange, indicating that some of the amide protons are accessible to the solvent and exchange rapidly. Further exchange is slow and is not complete after 24 h. Comparison of the ratio of the amide II′/amide I′ band intensities indicates that these slow-exchanging amide protons corresponds to approximately 30–40% of the total peptidic bonds. This agrees with reports on $H/^2H$ exchange of the coat protein in SDS micelles using ^1H-NMR spectroscopy. The slow-exchanging amide protons were suggested in this case to correspond to an 11 amino acid long hydrophobic part of the Pf1 coat protein (Schiksnis et al., 1987).

Coat Protein in a Lipid Membrane Environment. The spectrum of the phage and that of a suspension of vesicles in H_2O solution prepared by the deoxycholate–detergent dialysis method are shown in the top panel of Figure 4. Three bands occur; the first one at around 1740 cm^{-1} corresponds to the stretching vibrations of carbonyl groups of the acyl esters of the phospholipid. The amide I band maximum of the protein is observed at 1658 cm^{-1}, and its contour is similar to that in the phage, though slightly broader due to the presence of the band associated with the lipid ester group. The shape and frequency of the amide I band are similar to those observed with the coat protein in SDS micelles, indicative that the protein secondary structures are similar. The α-helical content determined by factor analysis (Lee et al., 1990) was approximately 90–100%, similar to that observed in the protein in the phage and detergent micelles.

In ^2H_2O solution (Figure 4, bottom panel), the amide I′ band maximum of the protein in the lipid membrane appears at 1658 cm^{-1}. Deconvolution of the spectrum indicates additional bands are present at 1630–1640 cm^{-1}, attributed to random-coil structures (Jackson et al., 1989). Comparison

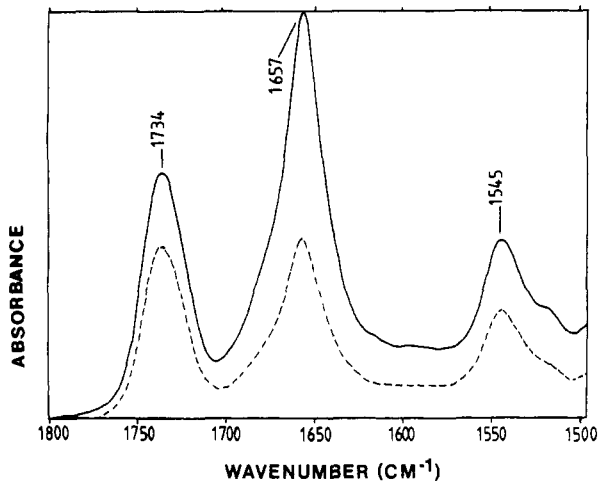

FIGURE 5: Internal reflectance infrared spectra of lipid films with reconstituted Pf1 protein, obtained with beams polarized perpendicular (solid trace) and parallel (dashed trace) to the plane of the film.

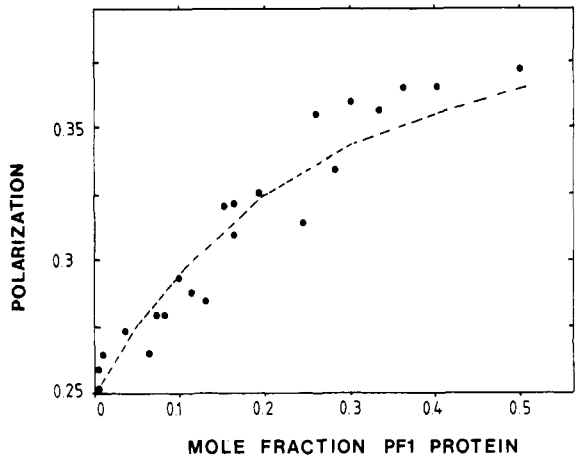

FIGURE 6: Plot of the fluorescence depolarization values of the probe DPH in aqueous suspensions of DMPC with reconstituted Pf1 protein at increasing molar fractions. The dashed line is the best-fit obtained with an exponential curve of the form $P = 1 - e^{-MX}$ for $M = 5$ (see Materials and Methods).

of the ratio of the amide I′/amide II′ band intensities to that observed in the H_2O spectrum indicates that approximately 60% of the amide protons undergo rapid $H/^2H$ exchange and the remaining amide protons showing slow exchange.

