Eur Biophys J 21, 71–76 (1992)

European Biophysics Journal

The thermal transition in crude myelin proteolipid has a lipid rather than protein origin

J. Ruiz-Sanz [1], J. Ruiz-Cabello [2], P.L. Mateo [1] and M. Cortijo [2]

[1] Departamento de Química Física (Facultad de Ciencias) e Instituto de Biotecnología, Universidad de Granada, E-18071 Granada, Spain
[2] Departamento de Química Física II, Facultad de Farmacia, Universidad Complutense de Madrid, E-28039 Madrid, Spain

Received July 29, 1991/Accepted November 13, 1991/Accepted in revised form November 13, 1991

Abstract. Myelin proteolipid has been isolated from bovine brain and purified using organic solvents according to conventional procedures. The protein content of the purified sample, or crude proteolipid, contains a minimum of 75% w/w of proteolipid, with DM-20, a proteolipid molecule with an internal deletion of 35 out of 276 amino acid residues, as the only other component. Biochemical analysis has shown the differences in lipid composition between brain white matter, myelin and crude proteolipid preparations. The latter contained practically no cholesterol, while the other two samples had about 22–23% w/w. High-sensitivity differential scanning calorimetry experiments with both crude proteolipid and its extracted pool of lipids have shown similar reversible thermal transitions at 52 °C and 48 °C. The effect of increasing amounts of cholesterol on the two calorimetric transitions led in both cases to a continuous decrease in the melting temperature and in the transition enthalpy. Parallel Fourier-transform infrared spectroscopy studies of crude proteolipid have detected a reversible, co-operative lipid transition centred at 49 °C, with no detectable change in the amide region between 20 °C and 60 °C. Once more an increase in cholesterol content led to a decrease in the sharpness of this transition. It is concluded that the thermal transition detected in crude proteolipid, which has in the past been attributed to proteolipid thermal denaturation (Mateo et al. 1986), actually corresponds to a thermotropic phase transition of the lipids included in the crude proteolipid sample.

Key words: Myelin proteolipid – High-sensitivity differential scanning calorimetry – Fourier-transform infrared spectroscopy – Lipid phase transition – Cholesterol

Introduction

High-sensitivity differential scanning calorimetry (DSC) has become one of the most useful techniques for analysing protein thermal denaturation and stability (Privalov and Potekhin 1986). Many studies have been carried out with globular proteins, which undergo reversible thermal denaturation by following either a simple two-state mechanism or a multi-step denaturation pathway (Privalov 1979, 1982). Biological membranes and their constituent proteins are also being investigated by DSC (Sánchez-Ruiz and Mateo 1987). Besides the obvious difficulties in the purification and reconstitution on this type of proteins, the irreversibility of their thermal denaturation imposes certain limitations on their study (Sánchez-Ruiz et al. 1988). To our knowledge the only exception to this appears to be with mammalian brain myelin proteolipid (PLP), the major intrinsic structural protein in both brain white matter and myelin (Lees and Brostoff 1984), the reversible thermal denaturation of which has been reported elsewhere (Mateo et al. 1986). We have consequently undertaken a more systematic study of the thermal properties of PLP by DSC and Fourier-transform infrared spectroscopy (FTIR), taking into account the effect of cholesterol on its thermal behaviour. The most important conclusion to be drawn from this investigation is that the DSC thermal transition tentatively assigned to PLP denaturation actually corresponds to a thermotropic phase transition of the lipids present in the crude PLP preparation.

Offprint requests to: M. Cortijo

Experimental

Materials

Calf brains were obtained from a local slaughterhouse. Acrylamide, Coomassie Brilliant Blue R, *Tris*, the molecular weight standards for electrophoresis, BSA, cholesterol and the enzymatic kit to determine cholesterol content came from Sigma. TEMED and SDS were purchased from Fluka, and Folin-Ciocalteou's reagent, ANSA and HPLC grade n-hexane and isopropanol were from Merck. Lipids used as standards for HPLC experiments were of analytical grade and were bought from

Sigma and Avanti. All the other chemicals and organic solvents used were also of analytical grade. Distilled, deionized water was used throughout.

Fresh brain white matter was obtained from calf brains immediately after slaughter. Crude proteolipid (PLP) was purified from brain white matter by extraction with a mixture of chloroform-methanol (2 : 1 v/v) and precipitation with diethyl ether-ethanol (1 : 1 v/v) as described elsewhere (Monreal 1975). The PLP obtained was then lyophilized and stored at −20 °C.

