Solubilization and insertion into reverse micelles of the major myelin transmembrane proteolipid

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The Folch-Pi proteolipid has been isolated from bovine white matter and characterized with respect to phospholipid and glycolipid composition. The protein–lipid complex has been solubilized in aqueous reverse micelles of di(2-ethylhexyl) sodium sulfosuccinate and isooctane. Solubilization of this otherwise water-insoluble proteolipid requires small amounts of water, the percent of solubility being maximum for a low molar ratio of water to surfactant (w_o = 5.6). Unlike hydrophilic proteins, the extent of incorporation into the micellar system is negligible at 50 mM surfactant and reaches 90% only at 300 mM. However, the conformation of the proteolipid in reverse micelles as studied by fluorescence emission spectroscopy and circular dichroism was not affected by variations of the surfactant concentration. These results are consistent with the peculiar properties of the aqueous environment of the proteolipid within the reverse micelles and may reflect the membrane-like character of these bio-assemblies.

Reverse micelle *Myelin proteolipid complex* *Protein interacting lipid* *Integral transmembrane protein*

1. INTRODUCTION

Reverse micelles are aggregates of surfactants in hydrocarbon solvents. Although much is known about the physical chemistry of these organized assemblies, the peculiar properties of cosolubilized water are different from those of bulk water and are still being widely investigated [11]. Recently, interest in these membrane-mimetic agents has been broadened by the solubilization in the aqueous inner core (water pool of reverse micelles) of hydrophilic enzymes, the structural and catalytic properties of which have been described [2,3], and these studies have now been extended to nucleic acids [4].

Here we report, for the first time, evidence for the solubilization of a water-insoluble myelin membrane proteolipid complex and its insertion into reverse micelles formed by di(2-ethylhexyl) sodium sulfosuccinate (AOT), isooctane and water. Indeed, the Folch-Pi protein–lipid complex has been solubilized, but only in chloroform–methanol mixtures or in 2-chloroethanol solutions [5,6]. Previously it had been possible to study the properties of this major transmembrane protein (50% of total myelin protein) in aqueous media only after careful delipidation, a process leading to a water-soluble apoprotein [7].

Because reverse micelles represent an ordered system of aqueous and membrane-like character [1], it is hoped that these studies will provide a better characterization of the protein–lipid complex in the native state than can be provided using pure organic solvents. Here, in addition to chemical determination of specifically bound phospholipids and cerebrosides, we have characterized the conformation of the proteolipid by absorption, fluorescence and circular dichroism spectroscopy.

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2. MATERIALS AND METHODS

All the reagents used were of analytical grade. The solvents were spectroscopic or HPLC grade. Di(2-ethylhexyl)sodium sulfosuccinate (Aerosol OT 100 or AOT) was a generous gift from the Cyanamid Co. (France). It was purified as in [8]. The purity of AOT solution was carefully checked for each batch according to the criteria recommended in [9]. The HPLC of lipids was carried out with two Beckman Model 110 A pumps, and an LCR Spectromonitor III absorbance detector as in [10].

2.1. *Proteolipid preparation*

The Folch-Pi proteolipid was extracted together with all myelin lipids from bovine white matter using the chloroform–methanol (2:1, v/v) mixture described in [11] and separated from neutral lipids by precipitation with light petroleum ether (b.p.: 40–60°C) as in [12]. The precipitate was then dissolved in chloroform–methanol.

2.2. *Preparation of reverse micelles containing the proteolipid*

For solubilization in reverse micelles, the proteolipid at known concentration in chloroform–methanol is reprecipitated by isooctane, as in [12]. The precipitate is redissolved by stirring in a micellar solution of AOT at concentrations varying from 50 to 300 mM, containing from 0.5 to 10% water. Dissolution is achieved by a 2-min sonication followed by a final centrifugation at $5000 \times g$.