Internal Reflectance Infrared Studies. Figure 5 shows the infrared spectra of lipid films with reconstituted Pf1 coat protein recorded with polarized beams. The amide I and II bands are located at similar band frequencies to those observed in aqueous suspensions (see top panel of Figure 4). The amide I band at 1657 cm^{-1} is more intense in the spectra recorded with the beam polarized perpendicular to the plane of the lipid film. The amide II bands show similar absorbances in the parallel and perpendicular polarized spectra. The ratios of the amide I band intensities in both directions are 2.3–2.5, which is similar to those reported in ATR studies of bacteriorodopsin in films of purple membrane (Yang et al., 1987) and in our lab (unpublished results).

Fluorescence Polarization. Figure 6 shows the values of polarization, P, using the fluorescent probe DPH obtained with DMPC suspensions containing increasing concentrations of the Pf1 coat protein. The polarization value of the DPH probe increases as the Pf1 protein concentration increases, indicating that the presence of the protein restricts the motion of the probe. At high protein:lipid ratios, the polarization

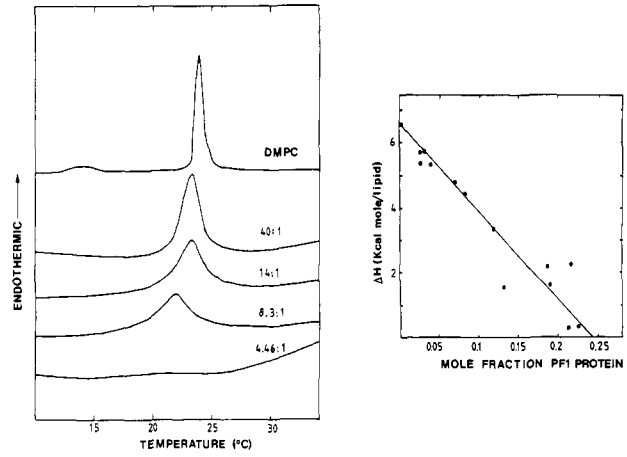

FIGURE 7: (Left) Calorimetric heating curves of aqueous suspensions of lipid, of pure DMPC, and with decreasing lipid:protein molar fractions (from top to bottom curves). The measured lipid sample was approximately 2 μmol. The scale for the curve obtained with pure DMPC aqueous suspension has been halved. (Right) Plot of the molar enthalpies of the phase transition of the DMPC aqueous suspensions with reconstituted Pf1 coat protein at different molar fractions.

values reach a limit in a similar manner to the polarization curves obtained with other aqueous lipid systems containing cytochrome oxidase, gramicidin A, and cholesterol (Hoffman et al., 1981). Hoffman et al. (1981) showed that these curves can be fitted to exponential curves of the form $P = 1 - e^{-MX}$, where M corresponds to the number of DPH chains surrounding the intrinsic molecule. A curve constructed for $M = 5$ is shown in Figure 6, which gives a best fit ($r^2 = 0.9$) for the experimental values.

Calorimetric Studies of the Pf1 Coat Protein Reconstituted into Phospholipid Aqueous Systems. The effect of the presence of the Pf1 protein on the lipid chain organization was also investigated by differential scanning calorimetry (Figure 7). The phase transition of the pure DMPC suspension exhibits a narrow peak, indicative of a highly cooperative phase transition. The incorporation of the coat protein causes a a marked reduction of the enthalpy of the phase transition of the lipid, indicative of the insertion of the protein within the lipid bilayer. This reduction in enthalpy was linearly dependent on the protein:lipid ratio of the DMPC suspension. The value of the protein:lipid ratio at which the phase transition enthalpy reaches zero was extrapolated to a value of 0.24, corresponding to a lipid:protein molar ratio of approximately 4.1:1. This is the amount of lipid molecules withdrawn from the cooperative transition per molecule of Pf1 coat protein present in the lipid membrane suspension.