Lipids were extracted from PLP or white matter using the method described by Santiago et al. (1964) and their individual composition was analysed by HPLC according to Yandrasitz et al. (1981) using a Lichrosorb Si 60 column (250 × 46 mm) provided by Merck. Bidimensional thin-layer chromatography was performed according to Rouser et al. (1970). Phospholipid content was determined by the method described by Bartlett (1959), using an average molecular weight of 775. Glycolipid content was ascertained by the method of Kushwaha and Kates (1981), using an average molecular weight of 850, while that of cholesterol was determined by enzymatic assay (Siedel et al. 1983). Protein concentration was assayed by Lowry's method (Lowry et al. 1951) as modified according to Wang and Smith (1975), using BSA as the standard. Suspensions of PLP or of PLP lipids were prepared by adding an appropriate volume of 20 mM phosphate buffer solution at pH 7.0. Samples of the PLP-cholesterol or lipid-cholesterol mixtures were prepared by dissolving weighed amounts of lipids or PLP and cholesterol in chloroform and evaporating to dryness first under nitrogen and then in a vacuum for several hours. An appropriate volume of phosphate buffer, pH 7.0, was then added and the mixture vortexed at room temperature. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using the discontinuous buffer system described by Laemmli (1970), but, to prevent PLP aggregation, the samples were incubated with the sample electrophoresis buffer at room temperature for 30 min without heating. Samples were then run in 10%–15% acrylamide gels.

Methods

Calorimetric experiments were carried out in a DASM-1M high-sensitivity differential scanning microcalorimeter (DSC) with 1 ml volume cells (Privalov et al. 1975) at a scanning rate of 1 K/min. The DSC was interfaced to a microcomputer equipped with a Data Translation DT-2801 A/D converter board for automatic data collection. The lipid concentration in the samples was 4 to 5 mg/ml, while protein concentration was about 1 mg/ml. The DSC transitions were corrected for the instrumental and chemical base lines in the way described by Galisteo et al. (1991). Reversibility of the transitions was checked by reheating the samples after the first run.

Samples for Fourier-transform infrared spectroscopy (FTIR) experiments were put into a thermostatically controlled (Specac) 20 500 cell for liquids, fitted with CaF_2 windows and with a spacer thickness of 6 μm. The crude samples were resuspended in 20 mM phosphate buffer, pH 7.0, to obtain a protein concentration of around 10 mg/ml (about 50 mg of lipid/ml). FTIR spectra were recorded using a 20sxb Nicolet instrument equipped with a TGS detector and with a resolution of 2 cm^{-1}. Water contribution was subtracted by keeping a straight line between 1 900–1 800 cm^{-1} using the highest possible subtraction factor. To eliminate any spectral contributions from atmospheric water vapour, the instrument was continuously purged with air dried in an adsorption Ecodry desecator. Some of the FTIR experiments were also carried out with a Nicolet 10 DX spectrometer at the Basque Country University (Bilbao). The techniques of Fourier deconvolution and fourth derivatives were used to resolve overlapping infrared bands (Kauppinen et al. 1981; Surewicz and Mantsch 1988).

Results

Protein and lipid content

The myelin protein composition found from densitometry of numerous gel electrophoresis analyses was 41% to 42% of both PLP and MBP and 8% to 9% of both DM-20 and Wolfgram fraction. SDS polyacrylamide gel electrophoresis of crude PLP indicated a high proportion of PLP in all the preparations. The minimum PLP content found was 75%, the only other protein constituent being DM-20, with negligible amounts of myelin basic protein (MBP). The total protein content in lyophylized PLP samples constituted about 14% w/w, which leads to a lipid/protein molar ratio of 240/1 assuming an average lipid molecular weight of 775. The high DM-20 content in crude PLP was not unexpected given the high similarity between the two proteins (see Discussion).

Two-dimensional thin-layer chromatography of the pool of lipids extracted from both brain white matter and crude PLP indicates a lower variety of lipids in crude PLP, which does not seem to contain any plasmalogens or cerebrosides. HPLC experiments with lipids from both sources proved (Fig. 1) that the relative free fatty acid, phosphatidylcholine and sphingomyelin contents in crude PLP are lower than in brain white matter; the former contains practically no cholesterol nor plasmalogens, but these are nevertheless present in high proportions in the lipids extracted from the white matter. On the other hand, analysis of the three pools of lipids extracted from brain white matter, myelin itself and crude PLP shows similar phospholipid (40–50% w/w) and galactolipid (32–34% w/w) contents in all three samples; cholesterol was once more practically absent in the lipids of crude PLP, while it was present at about 22–23% w/w in the other two pools of lipids.

