Secondary Structure of M13 Coat Protein in Phospholipids Studied by Circular Dichroism, Raman, and Fourier Transform Infrared Spectroscopy†

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ABSTRACT: There is considerable uncertainty about the precise secondary structure adopted by the M13 coat protein when embedded in a phospholipid bilayer. Circular dichroism (CD) spectroscopy suggests that a major change in the structure of the coat protein occurs upon membrane insertion. It is reported that the structure of the protein in the membrane has only about 50% α-helix, the rest being mainly in a β-sheet conformation, whereas the protein is almost completely α-helical when intact in the phage. In this study we have undertaken a spectroscopic analysis using Fourier transform infrared, Raman, and CD spectroscopy to characterize the secondary structure of M13 coat protein when present in membranes consisting of dioleoylphosphatidylglycerol and dimyristoylphosphatidylglycerol. In sharp contrast to earlier CD studies, our results indicate that the coat protein in its membrane-embedded state has a very high α-helical content with virtually no β-sheet structures present. This result indicates that the structures of the coat protein when intact in the phage or when embedded in the membrane are similar. Although our results differ from earlier CD studies, they are consistent with a recent NMR study, which showed that the M13 coat protein in sodium dodecyl sulfate micelles is primarily α-helical with no evidence for β-sheet structure [Henry, G. D., & Sykes, B. D. (1992) *Biochemistry 31*, 5284–5297]. These results lead to the conclusion that the M13 coat protein can insert from the membrane-bound state into a virus particle with a similar secondary structure, without large energy implications. Under certain conditions a high β-sheet content can occur for the M13 coat protein in phospholipid membranes. The detailed secondary structure of this state is also investigated. The results obtained show over 50% β-sheet structure and only about 10–15% α-helical structure. The β-sheet structure observed is a reflection of highly aggregated protein molecules.

M13 bacteriophage is a filamentous phage, which infects *Escherichia coli* cells. The circular stranded DNA of the phage is surrounded by a protein coat, which predominantly consists of the gene 8 product, the major coat protein. During infection this coat protein is inserted into the host membrane. Newly synthesized coat protein is also inserted into the host bilayer, where it is assembled together with old coat protein around DNA to form new M13 bacteriophage particles (Smilowitz, 1974; Wickner, 1975). The major coat protein is composed of three domains: a 19 amino acid long hydrophobic core, an N-terminal region (residues 1–20), and a C-terminal region (residues 40–50). The hydrophobic core is the membrane-spanning part when the protein is in the host bilayer.

From high-performance size-exclusion chromatography experiments in combination with CD spectroscopy it has been found that M13 coat protein reconstituted into phospholipid systems can adopt two different conformations, which have quite different aggregation behaviors (Spruijt et al., 1989). M13 coat protein in a predominant α-helix conformation can form reversible small aggregates (α-oligomeric protein). The coat protein in a predominant β-sheet conformation appears to be aggregated in an irreversible way (β-polymeric protein). The presence of either one of these M13 coat protein forms depends on the protein purification procedure, the reconstitution method, the L/P ratio, the phospholipid headgroup type, the length and degree of saturation of the acyl chains of the lipids used, and the salt concentration of the buffer. The α-oligomeric state of the coat protein is stabilized by an increased ionic strength of the buffer. Without salt, the α-oligomeric state of the coat protein is maintained only in phospholipid systems containing at least one unsaturated acyl chain, and then only at high L/P ratios (Spruijt et al., 1989; Spruijt and Hemminga, 1991). M13 coat protein in the β-polymeric state is strongly aggregated in an irreversible way, and the corresponding conformation is characterized by a high amount of β-sheet and a lack of α-helix (Nozaki et al., 1976; Williams & Dunker, 1977; Nozaki et al., 1978; Fodor et al., 1981; Spruijt et al., 1989; Dunker et al., 1991a). Protein in the β-polymeric state is characterized by an extended intermolecular hydrogen bond network in the plane of the membrane (Hemminga et al., 1992).

Although the smallest aggregational entity of the α-oligomeric coat protein is a monomer, α-oligomeric coat protein can aggregate reversibly and can occur in aggregates of variable size, depending on the conditions applied. The formation of β-polymers requires an aggregated state of the coat proteins

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[1] Abbreviations: CD, circular dichroism; DOPG, 1,2-dioleoyl-*sn*-glycero-3-phosphoglycerol; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol; FTIR, Fourier transform infrared; L/P, lipid to protein molar ratio; NMR, nuclear magnetic resonance.

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involved. The aggregation-related conformational change of the α-oligomeric coat protein into β-polymeric coat protein is irreversible. The β-polymers can consist of about 20 up to several hundred monomers. These β-polymers are able to associate into even larger aggregates in a reversible way (Spruijt & Hemminga, 1991).

The coat protein, when associated with DNA in the virion, is almost completely α-helical, as judged by X-ray diffraction techniques (Marvin & Wachtel, 1975; Banner et al., 1981; Glucksman et al., 1992), circular dichroism (Marvin et al., 1974; Nozaki et al., 1976; Williams & Dunker, 1977; Fodor et al., 1981; Clack & Gray, 1989; Arnold et al., 1992), and laser Raman spectroscopy (Thomas et al., 1983; Williams et al., 1984). While the structure of the protein intact in the virus is agreed upon, there are several uncertainties concerning the precise secondary structure of the different types of membrane-bound M13 coat protein. This is due to the fact that, in the case of optical spectroscopy (FTIR, CD, Raman), information about secondary structure comes from databases with known protein structures that are not specifically suitable for small membrane-bound proteins such as M13 coat protein. Two-dimensional proton NMR spectroscopy, which is the most powerful method to date to obtain detailed structural information about the secondary structure of proteins, has only been successfully applied to M13 coat protein reconstituted in micellar systems (Henry & Sykes, 1992).

Since CD spectroscopy of the protein in the two membrane-bound forms showed it to be in a less α-helical state compared to the virus-bound protein, it has been suggested that M13 coat protein should undergo major conformational changes upon virus formation (Nozaki et al., 1976, 1978). This raises several questions concerning the energy implications of this change in conformational state. An additional problem was related to the stability of the protein in the lipid–protein preparations, which caused the protein to convert irreversibly from the α-oligomeric state into the β-polymeric state (Spruijt & Hemminga, 1991).

In this paper, we have undertaken spectroscopic studies to determine the detailed secondary structure (both qualitative and quantitative) of the two forms of M13 coat protein in its membrane-bound state. For the first time three different optical techniques (CD, Raman, and FTIR spectroscopy) commonly used for secondary structural analysis have been applied to M13 coat protein in the α-oligomeric and the β-polymeric state. We have carefully controlled and checked the state of the coat protein, leading to a consistent set of spectroscopic data of each protein form. This information is crucial to understand the way these different protein forms interact with phospholipid bilayers and their role in the viral infection process.

