Circular Dichroism Studies on Lipid–Protein Complexes of a Hydrophobic Myelin Protein†

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ABSTRACT: Circular dichroism spectra of lipophilin (a hydrophobic protein purified from human central nervous system myelin) were analyzed by the method of Chen et al. (1974) to obtain information on its secondary structure in aqueous and lipid environments. When introduced into phosphatidylcholine vesicles by dialysis from 2-chloroethanol, the protein possessed about 75% α helix. A new water-soluble form of lipophilin also containing over 70% α helix was obtained by a similar dialysis in the absence of lipid. This product had a higher helical content than two other water-soluble preparations derived by dialysis from phenol–acetic acid–urea. Interaction of all three aqueous forms of the protein with lysolecithin micelles resulted in increases in total helical content or in the average length of helical segments. The amount of β sheet was at a minimum for lipophilin incorporated into vesicles, where the presence of lipid also provided some protection against thermal denaturation.

Lipophilin is a homogeneous delipidated protein purified from the "proteolipid fraction" of central nervous system myelin, and previously termed N-2 for convenience (Gagnon et al., 1971). As its name implies, lipophilin is a hydrophobic protein with a pronounced affinity for lipids, and is thus regarded as an intrinsic membrane protein (Moscarello, 1976). When recombined with model phospholipid vesicles it exerts appreciable effects on the organization of the lipid bilayer (Vail et al., 1974; Papahadjopoulos et al., 1975; Boggs et al., 1976, 1977); however, no information is currently available on the conformational state of the protein in these systems or in myelin itself. Two distinct water-soluble forms were previously obtained by dialysis from phenol–acetic acid–urea, which on the basis of optical properties appeared to comprise mainly α helix and β structure, respectively (Anthony and Moscarello, 1971; Moscarello et al., 1973). In this paper we describe a new, more helical modification prepared by simple dialysis from 2-chloroethanol into water.

In a previous study (Boggs et al., 1976), it was shown that lipophilin may be incorporated uniformly into phosphatidylcholine vesicles by dialyzing mixtures of the components from 2-chloroethanol solution into water. At high protein concentrations, these vesicles become single layered and form clear suspensions which do not precipitate on standing. We have now found that the circular dichroism of such preparations appears little affected by the serious distortions that normally characterize CD[1] spectra of particulate material, such as suspensions of myelin itself (Moore and Wetlaufer, 1973). We have thus been able to estimate that around 75% of the protein consists of helical structures, comparable to the value recently determined for the purple membrane protein bacteriorhodopsin (Henderson and Unwin, 1975). We have also studied the binding of all water-soluble forms of lipophilin to lysolecithin micelles, and observed an increase in helical character in each case. These results are discussed in terms of variability in protein–protein and protein–lipid interactions, with regard to the possible conformational state of lipophilin in the myelin membrane. The accompanying paper (Cockle et al., 1978) describes parallel studies on the intrinsic fluorescence of lipophilin, to obtain further insight into the internal structure of the protein and the changes induced by phospholipids.

Materials and Methods

Preparation of Lipophilin. Myelin was isolated from normal human white matter by the method of Lowden et al. (1966). Lipophilin was purified by chromatography on Sephadex LH-20 in chloroform–methanol (1:1) containing 5% of 0.1 N HCl (Gagnon et al., 1971) and lyophilized for storage.

Preparation of Water-Soluble Forms. The original α and β forms of lipophilin were derived by dialysis from phenol–acetic acid–urea as previously described (Moscarello et al., 1973). These are henceforth referred to as forms A and B, respectively, since the new form C proved to contain a particularly high proportion of α helix. The latter was prepared by dialysis of a solution of protein in 2-chloroethanol (5 mg/mL) against repeated changes of distilled water at 5 °C.

Lipids and Other Materials. Lysolecithin and phosphatidylcholine (egg yolk lecithin) were purchased from Sigma Chemical Co. and Serdary Research Laboratory (London, Ontario), respectively. The former was received as the solid, and the latter in chloroform solution, which was maintained under nitrogen is a sealed ampoule at −70 °C. Each gave a single spot on thin-layer chromatography.

