Fourier Transform Infrared Spectroscopic Investigation of the Interaction between Myelin Basic Protein and Dimyristoylphosphatidylglycerol Bilayers†

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ABSTRACT: The interaction of basic protein from human myelin with dimyristoylphosphatidylglycerol (DMPG) bilayers was investigated by Fourier transform infrared spectroscopy. The effect of protein on the lipid conformation and also the effect of lipid on the protein secondary structure were examined. The association of myelin basic protein with DMPG results in a broadening of the lipid phase transition, accompanied by an increase in the conformational order of the acyl chains at temperatures below the phase transition. While the direct contact between myelin basic protein and acidic phospholipids is believed to involve the polar region of the bilayer, infrared spectra indicate that the association between DMPG head groups and protein does not result in drastic changes in the conformation of the lipid phosphate moiety. Infrared spectra of myelin basic protein in the amide I region were analyzed quantitatively by using resolution enhancement and band fitting procedures. The above analysis suggests that in aqueous solution the protein is devoid of α-helical and β-conformations but that it contains a significant amount of turns. Upon binding to DMPG bilayers, the secondary structure of the protein is dramatically altered. The lipid-complexed basic protein adopts a highly ordered secondary structure. According to the quantitative infrared analysis, the main components of this structure are antiparallel β-sheet (53%), α-helix (15%), and turns (15%).

Studies of lipid–protein interactions in reconstituted model systems provide basic information about the molecular organization of biological membranes. Spectroscopic investigations have concentrated predominantly on integral membrane proteins, providing detailed characterization of the effect of hydrophobic surfaces of these proteins on the conformation and mobility of lipid acyl chains [see reviews by Chapman (1982) and Devaux and Seigneuret (1985)]. Much less is known about the molecular mechanism of the interaction between lipid bilayer surfaces and extrinsic proteins. In view of recent reports, this interaction seems to be complex and highly specific, varying considerably from system to system (Boggs et al., 1982b; Sixl et al., 1984; Devaux & Seigneuret, 1985; Devaux et al., 1986).

The extrinsic protein which received much attention in recent years is the basic protein of the central nervous system myelin. This protein is believed to play a major role in the organization of the myelin sheath (Boggs et al., 1982b). The particular interest in the interaction of myelin basic protein (MBP)[1] with lipids is further stimulated by the implications of this interaction in the etiology of demyelinating diseases such as multiple sclerosis. Although the interaction of MBP with phospholipids has been studied by various techniques (Boggs et al., 1982b; Epand, 1986), many aspects of this interaction still remain poorly understood.

In this paper, we report the results of a Fourier transform infrared (FT-IR) spectroscopic investigation of the interaction between myelin basic protein and the acidic phospholipid dimyristoylphosphatidylglycerol (DMPG). The advantage of infrared spectrometry is that, without introducing perturbing probe molecules, it provides a detailed picture of the packing and conformation of lipid molecules (Casal & Mantsch, 1984). In addition to the acyl chain conformation, probed by C–H stretching vibrations, the structure of both interfacial and head-group regions can be examined via the carbonyl ester and phosphate moiety vibrations, respectively. Moreover, with the aid of recently developed band resolution enhancement methods (Mantsch et al., 1986), the conformation of membrane-bound proteins can be studied in detail. While FT-IR spectrometry has been successfully used to explore molecular details of lipid interaction with various integral membrane proteins (Mendelsohn & Mantsch, 1986; Lee & Chapman, 1986), we are aware of only one application of the method to a model system involving an extrinsic protein (Zand & Randall, 1986).

Materials and Methods

Materials. Dimyristoylphosphatidylglycerol was obtained from Avanti Polar Lipids (Birmingham, AL). The lipid was pure by thin-layer chromatographic criteria. The myelin basic protein was extracted from isolated human myelin by the method of Lowden et al. (1966) and was stored in the lyophilized form; the buffer was 60 mM HEPES, pH 7.6, prepared either in D_2O or in H_2O.

