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LASER RAMAN STUDIES OF LIPID DISORDERING BY THE B-PROTEIN OF fd PHAGE

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Summary

Complexes of the B-protein of fd phage with the model lipid dipalmitoyl phosphatidylcholine (DPPC) were made by sonication of the fd phage in the presence of dipalmitoyl phosphatidylcholine. Both laser Raman spectra and circular dichroism show the protein in the membrane to be almost entirely in the β-sheet conformation. This β-sheet conformation is found to be independent of the temperature between 10°C and 50°C. On the other hand, the protein has a very dramatic effect on the organization of the lipid bilayer. An aqueous dispersion of 1 : 1 lipid/protein mixture gives a broad conformational transition of DPPC which occurs between 10°C and 30°C. This contrasts markedly with simple aqueous DPPC dispersions which show a sharp transition at 41°C. This appears to be the first reported example of the lowering of the conformational transition of a membrane bilayer by an intrinsic membrane protein.

Introduction

Laser Raman spectroscopy offers unique potential for studying biological membranes because both the protein [1—3] and lipid [4,5] components exhibit conformationally sensitive Raman peaks. In this paper we present the results of a Raman study of the conformational transitions of a lipid-protein

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complex as a function of temperature. The important result is that the lipid conformational transition, which corresponds in the pure lipid to the melting transition at 41°C, is lowered by nearly 20°C.

The major coat protein (B-protein) from the filamentous phage fd can be isolated in pure form in abundance [6], is extraordinarily hydrophobic [7,8], has a known sequence of just 50 amino acids [9], and forms tight associations with purified lipids [10—12]. Furthermore, we [12] and others [11] have shown that the B-protein can assume two distinct conformational states when associated with lipid. In this communication we report the results of studies on lipid in association with B-protein in the c-state conformation [12], which has almost no helical structure.

Materials and Methods

Preparation and purification of fd phage

The fd phage were grown and titered on *E. coli* CGSC No. 4620 kindly provided by Dr. Barbara Bachman (*E. coli* Genetic Stock Center, Department of Human Genetics, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510). After growth on tryptone broth for about 7 h after infection in 20 l New Brunswick fermentors, cells were collected by centrifugation. The phage containing growth medium was made to 3% polyethylene glycol, 0.5 M NaCl. After standing 3—4 days in the cold, the supernatant containing less than 1% of the phage was siphoned off.

After the fd phage was redissolved in 0.02 M Tris-HCl (pH 8.1), it was purified by repeated cycles of centrifugation at 10 000 × *g* for 2 h to remove cells and debris followed by polyethylene glycol phase separation using 0.5 M NaCl, 3% polyethylene glycol and centrifugation at 5000 × *g* for 1 h. The cycles were repeated as necessary (generally 4—6 cycles) to obtain a white phage phase, free of colored material. The last polyethylene glycol phase separation was followed by three cycles of differential centrifugation to remove the residual polyethylene glycol. Finally, the phage was sedimented to equilibrium on KBr density gradients and the KBr was removed by dialysis. The fd phage prepared in this way is pure judging by the Emax/Emin ratios of 1.52—1.62 at pH 7 for several different phage preparations.

Preparation of protein/lipid complexes

To induce protein/lipid association, mixtures of dipalmatoyl phosphatidylcholine (DPPC) and fd phage were sonicated at maximum power at 45—50°C for 5—10 min, in 1-min bursts each followed by a 30-s pause, with the sonication probe immersed directly into the solution. Under comparable conditions, the lipid alone would be mixtures of dispersions and vesicles [13]. Whether the protein alters the relative amounts of vesicles and dispersions obtained by these methods has not yet been determined, but in any case no attempt was made to fractionate the lipid/protein complex. Lipid/protein association was confirmed by sucrose density centrifugation [12]. The protein was found to be in the β-sheet-rich c-state conformation as determined by circular dichroism, which was the method originally used to identify the various conformational states of the B-protein [10,12].

In separate experiments, the phage DNA was found to be separate from the protein-lipid complexes. Since the phage is about 12% DNA [6], its contributions to the Raman spectra are barely detectable.

SDS-urea polyacrylamide gels and CD spectra of sonicated fd coat protein are identical to gels and spectra of coat protein that has not been sonicated, indicating that sonication does not disrupt the protein backbone.

Laser Raman spectroscopy

Raman spectra were obtained with a system consisting of a Spex 1301 double monochromater, a Spectra-Physics model 165 argon ion laser, photon-counting electronics coupled to a Varian 620/i computer, and a sample holder temperature regulated to ±0.2 degrees. The multi-scan spectra were smoothed [14], water was subtracted and (where necessary) baseline was adjusted to correct for slight fluorescence. The difference spectrum was determined by methods described previously [15].