2-Chloroethanol was obtained from BDH Chemicals (Toronto) and purified by vacuum distillation over Tris and Girards Reagent T (4 g of each per L); it was stored in the cold and dark, and periodically redistilled.

Preparation of Protein–Lipid Complexes. The interaction of lipophilin with lysolecithin was studied by mixing aqueous solutions of the components in appropriate proportions; protein/lipid ratios ranged from 1:1 to 1:20 by weight, and the mixtures remained optically clear in all cases.

Lipophilin was combined with phosphatidylcholine (PC) by dialysis of protein–lipid mixtures from 90% v/v 2-chloroethanol into 10 mM NaCl–1 mM EDTA adjusted to pH 7.4 with NaOH (cf. Boggs et al., 1976). Freeze fracture electron microscopy has shown that this technique produces homoge-

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‡ Assisted by a travel grant from the International Cell Research Organization.

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[1] Abbreviations used: CD, circular dichroism; Tris, tris(hydroxymethyl)aminomethane; PC, phosphatidylcholine.

0006-2960/78/0417-0624$01.00/0

neous incorporation of protein into the lipid bilayer, giving rise to single-walled vesicles of diameter about 200 nm when the protein content is around 50% by weight (Boggs et al., 1976). For the present purposes, the initial mixture contained 70–80% lipophilin, since the resulting suspensions were only faintly opalescent; after dilution to an appropriate concentration with 10 mM NaCl, these samples were used directly for CD measurements. Seventy-five percent of the material could be sedimented on centrifugation at 35 000 rpm for 1 h at 4 °C in an SW 50.1 rotor, but, at these high protein concentrations, the resulting pellet was very sticky and not suitable for examination by electron microscopy. However, centrifugation of the pellet in a discontinuous sucrose gradient revealed that the protein and lipid were present in a single band, and that all the protein was incorporated into lipid-containing structures. The composition of samples was verified by protein and phospholipid (phosphate) analysis, which gave protein/lipid ratios close to 3:1 by weight.

Protein Determinations. Protein concentrations were routinely estimated from the ultraviolet absorption at 280 nm, using extinction coefficients based on protein determinations by amino acid analysis (see the accompanying paper, Cockle et al., 1978) and with the Folin–Ciocalteau reagent as described by Hess and Lewin (1965) for proteolipid proteins.

Gel Electrophoresis. Polyacrylamide gels (5 or 7.5%) were prepared in 35% acetic acid, with or without 5 M urea, and run in 10% acetic acid (cf. Takayama et al., 1966). The water-soluble forms of the protein (40–50 μg) were layered on top of the gels in 0.4 M sucrose. Electrophoresis was conducted for 1 h at 2–3 mA/tube, followed by staining with 1% Amido Black in 7% acetic acid.

Analytical Ultracentrifugation. A Beckman Model E instrument was employed, with detection by ultraviolet absorption at 280 nm. Equilibrium runs were performed at 60 000 rpm for lipophilin dissolved in 2-chloroethanol and at 9000 rpm for form C in water. A sedimentation velocity run was also carried out for form C at 60 000 rpm; the operating temperature was 20 °C in all cases. The partial specific volume of the protein was estimated to be 0.74 cm^3/g from the amino acid composition (McMeekin and Marshall, 1952).

Circular Dichroism. CD spectra were recorded in the wavelength range 250–190 nm on a Cary 61 spectropolarimeter equipped with a thermostated cell holder; the normal operating temperature was 25 °C. Protein concentrations were 0.15–1.0 mg/mL (mostly 0.3–0.5 mg/mL) in cells of path length 1.0 or 0.5 mm. The spectrum of each protein or protein–lipid system was generally determined at least three times with two or more preparations, and the results were averaged. The CD data were expressed as the mean residue ellipticity $[\theta]$ (deg cm^2 $dmol^{-1}$), using 107.5 as the calculated mean residue weight of the protein.