2.3. *Spectral measurements*

The absorption spectra were measured on a Cary model 118 spectrophotometer. The molar extinction of the proteolipid at 278 nm was taken as $4.05 \times 10^4\ M^{-1} \cdot cm^{-1}$ for an M_r of 30000 [13]. The circular dichroism spectra were carried out on a Cary 60 spectropolarimeter equipped with a 6001 CD attachment. Fluorescence measurements were done on a Jobin et Yvon model J.Y.3 fluorometer coupled to a Hewlett-Packard 9815 A calculator and a 9826 plotter.

3. RESULTS AND DISCUSSION

The proteolipid complex has been analyzed for bound phospholipids and glycolipids present after precipitation by isooctane. The lipid composition is shown in table 1. Only negatively charged lipids remain attached to the apoprotein and the ratio phospholipids/glycolipids was found constant from batch to batch.

The extent of solubilization of the Folch-Pi proteolipid is a function of the water content of the micellar system, expressed in terms of w_o, the ratio of water molecules to surfactant molecules. As shown in fig.1A, the proteolipid is totally insoluble in the absence of water. At both values of the AOT concentration shown, the percent of solubilization increases with an increasing $[H_2O]/[AOT]$ ratio up to a value of 5.56 and then decreases. The maximum of the solubility curve accordingly does not depend on AOT concentration, w_o having an identical value at 200 and 300 mM AOT. It has to be pointed out that the optimum solubilization of hydrophilic enzymes is often attained at considerably higher values of w_o [17].

The fact that maximal solubilization at either of the two AOT concentrations occurs at the same low value of w_o reflects the dual nature of the interaction of the proteolipid with water. A definite amount of water is required for the phospholipid charges and perhaps also for limited surface charge on the protein, whereas excessive water generates unfavorable interactions within the hydrophobic parts of the protein. In connection with these results it is interesting to point out that what is

Table 1

Lipid composition of the Folch-Pi proteolipid

Phospholipids[a]	5 ± 0.5 mol[b]
Phosphatidylserine	
Phosphatidylinositol	
Diphosphatidylinositol	
Triphosphatidylinositol	
Galactolipids (as sulfatides)	3.5 ± 0.5 mol[c]

[a] The relative amounts of phospholipids have not been estimated at present due to the well established instability of polyphosphoinositides during the extraction process [14]

[b] Results expressed in mol phosphoric acid [15]/mol protein (M_r 30000)

[c] Expressed in mol galactose/mol protein [16]

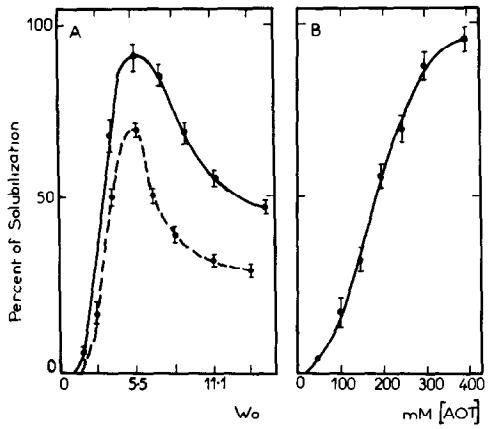

Fig.1. Solubilization of the Folch-Pi proteolipid in isooctane–AOT–water reverse micelles. (A) Percentage of solubilization plotted against w_o = [H_2O]/[AOT] at 300 (——) and 200 (– – –) mM AOT concentration. (B) Percentage of solubilization against AOT concentration, at a constant w_o value of 5.56. The bars represent experimental error. The percentage of incorporation is calculated from the final absorption of a clear micellar solution (1 ml) compared to the absorption of the initial chloroform–methanol solution of the proteolipid (20 nmol in 0.5 ml), taking into account the respective volumes.

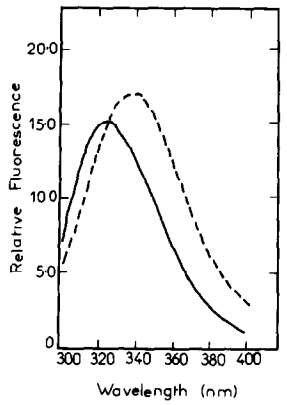

Fig.2. Fluorescence emission spectra of the proteolipid in reverse micelles (——) and in 2-chloroethanol (– – –). The excitation wavelength was 295 nm. Wavelength dependent corrections for optical and photomultiplier tube distortions were made with rhodamine B.

referred to as 'hydrophobic hydration' is more important in the interior of membranes than previously suspected [18].