Results and Discussion

Fig. 1A shows a Raman spectrum of the DPPC-B-protein complexes prepared using equal weights of protein and lipid, which corresponds to a lipid/protein molar ratio at about 7 : 1. We are in the process of examining various lipid/protein stoichiometries. Preliminary results at much higher lipid/protein molar ratios (approx. 30/1) give results similar to those that follow. Thus, what we report here is not an artifact of the low lipid/protein ratio. Furthermore, such low ratios make analysis of the protein specific peaks much easier. From previously published Raman spectra of protein [1—3] and lipid [4,5], we have been able to assign all of the major peaks as indicated in Table I. The spectrum of Fig. 1A was taken at 15°C.

Fig. 1B shows a spectrum of the same B-protein-DPPC complex taken at 37°C. Fig. 1C shows the spectrum at 15°C (Fig. 1A) minus the spectrum at 37°C (Fig. 1B). The difference spectrum of Fig. 1C serves to illustrate the spectral changes that occur upon heating.

A characteristic of the different conformational states of the B-protein is the wide variation in amounts of helix and sheet. Thus, these two conformations merit particular attention. In a difference spectrum such as that of Fig. 1C a β-sheet to α-helix conversion would result in a negative peak near 1650 cm^{-1} and a positive peak near 1670 cm^{-1}; the signs of the peaks would be reversed for an α-helix to β-sheet conversion. The absence of such coupled negative and positive peaks in Fig. 1C suggests little or no conversion between α-helix and β-sheet over the 15—37°C range. In other difference spectra covering the 7—50°C range, we have observed no discernable β-sheet to α-helix conversion.

We are currently examining our spectra for evidence of other, more subtle, indications of changes in protein conformation, but to date we have not been able to detect any clear demonstration of protein conformational changes due to temperature variations. Furthermore, we have also studied the conformation of protein prepared by sonication of fd phage in the absence of lipid. We cannot detect any consistent difference in various spectra of that protein as compared to spectra of DPPC-associated protein. Thus, the association of B-

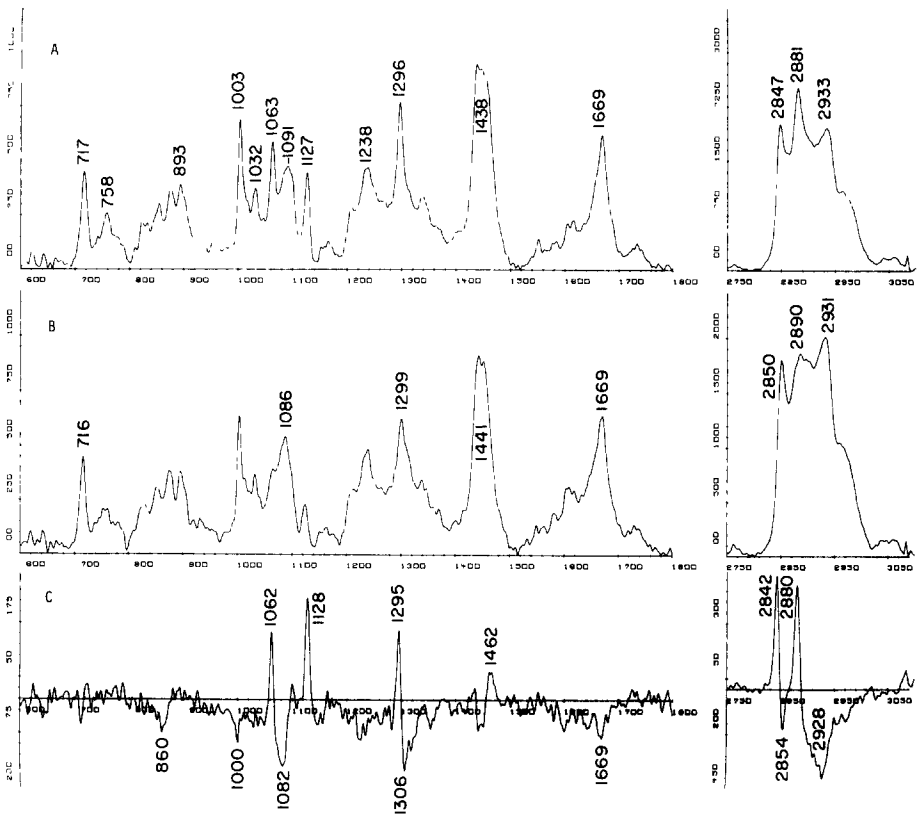

Fig. 1. Laser Raman spectra of B-protein-DPPC complexes. A 1 1 (mass ratio) DPPC/B-protein complex was prepared, placed in a melting point capillary, and sedimented for about 15 min in an I.E.N. Hematocrit centrifuge at full speed. A clearer lower phase and a more turbid upper phase exhibited virtually identical Raman spectra, except for a prominent grading ghost that arose due to the light scatter of the turbid upper phase Thus, the clearer lower phase was used throughout for our studies. A, the Raman spectrum of protein-lipid vesicles at 15°C; B, the Raman spectrum of the same sample at 37°C, C, the difference spectrum of A — B. This particular difference spectrum may be slightly over-subtracted.

protein in the c-state with DPPC does not appear to lead to changes in the protein conformation. The lack of a change in the Raman spectrum upon lipid association agrees with our earlier findings that were based on circular dichroism [12].