Method of Analysis. The secondary structure composition of lipophilin was calculated assuming that the total ellipticity at any wavelength λ in the peptide region can be represented as a linear combination of three conformation-specific components (Saxena and Wetlaufer, 1971; Chen et al., 1972):

$$[\theta] = f_\alpha[\theta]_\alpha + f_\beta[\theta]_\beta + f_r[\theta]_r \quad (1)$$

The subscripts α, β, and r refer respectively to α helix, β sheet, and unordered ("remainder") structures. The three fractions f_i should obey the relations $\Sigma_i f_i = 1$ and $f_i \leq 1$. Any nonpeptide contributions to $[\theta]$ are neglected.

The procedure of Chen et al. (1974) was adopted, whereby the helical component $[\theta]_\alpha$ is expressed as the sum of three Gaussian bands whose rotational strengths are chain-length-dependent. Experimental spectra were digitized at 0.9-nm intervals in the range 245–205 nm (46 points). Values for $[\theta]_\beta$ and $[\theta]_r$ were interpolated from results very kindly provided by J. T. Yang. The unknown parameters f_α, f_β, $\bar{n}$ (the average length of a helical segment), and the bandwidths of the three helical transitions were extracted by an iterative nonlinear least-squares curve-fitting analysis. Calculations were carried out on a CDC 6400 computer using a library Fortran program NLWOOD (McMaster University Computer Centre).

This method tends to produce unreliable values for $\bar{n}$. To eliminate $\bar{n}$ as a variable, an explicit relationship with f_α was sought from the observed ellipticity at the calculated isodichroic point near 224 nm for the β and unordered forms, at which wavelength $[\theta]_{\beta r} = 600$ and $[\theta]_\alpha = -39\,000$ for an infinite α helix (from Chen et al., 1974). Hence

$$[\theta]_{224} = -39\,000(1 - 2.54/\bar{n})f_\alpha + 600(1 - f_\alpha) \quad (2)$$

In nearly all cases, agreement with the six-variable analysis was good (see Results section).

Results

Characterization of the Water-Soluble Form C of Lipophilin. The homogeneity of the new form of lipophilin obtained by dialysis from 2-chloroethanol was assessed by gel electrophoresis and by equilibrium ultracentrifugation. The gel system of Takayama et al. (1966) was earlier applied to lipophilin with some success, but sharp bands were difficult to obtain (Gagnon et al., 1971; Moscarello et al., 1973). Forms C and A migrated the same distance in both 7.5% and 5% polyacrylamide gels, in contrast to the slower moving form B. Omission of urea from the gel medium had no appreciable effect. In all cases form C migrated as a single component, evidently of similar size to the previously characterized form A (Moscarello et al., 1973).

The sedimentation coefficient $s_{20,w}$ of form C in water was 6.17 S, also comparable to the result of 6.0 S found for form A (Gagnon, 1976). Sedimentation equilibrium runs resulted in linear plots of log c vs. r^2, where c is the concentration at radius r from the center of rotation, consistent with sample homogeneity. The estimated molecular weight was 79 000, in comparison with the previously reported values of 86 000 and about 500 000 for forms A and B, respectively, and 24 000 and 28 000 for presumably monomeric forms in dissociating solvents (Moscarello et al., 1973). From these data it seems likely that forms C and A both comprise three protein subunits, whereas form B is an aggregate of many subunits.

Lipophilin dissolved in 2-chloroethanol also appeared homogeneous, with an apparent molecular weight of 34 000, using the same partial specific volume as before (0.74 cm^3/g). While the protein may indeed have a similar partial specific volume in water and 2-chloroethanol (Inoue, 1973), any error has a particularly serious effect on the calculated molecular weight in this instance, since the density of 2-chloroethanol is 1.20 g/cm^3. Thus we can only infer that lipophilin is probably largely monomeric in this solvent.