Calorimetric measurements

DSC experiments with crude PLP in 20 mM sodium phosphate, pH 7.0, at 1 K/min gave rise to a highly reversible transition centred at around 52 °C (Fig. 2), similar to that reported by Mateo et al. (1986). DSC runs with the pool of lipids extracted from crude PLP under the

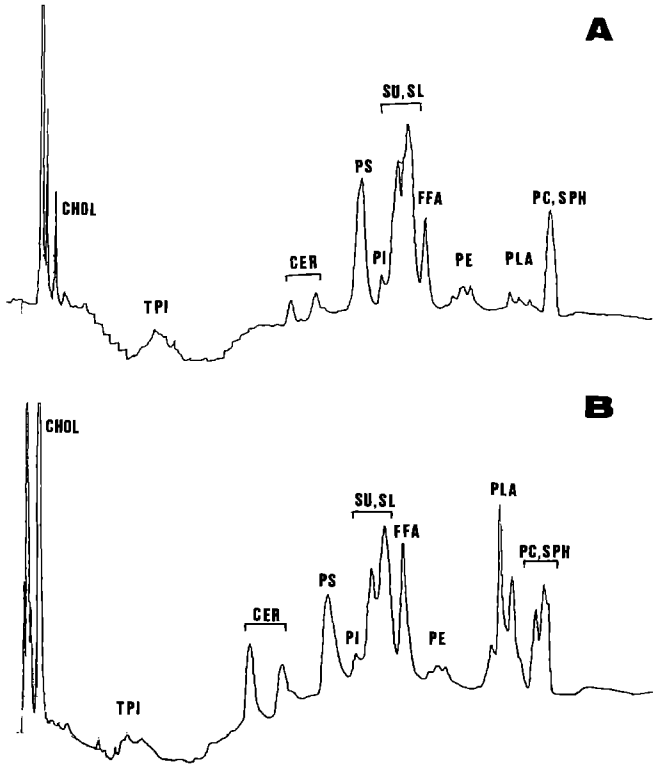

Fig. 1. HPLC recordings of **A** the mixture of lipids extracted from crude PLP and **B** the mixture of lipids extracted from brain white matter. FFA, free fatty acids; CE, cerebrosides; CHOL, cholesterol; PC, phosphatidylcholines; PE, phosphatidylethanolamines; PI, phosphatidylinositols; PLA, plasmalogens; PS, phosphatidylserines; SL, sulfolipids; SPH, sphingomyelins; SU, sulfocerebrosides; TPI, triphosphoinositols

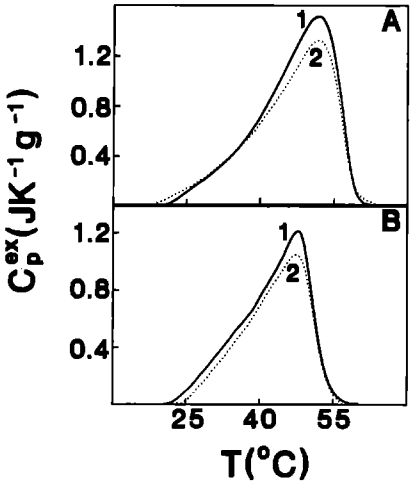

Fig. 2. Excess heat capacity values of **A** crude PLP with 4.3 mg/ml of lipid and 0.7 mg/ml of total protein and **B** the pool of lipids, 5 mg/ml, extracted from crude PLP. (*1*) First heating, (*2*) reheating of the sample. Both samples were in 20 mM phosphate buffer, pH 7.0. Scan rate 1 K/min. Excess Cp values are stated per g of lipid in the samples

Table 1. Values for the specific enthalpy, Δh (J/g of lipid), and the transition temperature, Tm (°C), of crude PLP and the pool of lipids extracted from crude PLP, with different cholesterol contents in % (mg of cholesterol added/100 mg of total lipids including the cholesterol added). Scan rate 1 K/min

