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Circular Dichroism and Fluorescence Studies on a Cation Channel Forming Plasma Membrane Proteolipid†

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ABSTRACT: The structure of a plasma membrane proteolipid complex that forms a voltage-dependent cation channel was probed by using circular dichroism and fluorescence in water, in liposomes, and in organic solvents. The protein in water exhibited one peak of tryptophan emission at 335 nm, suggesting that in water this amino acid is restricted to a hydrophobic environment. In chloroform–methanol and in liposomes, the spectrum had emission maxima at 345 nm as well as at 335 nm. These data suggest that in chloroform–methanol or after introduction into liposomes the protein undergoes a conformational shift from the water-soluble form; some or all of the tryptophan residues (or a population of tryptophan residues of the proteolipid) appear to be shifted to a more hydrophilic environment. Analysis of the extrinsic fluorescence obtained through energy transfer to 8-anilino-1-naphthalenesulfonate suggests that the proteolipid complex exists in different states of oligomerization in organic solvents and in water. Circular dichroism studies of the water-soluble form indicated that the protein is 73% α helix, 14% β structure, and 12% random coil, indicative of the presence of a large hydrophobic interior. Addition of sodium dodecyl sulfate to the proteolipid in water did not greatly reduce the percent α helix and only slightly decreased the contribution of β structure, suggesting that sodium dodecyl sulfate has little access to the helix-forming hydrophobic interior. Trifluoroethanol induced a predictable increase in the helical content. Most significant was the change in secondary structure after introduction of the protein into phosphatidylcholine–cholesterol liposomes; a small reduction in helical content was accompanied by an almost total elimination of β structure with a concomitant increase to 35% of the contribution by the random structure.

Proteolipids are a class of hydrophobic membrane proteins characterized by their solubility in chloroform–methanol (Lees et al., 1979; Schlesinger, 1981). The delipidated apoproteins retain their solubility in chloroform–methanol but can be converted to water-soluble forms by several methods (Lees & Sakura, 1978; Cockle et al., 1978a). Proteolipid proteins were first described in central nervous system myelin (Folch & Lees, 1951) where the proteolipid accounts for 50% of the total protein; since then, different proteolipids have been isolated from mitochodria (Beechey et al., 1975; Enna & Criddle, 1977; Sebald & Wachter, 1978), sarcoplasmic reticulum (Maclennan et al., 1973; Eytan & Racker, 1977), and plasma membranes (Tosteson & Sapirstein, 1981). Although the proteolipids from these different membranes are distinct, they do share certain characteristics in addition to their solubility properties. The myelin (Ting-Beall et al., 1979), mitochondrial (Miller & Racker, 1976), sarcoplasmic reticulum (Racker & Eytan, 1975), and plasma membrane proteolipids (Tosteson & Sa-

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pirstein, 1981) have all been implicated in ion channel formation. Evidence has been presented suggesting that for the myelin and plasma membrane proteolipids oligomerization may be involved in the ion channel formation by these proteins. Oligomers of the mitochondrial proteolipid from the oligomycin-sensitive ATPase have been shown to exist (Tzagaloff et al., 1976), and we have postulated (Lees et al., 1979) that monomer–oligomer transitions may play an important role in the ionophoric functions of this protein.

The plasma membrane proteolipid complex consists of two subunits, α and β, with molecular weights of 11 500 and 13 200, respectively. Analysis of the voltage-dependent channels formed by this protein complex indicates they are cation specific, K^+ selective ($K^+ > Na^+$) and that both subunits must recombine as oligomers to form a functional unit. At least three monomers are required for channel formation. Because both α and β subunits are required for activity, the functional unit may represent either a trimer or a hexamer consisting of trimers of recombinants. A related feature of these proteins is that depending on the method of preparation the proteins migrate either as monomers or as monomers, dimers, and trimers on sodium dodecyl sulfate gels. Studies from other laboratories have suggested that similar proteolipids are subunits of (Na^+, K^+)-ATPase (Forbusch et al., 1978; Reeves et al., 1980). Although a channel-forming role for the plasma membrane proteolipids in this important cation pump would be intriguing, the possibility still exists that these proteins copurify with the ATPase and are not truly subunits.