Sample Preparation. Lipid suspensions were prepared by adding buffer to powder lipid and dispersing the mixture by vortex shaking at a temperature slightly above that of the lipid phase transition. For preparation of lipid–protein complexes, a protein solution in buffer was added to the lipid suspension so that the appropriate lipid:protein ratio was reached. The mixture was vortexed for approximately 10 min during which time the sample was warmed and cooled repeatedly through the transition temperature. The suspension was then centrifuged at about 10000 rpm in an Eppendorf microcentrifuge,

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[1] Abbreviations: FT-IR, Fourier transform infrared; DMPG, dimyristoylphosphatidylglycerol; MBP, myelin basic protein; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid.

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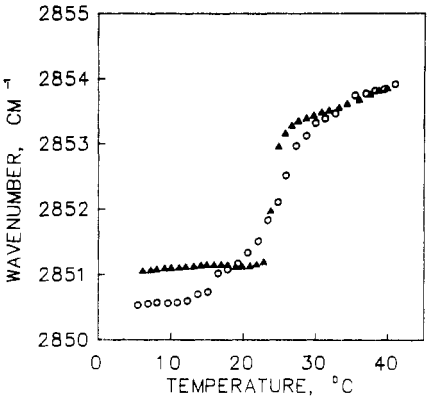

FIGURE 1: Temperature dependence of the position of the CH_2 symmetric stretching band in pure DMPG multibilayers (▲) and DMPG in the presence of 31% (w/w) myelin basic protein (○).

the supernatant was removed, and the lipid–protein complex was resuspended in buffer to give a final lipid concentrations of approximately 100 mg/mL. The ratio of protein to lipid in the DMPG–MBP complex was estimated by analyzing the supernatant for unbound protein (Lowry et al., 1951) and unsedimented lipid (Chen et al., 1956). Although this represents a reasonable estimation, the actual amount of lipid-bound protein in the final preparation may be slightly smaller as a fraction of bound protein may dissociate when fresh buffer is added to resuspend the pellet at 100 mg/mL.

Fourier Transform Infrared Spectroscopy. Samples for FT–IR spectroscopy were examined in a demountable Harrick cell fitted with CaF_2 or BaF_2 windows. the thickness of the spacer was 0.012 mm in the case of lipid–containing samples and 0.050 mm in the case of pure protein samples. Spectra were recorded by using a Digilab FTS–15 instrument equipped with an HgCdTe detector. For each spectrum, between 250 and 1000 interferograms were collected to ensure an adequate signal-to-noise ratio. The interferograms were coadded, apodized with a triangular function, and Fourier transformed to give a resolution of 2 cm^{-1}. Temperature was controlled by the computer, and during data acquisition, it was stable within 0.1 °C. The average heating rate was about 4 °C/h. In order to eliminate spectral contributions of atmospheric water vapor, the instrument was continuously purged with dry nitrogen. Frequencies were determined with an accuracy of ±0.01 cm^{-1} by using a center of gravity algorithm (Cameron et al., 1982). The techniques of spectral deconvolution and derivative spectroscopy were used to resolve overlapping infrared bands (Mantsch et al., 1986; Moffatt et al., 1986). Curve fitting was performed by using standard procedures (Fraser & Suzuki, 1966).

RESULTS

Lipid Structure. The carbon–hydrogen stretching vibrations of acyl chains give rise to a number of well-defined bands in the 3100–2800 cm^{-1} region of the infrared spectrum of lipids. The antisymmetric and symmetric methylene stretching modes appear as strong bands near 2920 and 2850 cm^{-1}, respectively. The sensitivity of these bands to the conformation of acyl chains is well recognized. Frequencies of C–H stretching modes respond in a regular manner to changes in the trans:gauche ratio in lipid acyl chains and can be used as a sensitive index of lipid conformational order (Casal & Mantsch, 1984; Mendelsohn & Mantsch, 1986; Lee & Chapman, 1986). The vibration most suitable for following lipid structural changes in the presence of proteins is the CH_2 symmetric mode near 2850 cm^{-1}. In contrast to the antisymmetric mode, this vibration contains very little, if any, underlying contribution from the protein component.

