Biochimica et Biophysica Acta 863 (1986) 45–52
Elsevier

BBA 72965

Modulation of myelin basic protein-induced aggregation and fusion of liposomes by cholesterol, aliphatic aldehydes and alkanes

Witold K. Surewicz [a], Richard M. Epand [a], Raquel F. Epand [a], F. Ross Hallett [b] and Mario A. Moscarello [c]

[a] *Department of Biochemistry, McMaster University, Health Sciences Centre, Hamilton, Ontario,* [b] *Department of Physics, University of Guelph, Guelph, Ontario and* [c] *Research Institute, The Hospital for Sick Children, Toronto, Ontario (Canada)*

(Received 23 June 1986)

Key words: Liposome aggregation; Myelin basic protein; Cholesterol; Aliphatic aldehyde; Alkane; (QELS)

The effect of cholesterol on myelin basic protein-induced aggregation of zwitterionic phospholipid vesicles was studied by turbidimetry, quasi-elastic light scattering and centrifugation techniques. Without cholesterol, the degree of vesicle aggregation caused by myelin basic protein is relatively low and is only slightly increased using cholesterol concentrations up to approx. 25–30 mol%. When the cholesterol content in the bilayer exceeds approx. 30 mol%, there is a dramatic increase in the susceptibility of the vesicles to aggregation in the presence of myelin basic protein. Palmitoyl aldehyde and eicosane, substances resembling products of lipid degradation, increase myelin basic protein promoted fusion of vesicles. The fusion is accompanied by increased leakage of entrapped carboxyfluorescein. In the presence of cholesterol, myelin basic protein-induced fusion of the liposomes becomes much more sensitive to the presence of aliphatic aldehydes or alkanes. The results suggest that cholesterol has an important role in promoting membrane adhesion in biological systems but these structures become unstable in the presence of small amounts of products of lipid degradation. The findings have important implications to the understanding of the stability of the myelin membrane.

Introduction

Interaction of myelin basic protein (MBP) with a lipid bilayer is thought to play a crucial role in membrane adhesion in the myelin sheath [1–6]. Protein-induced aggregation of phospholipid vesicles has received much attention as a model of membrane-membrane adhesion that is required for the stabilization of the structure of myelin [2,6–10]. It is well established that myelin basic protein induces rapid and massive aggregation of vesicles which contain a sufficient amount of acidic phospholipids [6–8]. Vesicles prepared from a major zwitterionic phospholipid, phosphatidylcholine, are much more resistant to aggregation by myelin basic protein. With these vesicles, increased membrane-membrane interaction may be observed only when relatively very high protein concentrations and long incubation times are employed [2,9]. The susceptibility of phosphatidylcholine vesicles to aggregation in the presence

Abbreviations: MBP, myelin basic protein; PC, egg yolk phosphatidylcholine; DOPE, dioleoylphosphatidylethanolamine; N-NBD-PE, *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)phosphatidylethanolamine; N-Rh-PE, *N*-(lissamine rhodamine B sulfonyl)dioleoylphosphatidylethanolamine; Pipes, 1,4-piperazinediethanesulfonic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Correspondence: Dr. R.M. Epand, Department of Biochemistry, McMaster University, Health Sciences Centre, 1200 Main Street West, Hamilton, Ontario, Canada L8N 3Z5.

of MBP is somewhat increased when another zwitterionic phospholipid, phosphatidylethanolamine, is present in the bilayer [10].

In addition to phospholipids, cholesterol is a major lipid component of myelin as well as many other biological membranes. Many properties of cholesterol, such as its effects on membrane structure, have been very extensively studied (Ref. 11 and references therein). The possible role of cholesterol in membrane-membrane interactions and particularly its role in bilayer adhesion in myelin, have received much less attention. Here we present experimental evidence that cholesterol strongly promotes MBP-induced aggregation of vesicles prepared from the two zwitterionic phospholipids, phosphatidylcholine and phosphatidylethanolamine.