Spectroscopic studies of proteolipid proteins have been confined to the myelin proteolipid. This protein exhibits a conformational flexibility that depends upon the means of preparation as well as the solvent environment (Cockle et al., 1978b; Sherman & Folch-Pi, 1970). Early studies suggested 16% helix for the water-soluble form with 60–75% α helix in organic solvents. More recent studies obtained a higher degree of α helix for the aqueous forms with values ranging from 53 to 74% depending on the method of preparation (Cockle et al., 1978b). Intrinsic fluorescence studies of the tryptophan moieties indicate that these residues are buried in a hydrophobic environment with an emission spectrum consisting of one peak at <340 nm (Feinstein & Felsenfeld, 1975; Cockle et al., 1978b; DeForesta et al., 1979).

In the present study on the plasma membrane proteolipid, we have utilized circular dichroism and fluorescence to probe the structure of the plasma membrane proteolipid in water, in organic solvents, and in liposomes. The data reveal a protein complex with significant conformational flexibility as well as considerable stability under certain denaturing conditions.

Materials and Methods

Membrane Preparation. Bovine kidney was dissected free of fatty and connective tissue and cut into 0.5 cm^3 pieces. The tissue was homogenized in 3 volumes of 0.25 M sucrose by a two-step procedure: first, the tissue was disrupted in a Waring blender by using 2 × 30 s bursts. The material was passed through two layers of gauze and further homogenized with three strokes of a motor-driven Teflon–glass homogenizer. The homogenate was initially centrifuged at 10000*g* for 15 min in a Sorvall RC2B refrigerated centrifuge. The supernatant was used for crude plasma membrane and microsomal fractions. The supernatant (S1) was centrifuged at 15000*g* for 15 min and the supernatant (S2) removed with gentle swirling to disperse the large plasma membrane fragments sedimenting on top of the mitochondria. S2 was centrifuged at 25000*g* for 30 min. The resultant pellet (P2) was used for crude plasma membrane isolation. P2 was partially resuspended in 0.32 M sucrose with care taken not to disturb the dense brown mitochondrial pellet while removing the pinkish overlying plasma membrane fraction. The plasma membrane were homogenized in a glass–Teflon homogenizer, and the centrifugation resuspension process was repeated until a membrane fraction was obtained which was free of the denser mitochondrial pellet. By repeating this centrifugation–resuspension procedure, we obtained a membrane preparation which is free of the dense brown mitochondrial pellet.

Extraction and Chromatography of Proteolipids. Membranes were extracted with 15 volumes of chloroform–methanol (2:1 v/v). The extract was filtered and partitioned with 0.2 volume of H_2O, and the phases were separated by centrifugation. The lower phase was placed at 4 °C for several hours in order to exclude more H_2O from the lower phase. These steps to remove excess H_2O were necessary to ensure stability of the protein during concentration. The extract was concentrated first in a rotary evaporator and then under N_2.

LH-60 (Pharmacia Fine Chemicals) chromatography was performed on an 80 × 2 cm column equilibrated with chloroform–methanol–acetic acid (2:1:0.03 v/v/v). The sample was also acidified with 0.01 N HCl in order to dissociate the protein from acidic lipids. All columns were monitored at 280 nm with a UV 1 monitor (Pharmacia Fine Chemicals).

Proteolipid apoproteins, free of complex lipids (<0.05% lipid phosphorus) as determined by the method of Bartlett (1959), were converted to the water-soluble form as described by Lees & Sakura (1978). The protein either was directly converted to the water-soluble form or was rechromatographed on a neutral LH-60 column in order to remove the acetic acid. In the cases where the protein was directly converted to the water-soluble form, the acid was removed by dialysis. Our preliminary results indicated that these two methods of preparation gave proteins with identical spectroscopic properties. In experiments in which a direct comparison of the water-soluble and chloroform–methanol-soluble forms was made, the aqueous form was prepared from neutral organic solvents. In experiments examining the protein complex in trifluoroethanol (TFE), the water-soluble form was diluted with trifluoroethanol.