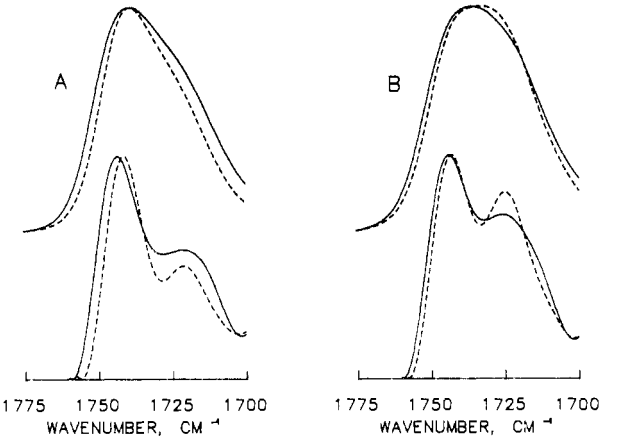

FIGURE 2: Infrared spectra in the region of the C=O bands of pure DMPG multibilayers (- - -) and DMPG complexed with 31% (w/w) myelin basic protein (—). Upper traces represent original spectra, and lower traces represent the same spectra after resolution enhancement by Fourier deconvolution using a 30 cm^{-1} half-width Lorentzian line and a k value of 2.2 [see Mantsch et al. (1986)]. (A) Spectra recorded at 10 °C; (B) spectra recorded at 36 °C.

Figure 1 shows frequency vs. temperature plots of the CH_2 symmetric vibration for DMPG alone and for DMPG in the presence of myelin basic protein. The sharp increase in the CH_2 stretching frequency, observed for pure lipid around 24 °C, reflects the cooperative gel-to-liquid-crystalline transition. In the presence of protein, the transition shows considerable broadening. The conformational melting occurs now in the temperature range between approximately 15 and 30 °C, and there is an indication that it happens in two stages. The temperature profile in Figure 1 was found to be reproducible with different sample preparations. The midpoint of the broad transition, though difficult to determine with high accuracy, does not seem to shift considerably with respect to the phase transition temperature of the pure lipid.

Apart from showing the effect of protein on the phase transition behavior of lipid molecules, FT–IR spectra provide information about the physical state of acyl chains at temperatures beyond the transition region. Data of Figure 1 show that at temperatures below the phase transition, in the presence of basic protein, the frequencies of the CH_2 symmetric stretching vibrations are shifted to lower values. On the other hand, no significant changes in frequency are observed at temperature above the melting region. From this, it may be concluded that the basic protein produces an increase in the conformational order of gel phase DMPG but has no significant effect on the acyl chain conformation in the liquid-crystalline state.

Information about the structure of the interfacial region of the bilayer is provided by vibrational modes of phospholipid ester groups. The ester C=O stretching bands of DMPG in the absence (dashed curves) and in the presence of myelin basic protein (solid curves) are shown in Figure 2. Resolution enhancement of the broad band centered at 1739 cm^{-1} in the gel phase and at 1735 cm^{-1} in the liquid-crystalline phase reveals two spectral features. Splitting of the carbonyl ester band into two components is believed to reflect conformational nonequivalence of the two acyl chains, the higher frequency band being assigned to the carbonyl group of the *sn*-1 chain with a trans conformation in the C–C bond adjacent to the ester group, and the lower frequency band to the carbonyl group of the *sn*-2 chain which has a gauche bend in that position (Bush et al., 1980; Mushayakarara & Levin, 1982).

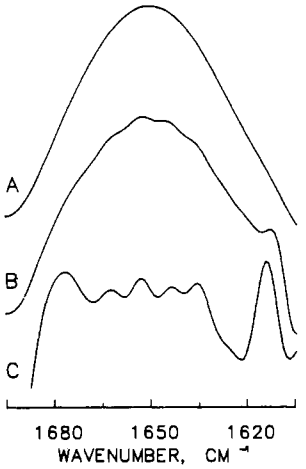

FIGURE 3: Infrared spectra in the amide I region of myelin basic protein associated with DMPG multibilayers. (A) Original absorbance spectrum; (B) same spectrum after resolution enhancement by Fourier deconvolution using a 15 cm^{-1} half-width Lorentzian line and a k value of 2 [see Mantsch et al. (1986)]; (C) same spectrum after resolution enhancement by Fourier derivation using a power of 3 and a breakpoint of 0.27 [see Moffatt et al. (1986)].