DISCUSSION

Pf1 Phage. Previous studies of the molecular structure of Pf1 filamentous phage have suggested a model where the coat protein subunits are arranged as rods comprised of two helical segments joined by a loop-connector of five amino acids (Nambudripap et al., 1991). Our FT-IR spectroscopic studies show that the phage contains a high amount of α-helical structure with the α-helical segments being oriented in the direction of the filament.

Both solution and dried films (see Figure 1 and the bottom panel of Figure 2) provide evidence for the existence of some nonhelical structures. We have assigned the band at 1675 cm^{-1} to β-turn structures (Krim & Bandekar, 1986; Jackson et al., 1989). The transmission polarization studies of Pf1 phage films show that this band at 1675 cm^{-1} has a net

orientation parallel to the direction of the axis of the phage. It is plausible that the infrared band at 1675 cm^{-1} originates from a loop-connector structure (Asp14–Asp18) present in the coat protein subunits in the phage as proposed by Nambudripap et al. (1990). In most known secondary structures, e.g., α-helix, 3_{10}-helix, and β-sheet, in proteins and peptides (Krim & Bandekar, 1986), the direction of the vibrational transition moments of the peptidic groups coincides with the axis of the α-helix and the 3_{10}-helix and is perpendicular to that exhibited by the β-sheet. However, in a β-turn, this is different since most are compared of paired amide bonds (β-sheet-like) and nonbonded amide groups which vary in their dihedral angles, giving randomized orientations. Therefore, no defined orientation for a β-turn can be deduced from our polarization studies of the oriented films of the Pf1 phage. In agreement with Raman spectroscopy (Day et al., 1988), the parallel orientation of the tyrosine band at 1515 cm^{-1} provides evidence that the benzene ring planes are aligned with the filamentous axis. Tyrosine residues are found in positions 25 and 40 in the amino acid sequence of the coat protein. The alignment of the tyrosine side chains may arise from a restricted motion due to the stacking with the DNA bases or from space constaints in the interior of the filament (Nakashima et al., 1975).

Pf1 Coat Protein in Detergent Micelles and Lipid Membranes. Previous studies using CD spectroscopy have indicated that the Pf1 coat protein in detergent micelles and in lipid membranes is predominantly α-helical with perhaps a minimal reduction in the content of α-helical structure compared with that present in the phage (Schiksnis et al., 1987). FT-IR spectroscopy techniques enable a direct comparison to be made of the three structural forms of the coat protein: in detergent micelles, artificial membranes, and filamentous phage. The amide I band maximum in the detergent micelles and in the lipid membrane reveals a frequency of 1656–1658 cm^{-1}, which is higher than that observed with the phage. This is indicative of the presence of a weaker hydrogen-bonding α-helix of the protein in detergent and membrane systems. A similar phenomenon occurs with soluble proteins compared to membrane proteins. The amide I band frequency of the former at 1654–1651 cm^{-1} is usually lower than with membrane proteins at 1658–1656 cm^{-1} (Haris & Chapman, 1992).

The rate of amide $H/^2H$ exchange in SDS micelles of the Pf1 coat protein agrees with previous findings made by ^1H-NMR spectroscopy (Schiksnis et al., 1987). The percentage of nonexchangeable amide protons of about 30–40% corresponds well with that deduced from the ^1H-NMR studies. These amide protons are considered to encompass the amino acid stretch Leu30–Tyr40 that forms an α-helical stretch in SDS micelles (Schiksnis et al., 1987). NMR spectroscopic studies of the $H/^2H$ exchange of the M13 coat protein in SDS micelles show similar results (Henry & Sykes, 1992). In the latter study, the slow-exchanging amide protons correspond to a continuous α-helical segment between Met28 and Phe42.