CD Spectra of Lipophilin. Figure 1 (full lines) depicts the CD spectra of lipophilin in 2-chloroethanol and of the three water-soluble forms; the extrema measured for these and the lipid-containing systems are listed in Table I. The spectrum of the protein in 2-chloroethanol is characteristically that of an α helix, with double minima at 222 and 211 nm and ellipticities approaching $-40\,000$ deg cm^2 $dmol^{-1}$. On transfer to water, the protein evidently retains a large proportion of helical structure; simple dilution of a 2-chloroethanol solution also produced the same drop in ellipticity. Inspection of the spectra

TABLE I: Extrema of Lipophilin CD Spectra.

Protein system[a]	λ (nm)	$-[\theta] \times 10^{-3}$ [b] (deg cm^2 dmol^{-1})	λ (nm)	$-[\theta] \times 10^{-3}$ [b] (deg cm^2 dmol^{-1})
2-Chloroethanol solution	222	37.9 ± 0.1	211	37.8 ± 0.1
Aqueous solutions				
Form C	222	20.8 ± 0.4	211	20.1 ± 0.4
Form A	219	16.6 ± 0.3	211	15.9 ± 0.3
Form B	217	15.0 ± 0.2		
Lysolecithin complexes				
Form C	220	25.9 ± 0.8	209	27.8 ± 0.8
Form A	220	21.4 ± 0.6	209	22.2 ± 0.6
Form B	217	20.5 ± 0.7	211	20.1 ± 0.7
PC vesicles	222	24.2 ± 0.1	211	22.6 ± 0.1

[a] The aqueous medium was distilled water in all cases except for the PC vesicles, which were suspended in 10 mM NaCl. The compositions of the protein-lipid samples are given in the legend to Figure 3. [b] Mean values with their standard errors.

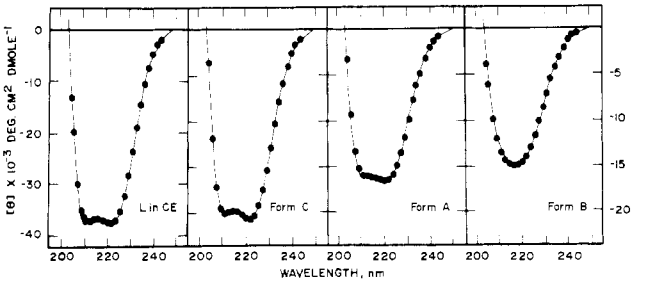

FIGURE 1: Comparison of experimental (—) and computed (●) CD spectra of lipophilin (L) in 2-chloroethanol (CE) and water at 25 °C.

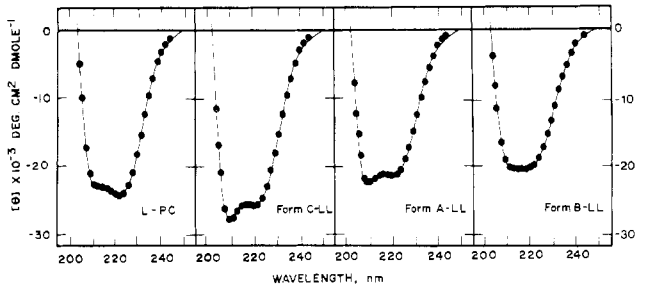

FIGURE 2: Comparison of experimental (—) and computed (●) CD spectra of lipophilin (L) in the presence of phosphatidylcholine vesicles (PC) and lysolecithin micelles (LL). Protein concentrations were in the range 0.3–0.5 mg/mL, and protein/lipid ratios (w/w) were as follows: L-PC, 3:1; form C-LL, 1:12; form A-LL, 1:7; form B-LL, 1:4.