Crude PLP			Pool of lipids		
%	Tm	Δh	%	Tm	Δh
0.0	51.9	27.6	0.0	47.7	18.3
4.4	48.2	20.8	3.9	45.7	17.8
10.4	47.0	17.1	12.3	38.0	13.1
18.9	45.9	10.4	16.7	32.1	2.6
31.7	45.4	8.2	23.1	30.1	1.6
41.1	47.3	5.3			

same experimental conditions as those with crude PLP also showed a comparable reversible thermal transition centred at 48 °C (Fig. 2). The van't Hoff to calorimetric enthalpy ratios (per mol of lipid) were 10 and 15 for crude PLP and the pool of lipids respectively. This ratio provides the average size of the co-operative unit (Mabrey and Sturtevant 1978), i.e. the average number of molecules to undergo the transition in a co-operative manner.

The effect of cholesterol on both transitions depicted in Fig. 2 was investigated further. It was found that increasing amounts of cholesterol originated a similar trend in both crude PLP and its pool of lipids, that is, a clear decrease in the enthalpy of the transition (Table 1), which is a well known effect of cholesterol in lipid transitions (Mabrey et al. 1978; Mabrey and Sturtevant 1978). In addition, the melting temperature of the transition, Tm, also decreased concomitantly with cholesterol content, although this effect was more pronounced for the pool of lipids (Table 1). Furthermore, the DSC recording of the pool of lipids extracted from crude PLP with the addition of 1.5 mg/ml of cholesterol (23% w/w) was very similar to the recordings of the pool of lipids extracted from both the white matter and myelin, which in fact contain about 23% w/w of cholesterol (results not shown). The transitions were always reversible with an average reversibility value (i.e. the ratio of the transition enthalpy of the first run to that of the consecutive heating of the same sample) higher than 80% and 70% for crude PLP and its pool of lipids respectively.

FTIR spectroscopy

Fourier deconvolution and fourth derivative analysis of the FTIR spectra were carried out to distinguish between the characteristic bands corresponding to the secondary structure of proteins. Figure 3 shows the original spectrum after solvent subtraction, its fourth derivative and the deconvolved amide I region corresponding to a crude PLP suspension in 20 mM phosphate buffer, pH 7.0, at 20 °C. The sample shows the usual two components in the amide I region at 1 656 cm^{-1} and 1 630 cm^{-1} wavenumbers, corresponding to the α-helix and β-structure respectively (Dong et al. 1990), with a minor component at 1 685 cm^{-1} characteristic of β-turns. These results

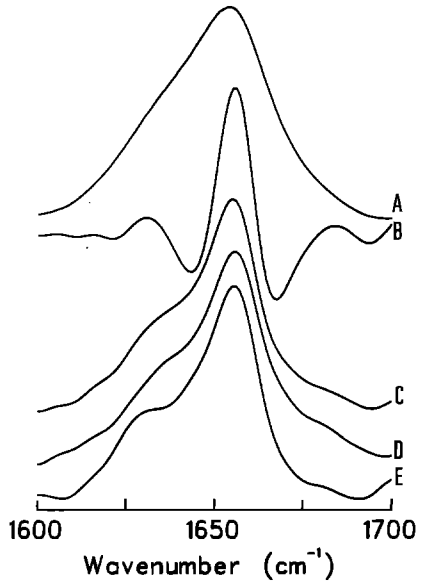

Fig. 3. The amide I region of the FTIR spectrum of crude PLP in 20 mM phosphate buffer, pH 7.0. (*A*) Original spectrum at 20 °C after solvent subtraction. (*B*) Fourth derivative of the original spectrum. Band narrowing by Fourier deconvolution of the original spectrum at (*C*) 20 °C, (*D*) 60 °C and (*E*) 20 °C after heating the sample to 80 °C

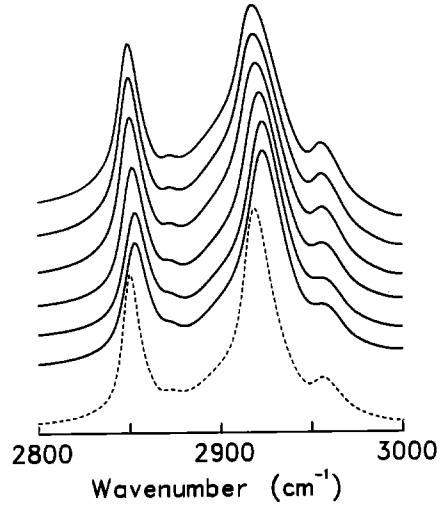

Fig. 4. FTIR spectra between 2 800 and 3 000 cm^{-1} of crude PLP in 20 mM phosphate buffer, pH 7.0, at different temperatures. Temperature values are from top to bottom 20, 30, 40, 50, 70 and 80 °C. Dashed line corresponds to the spectrum at 20 °C of the same sample previously heated to 80 °C

compare well on the whole with those obtained by various authors for the brain proteolipid apoprotein also purified using organic solvents (Surewicz et al. 1987; Ayala et al. 1987; Carmona et al. 1988).