MATERIALS AND METHODS

Chemicals. Dioleoylphosphatidylglycerol (DOPG) and dimyristoylphosphatidylglycerol (DMPG) were obtained from Sigma (St. Louis, MO) and used without further purification.

Protein Purification and Reconstitution. Bacteriophage M13was grown and purified as described previously (Spruijt et al., 1989). After the chloroform was removed with nitrogen gas, the desired amount of DOPG was lyophilized for at least 12 h. DMPG and DOPG were solubilized in buffer (Buffer A for the M13 coat protein in the β-polymeric form: 8.0 M urea, 5 mM Tris, 0.1 mM EDTA, 20 mM ammonium sulfate, and 140 mM NaCl, pH 8.0. Buffer B for the coat protein in the α-oligomeric form: 50 mM cholate, 10 mM Tris, 0.2 mM EDTA, and 140 mM NaCl, pH 8.0.) To buffer A was added the desired amount of protein, purified as described by Knippers and Hoffmann-Berling (1966), to obtain the protein in the β-polymeric form. To obtain the protein in the α-oligomeric form, the protein, purified as described by Spruijt et al. (1989), was added to buffer B. This was followed by dialysis at room temperature against 100-fold excess buffer (10 mM Tris, 0.2 mM EDTA, and 140 mM NaCl, pH 8.0) for both protein preparations for a total of 48 h, changing the buffer every 12 h. Directly after the dialysis procedure the reconstituted lipid–protein complexes were concentrated using an Amicon stirring cell. The samples were divided into three parts. From each of these parts the secondary structure of the M13 coat protein was determined using either CD, Raman, or FTIR spectroscopy. The aggregation state, the L/P ratio, and the incorporation of the protein were checked as described by Spruijt et al. (1989).

CD Spectroscopy. CD measurements were performed at 30 °C on a Jobin-Yvon Dichograph Mark V in the wavelength range 190–290 nm, using a 0.1-cm path length. The scan time for one scan was 1500 s with a 2-s time constant. The samples for the CD measurements were diluted to an OD_{280} of about 0.1. The temperature was controlled using a thermostated water bath and maintained at 30 °C. Background spectra, from samples consisting of buffer with the same lipid concentration as used in the corresponding protein sample spectrum, were recorded under the same experimental conditions. Difference spectra were generated by subtracting the background spectra from the corresponding protein spectra. The difference spectra were transferred to a VAX computer. Spectra in the wavelength region from 195/200 to 240 nm were analyzed using a fitting program supplied by Provencher and Glöckner (1981). In the fitting procedure the experimental ellipticity values were used, and no normalization was applied (constrained analysis).

Raman Spectroscopy. Raman spectra were obtained with a Jobin-Yvon HG2S monochromator. A Hamamatsu photomultiplier tube, r942-02, was used in photon-counting mode. The tube voltage was 1750 V. The Raman spectra were excited with 514.5-nm light from a Spectra Physics Ar^+ laser (type 2025). The power at the sample was 400 mW. The slit widths of the spectrometer were adjusted to produce a resolution of 4 cm^{-1}. The scan interval ran from 400 to 1800 cm^{-1} with a step width of 1 cm^{-1}. The temperature of the sample was regulated with a thermostated water bath at 30 °C. Spectra were obtained from phospholipid/M13 coat protein complexes, from pure phospholipid systems, and from the buffer solution. Prior to the analysis, the protein spectrum was obtained in the following way:

$$[\text{protein}] = [\text{complex}] - c_1 \times [\text{buffer}] - c_2 \times [\text{lipid}]$$

where the brackets indicate the spectrum of each component. The constants c_1 and c_2 were adjusted in such a way that a flat baseline was obtained between 1500 and 1730 cm^{-1}. The position of the phenylalanine band in the analyzed spectra was at 1002 cm^{-1}. The fit of the amide I band was performed using a singular value decomposition routine. A basis set of 15 reference proteins obtained by Williams (1983) was used for this purpose.

FTIR Spectroscopy. FTIR spectra were recorded on a Perkin-Elmer 1750 FTIR spectrometer equipped with a fast-recovery TGS detector and a Perkin-Elmer data station. Aqueous samples were recorded in a temperature-controlled Specac cell fitted with either a 6-μm tin spacer for studies in H_2O or a 50-μm Teflon spacer for studies with D_2O. The samples in D_2O were prepared by dialysis of a part of the sample solution against D_2O. The temperature was maintained at 30 °C. The spectrometer was continuously purged

with dry air to eliminate interference from water vapor. A sample shuttle was used to allow the background spectrum to be signal averaged over the same time period as the sample spectrum. For the H_2O samples 400 scans were co-added and apodized, giving a resolution of 4 cm^{-1}. For samples in D_2O 256 scans were co-added and processed as for the samples containing H_2O.

Absorbance spectra of the protein were generated by subtracting the appropriate aqueous buffer spectrum from the sample spectrum. Details about solvent subtraction are described elsewhere (Haris et al., 1986; Lee et al., 1990). Second-derivative spectra were generated from the difference spectra using the Perkin-Elmer DERIV routine as described previously (Haris et al., 1986). Quantitative analysis in terms of secondary structural elements was performed with the program CIRCOM (Lee et al., 1990). This program uses 18 water-soluble proteins as a calibration set. The area under the amide I band (i.e., from 1700 to 1600 cm^{-1}) was made constant, and also the set ordinate at 1700 cm^{-1} was set to a constant value. This procedure is applied to adjust for any variation in baseline and absorbance due to variation in path length and concentration (Lee et al., 1990).

RESULTS

Protein Checks. The protein in the β-polymeric form is characterized as being highly aggregated, as revealed by the high-performance size-exclusion chromatography elution profiles, whereas the protein in the α-oligomeric form shows no aggregation. This is in agreement with the findings of Spruijt et al. (1989). Both samples were checked for homogeneity and incorporation of the protein by sucrose gradient centrifugation. The sucrose gradient centrifugations of samples with and without protein show only one band, demonstrating sample's homogeneity. The band of the samples with protein is at a significantly lower position in the sucrose gradient, indicating a higher density due to the incorporation of the protein. The L/P ratio was checked after the dialysis procedure and was found to be 20 for the samples with the M13 coat protein in both states.

CD Measurements. The CD spectra of the M13 coat protein in the β-polymeric form and in the α-oligomeric form in DOPG are given in panels a and b, respectively, of Figure 1. The secondary structure of the M13 coat protein in the β-polymeric form is predominantly β-sheet in DOPG and DMPG. However, smaller amounts of other structures are also found from a secondary structure analysis (Table I). The α-helix content for the protein in the α-oligomeric form is calculated to be 95% and 91% in DOPG and DMPG, respectively. In both phospholipid systems only a small number of other secondary structure arrangements are observed (Table I). Performing the analyses without taking into account the protein concentration or changing the temperature did not result in a change of the results. It is known, however, that the CD spectrum of an α-helix is length-dependent (Gans et al., 1991) and that the length of the helix found here is much longer than any of the reference proteins in the data set used. Using a set of reference proteins, the ellipticity for a pure helix is typically calculated to be about −31 000 to −32 000 (deg·cm^2)/dmol at 222 nm, whereas the observed CD spectrum for long helices, such as poly-L-lysine, is about −40 000 (deg·cm^2)/dmol. This effect may lead to a calculated percentage of α-helix in Table I that is too high.