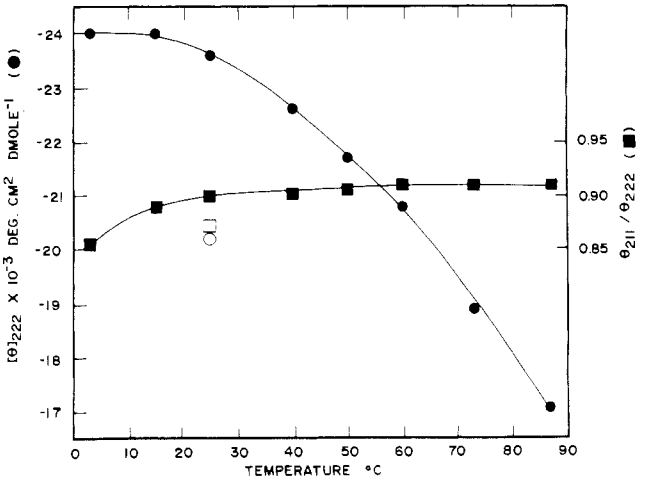

FIGURE 3: Effect on temperature on the CD spectrum of the lipophilin-PC complex: changes in $[\theta]_{222}$ (●) and the peak ratio $\theta_{211}/\theta_{222}$ (■) on warming to 90 °C. The open symbols (○ and □) refer to the spectrum obtained on recooling to 25 °C.

in Figure 1 suggests that while lipophilin forms C and A are predominantly helical, form B contains a comparatively large proportion of β structure. However, the CD intensities of forms A and B are considerably greater than earlier reported (Moscarello et al., 1973), though the spectra have the same shapes; the discrepancy may be due to more accurate determinations of protein concentration in the present work.

Interactions with Lysolecithin and Phosphatidylcholine. Treatment of each of the three aqueous forms of lipophilin with lysolecithin micelles induced spectral changes generally consistent with increased helical content. Intensity enhancement increased with the amount of lysolecithin added up to a saturation point, as expected for the formation of discrete protein-lipid species. Saturation was reached at a lower lipid/protein ratio with form B than with the two "helical" modifications; this differential behavior has been studied in detail by fluorescence techniques (Cockle et al. 1978). The spectra presented in Figure 2 illustrate the maximal effects achieved using excess lysolecithin.

Attempts to introduce aqueous forms of the protein into pre-formed lipid vesicles simply by mixing and incubating the components resulted in aggregation and precipitation. However, by the dialysis technique it was possible to produce almost clear suspensions of lipophilin incorporated into PC vesicles. The CD spectrum (Figure 2) indicates a predominant helical contribution, though the conformational state appears not to be the same as in form C or its complex with lysolecithin. Some experiments were performed to determine whether the lipid matrix conferred any thermal stability on the protein conformation, since form A is readily converted to form B by heat (Moscarello et al., 1973). A sample of lipophilin-PC vesicles was gradually warmed up to 90 °C in the heating block of the CD instrument during repeated scanning of the spectrum. The results are summarized in Figure 3. The progressive loss of intensity ($[\theta]_{222}$), reminiscent of the behavior of the aqueous protein, was, however, accompanied by little change in spectral profile (the ratio $\theta_{211}/\theta_{222}$); 50% recovery occurred on recooling to 25 °C. Evidently the protein undergoes a partially reversible thermal transition, but does not aggregate to the polymeric form B; such a process may be impeded when the protein is embedded in lipid. In a second study, samples of the lipophilin-PC complex and the aqueous form C were incubated

TABLE II: Least-Squares Analysis of Lipophilin CD Spectra.