The FTIR spectra obtained do not change appreciably when crude PLP is heated to 60 °C (Fig. 3). When the sample is heated to 80 °C, however, and then cooled down to 20 °C (Fig. 3) there is an increase in the β-structure component (1 630 cm^{-1}), while that of the α-helix decreases slightly. This would indicate that the protein is undergoing some irreversible conformational change in the temperature range from 60 °C to 80 °C, although

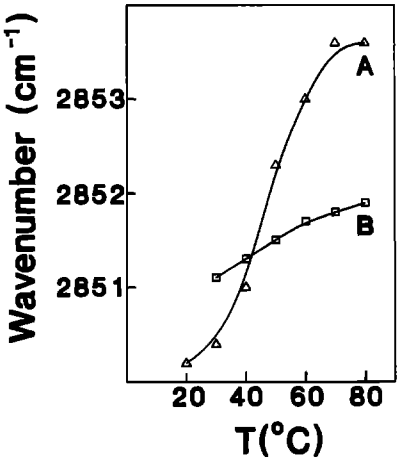

Fig. 5. Temperature dependence of the maximum wavenumber of the symmetric stretching vibration of the lipid C—H bonds of crude PLP in 20 mM phosphate buffer, pH 7.0. (*A*) In the absence of cholesterol; (*B*) in the presence of 3 mg/ml (41%, w/w) of cholesterol. Solid lines correspond to the data fitted to the two-state equilibrium model

there is no calorimetric evidence of such a change in the corresponding high-sensitivity DSC trace (Fig. 2).

FTIR experiments have also been carried out in the spectral region between 2 800 cm^{-1} and 3 000 cm^{-1}, corresponding to the symmetric (2 850 cm^{-1}) and asymmetric (2 920 cm^{-1}) stretching vibrations of the C—H bonds. This provides us with information about the static order of the hydrophobic chains in the membrane lipids (Cameron and Mantsch 1978; Cortijo and Chapman 1981; Brauner and Mendelson 1986; Jackson et al. 1989). Figure 4 shows how the wavenumber maxima for both stretching bands shift to higher values on increasing the temperature of the crude PLP sample. This shift is particularly sharper between 40 °C and 60 °C (Fig. 5). The maximum of the symmetric stretching band at 20 °C appears at 2 850 cm^{-1}, which is characteristic of a lipid gel phase, and shifts to 2 854 cm^{-1} at 80 °C (Fig. 5), indicating a liquid-crystal phase for the membrane lipids (Casal and Mantsch 1984; Jackson et al. 1989). This shift reveals a temperature-induced phase transition between 20 °C and 80 °C from a highly-ordered to a disordered conformation in the membrane lipids (Goñi et al. 1986; Muga et al. 1986), with a mid point at about 49 °C (Fig. 5). The transition is clearly reversible since the maxima in the spectra recover their position on cooling the sample to 20 °C after heating it to 80 °C (Fig. 4), which coincides with the reversible nature of the thermal transition detected by DSC within this temperature range (Fig. 2).

The effect of cholesterol on this transition is also similar to that found for the DSC transition (Table 1). Thus, increasing amounts of cholesterol in the crude PLP samples decrease the sharpness of the thermal transition as followed by FTIR spectra. Figure 5 shows the temperature dependence of the symmetric stretching wavenumber of crude PLP containing 3 mg of cholesterol per ml (about 40% cholesterol w/w). Here, for instance, the maximum of the spectrum is 2 851 cm^{-1} at 30 °C, which means that the lipids are already in an intermediate state between those of the gel and the liquid crystal. Compara-

ble spectroscopic results have been obtained for natural and synthetic lipids in the presence of increasing amounts of cholesterol (Jackson et al. 1989). FTIR lipid spectra of myelin itself also show a smooth temperature dependence (results not shown), similar to that of crude PLP in the presence of cholesterol.