Liposomes were formed with soybean phosphatidylcholine (PC) and cholesterol (Sigma Chemical Co.; 4:1 w/w). The lipids were dried from chloroform–methanol (2:1 v/v) under nitrogen and resuspended by sonication in an aqueous solution of the protein. The final lipid concentration was 1.2 mg/mL, and the protein concentration was 0.12 mg/mL. High-speed centrifugation of the liposomes indicated that >90% of the protein had been incorporated.

Analytical Techniques. Amino acid analysis was performed on a Beckman 119C amino acid analyzer after hydrolysis for 24 h in 6 N HCl. The extinction coefficient of the protein ($E_{1\%}$ = 1.25) was determined by comparing ultraviolet scans, performed on a Pye Unicam scanning spectrophotometer, with the amino acid analyses by using a value of 70% recovery from the Beckman 119C amino acid analyzer. Protein assays were routinely done by the procedure of Lowry et al. (1951) as modified for proteolipids by Lees & Paxman (1972) with bovine serum albumin as standard; comparison of Lowry results with ultraviolet scans gave a factor of 1.3 for conversion of the Lowry data to the proteolipid concentration. All chemicals were reagent grade or better and were purchased through Sigma Chemical Co., (St. Louis, MO) or Fisher Chemical Co. (Pittsburgh, PA).

Circular dichroism (CD) spectra were obtained on a Cary 60 spectropolarimeter equipped with a 6001 circular dichroism

Table I: Amino Acid Composition of Proteolipid Subunits α and β from Canine Kidney Plasma Membrane

amino acid	mol % ± SD[c]	residues/ mol[a]	mol % ± SD[c]	residues/ mol[b]
Asp	6.7 ± 0.4	8.0	6.4 ± 0.4	7.0
Thr	5.7 ± 0.2	7.0	5.5 ± 0.6	6.0
Ser	7.6 ± 0.5	9.3	8.0 ± 0.5	8.8
Glu	7.4 ± 0.2	9.0	6.9 ± 0.5	7.6
Pro	5.0 ± 1.0	6.0	5.0 ± 0.5	5.4
Gly	9.1 ± 0.5	11.1	9.8 ± 1.0	10.7
Ala	10.3 ± 0.9	12.5	13.2 ± 0.4	14.3
Val	6.5 ± 0.7	7.9	7.0 ± 0.2	7.2
Met	3.2 ± 0.3	3.8	2.4 ± 0.5	2.5
Ile	6.0 ± 0.7	7.2	4.8 ± 1.2	5.2
Leu	13.3 ± 0.7	16.0	12.0 ± 0.2	13.0
Tyr	3.6 ± 0.1	4.4	3.2 ± 0.1	3.5
Phe	6.4 ± 0.4	7.6	5.9 ± 0.2	6.4
His	2.3 ± 0.2	2.7	3.0 ± 1.0	3.2
Lys	4.1 ± 0.3	5.0	4.6 ± 0.5	5.2
Arg	3.2 ± 0.4	3.8	2.7 ± 0.2	2.9

[a] Based on the mean mole percent and a molecular weight of 13 200. [b] Based on the mean mole percent and a molecular weight of 11 800. [c] The mole percent totals 100.2% for α and 100.6% for β due to rounding off one decimal place. These values reflect the mole percent excluding tryptophan which was not determined. Values are the average of three determinations.

attachment. Protein concentrations were typically 0.3–0.5 mg/mL except in liposomes when the protein concentration was 0.12 mg/mL; cell path lengths of 0.2–1.0 mm were employed. All spectra were run at least 2 times on two different proteolipid preparations. Intensities are reported as mean residue ellipticities (degrees per square centimeter per decimeter) by using 115 as the average molecular weight for a residue. Data were analyzed in terms of contributory structures by the method of Chen et al. (1974). The observed spectra were fit by using an unconstrained linear least-squares routine, based on IMSL library subroutine IFLSQ. The percentages of α helix and β and random structures were the coefficients of those respective basis functions.