In contrast to the protein, the lipid-specific peaks in the Raman spectra show distinct changes due to temperature variation. In particular, a pair of peaks at approx. 1062 cm^{-1} and approx. 1131 cm^{-1}, which are due to hydrocarbon chains in the all-*trans* conformation [5], decrease in intensity as the temperature is raised, whereas a peak at approx. 1100 cm^{-1}, which is due to hydrocarbon chains in the *gauche* conformation [5], increases in intensity as the temperature is raised. The 15°C minus 37°C difference spectrum reveals decreasing intensities as positive peaks and increasing intensities as negative peaks and so the changes just described for the peaks at approx. 1062 cm^{-1}, approx. 1100 cm^{-1}, and 1131 cm^{-1} are clearly evident in Fig. 1C. A difference spectrum between the Raman spectra at 37°C and 50°C showed only a very

TABLE I

PEAK ASSIGNMENTS OF THE LASER RAMAN SPECTRA OF B-PROTEIN-DPPC COMPLEXES

Δcm^{-1}	Assignment	Δcm^{-1}	Assignment
621	Phe	1238	Amide III
644	Tyr	1296	Lipid CH_2 twist
717	Choline, N-CH_3 stretch	1340	Trp
758	O-P-O	1438	Lipid, protein CH_2 bend
830	Tyr	1550, 1583	Trp
850	Tyr	1604	Phe, Tyr
875	Trp, Lipid skeletal	1618	Phe, Tyr, Trp
893	C-C-stretch	1669	Amide I
930, 960	C-C-stretch	2847	Symmetric CH_2 stretch
1003	Phe, β-sheet C-C	2881	Asymmetric CH_2 stretch
1012	Trp	2993	Protein symmetric CH_3 stretch
1032	Phe		
1062	Lipid skeletal (*trans*)		
1091	Lipid skeletal (*gauche*)		
1127	Lipid skeletal (*trans*)		

low-count background with no prominant peaks or valleys, indicating that no detectable conformational change occurs as the temperature is raised from 37°C to 50°C. These results suggest that the lipid may be fully disordered by 37°C or at least that an undetectably small amount of lipid undergoes a conformational change between 37 and 50°C, which spans the normal melting temperature of the lipids used.

From the ratios of these and other peaks, we previously defined S_{trans} and $S_{lateral}$, quantitative measures of lipid order to be used to compare lipid under a variety of conditions [4]. At 15°C the protein-associated lipids exhibit an S_{trans} of 0.63, which is comparable to that of pure DPPC vesicles at 26°C and dispersions at 35°C. At 15°C, the $S_{lateral}$ is about 0.25, a value which falls between those of pure DPPC vesicles and dispersions at 30°C.

The behavior of the B-protein/lipid complexes stands in sharp contrast to the reported behavior of other lipid/protein complexes studied to date [16]. Valinomycin and alamethecin were found to increase the disorder of lipids at temperatures above the lipid phase transition temperature, but showed little effect on lipids below the phase transition temperature. The failure to detect an effect below the phase transition temperature could be due to a lack of protein insertion into the bilayer by the methods used previously.

From the changes in the intensities of the lipid-specific peaks as a function of temperature, lipid melting curves can be constructed. Fig. 2 shows such a lipid melting curve based on the peak height at approx. 1062 cm^{-1}. Using the changes in the *trans* peaks as the criteria for melting, pure dipalmatoyl phosphatidylcholine in dispersions shows a pre-transition temperature of about 34°C and a melting temperature of about 41°C. The latter transition is very narrow, extending from abut 40°C to 42°C [4].

The results of Fig. 2 therefore indicate that the B-protein greatly broadens the conformational transition of the phospholipid and also lowers the midpoint of the transition by 15—20°C. These results confirm the lipid/protein association inferred from the sucrose gradient centrifugation.

Fully deuterated DPPC melts about 6°C lower than does DPPC with protonated carbon atoms [17]. Fig. 3 shows a conformational transition curve obtained with fully deuterated DPPC complexes with B-protein at a lipid/protein mass ratio of 1 : 1. Although the data scatter in Fig. 2 weakens the conclusions somewhat, no difference in melting can be determined. In fact, if the curves of Figs. 2 and 3 are drawn on the same graph, the curve in Fig. 3 is found to lie in the center of the data points of Fig. 2. If there were a 6°C lowering of the conformational transition, one would expect the curve of Fig. 3 to be biased to the lower temperature side. One possible interpretation of such a result is that the protein-protein interactions dominate and thereby determine the conformational transition of the associated lipid.