Protein system	6-Variable method[a]					5-Variable method[a]				
	f_α	f_β	f_r	$\bar{n}$	i^b	f_α	f_β	f_r	$\bar{n}$	i^b
2-Chloroethanol solution	0.97 ±0.01	0.01 ±0.01	0.02 ±0.01	>100	1	0.94 ±0.02	0.00 ±0.02	0.06 ±0.03	>100	1
Aqueous solutions										
Form C	0.74 ±0.02	0.17 ±0.01	0.09 ±0.03	9.5 ±0.8	19 ±2	0.71 ±0.04	0.14 ±0.02	0.15 ±0.04	9.3 ±1.3	18 ±3
Form A	0.66 ±0.02	0.20 ±0.01	0.14 ±0.02	7.0 ±0.3	23 ±1	0.63 ±0.02	0.17 ±0.02	0.20 ±0.03	7.2 ±0.5	21 ±2
Form B	0.53 ±0.03	0.29 ±0.01	0.18 ±0.03	6.5 ±0.5	20 ±2	0.54 ±0.02	0.30 ±0.01	0.16 ±0.03	6.4 ±0.5	20 ±2
Lysolecithin complexes										
Form C	0.75 ±0.02	0.29 ±0.02	−0.04 ±0.03	19.0 ±3.1	9 ±2	0.66 ±0.04	0.19 ±0.03	0.15 ±0.05	39.5 ±18.0	4 ±1
Form A	0.67 ±0.02	0.26 ±0.02	0.07 ±0.03	13.2 ±1.7	12 ±2	0.61 ±0.03	0.21 ±0.02	0.18 ±0.04	16.0 ±0.6	9 ±3
Form B	0.68 ±0.02	0.28 ±0.01	0.04 ±0.03	8.8 ±0.7	19 ±1	0.66 ±0.02	0.26 ±0.02	0.08 ±0.03	9.3 ±1.3	17 ±2
PC vesicles	0.75 ±0.03	0.15 ±0.01	0.10 ±0.03	16.4 ±4.1	11 ±3	0.78 ±0.05	0.09 ±0.02	0.13 ±0.05	11.7 ±3.6	16 ±5

[a] The standard errors quoted for the parameters measure the reliability of single analyses based on mean experimental ellipticities, the precision of which may be judged from Table I; the overall uncertainty of the results is of course somewhat larger. [b] The number of helical segments per protein monomer, assuming a molecular weight of 26 000; if $\bar{M}$ = 107.5, then $\bar{N} \simeq 240$ residues.

together at 80 °C in a water bath, and their CD spectra recorded at intervals after cooling to 25 °C. The rate of decrease in ellipticity (θ_{222}) was two to three times greater for the free than for the lipid-associated protein; after 1 h the former was completely coagulated, while the latter still retained 40% of the original intensity. Thus the presence of phospholipid provides some protection against thermal denaturation.

Determination of Secondary Structure. The CD spectra were analyzed using both procedures described earlier. Table II lists the computed fractions f for the eight lipophilin systems studied and the corresponding values of $\bar{n}$ and i, where i is the number of helical segments and is equal to $f_\alpha N/\bar{n}$ (N is the total number of amino acid residues). In all cases the iterative process of error minimization converged for $0 < f_\alpha, f_\beta < 1$ (though in one instance $f_\alpha + f_\beta > 1$) and yielded reasonable bandwidths in the range 8.0–12.5 nm (not shown). f_α, f_β, and $\bar{n}$ estimated by the two methods generally agree well and are consistent with a qualitative interpretation of the spectra, thus providing a measure of confidence in the analysis. The largest discrepancies between the two methods, as well as the poorest precision in $\bar{n}$ according to the five-variable analysis, are observed among the protein–lipid complexes. The CD spectra of some of these systems in fact exhibit unusual features with regard to the relative intensities of the minima near 222 and 210 nm, which may be due to factors beyond the scope of the present three-component analysis (see the Discussion).

The calculated parameters were used to reconstruct the original CD spectra. Agreement between experimental and computed curves was excellent in every case (Figures 1 and 2, dots), and visible differences between the two methods of calculation were slight. However, it should be pointed out that, in view of the large number of unknown parameters to be fixed and the reservations mentioned above, a good fit does not necessarily indicate a unique solution.

Discussion

Lipophilin is a hydrophobic protein solubilized in water only indirectly by transfer from suitable organic solvents. In this and earlier work (Gagnon et al., 1971; Moscarello et al., 1973) we have used gel electrophoresis and equilibrium ultracentrifugation to establish the homogeneity of our water-soluble modifications. In contrast, the Folch-Pi proteolipid apoprotein isolated from bovine white matter has recently been shown to behave as a polydisperse system in water (Nguyen Le et al., 1976). During our present studies, we have found the CD intensities for the A and B forms of lipophilin to be consistently greater than first reported (Moscarello et al., 1973), though the spectra were unchanged in appearance; earlier errors in protein determination may account for the discrepancies since we have paid particular attention to this aspect in the current work.