Membrane association is the first step leading to membrane fusion (see, for example, Ref. 12). Membrane fusion is induced by myelin basic protein only in situations where the products of lipid degradation such as lysophosphatidylcholine [8] or aliphatic aldehydes [13] are present. When, in addition, cholesterol is present in the membrane, MBP-induced fusion becomes particularly sensitive to the presence of small amounts of the products of lipid degradation. Thus cholesterol has an important role in promoting membrane adhesion but the resulting structures become unstable in the presence of small amounts of aliphatic aldehydes or alkanes.

Experimental

Materials. Lipids were obtained from Avanti Polar Lipids, Inc. (DOPE, PC, N-Rh-PE, N-NBD-PE) and Nu-Chek-Prep, Inc. (cholesterol, eicosane). Palmitoylaldehyde was prepared by oxidation of hexadecanol (Sigma) according to the procedure of Corey and Suggs [14]. The cholesterol was recrystallized from methanol/water and showed only one spot on TLC in hexane/diethyl ether/acetic acid (70:30:1, v/v). 6-Carboxyfluorescein was from Eastman Kodak Co. Myelin basic protein was prepared according to the method of Lowden et al. [15]. Bovine pancreatic ribonuclease, poly(L-lysine)HBr (mol. wt. 40000) and poly(L-arginine)HCl (mol. wt. 40000) were obtained from Sigma Chemical Co. Bovine serum albumin (Fraction V) was from Boehringer Mannheim.

Preparation of vesicles. Small unilamellar vesicles for all experiments were prepared by dissolving the appropriate mixture of lipids in chloroform/methanol (2:1, v/v), evaporating the solvent to dryness and resuspending the sample in buffer. The mixture was subsequently sonicated under nitrogen to visual clarity in a bath-type sonicator at approx. 4°C. For assays of vesicle aggregation a buffer of 10 mM Pipes, 150 mM NaCl, 0.1 mM EDTA (pH 7.4) was used. In order to remove residual larger lipid aggregates, the resulting suspension was centrifuged at $40000 \times g$ for 1 h, and the clear supernatant was used for aggregation studies. Vesicles were kept at room temperature and used within a few hours after preparation.

Turbidimetric assay of vesicle aggregation. Vesicle aggregation was measured by an increase in the turbidity of the suspension at 500 nm [2,9,10,16]. Lipid concentration was 0.5 mg/ml and experiments were performed at room temperature using a Gilford, Model 2400 spectrophotometer. Results are expressed as a ratio of turbidity before addition of protein (zero time) to that after 30 min incubation in the presence of MBP.

Quasi-elastic light scattering. Quasi-elastic light scattering (see Ref. 17 for review) was used to determine the hydrodynamic diameters of the vesicles before and after aggregation. A 15 mW helium-neon laser (Jodon Eng. Assoc. Inc., Ann Arbor, MI) producing light of wavelength 632.8 nm was focused into the sample. The sample chamber has been described in detail elsewhere [18]. The detection system included a quantum photometer (PAR Instruments Inc., Princeton, New Jersey, Model 1140) and a dedicated autocorrelator (Langley-Ford, Amherst, MA, Model 1096). The scattering angle for all experiments was 90°.

Analysis of the experimental autocorrelation function was performed using the method of exponential sampling [19] to obtain an average correlation time for each function. Average hydrodynamic diameters of the vesicles and aggregates were obtained using the standard Stokes-Einstein relation.

Centrifugation assay for aggregation of vesicles. This assay made use of the change in sedimenta-

tion coefficient as a result of vesicle aggregation [20]. Vesicles (0.5 mg/ml total lipid) containing 1 mol% of N-Rh-PE were incubated without or in the presence of myelin basic protein for 30 min at room temperature. The suspension was then centrifuged at $10000 \times g$ for 15 min at 20°C and the supernatant was separated from the pellet. The fraction of lipid in the supernatant and in the pellet was determined by measuring the absorbance of the dye lipid in the supernatant at 570 nm after disruption of the vesicles with Triton X-100. This absorbance was compared with the total absorbance obtained from protein-free, non-centrifuged vesicles. Results are expressed as percent of total lipid recovered in the pellet.