The rate of amide proton exchange of the protein in the phospholipid membrane is comparable to that observed in SDS micelles. The rapid $H/^2H$ exchange indicates that at least half of the coat protein inserted in the membrane is available for exchange and therefore is accessible to the aqueous solvent. The $H/^2H$ slow-exchanging structures may correspond to the hydrophobic α-helical structures deeply buried within the lipid membrane. The exchange properties of bacteriorodopsin studied by FT-IR spectroscopy also show slow exchange of the hydrophobic α-helices embedded within the lipid bilayer (Lee et al., 1987; Earnest et al., 1990).

Previous NMR spectroscopic studies of the Pf1 protein in micelles and in lipid membranes have lead to a proposal of a model for the Pf1 protein in the lipid membrane (Shon et al., 1991). This model is based on the tertiary structure of the Pf1 protein in DPC detergent micelles determined by two-dimensional combined ^1H–^{15}N-NMR spectroscopy and maintains that the structure of the protein in the detergent micelles is unchanged in the lipid membrane. The similarity of the FT-IR spectra, and therefore the secondary structure, of the coat protein in detergent micelles and in phospholipid membrane systems in H_2O lends support to the conclusion of Shon et al. (1991) that the protein adopts α-helical structures in the detergent micelle and also in a lipid environment.

Shon et al. (1991) also proposed that an α-helical domain occurs with its axis perpendicular to the plane of the membrane and that another domain corresponding to the amino terminus lies parallel to the surface of the lipid bilayer. They suggest that this α-helix is amphipathic with its hydrophobic face immersed within the lipid chains and its hydrophilic face exposed to the water. The high dichroic ratio which we observe with the Pf1 coat protein in the lipid bilayer indicates that the protein has an α-helical structure which is predominantly perpendicular to the plane of the lipid bilayer. However, the fluorescent probe experiment provides evidence for the presence of only a single helix lying within the hydrocarbon region of the lipid bilayer. The fluorescence polarization studies indicate that the number of DPH probe molecules, M, affected by the presence of the Pf1 coat protein in the membrane is approximately 5 or 6. Recent electron-spin resonance spectroscopic studies of reconstituted rhodopsin in fluid lipid systems, where the protein exists in a monomeric form, indicate that 22 lipid molecules surround the perimeter of a monomer of rhodopsin (Ryba & Marsh, 1992). A monomer of rhodopsin is predicted to have seven transmembrane α-helices. Extrapolation of the number of lipids surrounding the perimeter of a monomer of rhodopsin of 3-nm diameter to that of a single transmembrane α-helix of 1-nm diameter gives an approximate value of 6–7 lipids for the perimeter of a α-helix. Hence, the perturbation of the lipid chains is consistent with the presence of only a single α-helix in the lipid membrane.

It might be expected that the amino-terminal polypeptide, if present as proposed with its hydrophobic face immersed among the lipid molecules, would affect the crystallization of the lipid chains with the lipid bilayer structure. The calorimetric studies are consistent with the Pf1 coat protein being present in the membrane only as a single α-helical membrane-spanning domain. The crystallization of the lipid chains necessary for the observation of the enthalpy change could perhaps squeeze out any amphipathic helix; however, the fluorescence depolarization experiment which was performed with the lipid in a fluid state, i.e., above the transition temperature of the phospholipid, shows a similar result.

In conclusion, our FT-IR studies of the coat protein in the Pf1 phage show that the protein has a high α-helical content of 90–100% in the phage. The α-helical structures and tyrosine residues show an orientation parallel to the axis of the phage. Upon solubilization in detergent micelles or reconstitution in lipid vesicles, the protein retains a high α-helical content, but there is a weakening of the hydrogen-bonding of the α-helix and also the appearance of some random structures. The $H/^2H$ exchange studies of the protein in the lipid bilayer are consistent with some 60% of the polypeptide undergoing rapid exchange, while the remaining 40% is probably embedded in the hydrophobic core of the lipid. FT-IR polarization studies show that the α-helical structure of the protein is oriented

mainly perpendicular to the membrane plane. Fluorescence polarization studies indicate that the structure of the coat protein which interacts with the lipid membrane is consistent with the presence of only a single membrane-spanning helix. Calorimetric studies are also consistent with this conclusion.