The phase transition in pure DMPG is accompanied by a change in the relative intensities of these two bands and by a shift of the position of the two component bands from 1742 and 1721 cm^{-1} in the gel phase to 1744 and 1725 cm^{-1} in the liquid-crystalline phase. Upon complexation with basic protein, the spectral differences between gel and liquid-crystalline states become significantly reduced. Above the transition temperature, the intensity of the lower frequency band is decreased relative to that in the pure lipid. In the gel phase, on the other hand, this band is more intense than that in pure lipid. The above spectral changes suggest that in the presence of MBP the interfacial region of DMPG adopts a conformation which is somewhat different from that of uncomplexed lipid and is less sensitive to temperature.

In order to address questions regarding the nature of the interaction between the positively charged MBP and the negatively charged lipid phosphate groups, we have also investigated the characteristic phosphate bands in DMPG and in the DMPG–MBP complex. Phospholipid head groups give rise to a number of bands between 1300 and 1000 cm^{-1} (Fringeli & Günthard, 1981; Arrondo et al., 1984). The most prominent of these vibrational modes is the PO_2^- antisymmetric stretching band which in DMPG is centered at 1213 cm^{-1}. Upon complexation of DMPG with myelin basic protein, the position of this band remains unaffected, although its width is slightly increased (spectra not shown).

Protein Conformation. The usefulness of infrared spectroscopy to probe the secondary structure of polypeptides and proteins is well recognized (Susi, 1972; Parker, 1983). In aqueous solution, the most useful band for conformational analysis is the amide I band which involves primarily C=O stretching vibrations. The exact frequency of this vibration depends on the particular secondary structure adopted by polypeptide chains, varying between approximately 1620 and 1690 cm^{-1}. The amide I band of proteins is usually broad. It consists of several overlapping components characteristic for specific secondary structures, such as α-helix, β-structure, turns, and nonordered segments. Because of the large intrinsic widths of the individual infrared bands, they cannot be resolved by increased instrumental resolution. In the past, this had severely limited the application of IR spectroscopy to protein conformation analysis. However, recently developed techniques for resolution enhancement, such as Fourier deconvolution (Kauppinen et al., 1981) and derivative spectroscopy (Susi & Byler, 1983; Lee et al., 1985), now provide a computational procedure to resolve overlapping infrared bands, thus permitting the identification of specific components of protein secondary structure.

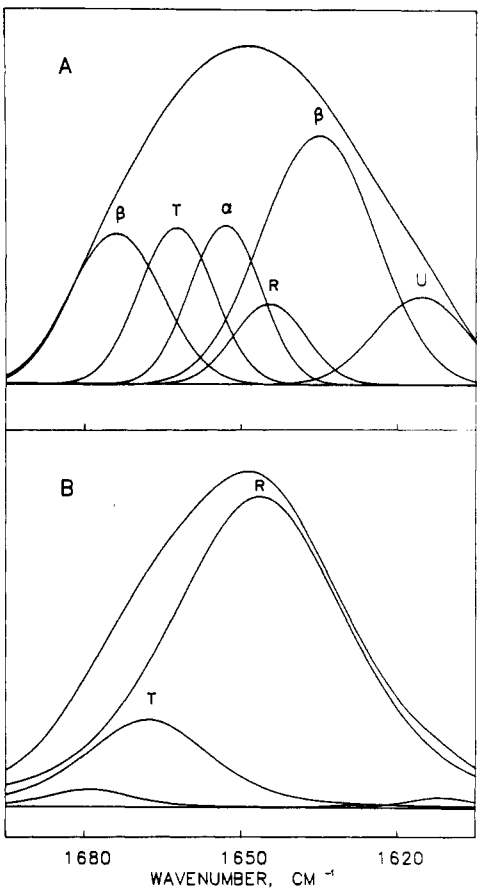

FIGURE 4: Amide I band contour with the best-fitted individual component bands for myelin basic protein complexed with DMPG (A) and for pure myelin basic protein (1% w/w) in buffer (B). The symbols α, β, T, R, and U stand for α-helices, β-structures, turns, random (nonordered) structures, and undefined origin, respectively.