Carboxyfluorescein release assay. Carboxyfluorescein (250 mM) in 10 mM Hepes buffer (pH 7.4), 0.1 M NaCl, 0.002% NaN_3 was entrapped in sonicated liposomes as previously described [10]. External carboxyfluorescein was removed by gel filtration through a Sephadex G-50 column (20×1 cm) using 10 mM Hepes (pH 7.4), 0.1 M NaCl, 0.002% NaN_3 as eluant. Leakage from vesicles was examined at 25°C by the technique of Weinstein et al. [21]. As the highly self-quenched entrapped carboxyfluorescein leaks from vesicles, it becomes diluted, and as a result, an increase in fluoresence is observed. Fluorescence changes corresponding to dye efflux were recorded with excitation and emission wavelengths of 490 and 520 nm, respectively. The carboxyfluorescein release is expressed as:

$$\% \text{ Release} = \frac{F_2 - F_1}{F_3 - F_1}$$

where F_1 is the initial fluorescence (in the absence of protein); F_2 is the fluorescence after an incubation period, F_3 is the fluorescence after disruption of the vesicles by addition of 100 μl of 5% Triton X-100 (100% dye release). The amount released from vesicles in the absence of protein was subtracted from the protein-induced release.

Carboxyfluorescein assay for leaky fusion. In order to determine if the carboxyfluorescein leakage depended on vesicle-vesicle interactions, we measured the effect of the addition of unlabelled sonicated vesicles on the rate of carboxyfluorescein leakage. These experiments were performed as described above except that to one set of curvettes an addition 1 mg/ml of unlabelled sonicated vesicles was added (about 20-fold higher concentration than the vesicles with carboxyfluorescein entrapped).

Resonance energy transfer assay of lipid intermixing. Lipid intermixing was assayed by the loss of resonance energy transfer between N-NBD-PE and N-Rh-PE incorporated at 1 mol% each into the same vesicle [22,23] as previous described [13]. The lipid intermixing assay, based on dilution of membrane-incorporated fluorophores, was carried out as follows: 50 μl of labelled vesicles (lipid concentration of 1.5 mg/ml) and 50 μl of unlabelled vesicles (lipid concentration of 7.5 mg/ml) were added to a cuvette containing 2 ml of buffer (10 mM Hepes (pH 7.4), 0.1 M NaCl, 0.002% NaN_3) at 25°C and the ratio of fluorescence emission at 530 and 591 nm was determined (excitation wavelength, 450 nm). The fluorescence ratio of the vesicles alone shows almost no change with time of incubation over a period of 30 min. The fluorescence was also measured as a function of time after the addition of protein. The extent of lipid intermixing was calculated as

$$\% \text{ Intermixing} = [(C_o/C) - 1]100/(D - 1)$$

where C_o is the initial fluorophore concentration in the membrane; C, the concentration of the fluorophore in the membrane after incubation with myelin basic protein; D, the maximal possible dilution (six in the present assay). Values of C were obtained from standard curves made from measurements of fluorescence emission intensity ratios for a series of liposomes prepared from lipid films containing the labelled lipids and varying amounts of unlabelled lipid which had been dissolved together in chloroform/methanol. Addition of myelin basic protein had little effect on the standard curve.

Results

Fig. 1 shows turbidity changes of PC/DOPE (2 : 1) vesicles, containing various amounts of cholesterol, upon 30 min incubation of the samples in the presence of various concentrations of myelin basic protein. Without protein there is no