Fig.1B shows the percent of proteolipid solubilization as a function of AOT concentration at a constant w_o value (5.56). The solubility of the proteolipid is very low at 50 mM where, for example, the solubilization of a protein such as lysozyme is extensive [17], levelling off at a relatively high value of 300 mM AOT. At the latter concentration of AOT, these results suggest the involvement of more than one micelle per protein molecule in the solubilization process of the proteolipid. Higher concentrations of surfactant were not systematically tried [19].

The UV absorption spectrum of the micellar solution of the proteolipid (not shown) was recorded. When the proteolipid was dissolved in freshly distilled 2-chloroethanol, a superposable spectrum was obtained. The characteristic maximum at 278 nm and the absence of scattering in the 350 nm region indicate a lack of aggregation and excellent solubility in both milieus.

Fluorescence emission spectra of the proteolipid, on the other hand, display distinctive features when measured in micellar solutions or in 2-chloroethanol. As seen in fig.2, both spectra consist of a single peak when the excitation wavelength is 295 nm and show comparable intensities. However, in reverse micelles, the wavelength of maximum fluorescence emission is located at 326 nm, whereas in 2-chloroethanol the maximum is at 339 nm. These spectral properties were unaffected by the concentrations of surfactant in the 50–200 mM range. These results most likely reflect substantial differences in physical properties between water encapsulated in reverse micelles and bulk water, such as dielectric constant, polarity and microviscosity [8,20]. However, a decrease in tryptophan accessibility to the solvent, due to increased contact with the hydrophobic portion of the protein, cannot be ruled out at this stage.

The circular dichroism spectra of the proteolipid in reverse micelles have been measured in the UV. In the far UV (250–190 nm), spectra were recorded at a constant w_o value of 5.56. The observed spectra were independent of AOT concentration in the 50–150 mM range. The spectrum illustrated in fig.3 is consistent with a secondary structure that is substantially α-helical. At 222 nm, an ellipticity of -16500 deg·cm^2·dmol^{-1} was measured. Calculations according to [21] yield a value of approx. 55% α-helix, the remaining struc-

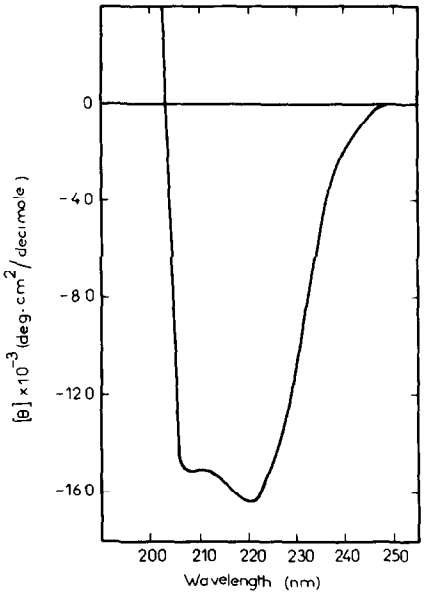

Fig.3. The far UV CD spectrum of the Folch-Pi proteolipid in reverse micelles at AOT concentrations in the 50–150 mM range. Cylindrical quartz cells of 1.0, 0.5 and 0.2 mm light paths were used. All spectra were measured at least in triplicate and average ellipticity [θ] was expressed on a mean residue basis. The mean residue weight of the proteolipid was taken as 109 according to the sequence of the apoprotein [13].

ture being random with negligible content of β-pleated sheet. Such a conformation is compatible with the model of the Folch-Pi proteolipid in [13] with 4 major α-helices penetrating the myelin bilayer. Measurements done in helix promoting solvents such as 2-chloroethanol or chloroform–methanol [7] yield higher values of the ellipticity, but it is known that such solvents often lead to artificially elevated α-helix content relative to aqueous solutions (see [13]).