Raman Measurements. The Raman spectrum of the amide I region of the protein in the β-polymeric form in DMPG is presented in Figure 2a. The spectrum of M13 coat protein in the α-oligomeric form in DOPG bilayers is shown in Figure 2b. The Raman spectra are obtained after the subtraction procedure described in the Materials and Methods section. The amide I region is of special interest for obtaining

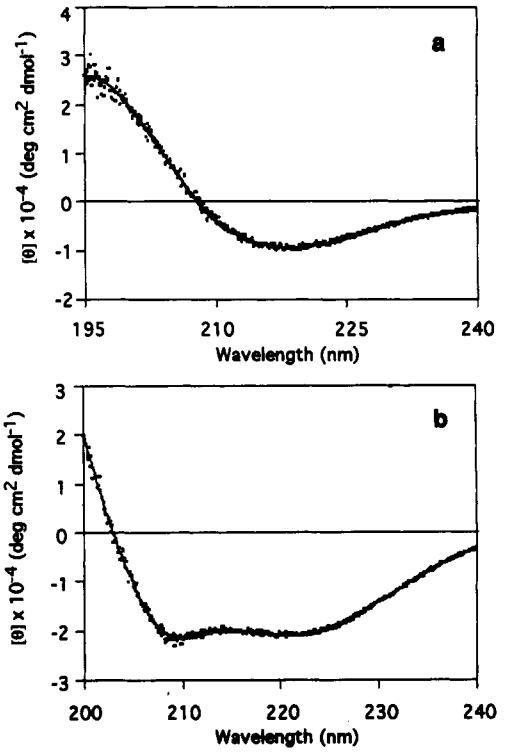

FIGURE 1: CD spectra of the M13 coat protein in the β-polymeric form (a) and the α-oligomeric form (b) in DOPG. In each panel, the squares represent the data points and the line through them represents the fitted spectrum.

Table I: Percentage of Secondary Structure Arrangements of M13 Coat Protein in the α-Oligomeric and the β-Polymeric Form, Incorporated in DOPG and DMPG[a]

protein form	lipid	tech-nique	% α-helix	% β-sheet	% turn	% remainder
α-oligomeric	DOPG	CD	95	0	2	3
α-oligomeric	DOPG	Raman	70	25	4	0
α-oligomeric	DOPG	FTIR	≈90[b]	0	0	0
α-oligomeric	DMPG	CD	91	0	7	2
α-oligomeric	DMPG	FTIR	≈90[b]	0	0	0
β-polymeric	DOPG	CD	10	60	2	28
β-polymeric	DOPG	FTIR	20	60	10	10
β-polymeric	DMPG	CD	0	69	11	20
β-polymeric	DMPG	Raman	6	46	38	10
β-polymeric	DMPG	FTIR	25	59	3	13

[a] The error estimate in the analyses is 10%. [b] Quantitative analysis gives >100%. The percentage given here is deduced by comparison of the spectrum with those of hemoglobin and myoglobin (see text).

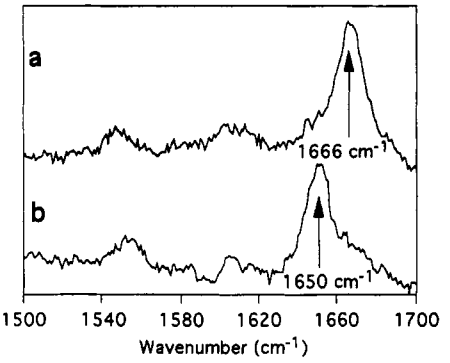

FIGURE 2: Raman spectra of the amide I region of M13 coat protein in the β-polymeric form in DMPG (a) and the α-oligomeric form in DOPG (b).

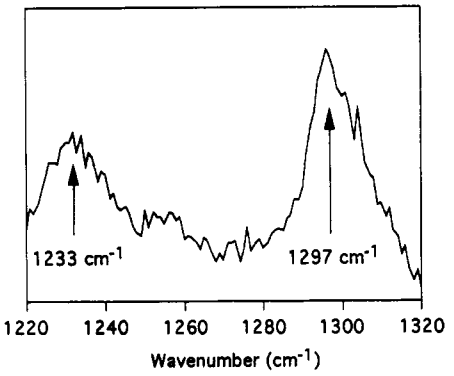

FIGURE 3: Raman spectrum of the amide III region of M13 coat protein in the β-polymeric form in DMPG.

information about the secondary structure. In the Raman spectra of the M13 coat protein in the β-polymeric form in DMPG, a band at 1666 cm^{-1} is observed, which can be assigned to a β-sheet structure (Tu, 1982). The broad shape at the high-frequency edge of the β-sheet band at 1666 cm^{-1} of the Raman spectrum of M13 coat protein in the β-polymeric form (Figure 2a) possibly indicates the presence of other secondary structures. This band could not properly be reduced by subtraction of the Raman spectrum of water or the phospholipid. Probably these contributions arise from the presence of turns (Williams, 1983). The band at 1233 cm^{-1} (Figure 3) arising from the amide III modes of the protein in the β-polymeric form suggests an antiparallel β-sheet conformation (Aslanian et al., 1986). In the amide III region (Figure 3), both turn and α-helix contributions can be found at 1297 cm^{-1}.

In the spectrum of M13 coat protein in the α-oligomeric form (Figure 2b) the amide I maximum is found to be at 1650 cm^{-1}, which is indicative of an α-helix (Tu, 1982). In spectra a and b of Figure 2, a band is observed near 1550 cm^{-1}, which is assigned to the single tryptophan of the M13 coat protein (Tu, 1982). The band at 1605 cm^{-1} observed in both Raman spectra is assigned to vibrations of the tyrosine and phenylalanine residues (Tu, 1982).