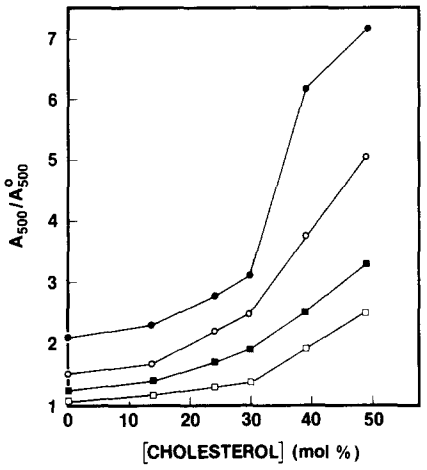

Fig. 1. Turbidity changes of the suspensions of PC/DOPE (2:1) vesicles containing various amounts of cholesterol upon 30 min incubation of the samples in the presence of various amounts of myelin basic protein (MBP). A_{500}/A^0_{500} is the ratio of turbidity at 500 nm of MBP-aggregated vesicles to that of protein-free vesicles. The concentration of myelin basic protein is 0.005 mg/ml (□), 0.01 mg/mll (■), 0.025 mg/ml (○) and 0.05 mg/ml (●). Each point represents the average of 4–6 independent experiments.

change in light scattered by the vesicles. Upon addition of myelin basic protein, a concentration-dependent increase in light scattering is observed, indicating protein-induced aggregation of small vesicles. With cholesterol-free vesicles the extent of this aggregation is relatively small. The presence of cholesterol in the membrane promotes MBP-induced aggregation in a concentration-dependent manner. At cholesterol concentrations of up to 25–30 mol%, only a small increase in the susceptibility of the vesicles to aggregation by myelin basic protein is observed. However, at higher cholesterol concentrations (30–49 mol%) the protein-induced increase in light scattering becomes much more dramatic, indicating a massive aggregation of the vesicles. The general shape of the turbidity increase vs. cholesterol content plots is observed for all protein concentrations employed although, of course, the extent of the aggregation increases with increasing concentrations of myelin basic protein.

The aggregated vesicles are no longer small, relative to the wavelength of light, and the turbidimetric measurements provide only a qualitative indication of the increase of particle size due to

TABLE I

PROPERTIES OF LIPID VESICLES IN THE PRESENCE OF MYELIN BASIC PROTEIN (MBP)

Lipid composition	[MBP] (μg/ml)	(D^{av}/D_0^{av}) [a]	% Lipid in pellet
PC/DOPE (2:1)	25	4±1	8±2
	50	– [b]	12±1
PC/DOPE/cholesterol (2:1:2.9)	25	20±4	42±5
	50	– [b]	60±6

[a] D^{av}/D_0^{av} is the ratio of the average particle diameter after 30 min incubation of the vesicles with myelin basic protein to that of protein-free vesicles. This ratio was determined by quasi-elastic light scattering experiments.

[b] Not determined.

vesicle aggregation. A better and more reliable measure of the increased size of the particles may be obtained from quasi-elastic light scattering experiments. The results of such experiments indicate that the increase in average particle size induced by myelin basic protein is several-fold larger for vesicles containing 49 mol% cholesterol compared to cholesterol-free vesicles (Table I). This confirms the conclusion reached from turbidimetric experiments that cholesterol promotes MBP-induced aggregation of PC/DOPE vesicles. The above conclusion is further supported by the observation that cholesterol-containing vesicles incubated with myelin basic protein are much more readily pelleted than cholesterol-free vesicles, indicating formation of larger structures with the cholesterol-containing vesicles (Table I). No lipid material was pelleted under present experimental conditions during centrifugation of the vesicles in the absence of basic protein.

We have previously demonstrated that the presence of aliphatic aldehydes in membranes promotes MBP-induced fusion [13]. Since the first step in the fusion of vesicles is their self-association, we would anticipate that cholesterol would further sensitize vesicle containing aliphatic aldehydes to fusion by myelin basic protein. Aliphatic aldehydes may promote fusion by partitioning predominantly into the hydrocarbon portion of the membrane and thereby destabilizing the bilayer phase in a manner analogous to the effects of alkanes [24]. In this work, we also tested the effect of the 20-carbon alkane, eicosane, on vesicle sta-

bility and fusion. By the criterion of the resonance energy transfer assay of lipid mixing, eicosane is at least as effective as palmitoyl aldehyde in promoting the MPB-induced fusion of vesicles (Fig. 2). The results also demonstrate that cholesterol promotes lipid mixing and that 6% eicosane promotes much greater lipid mixing in cholesterol-containing vesicles than does 10% eicosane in vesicles of the same phospholipid composition but without cholesterol.