Table I: Positions and Fractional Areas (in Percentages) of the Amide I Bands of Myelin Basic Protein

DMPG-complexed protein	lipid-free protein
1615 (10%)	1612 (1%)
1635 (36%)	1625 (1%)
1645 (7%)	1646 (80%)
1653 (15%)	1668 (16%)
1663 (15%)	1680 (2%)
1675 (17%)	

Figure 3A shows the amide I region of the infrared spectrum of myelin basic protein complexed with DMPG in D_2O buffer. Spectra recorded at 10 and 36 °C (below and above the lipid phase transition, respectively) were practically indistinguishable in this region. The original infrared spectrum of MBP reconstituted with DMPG consists of a single broad and featureless infrared band that extends from 1600 to 1690 cm^{-1}. After Fourier deconvolution of this broad-band contour, six components can be identified in this region (Figure 3B); these components can be seen even more clearly after Fourier derivation, as illustrated in Figure 3C. The frequencies of the component bands obtained after resolution enhancement can be used subsequently as input parameters for curve-fitting analysis of the original spectrum (without knowing approximate band positions, any curve fitting would be meaningless). The result of such a curve-fitting procedure applied to the

spectrum of DMPG-bound myelin basic protein is shown in Figure 4A. The frequencies of the component bands, along with their fractional areas, are given in Table I. The areas derived from curve-fitting analysis reflect relative populations of various conformational states assigned to individual bands.

The strongest feature in the amide I region of lipid-complexed basic protein is the band at 1635 cm^{-1}. The frequency of this band is characteristic for protein segments in the β-conformation (Susi et al., 1967; Susi & Byler, 1983; Byler & Susi, 1986). The band at 1674 cm^{-1} is also most likely associated with the β-structure; in fact, the simultaneous observation of a strong band around 1630 cm^{-1} and a weaker band around 1674 cm^{-1} is characteristic of proteins with antiparallel β-sheet structures (Susi et al., 1967; Byler & Susi, 1986). These two bands account for 53% of the total area of the amide I band, indicating that the major ordered conformation adopted by myelin basic protein after complexation with DMPG is a β-structure. The component bands at 1653 and 1645 cm^{-1} can be assigned to α-helices and unordered segments of protein (Susi et al., 1967; Byler & Susi, 1986), whereas the band at 1663 cm^{-1} is most likely due to some kind of turns (Byler & Susi, 1986). The origin of the minor band at 1614 cm^{-1} is at present not fully clear. In addition to protein backbone vibrations, it may contain unresolved contributions from amino acid side chains (Chirgadze et al., 1974).

The amide I band of MBP in the absence of lipid is markedly different. Compared to the spectrum of lipid-bound protein, the overall band contour of the amide I mode of the free protein shows considerable less intensity at frequencies below approximately 1640 cm^{-1} (cf. upper and lower spectra in Figure 4). Resolution enhancement and curve-fitting analysis identify five individual bands in the 1600–1700 cm^{-1} region of the IR spectrum of lipid-free MBP (Figure 4B and Table I). The major component, at 1646 cm^{-1}, can be unambiguously assigned to polypeptide chains lacking ordered secondary structure (Byler & Susi, 1986). This component accounts for 80% of the total area of the amide I band. Among the four remaining bands, at 1680, 1668, 1625, and 1612 cm^{-1}, only the band at 1668 cm^{-1} is of significant intensity (Table I). Like the 1663 cm^{-1} band in lipid-bound protein, this band can be assigned to turn structures. The other bands are very weak and most likely represent contributions from side chains (1612 cm^{-1}) (Chirgadze et al., 1975), residual β-structure (1625 cm^{-1}), and turns (1680 cm^{-1}) (Bandekar & Krimm, 1980; Byler & Susi, 1986).

Discussion

The effect of myelin basic protein on the phase behavior of synthetic phosphatidylglycerols has been previously studied in great detail by differential scanning calorimetry and electron spin resonance spin-labeling techniques (Boggs et al., 1981, 1982a,b; Boggs & Moscarello, 1982). The present observation of the broadening of the phase transition of DMPG in the presence of MBP is fully consistent with the calorimetric data obtained under similar experimental conditions (Boggs et al., 1982a). The specific novel information provided by the present FT-IR measurements concerns the effect of MBP on the bilayer order in different phase states of phospholipid. This aspect of the interaction has not yet been addressed with the use of membrane nonperturbing techniques. At temperatures below the phase transition, the conformational ordering of DMPG acyl chains is increased upon lipid complexation with basic protein. The observed increase in the amount of trans conformers in the already highly ordered gel-state lipid provides further experimental support for earlier conclusions (Boggs et al., 1981, 1982a) that in the presence of MBP the phosphatidylglycerol bilayers refreeze into a new gel state which is more ordered than that of the pure lipid. On the other hand, the FT-IR data indicate that the ratio of trans to gauche conformers in the liquid-crystalline state DMPG is not perturbed to a significant extent by MBP. The latter observation suggests that the previously reported MBP-induced increase in the rotational correlation time of spin-labeled fatty acids in the liquid-crystalline-stated phosphatidylglycerol (Boggs et al., 1981) may be due to factors other than the increased conformational order of the lipid acyl chains.