The results of the secondary structure analysis are presented in Table I. The percentages of ordered and disordered helix types (Williams, 1983) are summed in Table I under the percentage of α-helix. Also the percentages of parallel and antiparallel β-sheet are taken together. Data manipulation of the protein spectra prior to the analysis of the amide I band gives rise to errors in the percentages of secondary structure. The subtraction of the lipid spectrum in the case of DMPG is reliable because strong lipid contributions to the spectrum in the amide I region are absent. However, in the case of DOPG a strong band arises at 1659 cm^{-1}. This band results from the double C=C stretch motion in the acyl chains. Subtraction of the lipid spectrum from the complex spectrum influences the shape of the amide band at the high-frequency side. The best subtraction was finally obtained by eye. The presence of the Raman scattering of water around 1630 cm^{-1} influences most noticeably the percentage of α-helix in the amide I analysis. The part of the water spectrum at the low-frequency edge, however, does not coincide with the amide I band. This allows for a reasonable subtraction criterion. The remaining background was fitted by a straight line drawn between minima around 1525 and 1725 cm^{-1}. All these data manipulations give rise to the error indicated in footnote *a* of Table I.

FTIR Measurements. FTIR spectra of M13 coat protein in both conformations in DOPG bilayers in H_2O are displayed in Figures 4 and 6. The spectrum of the protein in the

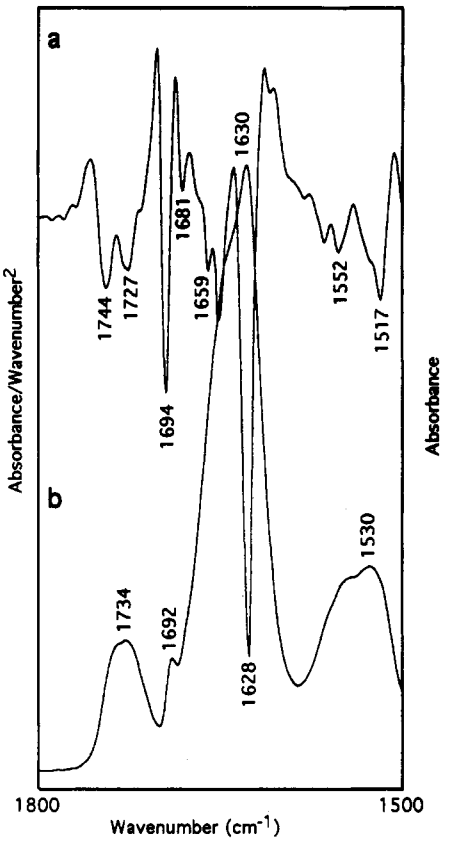

FIGURE 4: FTIR absorbance spectrum in H_2O (b) and second-derivative spectrum (a) of M13 coat protein in the β-polymeric form in DOPG.

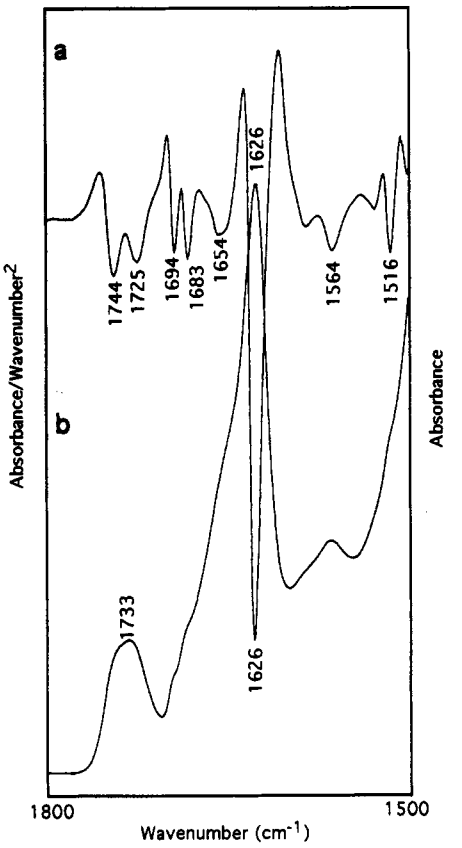

FIGURE 5: FTIR absorbance spectrum in D_2O (b) and second-derivative spectrum (a) of M13 coat protein in the β-polymeric form in DOPG.

β-polymeric form shows an amide I absorption maximum at 1630 cm^{-1}, while the amide II band is located at 1530 cm^{-1}

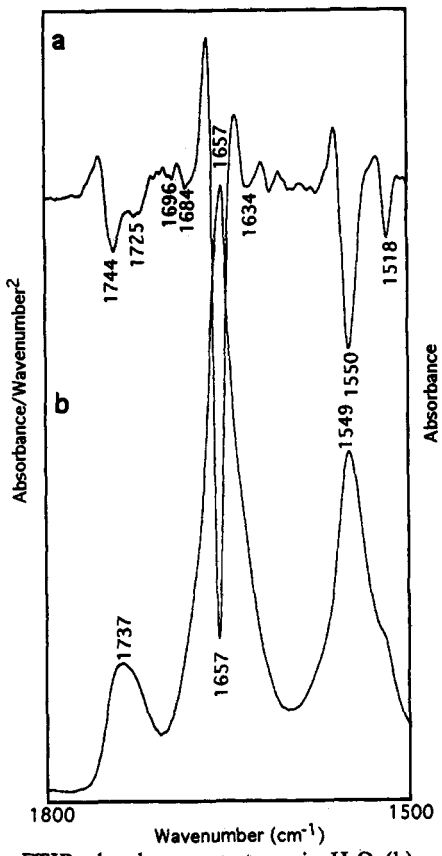

FIGURE 6: FTIR absorbance spectrum in H_2O (b) and second-derivative spectrum (a) of M13 coat protein in the α-oligomeric form in DOPG.

(Figure 4b). These bands correspond to a β-sheet structure (Surewicz & Mantsch, 1988). It is remarkable that the intensity of this band is strongly reduced compared to what normally is observed for proteins in H_2O. Previously, it has been noted that the intensity ratio of the two amide bands, A_{II}/A_I, was associated with changes in conformation, temperature, or solvent polarity (Nedelec et al., 1989). The abnormal low A_{II}/A_I ratio for the M13 coat protein in the β-polymeric form could possibly be due to aggregation of the protein in this form, giving rise to a very distinct amide environment. The band at 1734 cm^{-1} is assigned to the lipid carbonyl ester vibration. From the second-derivative spectrum (Figure 4a) it can be observed that in addition to the main band at 1628 cm^{-1}, which denotes a β-sheet structure, a weaker band is observed at 1694 cm^{-1}. This component has been attributed to the high-frequency component of the vibration of an antiparallel β-sheet structure (Haris et al., 1986; Jackson et al., 1989). The band at 1659 cm^{-1} most likely represents α-helical structure, although overlap of absorbance from a disordered structure is also possible. The results obtained from a quantitative analysis performed with the program CIRCOM are presented in Table I. Similar secondary structure contributions are observed for M13 coat protein in the β-polymeric form in DMPG bilayers (Table I).

The protein in the α-oligomeric form shows a very sharp, narrow, and nearly symmetric amide I band at 1657 cm^{-1} (Figure 6b), while the amide II band is centered at 1549 cm^{-1}. This is characteristic for an α-helix conformation (Lee et al., 1990). Also, the ratio A_{II}/A_I (0.6) is in agreement with a high α-helix content (Nedelec et al., 1989).