The extent of lipid mixing promoted by substances other than myelin basic protein was measured using vesicles composed of cholesterol/PC/DOPE/eicosane (molar ratio 0.4 : 0.3 : 0.3 : 0.06). Addition of 10 mM $CaCl_2$ did not induce lipid mixing nor did it affect MBP-induced lipid mixing. Neither bovine pancreatic ribonuclease, a protein with an alkaline p*I*, nor bovine serum albumin, a protein with some lipid-binding properties, induced lipid mixing in this system. However, the basic poly-amino acids, poly(L-lysine) and poly(L-arginine) were more effective than myelin basic protein in inducing lipid mixing, particularly during the first few minutes after peptide addition. The time-course of the poly(amino acid)-induced fusion is not presented because incubation of these samples resulted in the formation of large aggregates with apparent quenching of fluorescence.

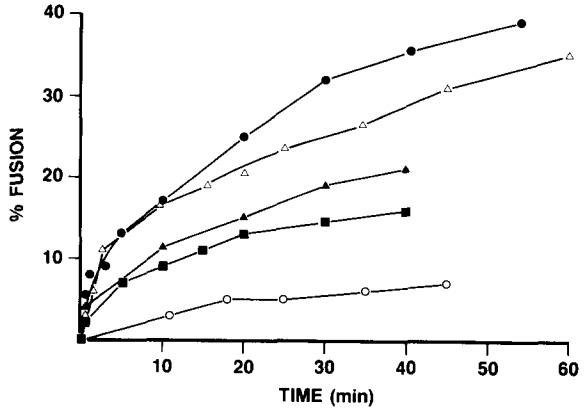

Fig. 2. Resonance energy transfer assay of lipid intermixing. Myelin basic protein (final concentration 5 μg/ml) was added to a solution containing unlabelled vesicles (187 μg/ml) and vesicles of identical lipid composition but containing 1 mol% each of the fluorescent labelled lipids N-NBD-PE and N-Rh-PE (37.5 μg/ml) at 25°C. The lipid compositions of the vesicles are as follows: ●, Cholesterol/PC/DOPE/eicosane (molar ratio 0.4 : 0.3 : 0.3 : 0.06); △, Cholesterol/PC/DOPE/palmitoyl aldehyde (molar ratio 0.4 : 0.3 : 0.3 : 0.06); ▲, Cholesterol/PC/DOPE (molar ratio 0.4 : 0.3 : 0.3); ■, PC/DOPE/palmitoyl aldehyde (molar ratio 0.5 : 0.5 : 0.1) (The curve for PC/DOPE/eicosane (molar ratio 0.5 : 0.5 : 0.1) is superimposable on this curve.); ○, PC/DOPE (molar ratio 1 : 1).

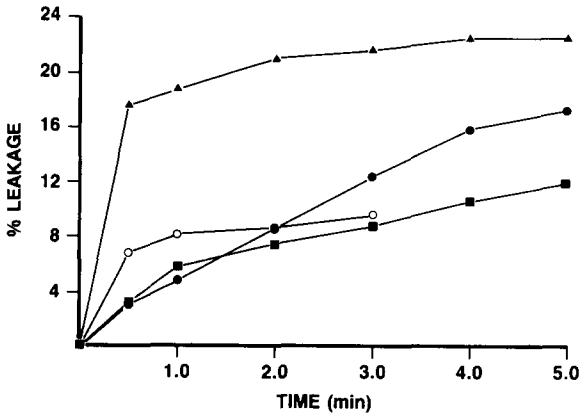

Fig. 3. Carboxyfluorescein leakage and leaky fusion assay. Myelin basic protein (7 μg/ml) added at zero time. PC/DOPE/cholesterol/eicosane in 0.4 : 0.3 : 0.3 : 0.06 molar ratio at a labelled vesicle concentration of 50 μg/ml (●) and with the addition of 1 mg/ml unlabelled vesicles (▲). PC/DOPE/cholesterol in a 0.4 : 0.3 : 0.3 molar ratio at a labelled vesicle concentration of 50 μg/ml (■) and with the addition of 1 mg/ml unlabelled vesicles (○).