Discussion

In a previous paper a reversible thermal transition detected by DSC in crude PLP centred at about 60 °C was attributed to protein denaturation (Mateo et al. 1986). We have obtained here an equivalent transition at 52 °C using a similar PLP purification procedure with organic solvents (Mateo et al. 1986; Monreal 1975; Aguilar et al. 1983). In our PLP preparations we found 14% total protein content, about 20% of which corresponds to DM-20 with no detectable MBP or Wolfgram fraction; that is to say, PLP represents about 80% of the total protein composition. The presence of DM-20 has also been detected before in PLP preparations obtained using organic solvents (Brophy 1977; Helynck et al. 1983; Bizzozero et al. 1987; Ross and Braun 1988).

The enthalpy of the transition found in our DSC experiments and that published by Mateo et al. (1986) are apparently very different, 28 J/g and 50 J/g respectively. These values refer, however, to different components, i.e. our DSC value is expressed in terms of g of lipid while the latter one is per g of protein. If we expressed our value in terms of g of protein, the enthalpy would become 172 J/g, a value seven times higher than that of globular proteins (Privalov 1979) and, therefore, clearly preposterous since membrane proteins are known to have a specific denaturation enthalpy two to four times lower than that of globular proteins (Sánchez-Ruiz and Mateo 1987). On the other hand, when the value reported by Mateo et al. (1986) is normalized to g of lipid the resulting enthalpy value is 33 J/g, which compares well with that found here (28 J/g of lipid) and is also similar to that found for the phase transitions in lipid bilayers (Mabrey and Sturtevant 1978). Hence, both enthalpy values agree with each other and seem to be much more reasonable when they are stated per g of lipid.

There are several reasons to conclude that the reported DSC endotherm for crude PLP (Mateo et al. 1986) actually corresponds to a lipid transition. Firstly, the pool of lipids extracted from crude PLP undergoes a reversible thermal transition similar to that of crude PLP (Fig. 2). The difference in the co-operative unit size, 10 for the crude PLP and 15 for the pool of lipids, agrees with the effect of proteins on the magnitude of the lipid co-operative unit (Papahadjopoulos et al. 1975; Chapman et al. 1979; Goñi 1987). Curatolo et al. (1977) studied the effect of PLP on the thermal transition of DMPC by DSC, an investigation which has also been carried out in one of our laboratories with PLP reconstituted in DMPC as well as in DPPC (López-Lacomba 1987), and in no case was there any evidence of a transition at higher temperatures that could be related to PLP denaturation.

Secondly, it is also well known that cholesterol diminishes both the Tm and enthalpy values in lipid thermal transitions, while decreasing the lipid static order below Tm and increasing it above Tm (Mabrey et al. 1978; Mabrey and Sturtevant 1978; Chapman et al. 1979). This behaviour has also been found here for both crude PLP and its pool of lipids (Table 1 and Fig. 5). DSC of lipids extracted from either brain white matter or myelin do not show any thermal effect however (results not shown). This could well indicate that some particular lipids with the capacity to nullify the DSC transition (e.g. cholesterol) might be lost during the isolation of crude PLP. In fact, our lipid analyses (see Results) show the virtual disappearance of cholesterol and plasmalogens as well as a lower proportion of free fatty acids, phosphatidylcholine and esphingomyelin in crude PLP compared to the lipid composition of white matter and of myelin. A similar conclusion had already been arrived at by Ladbrooke et al. (1968), who observed no thermal transition in lipids extracted from myelin; after eliminating cholesterol from their lipids, however, a thermal transition was found at 50 °C with an enthalpy of 12 J/g. These values compare well with ours for the lipids extracted from crude PLP (Table 1).

Thirdly, FTIR experiments of crude PLP have shown a reversible lipid phase transition centred at 49 °C (Fig. 5), comparable to that found by DSC. Furthermore, the addition of cholesterol to crude PLP lessens the shift with temperature in the symmetric stretching wavenumber of the lipid chains (Fig. 5), normal behaviour for a typical lipid phase transition (Mabrey et al. 1978; Chapman et al. 1979). Moreover, there is no noticeable change in the amide I region between 20 °C and 60 °C. These results confirm that the DSC transition detected here for crude PLP corresponds to a lipid transition with no appreciable effect deriving from the thermal denaturation of PLP.