The absence of any additional features in the amide I region suggests a homogeneous structural composition for the protein. The fact that the amide I maximum remains at the same frequency (approx. 1657 cm^{-1}) in D_2O (see later) indicates that it arises from the absorption of α-helical structure. Furthermore, the position of the amide I maximum at 1657 cm^{-1} is similar to those observed for a number of membrane proteins (Haris & Chapman, 1992). Detailed analysis using the second-derivative method only shows the presence of weak bands near 1630–635 cm^{-1} and 1670–1680 cm^{-1} (Figure 6a). The spectrum of the M13 coat protein has some similarity with highly helical proteins such as hemoglobin and myoglobin. Both of these proteins show weak bands in these same regions as observed for the M13 coat protein. However, myoglobin and hemoglobin contain 88% and 86% α-helical structure, respectively, and they do not contain any β-sheet structure (Lee et al., 1990). In this context the bands observed near 1630–1635 and 1670–1680 cm^{-1} for M13 coat protein may reflect structures other than α-helix and β-sheet. In any case the contribution of these bands in the spectra of the protein is very small. In addition, a visual comparison of the spectra of hemoglobin and myoglobin with that of M13 coat protein suggests that the latter is the most helical. This would mean M13 coat protein is over 88% α-helix in its lipid-embedded state. Quantitative analysis using the factor analysis method (CIRCOM program) gives the unusual result of over 100% α-helix. The reason for this is probably a reflection of the spectral properties of M13 coat protein lying outside the range of properties defined by the calibration set. For example, the α-helical content in M13 coat protein appears to be even higher than the most helical protein (myoglobin) used in the calibration set for the quantitative analysis method. Furthermore, the calibration set is composed of soluble proteins, whereas M13 coat protein is a membrane protein. It should be noted, however, that the α-helix region (1650–1658 cm^{-1}) and the random coil region (1640–1648 cm^{-1}) are very close to each other (Surewicz & Mantsch, 1988) and that a shift of the bands due to the effects mentioned may result in an overestimation of the α-helix content. The results obtained here indicate about 90% helical structure in M13 coat protein, taking into consideration the comparison with hemoglobin and myoglobin, and any significant presence of β-sheet in its structure can be discounted.

The FTIR spectra of the M13 coat protein of the two forms in D_2O are shown in Figures 5 and 7. The main amide I band in the spectra with M13 coat protein in the β-polymeric form is centered at 1626 cm^{-1} (Figure 5b). Second-derivative analysis reveals a main band at 1626 cm^{-1} with weak components at 1694, 1683, and 1654 cm^{-1} (Figure 5a). The main band is attributed to β-sheet structure (Haris et al., 1986; Jackson et al., 1989; Lee et al., 1990). The component at 1694 cm^{-1} probably reflects a high-frequency vibration of an antiparallel β-sheet (Haris et al., 1986; Jackson et al., 1989). The bands at 1683 and 1654 cm^{-1} are assigned to turns and α-helices, respectively (Haris et al., 1986; Jackson et al., 1989).

The spectrum of protein in the α-oligomeric form in DOPG shows an amide I band centered at 1654 cm^{-1} and an amide II band located at 1547 cm^{-1} (Figure 7b). The intensity of the amide II band is reduced as a result of H–D exchange. The band at 1654 cm^{-1} is fully consistent with the presence of α-helix structure (Jackson et al., 1989; Nedelec et al., 1989).

DISCUSSION

In this paper a study of the secondary structure of the M13 coat protein in the α-oligomeric and β-polymeric forms has been carried out using optical spectroscopy. The conformation of the M13 coat protein has been determined from a quantitative computer analysis of the secondary structure data. The advantage of the optical secondary structure experiments

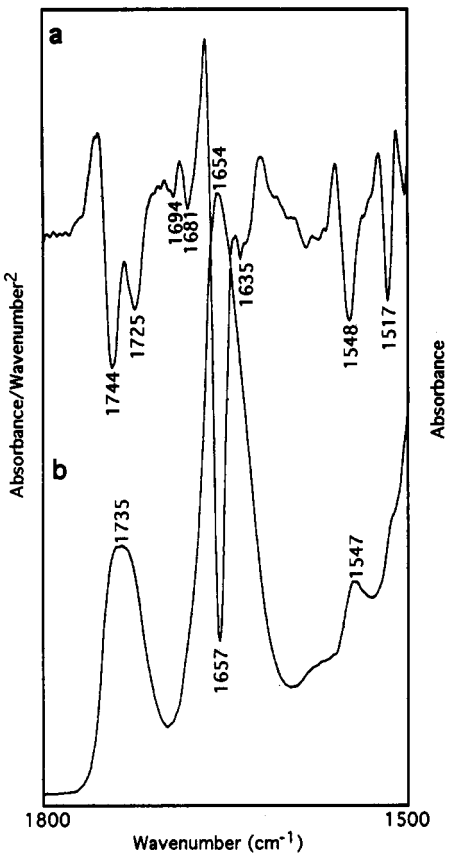

FIGURE 7: FTIR absorbance spectrum in D_2O (b) and second-derivative spectrum (a) of M13 coat protein in the α-oligomeric form in DOPG.

is that they require relatively small amounts of sample and give a direct determination of the overall protein conformation in phospholipid bilayer systems. However, it is not possibleto locate the secondary structure elements in the polypeptide chain. Because the secondary structure might reflect the influence of M13 coat protein on phospholipid bilayers, the aim of this investigation is to obtain a quantitative determination of the appearance of the various structural arrangements in both protein forms in phospholipid bilayers. As some controversy surrounds the precise secondary structure of the protein in lipid membranes, for the first time three different established techniques for protein secondary structure analysis (CD, Raman, and FTIR spectroscopy) have been simultaneously applied to the study of M13 coat protein in lipid membranes to enhance the reliability of the results.

Although in our study we have used the factor analysis method for quantitative analysis of FTIR spectra, there are other methods available, such as the curve-fitting procedure. Recently a review has been published that discusses the problems as well as the advantages and disadvantages associated with the different techniques available for quantitative analysis of the secondary structure of proteins from their infrared spectra (Surewicz et al., 1993). However, the present paper was not intended to deal with the various methods that exist to obtain quantitative parameters from spectroscopic data. The most successful methods have been used for each spectroscopic technique.