All fluorescence spectra were obtained on a Perkin-Elmer MPF-2A spectrofluorometer equipped with a thermostated cell holder. Excitation and emission slit widths were held constant at 6 mm for every spectrum. Typical protein concentrations were 0.05 mg/mL in water and 0.12 mg/mL in liposomes and in chloroform–methanol. Recrystallized 8-anilino-1-naphthalenesulfonate (Eastman Kodak, Rochester, NY) was dissolved in H_2O at stock concentrations of 10^{-2} and 10^{-3} M. Aliquots were added directly to cuvettes containing solutions of the proteolipid. Increasing concentrations of 8-anilino-1-naphthalenesulfonate represent serial additions.

Results

Protein Characterization. The proteolipids extracted from the plasma membrane were delipidated on Sephadex LH-60 and then characterized by electrophoresis; as was previously reported by Tosteson & Sapirstein (1981), this fraction consisted of two subunits with molecular weights of approximately 11 500 and 13 200. The amino acid compositions of the apoproteins are tabulated in Table I. Both subunits contain a high percentage of hydrophobic amino acids, with glycine, alanine, leucine, isoleucine, and phenylalanine comprising 50% of the residues. The difference in molecular weight between these species can in part be accounted for by an increase, based on residues per mole, of hydrophobic residues in the α subunit. However, the smaller β subunit has more alanine per mole, suggesting that more significant differences may exist. The requirement for both subunits in the formation of ion channels

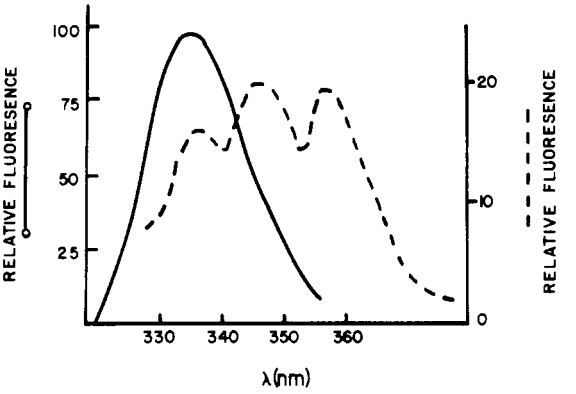

FIGURE 1: Fluorescence spectra of plasma membrane proteolipid in water and in trifluoroethanol. Excitation in water (—) was at 280 nm with a protein concentration of 0.05 mg/mL. The spectrum was obtained as described under Materials and Methods. The peak fluorescence was arbitrarily taken as 100%. In trifluoroethanol (---), the excitation was at 295 nm with a protein concentration of 0.05 mg/mL. Note the peak relative fluorescence is only 20%.

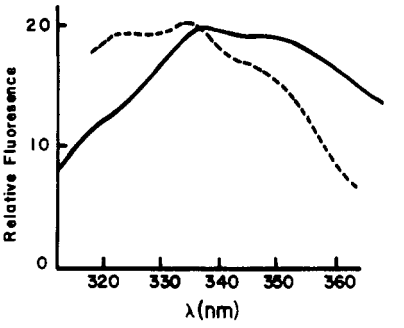

FIGURE 2: Fluorescence spectra of plasma membrane proteolipid in chloroform–methanol and in liposomes. The excitation was at 295 nm with a protein concentration of 0.12 mg/mL in both chloroform–methanol (—) and liposomes (---). Note the peak relative fluorescence is <20%.

supports this contention. Since the active form of the proteolipid requires both subunits, the structural studies carried out here were on the unfractionated species.