Finally, there is a possible question concerning alterations to the native PLP conformation caused by the use of organic solvents, a fact already reported for other membrane proteins (Helenius and Simons 1975; Tanford and Reynolds 1976; Scopes 1982). The absence of any DSC endotherm attributable to PLP denaturation either in the crude PLP sample, as we have seen here, or in PLP reconstituted in synthetic lipids (Curatolo et al. 1977; López-Lacomba 1987) would seem to indicate that PLP purified using organic solvents might be already denatured. As a matter of fact, we have very recently detected a distinct DSC transition at 80 °C in myelin corresponding to PLP denaturation, which disappears after treatment with organic solvents (manuscript submitted).

Acknowledgements. This research was supported by Grants PB87-0871 (P.L.M.) and PB87-0083 (M.C.) from DGICYT, Ministerio de Educación y Ciencia (MEC), Spain. J.R-S and J.R-C acknowledge predoctoral fellowships from the MEC, Spain.

References

Aguilar JS, Cózar M, Criado M, Monreal J (1983) The delipidation of brain protein by ultrafiltration. J Neurochem 40:585–588

Ayala G, Carmona P, Cózar M, Monreal J (1987) Vibrational spectra and structure of myelin membranes. Eur Biophys J 14:219–225

Bartlett GR (1959) Phosphorus assay in column chromatography. J Biol Chem 234:466–468

Bizzozero OA, McGarry JF, Lees MB (1987) Acylation of endogenous myelin proteolipid protein with different acyl-coAs. J Biol Chem 262:2138–2145

Brauner JW, Mendelson R (1986) A comparison of differential scanning calorimetric and Fourier transform infrared spectroscopic determination of mixing behavior in binary phospholipid systems. Biochim Biophys Acta 861:16–24

Brophy PJ (1977) Association of proteolipid apoproteins from bovine myelin with phospholipid in bilayer vesicles. FEBS Lett 84:92–96

Cameron DG, Mantsch HH (1978) The phase transition of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine as seen by Fourier transform infrared difference spectroscopy. Biochem Biophys Res Commun 83:886–892

Carmona P, Cózar M, García-Segura LM, Monreal J (1988) Conformation of brain proteolipid apoprotein. Effects of sonication and n-octyl-β-D-glucopyranoside detergent. Eur Biophys J 16:169–176

Casal HL, Mantsch HH (1984) Polymorphic phase behaviour of phospholipid membranes studied by infrared spectroscopy. Biochim Biophys Acta 779:381–401

Chapman D, Gómez-Fernandez JC, Goñi FM (1979) Intrinsic protein-lipid interactions. Physical and biochemical evidence. FEBS Lett 98:211–223

Cortijo M, Chapman D (1981) A comparison of the interactions of cholesterol and gramicidin A with lipid bilayers using an infrared data station. FEBS Lett 131:245–248

Curatolo W, Sakura JD, Small DM, Shipley GG (1977) Protein-lipid interactions: recombinants of the proteolipid apoprotein of myelin with dimyristoyllecithin. Biochemistry 16:2313–2319

Dong A, Huang P, Caughey WS (1990) Protein secondary structures in water from second-derivative amide I infrared spectra. Biochemistry 29:3303–3308

Galisteo ML, Mateo PL, Sánchez-Ruiz JM (1991) Kinetic study on the irreversible thermal denaturation of yeast phosphoglycerate kinase. Biochemistry 30:2061–2066

Goñi FM (1987) Cell membrane protein. An overview. Rev Biol Chem 11:3–14

Goñi FM, Urbaneja MA, Arrondo JLR, Alonso A, Durrani AA, Chapman D (1986) The interaction of phosphatidylcholine bilayers with Triton X-100. Eur J Biochem 160:659–665

Helenius A, Simons K (1975) Solubilization of membranes by detergents. Biochim Biophys Acta 415:29–79

Helynck G, Luu B, Nussbaum JL, Picken D, Skalidis G, Trifilieff E, Dorsselaer AV, Seta P, Sandeaux R, Gavach C, Heitz F, Simon D, Spach G (1983) Brain proteolipids. Isolation, purification and effect on ionic permeability of membranes. Eur J Biochem 133:689–695

Jackson M, Haris PI, Chapman D (1989) Fourier transform infrared spectroscopic studies of lipids, polypeptides and proteins. J Mol Struct 214:329–355

Kauppinen JK, Moffat DJ, Mantsch HH, Cameron DG (1981) Fourier self-deconvolution: a method for resolving intrinsically overlapped bands. Appl Spectrosc 35:271–276