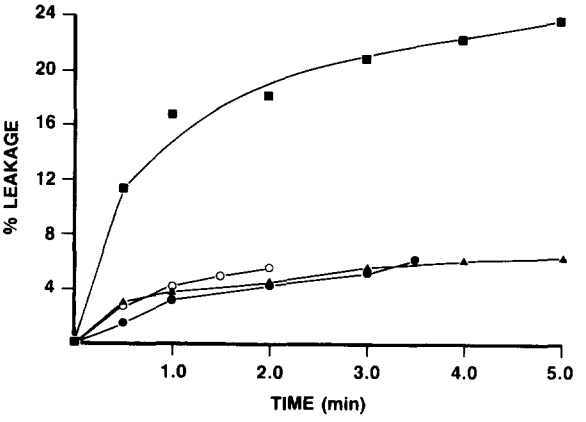

Fig. 4. Carboxyfluorescein leakage and leaky fusion assay. Poly(L-lysine) (7 μg/ml) added at zero-time. PC/DOPE/cholesterol/eicosane in 0.4 : 0.3 : 0.3 : 0.06 molar ratio at a labelled vesicle concentration of 50 μg/ml (●) and with the addition of 1 mg/ml unlabelled vesicles (■). PC/DOPE/cholesterol in a 0.4 : 0.3 : 0.3 molar ratio at a vesicle concentration of 50 μg/ml (▲) and with the addition of 1 mg/ml unlabelled vesicles (○).

The effect of myelin basic protein (Fig. 3) and poly(L-lysie) (Fig. 4) on carboxyfluorescein leakage was measured. Myelin basic protein is more effective than poly(L-lysine) in inducing vesicle leakage. The presence of additional unlabelled vesicles, particularly those containing eicosane, further enhanced vesicle leakage. These results demonstrate that vesicle aggregation is accompanied by bilayer destabilization which results in carboxyfluorescein leakage. Attempts to measure the mixing of aqueous contents by the Tb-dipicolinic acid assay [25] were unsuccessful, perhaps because in this system vesicle fusion is accompanied by increased vesicle permeability.

Discussion

The studies reported in this paper show that cholesterol promotes MBP-induced aggregation of small unilamellar vesicles prepared from zwitterionic phospholipids, as measured by turbidimetry, quasi-elastic light scattering and increased pelleting of the vesicles. The aggregation-promoting effect of cholesterol is particularly dramatic when the concentration of cholesterol in the bilayer exceeds approx. 30 mol%. Cholesterol comprises about 40 mol% of the lipid components of the myelin membrane [26]. The molecular mechanism of the above aggregation-promoting effect of cholesterol is at present not fully understood but it may be related to the fact that cholesterol in bilayers does not bind as much water as do phospholipids. As the removal of water is thought to be one of the major barriers to vesicle-vesicle contact [27], cholesterol-induced dehydration of the bilayer surface is likely to be an important factor in promoting vesicle contact. The aggregated vesicles will then be more prone to fuse through the formation of bilayer defects. Substances which promote hexagonal phase formation also induce membrane fusion. Several of the components of our system which promote vesicle fusion have also been shown to induce hexagonal phase formation. These substances include cholesterol [28], alkanes [24] and myelin basic protein [29]. The fusogenic activity of myelin basic protein is not unique to that protein but also occurs with poly(L-lysine) and poly(L-arginine). Polyamines such as spermine also promote the fusion of vesicles containing a high proportion of phosphatidylethanolamine [30]. Contact of vesicles containing an abundance of phosphatidylethanolamine has been shown to induce hexagonal phase formation followed by leakage of vesicle contents [31]. Such a phenomenon may occur with myelin basic protein or poly(L-lysine) induced fusion of vesicles containing PC, DOPE, cholesterol and eicosane (Figs. 3 and 4).