ACKNOWLEDGMENT

We kindly thank Dr. R. N. Perham (Cambridge, U.K.) for the supply of *Pseudomonas aeruginosa* strain K12 and Pf1 phage and Dr. S. J. Opella for providing us with samples of Pf1 coat protein solubilized in DPC detergent micelles. We thank Dr. P. I. Haris for helpful discussions and comments.

REFERENCES

Bartlett, G. R. (1959) *J. Biol. Chem. 234*, 466–471.
Byler, D. M., & Susi, H. (1986) *Biopolymers 25*, 469–487.
Day, L. A., & Wiseman, R. (1978) *J. Mol. Biol. 102*, 549–553.
Day, L. A., Marzec, S. A. R., & Casadevall, A. (1988) *Annu. Rev. Biophys. Chem. 17*, 509–539.
Earnest, T. N., Herzfeld, J., & Rothschild, K. J. (1990) *Biophys. J. 58*, 1539–1546.
Fringeli, U. P., & Gunthard, Hs. H. (1981) *Mol. Biol. Biochem. Biophys. 31*, 270–332.
Frizsche, H., Cross, T. A., Opella, S. J., & Kallenbach, N. R. (1981) *Biophys. Chem. 14*, 283–291.
Gomez-Fernandez, J. C., Goni, F. M., Bach, D., Restall, C. J., & Chapman, D. (1980) *Biochim. Biophys. Acta 598*, 502–516.
Haris, P. I., & Chapman, D. (1992) *Trends Biochem. Sci.*, 328–333.
Henry, G. D., & Sykes, B. D. (1992) *Biochemistry 31*, 5284–5297.
Hoffman, W., Pink, D. A., Restall, C., & Chapman, D. (1981) *Eur. J. Biochem. 114*, 585–589.
Jackson, M., Haris, P. I., & Chapman, D. (1989) *J. Mol. Struct. 214*, 329–355.
Krimm, S., & Bandekar, J. (1986) *Adv. Protein Chem. 38*, 181–364.
Lakowicz, J. R. (1983) in *Principles of fluorescence spectroscopy*, pp 112–152, Plenum Press, New York and London.
Lee, D. C., Herzyk, E., & Chapman, D. (1987) *Biochemistry 26*, 5775–5783.
Lee, D. C., Haris, P. I., Chapman, D., & Mitchell, R. C. (1990) *Biochemistry 29*, 9185–9193.
Marvin, D. A., & Wachtel, E. J. (1975) *Nature 253*, 19–23.
Nakashima, Y., Wiseman, R. L., Konisberg, W., & Marwin, D. A. (1975) *Nature 253*, 68–71.
Nambudripap, R., Stark, W., Opella, S. J., & Makowski, L. (1991) *Science 252*, 1305–1307.
Rath, P., Bousché, O., Merrill, A. R., Cramer, W. A., & Rothschild, K. J. (1991) *Biophys. J. 59*, 516–522.
Ryba, N. J. B., & Marsh, D. (1992) *Biochemistry 31*, 7511–7518.
Schiksnis, R. A., Bogusky, M. J., Tsang, P., & Opella, S. J. (1987) *Biochemistry 26*, 1373–1381.
Shinitzky, M., Dianoux, A. C., & Weber, G. (1971) *Biochemistry 10*, 2106–2113.
Shon, K.-J., Kim, Y., Colnago, L. A., & Opella, S. J. (1991) *Science 252*, 1303–1305.
Smilowitz, H., Carson, J., & Robbins, P. W. (1972) *J. Supramol. Struct. 1*, 8–18.
Susi, H. (1969) *Infrared spectra of biological macromolecules and related systems in structure and stability of biological molecules* (Timasheff, S. N., & Fasman, G. D., Eds.) Marcel-Dekker, New York.
Venyaminov, S. Yu., & Kalmin, N. N. (1991) *Biopolymers 30*, 1243–1257.
Webster, R. E., & Cashman, J. S. (1973) *Virology 55*, 20–38.
Yang, P. W., Stewart, L. C., & Mantsch, H. M. (1987) *Biochem. Biophys. Res. Commun. 145*, 298–302.