According to a recent X-ray diffraction study, there should be no direct contact between lipid acyl chains and myelin basic protein (MacNaughton et al., 1985). The question thus arises what is the mechanism of the observed increase in the conformational order of the acyl chains below (but not above) the lipid phase transition temperature. One possible explanation could be that the increased order of acyl chains simply reflects the immobilization of the whole lipid molecules due to their strong attachment to positively charged regions on the protein surface. It is difficult to imagine, however, how such binding could produce further ordering of lipids in the gel state, leaving unaffected the conformation of acyl chains in the liquid-crystalline state. The present results fully support the model proposed previously by Boggs and co-workers (Boggs et al., 1981; Boggs & Moscarello, 1982) to explain differential scanning calorimetry and electron spin resonance data. According to this model, basic protein induces interdigitation of phosphatidylglycerol acyl chains in the gel phase. The currently observed increase in the ratio of trans to gauche conformers at temperatures below the lipid phase transition appears as an expected consequence of such an interdigitation of fatty acid chains. The interdigitation of acyl chains in the gel phase could arise from the increased head-group separation due to the intercalation of protein fragments into the polar region of the bilayer (Boggs & Moscarello, 1982; Simon & McIntosh, 1984). Upon transition to the liquid-crystalline phase, the increased separation between head groups would be compensated by the increased volume occupied by fluid hydrocarbon chains. This could result in deinterdigitation of protein-containing bilayers and in restoration of acyl chain conformation similar to that found in the pure lipid.

Previous NMR studies indicate that MBP produces a perturbation in the mobility of DMPG head groups (Sixl et al., 1984). The usefulness of IR spectroscopy to explore this aspect of the interaction is limited as the position of bands due to phosphate group vibrations is not very sensitive to subtle changes in the mobility of these groups. However, the increased band width of the PO_2^- stretching vibration upon complexation with protein is compatible with an increased rate and/or amplitude of motion of the phosphate moiety in the liquid-crystalline phase (Sixl et al., 1984). On the other hand, the lack of change in the position of the phosphate bands upon lipid binding to basic protein suggests that the interaction between DMPG head groups and protein neither results in the formation of a strong complex in which the electronic environment of phosphate groups is altered nor produces a significant dehydration of the head group. Both effects would lead to shifts and/or splitting of the phosphate bands, as was observed upon complexation of phosphatidylserine with calcium (Dluhy et al., 1983). Also, the bands due to carbonyl ester vibrations are relatively little affected by the protein. Although the conformation of the interfacial region of the protein-complexed lipid seems to be slightly different from that of pure lipid, the spectra argue against any strong direct interaction between the protein and C=O groups [cf., for ex-

ample, Dluhy et al. (1983) and Mendelsohn et al. (1984)]. The complex between DMPG head groups and myelin basic protein seems thus to be relatively loose, the interaction being mostly of a polar nature with no strong bonding between specific chemical groups. This may explain the rapid exchange of DMPG molecules between the bulk and protein-associated phases (Sixl et al., 1984).