The aggregation-promoting effect of cholesterol found in a simple model vesicle system may be of direct relevance to the physiological role of cholesterol. Particularly, in view of the present results, it is tempting to speculate about the possible functional role of cholesterol in myelin. In addition, it has recently been reported that cholesterol increases the ability of myelin basic protein to aggregate vesicles of phosphatidylserine [32]. The high level of cholesterol in this membrane may contribute to the factors responsible for the interlamellar adhesive forces which are necessary for the formation and maintenance of the compact multilamellar structure of the myelin membrane. The role of cholesterol in the MBP-induced aggregation of the vesicles may, however, reflect a more general property of cholesterol. Recently, it has been reported [16] that zwitterionic phospholipid vesicles containing equimolar amounts of cholesterol are readily aggregated by calcium phosphate. No calcium phosphate-induced aggregation was, however, observed with cholesterol-free vesicles. The increased susceptibility of the vesicles to aggregation in the presence of cholesterol may also have important implications for the mechanism of biomembrane fusion, as aggregation of the membranes is a necessary condition for any fusion event to occur. Several reports have appeared which document the requirement for cholesterol in certain types of membrane fusion [33–35].

Non-leaky vesicle fusion can only be proven by demonstrating mixing of the aqueous contents of the vesicles without exposure to the external environment. However, model systems of membrane fusion result in intermediates or products which are at least somewhat leaky. It is the balance between increased vesicle permeability and the rate of fusion which determines whether mixing of aqueous contents can be detected in the initial

stages of fusion. In the present case, the vesicles become too permeable in the presence of the basic protein for mixing of aqueous contents to be detected. Nevertheless, it is likely that fusion is taking place. Using vesicles containing both cholesterol and eicosane the mixing of lipids upon addition of basic protein is rapid and extensive making it unlikely that the mechanism is via lipid exchange. We have previously shown by freeze-fracture electron microscopy that the product of the interaction of myelin basic protein with palmitoylaldehyde-containing vesicles is fused [13]. Finally, the leakiness of the membrane increases with increased concentration of vesicles (Fig. 3) demonstrating that the bilayer is destabilized at the point of vesicle-vesicle contact. This destabilization of the bilayer is promoted by the presence of palmitoyl aldehyde or eicosane. These substances were chosen for the present study because they represent the types of molecules which could accumulate in the myelin membrane as a result of membrane degradation. Aliphatic aldehydes are produced by the action of plasmalogenase on the myelin membrane. The increase in the activity of this enzyme is thought to lead to demyelination [36,37]. Alkanes are products of lipid peroxidation. Lipid peroxidation has been suggested to be of primary importance in inducing alterations in the physical properties of the myelin membrane [38,39]. Thus, cholesterol and myelin basic protein normally stabilize the compact multilamellar structure of myelin but in the presence of products of lipid degradation such as lysophosphatidylcholine [8], alkanes or aliphatic aldehydes the bilayer is destabilized and fusion processes are accelerated. This fusion would lead to a breakdown of the permeability barrier of myelin [1].

Acknowledgement

This work was supported by Grant MT7654 from the Medical Research Council of Canada.