In the literature, it has been reported that due to different protein isolation procedures, reconstitution methods, amphiphiles employed, and conditions applied one of the two defined states of the coat protein can be preferentially present, but also mixtures of the two states of the coat protein can occur (Williams & Dunker, 1977; Chamberlain et al., 1978; Fodor et al., 1981; Bayer & Feigenson, 1985; Spruijt et al., 1989; Spruijt & Hemminga, 1991). Because of this property of M13 coat protein, in several biophysical studies the state of M13 coat protein has not been well defined, giving rise to incomplete, incomparable, or even conflicting results (Wickner, 1976; Hagen et al., 1978; Wolber & Hudson, 1982; Bogusky et al., 1987; Leo et al., 1987; Weiner et al., 1987). In some other cases, it can be inferred which form of the coat protein was studied on the basis of the results that have been obtained. For example, the studies of Smith et al. (1980), Kimelman et al. (1979), and Johnson and Hudson (1989) showed that the coat protein appeared to be in a less aggregated state, indicating that they were studying the α-oligomeric form of the coat protein. Other authors found that the coat protein was aggregated, suggesting that these authors were studying the β-polymeric form of the coat protein (Datema et al., 1987a,b; De Jongh et al., 1990; Peng et al., 1990a,b). In the present paper, we have carefully controlled and checked the state of the M13 coat protein, leading to a consistent set of spectroscopic data of each protein form.

The secondary structure analysis of the data obtained from CD, Raman, and FTIR spectroscopy of M13 coat protein and presented in Table I shows that similar results are found for the two protein forms, independent of the phospholipids used. This indicates that the type of acyl chain is not a critical variable in the formation of the two different forms of the M13 coat protein, as has been suggested by Fodor et al. (1981) for the similar fd coat protein. Although fd coat protein differs from M13 coat protein in that it has a carboxylic acid side chain instead of an amide at position 12, so that the net total charges are different, this effect is assumed not to be of importance for the interactions in the hydrophobic region.

β-Polymeric Form. In agreement with previous results (Datema et al., 1987a), the β-polymeric form consists predominantly of β-sheet structure (Table I). It has been shown, using molecular dynamics simulations (Sanders et al., 1991), that the amount of β-sheet in the aggregated protein after a 100-ps simulation is about 60%. This value is in good agreement with the values found for β-polymeric protein.

A rather large percentage of turn is obtained in the analysis of the Raman spectra of this protein form. This finding is probably due to the overlap of the Raman scattering of turn structure with that of β-sheet, so that it is not possible to discriminate between β-sheet and turn (Berjot et al., 1987). From the strong band at 1237 cm^{-1} in the Raman spectra and the band at 1694 cm^{-1} in the FTIR spectra, it can be concluded that the β-sheet structure is an antiparallel β-sheet structure (Aslanian et al., 1986). The amount of α-helix for the M13 coat protein in the β-polymeric state as obtained from FTIR measurements is higher than the amount of α-helix obtained from CD measurements. This effect may be due to an underestimation of α-helix structure in CD, arising from absorption flattening, which is a consequence of the nonrandom distribution of the chromophores in these lipid samples (Wallace & Mao, 1984; Wallace & Teeters, 1987).

The antiparallel β-sheet region is proposed to be located in the hydrophobic part of the membrane. This finding is in agreement with the complete β-structured transmembrane domain that remains after proteolytic digestion of both hydrophilic terminal parts of the coat protein (Chamberlain et al., 1978). From NMR experiments carried out on phospholipid systems containing β-polymeric coat protein, it is found that the lipids close to the protein polymers are arranged in a non-bilayer structure that is caused by a misfitting of the hydrophobic core of the protein and the membrane bilayer (Sanders et al., 1992a). The antiparallel β-sheet structure of the polymers would leave about 10 amino

acids of the N-terminus unpaired. This unpaired region in the M13 coat protein in the β-polymeric form could be either in α-helix or in a random coil conformation. On the basis of the amount of α-helix in the protein in the β-polymeric form (Table I), this part is proposed to be in an α-helix conformation.

α-Oligomeric Form. M13 coat protein in the α-oligomeric form consists predominantly of α-helix, as can be seen from Table I. After a 100-ps molecular dynamics simulation (Sanders et al., 1991) the amount of α-helix in the α-oligomeric form was found to be 80–90%. This percentage agrees well with the values found for α-helix structure (Table I), especially taking into account the difficulty of assigning length-dependent effects in the CD spectra and the possible overlap of α-helix and random coil bands in the FTIR spectra. These effects may result in the calculated amount of α-helix in the α-oligomeric form in Table I being too high. It is generally agreed that M13 coat protein, when associated with DNA in the phage, is about 90% α-helical. If the unusually large tryptophan contributions are removed, the ellipticity values in the 210–220 nm region are −35 000 (deg·cm^2)/dmol (Arnold et al., 1992) or −37 000 (deg·cm^2)/dmol (Dunker et al., 1991b). The phage Pf1, which does not have the unusual tryptophan contributions, has ellipticities around −40 000 (deg·cm^2)/dmol (Arnold et al., 1992). These values are very close to those reported for α-helical poly-L-lysine (Greenfield & Fasman, 1969). The ellipticity found in the present work is about −20 000 (deg·cm^2)/dmol in the 210–220 nm range, which corresponds to an α-helix content for the protein of about 90% in DOPG and DMPG. This discrepancy regarding the ellipticity values for 90% helix probably arises from the fact that the relatively short helices (average length about 10 amino acid residues) of the reference proteins are appropriate neither for the very long helices found in the bacteriophage (40 amino acid residues in length for 90% helix) nor for the long helices of membrane-bound protein (about 20 amino acid residues for a membranes-spanning helix).

Compared to CD and FTIR, a relatively low contribution of α-helix and a large contribution of β-sheet are observed in the Raman spectra of this protein form in DOPG bilayers (Table I). However, in the case of DOPG bilayers a strong band is present at 1659 cm^{-1}, which is close to the amide I band of the α-helix. This band might be influenced by the protein, giving rise to a change in shape and band position, resulting in spectral subtraction errors.

As a result of the high amount (about 90%) of α-helix observed with virtually no β-sheet structure for the α-oligomeric form of the M13 coat protein, this type of coat protein is predominantly in an α-helical conformation, in the transmembrane part as well as in the C- and N-terminal regions. This is in contrast with previous suggestions where the α-helix was proposed to be located in the hydrophilic part of the protein (Chamberlain et al., 1978). Generally, it is accepted that a single transmembrane α-helix with about 20 hydrophobic amino acids will not disturb the lipid bilayer to a large extent. This is in agreement with NMR results obtained from the protein in this α-oligomeric form where only a small perturbation of the phospholipid headgroups and chains was observed (Sanders et al., 1992b).

High-resolution and solid-state NMR experiments of coat proteins in detergent micelles and phospholipid bilayers, respectively, support these conclusions. For M13 coat protein and the coat proteins of the related fd and Pf1 bacteriophages it is found that the backbone of the hydrophobic transmembrane amino acid residues is rigid on the picosecond to microsecond time scale and has a stable helical secondary structure, whereas the N- and C-terminal regions show an increasing mobility toward the ends of the polypeptide chain (Leo et al., 1987; Bogusky et al., 1988, 1990; Schiksnis et al., 1988; O'Neil & Sykes, 1989; Henry & Sykes, 1990a,b,c, 1992; Shon et al., 1991).