Kushwaha SC, Kates M (1981) Modification of phenol-sulphuric acid method for estimation of sugars in lipids. Lipids 16:372–373

Ladbrooke BD, Jenkinson TJ, Kamat VB, Chapman D (1968) Physical studies of myelin. I. Thermal analysis. Biochim Biophys Acta 164:101–109

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

Lees MB, Brostoff SW (1984) Proteins of myelin. In: Morell P (ed) Myelin. Plenum Press, New York, pp 197–224

López-Lacomba JL (1987) Differential scanning calorimetry study on the thermal denaturation of several proteins. Ph.D. Thesis, University of Granada

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Mabrey S, Sturtevant JM (1978) High-sensitivity differential scanning calorimetry in the study of biomembranes and related model systems. Methods Membr Biol 9:237–274

Mabrey S, Mateo PL, Sturtevant JM (1978) High-sensitivity scanning calorimetric study of mixtures of cholesterol with dimiristoyl- and dipalmitoylphosphatidylcholines. Biochemistry 17:2464–2468

Mateo PL, López-Lacomba JL, Moreno MC, Cózar M, Cortijo M, Monreal J (1986) Reversible thermal transition of brain myelin proteolipid. A preliminary report on a high-sensitivity differential scanning calorimetry study. FEBS Lett 197:221–224

Monreal J (1975) Chromatographic fractionation of brain white matter proteolipid. J Neurochem 25:539–541

Muga A, Valpuesta JM, Arrondo JLR, Goñi FM (1986) Influence of temperature on the conformation of membrane proteins as seen by FT-IR. J Mol Struct 143:465–468

Papahadjopoulos D, Moscarello M, Eylar EH, Isac T (1975) Effects of proteins on thermotropic phase transitions of phospholipid membranes. Biochim Biophys Acta 401:317–335

Privalov PL (1979) Stability of proteins. Small globular proteins. Adv Prot Chem 33:167–241

Privalov PL (1982) Stability of proteins. Proteins which do not present a single cooperative system. Adv Prot Chem 35:1–104

Privalov PL, Plotnikov VV, Filimonov VV (1975) Precision scanning microcalorimeter for the study of liquids. J Chem Thermodyn 7:41–47

Privalov PL, Potekhin SA (1986) Scanning microcalorimetry in studying temperature-induced changes in protein. Methods Enzymol 131:4–51

Ross NW, Braun PE (1988) Acylation in vitro of the myelin proteolipid protein and comparison with acylation in vivo: acylation of a cystein occurs nonenzymatically. J Neurosci Res 21:35–44

Rouser G, Felischer S, Yamamoto A (1970) Two-dimensional thin-layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. Lipids 5:494–496

Sánchez-Ruiz JM, Mateo PL (1987) Differential scanning calorimetry of membrane proteins. Cell Biol Rev 11:15–45

Sánchez-Ruiz JM, López-Lacomba JL, Cortijo M, Mateo PL (1988) Differential scanning calorimetry of the irreversible thermal denaturation of thermolysin. Biochemistry 27:1648–1652

Santiago E, Mule S, Redman CM, Hokin MR, Hokin LE (1964) The chromatographic separation of poliphosphoinositides and studies on their turnover in various tissues. Biochim Biophys Acta 84:550–562

Scopes RK (1982) Separation by precipitation. In: Scopes (ed) Protein purification: principles and practice. Springer, New York Berlin Heidelberg, pp 39–66

Siedel J, Hägale EO, Ziegenhorn J, Wahlefeld AW (1983) Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. Clin Chem 29:1075–1080

Surewicz WK, Moscarello MA, Mantsch HH (1987) Secondary structure of the hydrophobic myelin protein in a lipid environment as determined by Fourier-transform infrared spectrometry. J Biol Chem 262:8598–8602

Surewicz WK, Mantsch HH (1988) New insight into protein secondary structure from resolution-enhanced infrared spectra. Biochim Biophys Acta 952:115–130

Tanford C, Reynolds JA (1976) Characterization of membrane proteins in detergent solutions. Biochim Biophys Acta 457:133–170

Wang CS, Smith RL (1975) Lowry determination of protein in the presence of Triton X-100. Anal Biochem 63:414–417

Yandrasitz JR, Berry G, Segal S (1981) High-performance liquid chromatography of phospholipids with UV detection: optimization of separations on silica. J Chromatogr 225:319–328