References

1 Epand, R.M. (1986) in Neurobiological Research, Vol. 2, Functional and Clinical Aspects of Neuronal and Glial Proteins (Marangos, P.J., Campbell, I. and Cohen, R.M., eds.), Academic Press, Orlando, FL, in the press
2 Smith, R. (1977) Biochim. Biophys. Acta 470, 170–184
3 Golds, E.E. and Braun, P.E. (1978) J. Biol. Chem. 253, 8162–8170
4 Rumsby, M.G. (1978) Biochem. Soc. Trans. 6, 448–462
5 Brady, G.W., Murthy, N.S., Fein, D.B., Wood, D.D. and Moscarello, M.A. (1981) Biophys. J. 34, 345–350
6 Young, P.R., Vacante, D.A. and Snyder, W.R. (1982) J. Am. Chem. Soc. 104, 7287–7291
7 Lampe, P.D., Wei, G.J. and Nelsestuen, G.L. (1983) Biochemistry 22, 1594–1599
8 Lampe, P.D. and Nelsestuen, G.L. (1982) Biochim. Biophys. Acta 693, 320–325
9 Sridhara, S.I., Epand, R.M. and Moscarello, M.A. (1984) Neurochem. Res. 9, 241–248
10 Epand, R.M., Dell, K., Surewicz, W.K. and Moscarello, M.A. (1984) J. Neurochem. 43, 1550–1555
11 Yeagle, P.L. (1985) Biochim. Biophys. Acta 822, 267–287
12 Nir, S., Stegmann, T. and Wilschut, J. (1986) Biochemistry 25, 257–266
13 Surewicz, W.K., Epand, R.M., Vail, W.J. and Moscarello, M.A. (1985) Biochim. Biophys. Acta 820, 319–323
14 Corey, E.J. and Suggs, J.W. (1975) Tetrahedron Lett. 31, 2647–2650
15 Lowden, J.A., Moscarello, M.A. and Morecki, R. (1966) Can. J. Biochem. 44, 567–577
16 Ohki, S. and Leonards, K.S. (1984) Biochemistry 23, 5578–5581
17 Schurr, J.M. (1977) CRC Crit. Rev. Biochem. 4, 371–431
18 Racey, T., Hallett, F.R. and Nickel, B. (1981) Biophys. J. 35, 557–571
19 Ostrowsky, N., Sornette, D., Parker, P. and Pike, E.R. (1981) Optica Acta 28, 1059–1070
20 Kumar, N., Blumenthal, R., Henkart, M., Weinstein, J.N. and Klausner, R.D. (1982) J. Biol. Chem. 257, 15137–15144
21 Weinstein, J.N., Yoshikami, S., Henkart, P., Blumenthal, R. and Hagins, W.A. (1977) Science (Washington, DC) 195, 489–492
22 Struck, D.K., Hoekstra, D. and Pagano, R.E. (1981) Biochemistry 20, 4093–4099
23 Conner, J., Yatvin, M.B. and Huang, L. (1984) Proc. Natl. Acad. Sci. USA 81, 1715–1718
24 Epand, R.M. (1985) Biochemistry 24, 7092–7095
25 Düzgüneş, N., Wilschut, J., Fraley, R. and Papahadjopoulos, D. (1981) Biochim. Biophys. Acta 642, 185–195
26 Rumsby, M.G. (1978) Biochem. Soc. Trans. 6, 448–462
27 Rand, R.P. (1981) Annu. Rev. Biophys. Bioeng. 10, 277–314
28 Tilcock, C.P.S., Bally, M.B., Farren, S.B. and Cullis, P.R. (1982) Biochemistry 21, 4596–4601
29 Smith, R. and Cornell, B.A. (1985) Biochim. Biophys. Acta 818, 275–279
30 Schuber, F., Hong, K., Düzgüneş, N. and Papahadjopoulos, D. (1983) Biochemistry 22, 6134–6140
31 Bentz, J., Ellens, H., Lai, M.-Z. and Szoka, F.C., Jr. (1985) Proc. Natl. Acad. Sci. USA 82, 5742–5745
32 Walter, A.G. and Rumsby, M.G. (1985) Neurochem. Int. 7, 441–447
33 Hope, M.J., Bruckdorfer, K.R., Hart, C.A. and Lucy, J.A. (1977) Biochem. J. 166, 255–263
34 Hsu, M.-C., Scheid, A. and Choppin, P.W. (1983) Virology 126, 361–369

35 Umeda, M., Nojima, S. and Inoue, K. (1985) J. Biochem. 97, 1301–1310
36 Horocks, L.A., Spanner, S., Mozzi, R., Fu, S.C., D'Amato, R.A. and Krakowka, S. (1978) Adv. Exp. Med. Biol. 100, 423–438
37 Fu, S.C., Mozzi, R., Krakowka, S., Higgins, R.J. and Horrocks, L.A. (1980) Acta Neuropathol. 49, 13–18
38 Chia, L.S., Thompson, J.E. and Moscarello, M.A. (1983) FEBS Lett. 157, 155–158
39 Chia, L.S., Tompson, J.E. and Moscarello, M.A. (1984) Biochim. Biophys. Acta 775, 308–312