Previously we found a correlation between thermal transitions measured by differential scanning calorimetry and the conformational transitions determined by laser Raman spectroscopy [4,15]. Such a result is not surprising since there is a positive ΔH associated with the *trans* to *gauche* conformational change; indeed, the ΔH associated with this transition is one of the major factors leading to the absorption of heat as lipids melt [18]. Thus, we feel justified in comparing the transitions we are measuring with thermal transitions

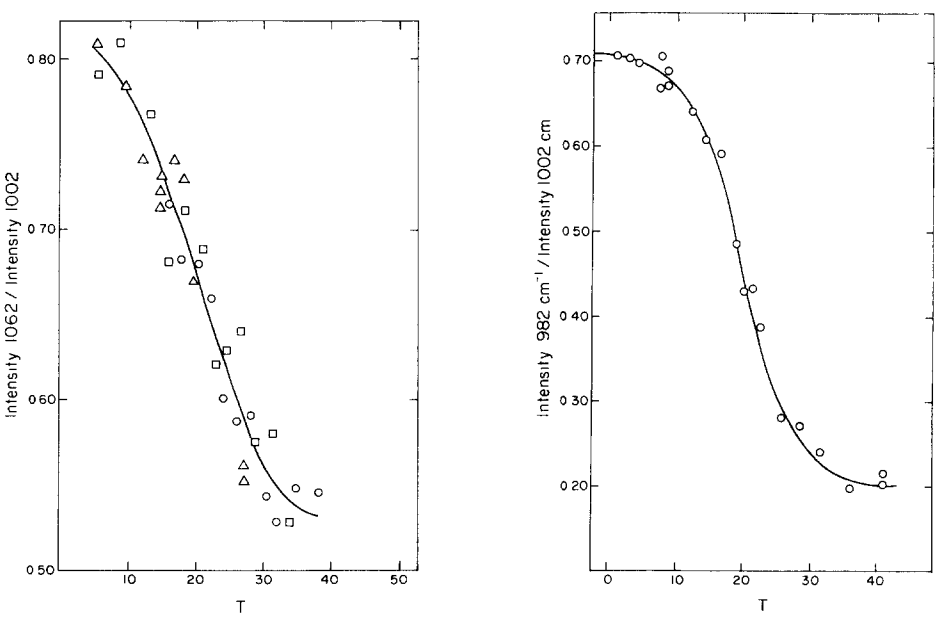

Fig. 2. Melting of fd B-protein-DPPC complexes. A 1 . 1 (mass ratio) lipid/protein complex was heated (○) starting at 15°C, cooled (△), and reheated (□). After 2—3 min equilibration at each temperature, the Raman spectrum over the 950—1200 cm^{-1} region was taken. The phenylalanine peak at 1002 cm^{-1}, which we had previously found to be invariant with temperature, was used as an internal standard. This convenience allows us to scan a very narrow frequency range, thus simplifying the experiment. The peak height near 1062 cm^{-1} divided by the phenylalanine peak height provides the given ratios.

Fig. 3. Melting of fd B-protein-perdeutero-DPPC complexes. A 1 : 1 (mass ratio) lipid/protein complex was heated according to the protocol described in Fig. 1, except that the complexes were initially cooled to 2°C and the 800—1040 cm^{-1} frequency range was scanned. The peak near 982 cm^{-1} is a skeletal optical mode for perdeutero DPPC in the all-*trans* conformation.

measured by calorimetry or with fluidity changes measured by spin label methods. However, we do suggest a note of caution with regard to such comparisons until calorimetry and spin label experiments have actually been performed on our protein-lipid complexes.

The present studies are the first to report a protein-lipid association that significantly lowers the conformational transition temperature of the associated lipids. In previous studies using spin label-electron spin resonance spectroscopy or differential scanning calorimetry, boundary lipids that melt at temperatures higher than bulk lipid have been found [19]. It is particularly interesting to compare our results with those obtained with the proteolipid isolated from brain white matter [20,21]. The proteolipid is very rich in α-helix when dissolved in organic solvents [22] and has been found to stabilize a very large boundary layer of lipid [21]. In contrast, the c-state B-protein is very rich in β-sheet and apparently destabilizes the surrounding lipids. This comparison suggests that hydrophobic β-sheets may destabilize boundary lipids, whereas hydrophobic α-helices may stabilize them. To determine if these are general effects of hydrophobic α-helices and β-sheets will require further study. But in any case, the destabilizing of boundary lipids by specific proteins, whether α-helix or β-sheet, suggests very interesting possibilities for locally altering membrane properties via such specific destabilizing proteins.

Acknowledgments

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