Our results demonstrate that the reverse micelle system solubilizes an integral myelin transmembrane proteolipid, and makes possible studies of the proteolipid in contact with an aqueous environment also present in myelin [22]. Judging from the physical measurements reported here, the solubilization in reverse micelles does not disturb the conformation of the proteolipid, which retains a high degree of periodicity in structure.

Many problems concerning the reactivity and properties of membrane proteins inserted into reverse micelles remain to be investigated, including the state of oligomerization and the precise nature of the interaction between these proteins, the aqueous interior and the surfactant molecules comprising the micelles. Moreover, it will be of interest to evaluate the applicability to hydrophobic membrane proteins of the models proposed in [23] to account for the properties of biological molecules in the reverse micelle system.

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REFERENCES

[1] Fendler, J.H. (1982) Membrane Mimetic Chemistry, pp.55–71, Wiley, New York.
[2] Luisi, P.L., Henninger, F., Joppich, M., Dossena, A. and Casnati, G. (1977) Biochem. Biophys. Res. Commun. 74, 1384–1389.
[3] Martinek, K., Levashov, A.V., Klyachko, N.L. and Berezin, I.V. (1978) Dokl. Akad. Nauk. SSSR (English edn.) 236, 951–953.
[4] Imre, V.E. and Luisi, P.L. (1982) Biochem. Biophys. Res. Commun. 107, 538–545.
[5] Folch-Pi, J. and Stoffyn, P.J. (1972) Ann. NY Acad. Sci. 195, 86–107.
[6] Vacher, M., Waks, M. and Nicot, C. (1984) Biochem. J. 218, 197–202.
[7] Boggs, J.M., Moscarello, M.A. and Papahadjopoulos, D. (1982) in: Lipid–Protein Interactions (Jost, P.C. and Griffith, O.H. eds) vol.2, pp.1–51, Wiley, New York.
[8] Wong, M., Thomas, J.K. and Grätzel, M. (1976) J. Am. Chem. Soc. 98, 2391–2397.
[9] Luisi, P.L., Meier, P., Imre, V.E. and Pande, A. (1984) in: Reverse Micelles (Luisi, P.L. and Strouts, B. eds) in press, Plenum, New York.
[10] Yandrasitz, J.R., Berry, G. and Segal, S. (1981) J. Chromatogr. 225, 319–328.
[11] Folch, J. and Lees, M. (1951) J. Biol. Chem. 191, 807–817.
[12] Nicot, C., Nguyen Le, T., Leprêtre, M. and Alfsen, A. (1973) Biochim. Biophys. Acta 322, 109–123.
[13] Stoffel, W., Hillen, H., Schroder, W. and Deutzmann, R. (1983) Hoppe-Seyler's Z. Physiol. Chem. 364, 1455–1466.
[14] Farese, R.V., Sabir, A.M. and Sandor, L. (1979) J. Biol. Chem. 254, 6842–6844.

[15] Ames, B.N. and Dubin, D.T. (1960) J. Biol. Chem. 235, 769–775.
[16] Hess, H.H. and Lewin, E. (1965) J. Neurochem. 12, 205–211.
[17] Grandi, C., Smith, R.E. and Luisi, P.L. (1980) J. Biol. Chem. 256, 837–843.
[18] Blume, A. (1983) Biochemistry 22, 5436–5442.
[19] Keh, E. and Valeur, B. (1980) J. Coll. Interf. Sci. 79, 465–478.
[20] Tsuji, K., Sunamoto, J. and Fendler, J. (1983) Bull. Chem. Soc. Jap. 56, 2889–2893.
[21] Chen, Y.H., Yang, J.T. and Chau, K.H. (1974) Biochemistry 13, 3350–3359.
[22] Braun, P.E. (1978) in: Myelin (Morell, P. ed.) pp.91–115, Plenum, New York.
[23] Bonner, F.J., Wolf, R. and Luisi, P.L. (1980) J. Solid-Phase Biochem. 5, 255–268.