Intrinsic Fluorescence. The excitation spectrum of the proteolipid in water exhibited a maxima at 280 nm, and this wavelength was used in subsequent studies carried out in water. In organic solvents and in liposomes, the excitation maximum was 295 nm. The emission spectrum in water (Figure 1) consisted of a single peak with a maximum at 335 nm and no detectable maxima at higher wavelengths. The spectrum in trifluoroethanol (Figure 1) clearly shows the presence of maxima at 345 and 355 nm as well as at 335 nm. Incorporation of the proteolipid into lecithin–cholesterol liposomes yielded emission spectra similar to those observed in chloroform–methanol with tryptophan emissions at 345 nm and approximately 355 nm (Figure 2). The relative tryptophan fluorescence intensity in liposomes and in chloroform–methanol was aproximately one-fifth that observed in water. The data suggest that the protein undergoes a conformational change which resembles the changes induced by organic solvents when incorporated into lipid. These structural alterations appear to include a shift to a more polar environment of a least one population of tryptophans in chloroform–methanol and liposomes and two populations in trifluoroethanol, hence the longer wavelength emission.

Extrinsic Fluorescence. Addition of 8-anilino-1-naphthalenesulfonate to the protein in water substantially quenched the tryptophan emission at 335 nm; the associated energy transfer yielded an 8-anilino-1-naphthalenesulfonate

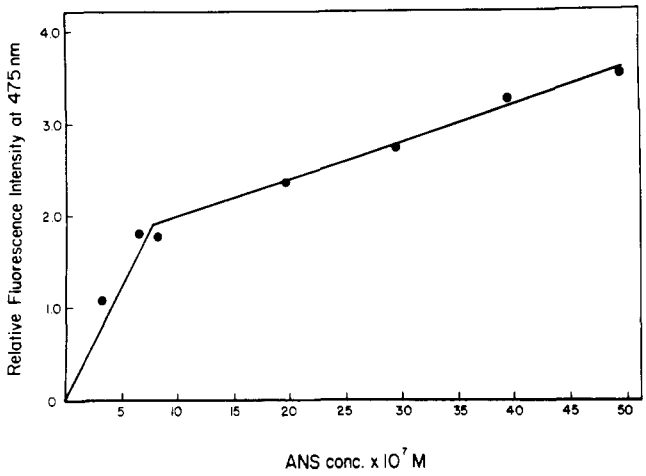

FIGURE 3: Titration of plasma membrane proteolipid with 8-anilino-1-naphthalenesulfonate in water. The titration of the protein with 8-anilino-1-naphthalenesulfonate was followed by excitation at 280 nm and determination of the emission at 475 nm. The molar concentration of the protein was 4.8 μM calculated on the basis of 12 300 as the average molecular weight of the two subunits; the 8-anilino-1-naphthalenesulfonate:protein ratio at saturation was based on the protein concentration and the inflection point on the graph which was estimated to correspond to an 8-anilino-1-naphthalenesulfonate concentration of 7.7×10^{-7} M.

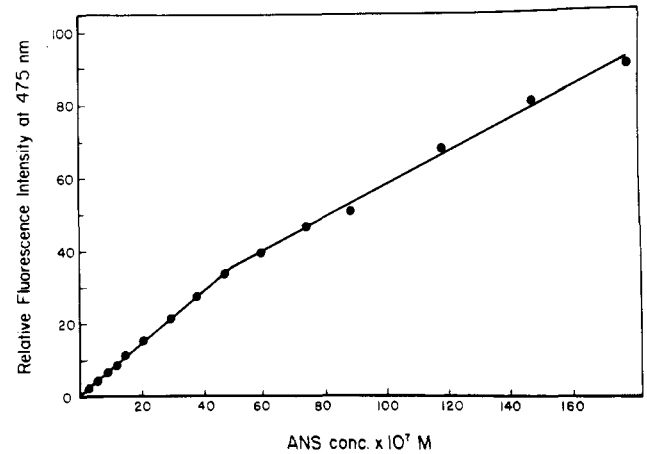

FIGURE 4: Titration of plasma membrane proteolipid with 8-anilino-1-naphthalenesulfonate in chloroform–methanol. The titration was carried out as described in Figure 2. The molar concentration of the protein was 8 μM. The 8-anilino-1-naphthalenesulfonate:protein ratio at saturation was based on the protein concentration and the inflection point on the graph which was estimated to correspond to an 8-anilino-1-naphthalenesulfonate concentration of 4.6×10^{-6} M. Linear regression analysis indicates that each line segment in this figure has a correlation coefficient of >0.995 with the slope in the low concentration region equal to 0.72 and that in the higher concentration region equal to 0.42.