The most detailed structure of membrane-bound M13 coat protein follows from recent NMR studies of the protein solubilized in perdeuterated sodium dodecyl sulfate. Henry and Sykes (1992) propose that micellar-bound M13 coat protein consists of two α-helices linked by a short region of uncertain conformation. The longer transmembrane helix extends through much of the hydrophobic section and the basic region of the protein, ending near the C-terminus. This part of the molecule is very stable. The N-terminal helix is suggested to reside outside the micelle and appears to be more structurally labile compared to the helix that extends through the hydrophobic section. The very ends of the polypeptide chain are disordered. This model is supported by the results found here, especially when it is taken into account that the amount of α-helix compared to random coil conformations may be overestimated. Shon et al. (1991) previously proposed a similar model for the 46-residue coat protein of the related phage Pf1 solubilized in dodecylphosphocholine micelles: two helices (residues 6–13 and 19–42) connected by a short loop. Solid-state NMR experiments on Pf1 coat protein reconstituted into phospholipid bilayers were used to determine the orientation of the N-terminal helix, which is found to be parallel to the surface of the membrane bilayer.

For M13 coat protein, the relative orientation of the two helices of M13 coat protein is not known, but it is possible that the N-terminal helix, which has an amphipathic nature, is similarly associated with the membrane surface (Henry & Sykes, 1992). An N-terminal helix parallel to the membrane surface is expected to have a strong steric effect on the aggregation of the transmembrane helix at increasing protein concentrations. Such an orientation of a helical N-terminal region along the membrane would increase the apparent diameter of the membrane-bound coat protein, thereby also increasing the number of annular or boundary phospholipid molecules at the lipid–protein interface and providing a larger action region for interactions. It can be estimated that the reach of a parallel N-terminal domain is about 1.5 nm. This corresponds to about three shells of surrounding phospholipid molecules. This would thus more strongly influence the properties of the phospholipids in the bilayer than in the case of a straight cylinderlike protein molecule.

Spectroscopic experiments carried out on reconstituted lipid–protein systems, however, do not give evidence for such a strong deviation from a cylindrical shape of the M13 coat protein (Sanders et al., 1991, 1992a,b; Hemminga et al., 1992). It may be possible that the orientation of the N-terminal part of the M13 coat protein changes from parallel to perpendicular to the membrane surface, when going from high to low L/P. An extended helix structure could be the conformation prior to assembly, since the coat protein in the intact virus has an extended, slightly bent, helical structure (Marvin, 1990). Such a conformational change could only involve a few amino acids that connect the two helices. This may be difficult to observe with the secondary structure determination methods described in this paper. In conclusion, it can be said that the orientation of the N-terminal part of reconstituted M13 coat protein with respect to the bilayer surface remains to be solved.

Biological Implications. The protein in both membrane-bound forms was previously found to be in a less α-helical state compared to the virus-bound protein. It was therefore suggested that this protein should undergo major conformational changes upon virus formation (Nozaki et al., 1976,

1978), and several questions were raised about the energy implications of this change in conformational state. This suggestion was already criticized by Williams et al. (1980) for M13 coat protein solubilized in sodium dodecyl sulfate micelles. The high α-helical content found for the α-oligomeric form of the M13 coat protein in the present work suggests that this form of M13 coat protein does not have to undergo a large conformational change upon formation of a new phage particle. This makes it also feasible that parental M13 coat protein can be used in the formation of new viruses. In contrast, the β-polymeric form has adopted a strongly changed conformation state compared to the protein in the intact virus. Probably this conformation arises as a result of protein–protein contacts, which are formed under conditions that favor these contacts (Spruijt & Hemminga, 1991).

NMR spectroscopic data from the micelle-bound form of Pf1 coat protein also show it to be predominantly α-helical, almost identical to its structure in the intact virus (Shon et al., 1991). This result is further supported by recent FTIR spectroscopic studies on the coat proteins of the bacteriophages Pf1 and Pf3 (W. F. Wolkers, R. B. Spruijt, P. I. Haris, D. Chapman, and M. A. Hemminga, unpublished data), which show that the protein in the intact virus and its membrane-embedded form have to a large extent an identical secondary structure. These results agree with the conclusion of this present study with M13 coat protein.

These results support the concept that insertion of coat proteins into phospholipid bilayers can take place without a major rearrangement of the polypeptide backbone structure and that the structure adopted by the coat proteins is primarily α-helical with no β-sheet. The presence of β-structure observed in many other studies may be a result of the experimental conditions that favor or induce the possibility of protein aggregation (Spruijt et al., 1989; Spruijt & Hemminga, 1991). The spectra of the β-oligomeric form of M13 coat proteins resemble those of denatured proteins, which also show similar amide I bands that are often assigned to intermolecular β-sheet structure (Jackson et al., 1989). The β-sheet structure observed for M13 coat protein may not be present *in vivo* and hence may not be relevant to an understanding of the mechanism of the viral assembly process.

REFERENCES

Arnold, G. E., Day, L. A., & Dunker, A. K. (1992) *Biochemistry 31*, 7948–7956.

Aslanian, D., Negrerie, M., & Chambert, R. (1986) *Eur. J. Biochem. 160*, 395–400.

Banner, D. W., Nave, C., & Marvin, D. A. (1981) *Nature 289*, 814–816.

Bayer, R., & Feigenson, G. W. (1985) *Biochim. Biophys. Acta 815*, 369–379.

Berjot, M., Marx, J., & Alix, A. J. P. (1987) *J. Raman Spectrosc. 18*, 289–300.

Bogusky, M. J., Schiksnis, R. A., Leo, G. C., & Opella, S. J. (1987) *J. Magn. Reson. 72*, 186–190.

Bogusky, M. J., Leo, G. C., & Opella, S. J. (1988) *Proteins: Struct., Funct., Genet. 4*, 123–130.

Bogusky, M. J., Leighton, P., Schiksnis, R. A., Khoury, A., Lu, P., & Opella, S. J. (1990) *J. Magn. Reson. 86*, 11–29.

Chamberlain, B. K., Nozaki, Y., Tanford, C., & Webster, R. E. (1978) *Biochim. Biophys. Acta 510*, 18–37.

Clack, B. A., & Gray, D. M. (1989) *Biopolymers 28*, 1861–1873.

Datema, K. P., Visser, A. J. W. G., van Hoek, A., Wolfs, C. J. A. M., Spruijt, R. B., & Hemminga, M. A. (1987a) *Biochemistry 26*, 6145–6152.

Datema, K. P., Wolfs, C. J. A. M., Marsh, D., Watts, A., & Hemminga, M. A. (1987b) *Biochemistry 26*, 7571–7574.