The conformation of myelin basic protein in solution has been studied extensively by using techniques such as circular dichroism, X-ray diffraction, ultracentrifugation, and nuclear magnetic resonance (Epand et al., 1974; Liebes et al., 1975; Krigbaum & Hsu, 1975; Deber et al., 1978; Martenson, 1978; Mendz et al., 1982). The picture emerging from numerous studies is, however, not totally coherent. Most of the data indicate that in aqueous solution the basic protein is largely unfolded and devoid of both α-helical and β-structures. Other experiments suggest, however, that its structure is not totally random. This is indicated, among others, by the sensitivity of MBP to denaturants such as guanidinium chloride (Epand et al., 1974; Randall & Zand, 1986). The interpretation of experimental results may be further complicated by the ability of MBP to self-associate in aqueous solutions. Recently, Smith (1985) argued that while the monomeric protein in dilute solutions has a random structure, its self-associated hexameric form adopts a more ordered conformation. Circular dichroism spectra of the hexameric form have been found to be compatible with a β-pleated sheet conformation, although, as pointed out by the author, these spectra do not fully match those of model proteins of known β-structure. The results of our FT-IR experiments are consistent with a picture of largely unfolded structure of myelin basic protein. Besides a main band at 1646 cm^{-1} (due to polypeptide chains lacking specific ordered secondary structure), the only significant contribution to the amide I region of pure myelin basic protein comes from vibrations around 1668 cm^{-1}. Our assignment of the latter to some type of turns seems to be consistent with the results of NMR studies (Mendz et al., 1982) that have identified reverse turns in MBP. Infrared spectra of MBP in aqueous solution, at least at the resolution achieved in this study, do not indicate a significant amount of β-structure. This, however, does not necessarily imply a discrepancy between our result and those of Smith (1985) but rather may reflect different experimental conditions. Despite the high concentration of myelin basic protein used in the IR measurements, the actual aggregation state of the protein under current experimental conditions is uncertain. It is possible that the lower ionic strength of the buffer used in our experiments (60 mM HEPES vs. 100 mM phosphate plus 200 mM NaCl used by Smith) may result in a smaller tendency of the cationic protein to self-associate than that observed in the study of Smith.

It should be noted that in an earlier FT-IR study of myelin basic protein several sharp peaks were identified in the amide I region, and specific substructures were assigned to these peaks (Randall & Zand, 1985). These sharp features, however, could be due to noise and/or atmospheric water vapor (Lee et al., 1985). In our experience, the amide I band contour of lipid-free and of lipid-complexed MBP is always featureless, and it is only after resolution enhancement that indivdual features become visible. Infrared bands arising from C=O stretching vibrations are generally quite broad, and any sharp features in the amide I region should be treated with great caution; they can be removed by increasing the signal-to-noise ratio and by subtracting the sharp bands characteristic of atmospheric water vapor.

In contrast to extensive studies of myelin basic protein structure in solution, much less attention has been given to the conformation of the protein in a membrane environment. This is probably because of experimental difficulties encountered in spectroscopic studies of membrane-bound proteins. In particular, the high turbidity arising upon binding of MBP (and many other extrinsic proteins) to acidic phospholipids severely limits the applicability of conventional optical techniques, such as circular dichroism or fluorescence spectroscopy. Results of previous circular dichroism experiments indicate that upon binding to acidic phospholipids the myelin basic protein adopts a partially ordered structure (Keniry & Smith, 1980). Quantitative analysis of circular dichroism spectra of MBP in the presence of various anionic phospholipids gave 20–27% α-helical structure, 10–12% β-structure, and about 50% random structure. The present infrared data suggest that the degree by which lipid binding affects protein secondary structure might be considerably larger. According to our curve-fitting analysis, the contribution from the band due to unordered protein segments drops from 80% in solution to only 7% in the presence of DMPG. Moreover, the IR spectroscopic data suggest that the main component of the secondary structure adopted by MBP upon binding to phosphatidylglycerol is not an α-helix, but a β-structure. Of particular relevance to this observation may be the recent report of Riccio et al. (1985), who isolated myelin basic protein using nonionic detergents. MBP purified by this novel procedure contains large amounts of bound lipid and, as indicated by preliminary circular dichroism experiments, exhibits a significant amount of β-structure, though no quantitative data are available.

Theoretical models of myelin basic protein folding in aqueous solution (Martenson, 1981; Stoner, 1984) have identified polypeptide segments with a potential for β-strand formation. Although the detailed analysis of infrared spectra does not support the presence of a significant amount of β-structure in the aqueous form of MBP, this analysis indicates that the propensity to fold into β-strands may be realized upon binding of the protein to the phospholipid bilayer. The question remains, of course, whether the conformation of MBP in its complex natural environment in myelin is similar to the highly ordered structure found in a simple model membrane system. If so, this may be of nontrivial biochemical consequences.

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