emission at 475 nm. Titration of the protein in H_2O with 8-anilino-1-naphthalenesulfonate was monitored by exciting at 280 nm and determining the fluorescence at 475 nm; the results suggest at least two populations of 8-anilino-1-naphthalenesulfonate binding sites (Figure 3). Similar studies in chloroform–methanol with an excitation of 295 nm also suggested the existence of at least two populations, with a substantially altered end point (Figure 4). These different end points suggest that the proteolipid may exist in different states of oligomerization, but any quantitative interpretation must await a detailed study of the 8-anilino-1-naphthalenesulfonate binding to the proteolipid.

Another possible explanation of these data is that the number of tryptophans quenched per mole of 8-anilino-1-naphthalenesulfonate is different in H_2O and in chloroform–methanol and not a result of different states of oligomerization of these solvents. However, the low residual tryptophan fluorescence at the respective inflective points (data not shown) in both solvent systems argues against this alternative explanation.

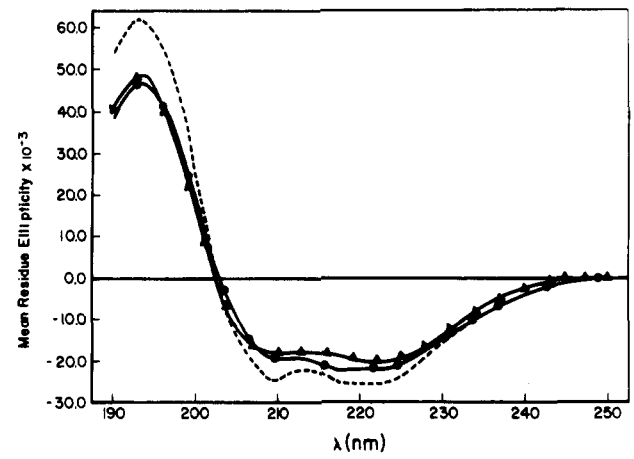

FIGURE 5: Circular dichroism spectra of the plasma membrane proteolipid in water, in 20% trifluoroethanol, and in 2% sodium dodecyl sulfate. The circular dichroism spectra were obtained as described under Materials and Methods. (●) Spectrum in water; (---) spectrum in 20% TFE; (▲) spectrum in 2% sodium dodecyl sulfate. The pH of the sodium dodecyl sulfate solution was 7.15.

Table II: Effects of Solvents on Secondary Structure of Plasma Membrane Proteolipid[a]

solvent	helix	β structure	random coil
H_2O	73	14	12
PC and cholesterol liposomes	62	2	35
H_2O and TFE	80	15	4
sodium dodecyl sulfate	68	22	10

[a] Average of two determinations.

Circular Dichroism. The circular dichroism spectra of the proteolipid complex were determined in H_2O, 0.1 M acetic acid, H_2O–trifluoroethanol, and liposomes. The observed spectrum in H_2O is illustrated in Figure 5 along with the observed spectrum obtained in H_2O–trifluoroethanol. The spectra in H_2O are consistent with a structure which is largely α helix. Utilizing the method of Chen et al. (1974), we calculate (Table II) 73% α helix with 14% β structure and 12% random coil. Addition of acetic acid to the proteolipid preparation at a final concentration of 0.1 M gave an identical spectrum (data not shown), suggesting that low pH has little or no effect on the secondary structure of this protein. However, trifluoroethanol at a final concentration of 20% (v/v) did exert structural effects (Figure 5 and Table II). Addition of trifluoroethanol produced an increase in the negative ellipticity at 225 and 208 nm and in the positive ellipticity at 193 nm. These changes are consistent with an increased order within the proteolipid as is reflected in the increase in helix and concomitant decreases in random coil (Table II) and are typical of the effect of fluorinated alcohols on the circular dichroism spectra of proteins in general.