De Jongh, H. H. J., Hemminga, M. A., & Marsh, D. (1990) *Biochim. Biophys. Acta 1024*, 82–88.

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.

Fodor, S. P. A., Dunker, A. K., Ng, Y. C., Carsten, D., & Williams, R. W. (1981) *Prog. Clin. Biol. Res. 64*, 441–455.

Gans, P. J., Lyu, P. C., Manning, M. C., Woody, R. W., & Kallenbach, N. R. (1991) *Biopolymers 31*, 1605–1614.

Glucksman, M. J., Bhattacharjee, S., & Makowski, L. (1992) *J. Mol. Biol. 226*, 455–470.

Greenfield, N. J., & Fasman, G. D. (1969) *Biochemistry 8*, 4108–4116.

Hagen, D. S., Weiner, J. H., & Sykes, B. D. (1978) *Biochemistry 17*, 3860–3866.

Haris, P. I., & Chapman, D. (1992) *Trends Biochem. Sci. 31*, 6279–6284.

Haris, P. I., Lee, D. C., & Chapman, D. (1986) *Biochim. Biophys. Acta 874*, 255–265.

Hemminga, M. A., Sanders, J. C., & Spruijt, R. B. (1992) in *Progress in Lipid Research* (Sprecher, H., Ed.) Vol. 31, pp 301–333, Pergamon Press, Oxford.

Henry, G. D., & Sykes, B. D. (1990a) *J. Mol. Biol. 212*, 11–14.

Henry, G. D., & Sykes, B. D. (1990b) *Biochemistry 29*, 6303–6313.

Henry, G. D., & Sykes, B. D. (1990c) *Biochem. Cell Biol. 68*, 318–329.

Henry, G. D., & Sykes, B. D. (1992) *Biochemistry 31*, 5284–5297.

Jackson, M., Haris, P. I., & Chapman, D. (1989) *Biochim. Biophys. Acta 998*, 75–79.

Johnson, I. D., & Hudson, B. S. (1989) *Biochemistry 28*, 6392–6400.

Kimelman, D., Tecoma, E. S., Wolber, P. K., Hudson, B. S., Wickner, W. T., & Simoni, R. D. (1979) *Biochemistry 18*, 5874–5880.

Knippers, R., & Hoffmann-Berling, H. (1966) *J. Mol. Biol. 21*, 281–292.

Lee, D. C., Haris, P. I., Chapman, D., & Mitchell, R. C. (1990) *Biochemistry 29*, 9185–9193.

Leo, G. C., Colnago, L. A., Valentine, K. G., & Opella, S. J. (1987) *Biochemistry 26*, 854–862.

Marvin, D. A. (1990) *Int. J. Biol. Macromol. 12*, 125–138; 335.

Marvin, D. A., & Wachtel, E. J. (1975) *Nature 253*, 19–23.

Marvin, D. A., Pigram, W. J., Wiseman, R. L., Wachtel, E. J., & Marvin, F. J. (1974) *J. Mol. Biol. 88*, 581–598.

Nedelec, J.-F., Alfsen, A., & Lavalle, F. (1989) *Biochimie 71*, 145–151.

Nozaki, Y., Chamberlain, B. K., Webster, R. E., & Tanford, C. (1976) *Nature 259*, 335–337.

Nozaki, Y., Reynolds, J. A., & Tanford, C. (1978) *Biochemistry 17*, 1239–1246.

O'Neil, J. D. J., & Sykes, B. D. (1989) *Biochemistry 28*, 6736–6745.

Peng, K., Visser, A. J. W. G., van Hoek, A., Wolfs, C. J. A. M., & Hemminga, M. A. (1990a) *Eur. Biophys. J. 18*, 285–293.

Peng, K., Visser, A. J. W. G., van Hoek, A., Wolfs, C. J. A. M., Sanders, J. C., & Hemminga, M. A. (1990b) *Eur. Biophys. J. 18*, 277–283.

Provencher, S. W., & Glöckner, J. (1981) *Biochemistry 20*, 33–37.

Sanders, J. C., Poile, T. W., Spruijt, R. B., van Nuland, N. A. J., Watts, A., & Hemminga, M. A. (1991) *Biochim. Biophys. Acta 1066*, 102–108.

Sanders, J. C., Poile, T. W., Wolfs, C. J. A. M., & Hemminga, M. A. (1992a) *Biochim. Biophys. Acta 1110*, 218–224.

Sanders, J. C., Ottaviani, M. F., van Hoek, A., Visser, A. J. W. G., & Hemminga, M. A. (1992b) *Eur. Biophys. J. 20*, 305–311.

Schiksnis, R. A., Bogusky, M. J., & Opella, S. J. (1988) *J. Mol. Biol. 200*, 741–743.

Shon, K. J., Kim, Y. G., Colnago, L. A., & Opella, S. J. (1991) *Science 252*, 1303–304.
Smilowitz, H. (1974) *J. Virol. 13*, 94–99.
Smith, L. M., Rubenstein, J. L. R., Parce, J. W., & McConnell, H. M. (1980) *Biochemistry 19*, 5907–5911.
Spruijt, R. B., & Hemminga, M. A. (1991) *Biochemistry 30*, 11147–11154.
Spruijt, R. B., Wolfs, C. J. A. M., & Hemminga, M. A. (1989) *Biochemistry 28*, 9158–9165.
Surewicz, W. K., & Mantsch, H. H. (1988) *Biochim. Biophys. Acta 952*, 115–130.
Surewicz, W. K., Mantsch, H. H., & Chapman, D. (1993) *Biochemistry 32*, 389–394.
Thomas, G. J., Jr., Prescott, B., & Day, L. A. (1983) *J. Mol. Biol. 165*, 321–356.
Tu, A. T. (1982) *Raman spectroscopy in biology, principles and applications*, John Wiley, New York.
Wallace, B. A., & Mao, D. (1984) *Anal. Biochem. 142*, 317–328.
Wallace, B. A., & Teeters, C. L. (1987) *Biochemistry 26*, 65–70.
Weiner, J. H., Dettman, H. D., Henry, G. D., O'Neil, J. D. J., & Sykes, B. D. (1987) *Biochem. Soc. Trans. 15*, 81–85.
Wickner, W. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 4749–4753.
Wickner, W. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 1159–1163.
Williams, R. W. (1983) *J. Mol. Biol. 166*, 581–603.
Williams, R. W., & Dunker, A. K. (1977) *J. Biol. Chem. 252*, 6253–6255.
Williams, R. W., Dunker, A. K., & Peticolas, W. L. (1980) *Biophys. J. 32*, 232–234.
Williams, R. W., Dunker, A. K., & Peticolas, W. L. (1984) *Biochim. Biophys. Acta 791*, 131–144.
Wolber, P. K., & Hudson, B. S. (1982) *Biophys. J. 37*, 253–262.