The reliability of the least-squares analysis depends both on the validity of the model and the suitability of the reference spectra. The assumption that proteins contain only three distinct conformational states, while obviously an oversimplification, has provided the foundation for most computational methods. The reference spectra proposed by Chen et al. (1974) are based on the CD spectra of various water-soluble globular proteins of known crystallographic structure, and while these are not obviously appropriate for membrane proteins, they are certainly superior to the only alternative references presently available, namely, synthetic poly(α-amino acids) (Greenfield and Fasman, 1969). One reservation with regard to our application of the curve-fitting procedure is the omission of data from the region of positive ellipticity below 200 nm; we felt that the desirability of including this information was outweighed by the uncertainty of amplitude measurements at low wavelength, due to deteriorating signal/noise ratio.

Our analysis shows that lipophilin in 2-chloroethanol exists almost entirely as extended helical chains, in accordance with the known tendency of halogenated alcohols to promote helix formation, and as indeed reported for the Folch-Pi apoprotein (Sherman and Folch-Pi, 1970). On dialysis of the protein into water, 75% of the helical content is retained, but the short segments and the substantial amount of unordered peptide indicate extensive tertiary folding, which together with the probable subunit association presumably serves to minimize

exposure of hydrophobic residues to the aqueous phase. The two other water-soluble forms of lipophilin also contain at least 50% α helix, and in the full series f_α decreases and f_β increases in the order of decreasing ellipticity. We obtain a value of only 0.3 for f_β in form B, although β sheet evidently contributes to the amide I and II infrared bands (Moscarello et al., 1973).

The significance of the differences in CD properties shown by two apparently trimeric forms of lipophilin prepared by different routes is uncertain, but the conformation and aggregation state of lipophilin in lipid environments becomes of great interest. The application of CD to the study of proteins in biomembranes or artificial vesicles is unfortunately hindered by the difficulty of obtaining optically clear samples, although experimental and computational procedures for overcoming the effects of absorption flattening and differential light scattering have been described (see Gitter-Amir et al., 1976; Long et al., 1977, and references quoted therein). Lysolecithin provides a convenient model system, since its aqueous solutions are transparent and contain micelles, which, insofar as they have a hydrophobic interior and a polar exterior, bear some resemblance to the lipid bilayer structure of membranes; the micelle size of lysolecithin has been reported as about 180 molecules (Lewis and Gottlieb, 1971).

Our experiments demonstrate that lysolecithin readily interacts with lipophilin, stabilizing the protein in highly helical conformations. Adoption of the α helix in a lipid environment serves the dual purpose of enhancing hydrophobic interactions between the many apolar amino acid side chains and the lipid alkyl groups, while diminishing the exposure of the hydrophilic peptide backbone (Nozaki and Tanford, 1971). Inspection of Table II reveals that the three protein modifications appear not to behave uniformly on interaction with lysolecithin, despite similar increases in ellipticity; the most pronounced changes are a rise in f_α for form B, but an elongation of helical segments for forms C and A. This variability is probably related to the two different aggregation states of the free protein, namely, a trimer (C and A) and a large polymer (B), which might lead to quite distinct protein–protein and protein–lipid interactions in the final complexes. A difference in the stoichiometry of complex formation for forms C and A compared with form B is in fact suggested by the observation that saturation of the CD changes was reached at a lower lipid/protein ratio for the latter than for the former two and has been corroborated by measurement of protein fluorescence during titration with lysolecithin (Cockle et al., 1978).