The circular dichroism spectra of the plasma membrane proteolipid in both H_2O and H_2O containing 2% sodium dodecyl sulfate are illustrated in Figure 5. It appears that addition of sodium dodecyl sulfate to an aqueous preparation of the proteolipid had little effect on the secondary structure of this protein complex; a small decrease in α helix and random

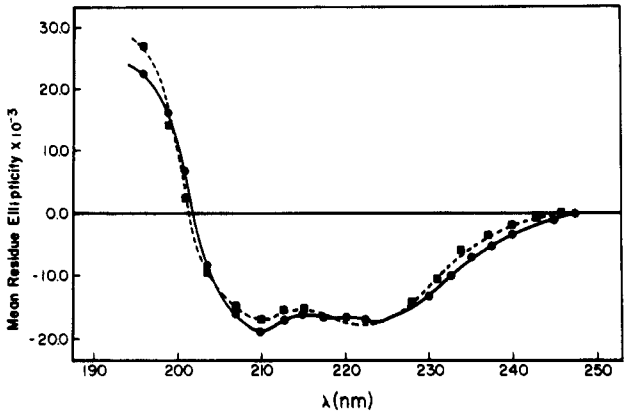

FIGURE 6: Observed and calculated circular dichroism spectra of the plasma membrane proteolipid in liposomes. The observed spectrum (■) was obtained, and the calculated spectrum (●) was determined as described under Materials and Methods.

coil was calculated along with a small increase in β structure (Table II). The introduction of the protein into phosphatidylcholine–cholesterol liposomes, however, was associated with a significant change in secondary structure. The observed and calculated spectra are illustrated in Figure 6. Recombination of the proteolipid with lipid bilayer allows for retention of much of the helical content with the spectra, indicating a small reduction in the contribution of this structure from 73 to 62% (Table II). However, β structure was virtually abolished with a concomitant increase in random coil from 12 to 35%.

Discussion

Proteolipid proteins such as the myelin proteolipid (Sherman & Folch-Pi, 1970; Cockle et al., 1978a; Lees & Sakura, 1978), the plasma membrane proteolipid (Tosteson & Sapirstein, 1981), and the *N,N'*-dicyclohexylcarbodiimide binding proteolipid of the oligomycin-sensitive mitochondrial proteolipid (V. S. Sapirstein, unpublished results) are soluble both in chloroform–methanol and in H_2O. This apparently wide solubility range probably is not the result of a unique structure compatible with both solvents but rather reflects two distinct conformational states of these proteins. In water, proteolipids presumably assume a conformation consisting of a large hydrophobic core; this globular-like structure should differ from similar structures in other globular proteins by the extent to which the core is lipophilic. The high percentage of α helix in H_2O in the plasma membrane proteolipid studied here and in the aqueous form of the myelin proteolipid (Cockle et al., 1978a) supports the concept of a lipophilic core which effectively excludes H_2O. The fluorescence spectrum is also compatible with such a structure with the tryptophan residues present in an extremely hydrophobic environment in the water-soluble form (Figure 1). The tryptophan spectrum in H_2O is similar to what has been observed for aqueous solutions of the myelin proteolipid with one peak between 330 and 340 nm (Cockle et al., 1978b; DeForesta et al., 1979). This wavelength range corresponds to a tryptophan emission of this amino acid when it is dissolved in nonpolar solvents.