The three lysolecithin systems also display considerable differences in the relative amplitudes of the α-helical extrema near 222 and 210 nm (Figure 2 and Table I) with the complex of form C particularly uncharacteristic of an "average" α helix. However, poly(α-amino acids) in the helical conformation have been shown to embrace a wide range of peak ratios above and below unity, as a result of variations in the precise geometry of the polypeptide backbone (Parrish and Blout, 1971). An alternative possibility for the form C complex is the emergence of a large contribution from the aromatic chromophores, which are thought to affect intensities more at 210 than at 220 nm (Filippi et al., 1976), arising from some especially favorable mutual orientation of several aromatic ring systems. These complications would introduce errors into any CD treatment based solely on peptide contributions from standard reference conformations; as noted earlier, the analysis for the complex of form C with lysolecithin was comparatively unsatisfactory.

The lack of turbidity in lipophilin–phosphatidylcholine suspensions containing high proportions of protein suggests that distortion of the CD spectra due to light scattering and absorption flattening might be small. Since the observed ellipticities are actually greater than for any water-soluble form of the protein on its own (Table I), spectral dampening is unlikely to be of significance. Moreover, no abnormal optical density due to scattering was detected in the range 340–400 nm. While it is not possible to dismiss these artifacts completely, we conclude that the observed asymmetry of the double minimum is only superficially analogous to the distortions frequently seen in the circular dichroism of membrane-bound proteins. More likely causes of asymmetry in the present case are perturbations in helix geometry as seen in poly(α-amino acids) (Parrish and Blout, 1971), or merely the predicted dependence of the CD profile on the helical chain length parameter n (Chen et al., 1974), an effect included in the current treatment. Indeed, the fact that the experimental spectrum can be fitted extremely closely by the three-component model, without any corrections for light scattering, shows that it can represent a genuine conformational state of the protein. According to our numerical analysis, the helical content of lipophilin incorporated into PC vesicles is about 75%, which is the highest value for any form of the protein examined, other than the solution in 2-chloroethanol; if, after all, our spectra do suffer from absorption flattening, then the helical content will in fact be underestimated. Significantly, the helical segments are also somewhat longer than in the free protein, the proportion of β structure reaches a minimum, and the overall conformation is stabilized toward heat by the presence of lipid. These results imply an extensive interaction between protein and lipid, consistent with the protein becoming embedded in the hydrocarbon region of the phospholipid bilayer (Papahadjopoulos et al., 1975).

Intrinsic membrane proteins are indeed thought to adopt appreciably helical structures, and the CD spectra of membranes which have been examined do exhibit the appropriate extrema at approximately 210 and 222 nm (Singer, 1971). However, the intensities of these bands are generally low, for example, $[\theta]_{222} = -10\,300$ deg cm^2 dmol^{-1} for whole myelin (Moore and Wetlaufer, 1973). Unless elaborate corrections are made to allow for the particulate nature of membrane suspensions, it is not possible to draw definite conclusions on protein conformation. By applying such techniques, it has been estimated that the average helical content of all proteins in the red blood cell membrane is 45% (Gitter-Amir et al., 1976), while bacteriorhodopsin, the single protein in the purple membrane of *Halobacterium halobium*, contains 73% α-helix (Long et al., 1977). The latter is in excellent agreement with an independent determination of 70–80% obtained by electron diffraction (Henderson and Unwin, 1975). A proteolipid protein from sarcoplasmic reticulum was also found to possess about 70% α-helix on reconstitution with sarcoplasmic reticulum phospholipids (Laggner, 1975). A bacteriophage coat protein, which appears to be completely helical in the intact virus, probably comprises at least 50% α-helix when absorbed into phospholipid vesicles and micelles, according to inspection of the published CD spectra (Nosaki et al., 1976).

It is therefore quite feasible that the high helical content, estimated for lipophilin introduced into phosphatidylcholine vesicles by the dialysis technique, is representative of the natural state in myelin. The lipid composition of myelin is of course extremely complex, so it is unlikely that a simple model system will faithfully reproduce all aspects of the native protein conformation. Indeed the conformational variability displayed by lipophilin in water may also be retained in the membrane; a possible role for a flexible protein in the dissipation of heat produced by the passage of nerve impulses has been discussed by Moscarello (1976).

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