The protein appears to have sufficient hydrophilic groups to hydrate the protein in H_2O. Although no sequence data are available for the plasma membrane proteolipids, the polar residues of the myelin and mitochondrial ATPase proteolipid appear to be organized into hydrophilic domains (Jolles et al., 1979; Lees et al., 1982; Sebald & Wachter, 1978). The myelin proteolipid forms oligomers in H_2O which are not observed in organic solvents (Moscarello et al., 1973); considering the number of hydrophobic residues in the myelin proteolipid and in the plasma membrane proteolipid, it is reasonable to assume that a sufficient number of nonpolar residues are present on the surface and that they might mediate hydrophobic interactions among monomers. The data on 8-anilino-1-naphthalenesulfonate binding in the present study suggest that in H_2O the proteolipid complex may consist of larger aggregates than it does in chloroform–methanol. However, no data are available on the composition of these oligomers, i.e., the number of α and β subunits per oligomer.

The addition of 2% sodium dodecyl sulfate to the H_2O-soluble form of the plasma membrane proteolipid did not effectively alter the secondary structure of this protein. Sodium dodecyl sulfate can exert different structural effects in neutral and acidic solutions (Wu et al., 1981), and care was taken to maintain our preparations at neutral pH. Preparation of the proteolipid as described in the present study gives rise to species which migrate on sodium dodecyl sulfate gels as monomers. Thus, it would appear that the helical content of the protein is not a result of oligomerization but may reflect the structure of the monomeric species. The stability of the proteolipid in sodium dodecyl sulfate is not unique; bacteriorhodopsin which also forms oligomers and promotes ion channels in lipid bilayers is an extremely hydrophobic protein which retains its biological activity even after treatment with sodium dodecyl sulfate.

Although the aqueous form retains its structure in sodium dodecyl sulfate and acetic acid, the circular dichroism and fluorescent data indicate that incorporation of the protein into liposomes or in solution in chloroform–methanol promotes a significant alteration in structure. Recombination of the proteolipid with phosphatidylcholine–cholesterol liposomes gives rise to a conformation devoid of β structure but increased in random coil. Under these conditions, the fluorescence spectra exhibit tryptophan emissions at 345 and 355 nm as well as at 335 nm. It is not clear whether this represents a shift of one of several tryptophans in the protein or two separate populations of proteins. The fluorescence results together with the circular dichroism data, however, suggest that a portion of the protein has been "melted" by the lipid environment, subsequently exposing the tryptophans to an environment more polar than the protein interior. Because the fluoresence spectrum in liposomes is so similar to that obtained in chloroform–methanol, we suggest that the structural changes observed upon incorporation into liposomes represent an alteration of the conformation of the protein and do not reflect an artifactual segregation of this protein into distinct populations with different interactions with lipid.

The circular dichroism spectra in liposomes suggest a protein structure consisting of α helix and random domains. When the proposed biological activity of this protein complex, e.g., ion channel formation, is considered, the liposome data may be explained by a protein traversing the bilayer as an α helix with the exposed segments assuming a random coil configuration. A similar structure has been proposed for bacteriorhodopsin. The latter protein traverses the membrane 7 times as an α helix with the extra membranous segments exhibiting random structure (Engelman et al., 1980). Both the plasma membrane proteolipid and bacteriorhodopsin form channels in bilayers. Bacteriorhodopsin exists in membranes as two trimers (Michel et al., 1980) while the plasma membrane proteolipid forms voltage-dependent channels which are comprised of trimers or hexamers consisting of trimers of heterodimers. Evidence indicates that bacteriorhodopsin monomers, which are equivalent in size to the plasma membrane proteolipid heterodimer, can pump protons (Dencher & Heyn,

1979); no data are available comparing the channels formed by different oligomeric states of the plasma membrane proteolipid.

The data in this study suggest that the proteolipid can exist in different oligomeric states. The data also suggest that changes in the state of oligomerization are accompanied by other changes in the secondary and tertiary structure which affect an alteration in tryptophan fluorescence. Although the data presented here do not demonstrate that the channel has a particular stoichiometry, they do indicate that the protein can self-associate to form an oligomer of some high order. The formation of oligomers may be a common feature of channel-forming membrane proteins of this type.

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