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Phase Separation of Acidic and Neutral Phospholipids Induced by Human Myelin Basic Protein†

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ABSTRACT: Differential scanning calorimetry was used to detect lipid phase separation induced in mixtures of acidic and neutral phospholipids by myelin basic protein from human central nervous system myelin. Phosphatidic acid, phosphatidylglycerol, and phosphatidylserine mixtures with phosphatidylcholine (PC) were used and were shown to be nearly randomly mixed in the absence of the protein. Incorporation of basic protein into these mixtures caused a shift in the phase transition temperature toward the temperature of the PC component, indicating that it binds and separates out the acidic lipid leaving a PC-enriched phase. In some cases, a transition due to the acidic lipid-basic protein complex was also observed. The shift toward the transition temperature of the PC component occurred regardless of whether the PC was the lower melting or the higher melting component of the mixture. Thus, the protein does not just bind to the lipid which melts first, but binds to the acidic lipid even if it melts at a much higher temperature than the neutral lipid. If enough acidic lipid is available, the protein can bind to 27-34 molecules of acidic lipid per molecule of protein. At pH 7.4 basic protein has 38 basic residues; thus, nearly all of these can be involved in electrostatic binding to acidic lipid polar head groups resulting in lipid phase separation.

There is abundant evidence which indicates that both lipids and proteins are asymmetrically distributed across certain cell membranes, but it is not clear how this asymmetry is induced and maintained (Rothman and Lenard, 1977). Early x-ray diffraction studies showed that the repeat distance of myelin was equivalent to the thickness of two membranes, indicating an asymmetric arrangement within each of the membranes (Schmitt et al., 1941; Blaurock and Worthington, 1969). Determination of the electron density profiles of myelin suggested that this asymmetry occurs both at the polar head group-aqueous interface and in the hydrocarbon region of the membrane (Caspar and Kirshner, 1971). It was suggested that the asymmetry in the hydrocarbon region was due to an asymmetric distribution of cholesterol, although it is equally possible that it is caused by an asymmetric distribution of other lipids and/or intrinsic proteins. Specific binding of proteins to certain lipid classes resulting in phase separation could be one way in which lipid asymmetry is induced and maintained. In order to investigate this possibility, we are studying the ability of myelin proteins to induce lipid phase separation when incorporated individually into lipid vesicles containing both acidic and neutral phospholipids randomly mixed. We reported earlier that a hydrophobic intrinsic protein, lipophilin, isolated from the proteolipid of human CNS myelin, is embedded in the hydrocarbon region and immobilizes some lipid surrounding it in the form of "boundary lipid" (Boggs et al., 1976). This protein causes phase separation by binding the acidic lipid preferentially to its boundary layer (Boggs et al., 1977).

In this study, we present evidence using differential scanning calorimetry (DSC) which indicates that the basic protein or encephalitogenic component from myelin, which interacts with lipid primarily by electrostatic interactions, can also cause lipid phase separation in a mixture of acidic and neutral lipids by binding to the polar head groups of acidic lipids.

Materials and Methods

Preparation of Basic Protein. Myelin was isolated from normal human white matter and basic protein extracted from it by the method of Lowden et al. (1966). The protein was stored in the lyophilized form.

Lipids. Phosphatidylserine (PS)[1] was isolated from bovine brain, phosphatidic acid (PA) was obtained from egg yolk phosphatidylcholine, and dimyristoylphosphatidic acid (DMPA) from dimyristoylphosphatidylcholine (DMPC) as described earlier (Papahadjopoulos and Miller, 1967; Papahadjopoulos et al., 1976). Phosphatidylglycerol (PG) was prepared enzymatically from egg phosphatidylcholine by a modification of the method of Dawson (1967). Dipalmitoylphosphatidylglycerol (DPPG) and dimyristoylphosphatidylcholine (DMPC) were synthesized as described earlier (Papahadjopoulos et al., 1973).

These lipids were stored in chloroform under nitrogen in sealed ampules at −50 °C. Dipalmitoylphosphatidylcholine (DPPC) was purchased from Serdary Research Laboratory

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[1] Abbreviations used: PS, phosphatidylserine; PA, phosphatidic acid; DMPA, dimyristoylphosphatidic acid; DMPC, dimyristoylphosphatidylcholine; PG, phosphatidylglycerol; DPPG, dipalmitoylphosphatidylglycerol; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; DSC, differential scanning calorimetry.

(London, Ontario) and was stored in the dry form under nitrogen at −50 °C. All lipids were chromatographically pure. [^{14}C]DPPC was purchased from Applied Science Lab Inc. with a specific activity of 25 mCi/mol.

2-Chloroethanol was obtained from Coleman and Bell, redistilled periodically, and stored in the dark at 4 °C. *N*-2-Hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) was obtained from Calbiochem.

Preparation of Vesicles. Basic protein was incorporated into lipid vesicles by one of three methods as described earlier (Papahadjopoulos et al., 1975; Boggs and Moscarello, 1977). These were by dialysis from 2-chloroethanol, by evaporation of the lipid and dispersion into buffer containing basic protein, or by addition of the protein dissolved in buffer to preformed sonicated vesicles. In all cases, the temperature at which the vesicles were prepared was 3–4 °C above the phase transition temperature of the highest melting component. The buffer contained NaCl (10 mM), Hepes (2 mM), and EDTA (1 mM) adjusted to pH 7.4. In all three procedures used, a precipitate consisting of multilayered aggregates was obtained in samples containing basic protein. For some of the lipid samples prepared by dialysis, only a small fraction of the initial lipid could be sedimented; however, enough sample was obtained for DSC. Multilameller vesicles of lipid prepared by evaporating lipid and dispersing in buffer were used as a control for those samples which were prepared by adding basic protein to sonicated liposomes since this results in fusion into multilayered aggregates.

The suspensions were centrifuged at 10 000 rpm for 5 min at room temperature in an Eppendorf centrifuge 3200 if a precipitate was obtained or at 40 000 rpm for 1 h at 4 °C in a SW 50.1 rotor if only a fine suspension was obtained.

For samples containing DPPC and prepared by dialysis, an aliquot of [^{14}C]DPPC was added to give a specific activity of 2×10^5 dpm/mg. Aliquots (50–100 μL) of the pellet suspended in buffer and the initial solution of lipids were evaporated in scintillation vials and counted for ^{14}C in a Nuclear Chicago Mark I liquid scintillation system to obtain the DPPC content, and similar aliquots were analyzed for total phospholipid content by measuring phosphate according to a modified Bartlett procedure (1959). The acidic lipid content was obtained by subtracting DPPC from total phospholipid. Protein content was measured by amino acid analysis on a Technicon TSM amino acid analyzer.

Differential Scanning Calorimetry. The temperature and heat content of the thermotropic transition of the phospholipids in vesicle preparations with and without protein was determined with a differential scanning calorimeter (Perkin-Elmer DSC-2) using scanning rates of 10 °C/min or 5 °C/min. Vesicle pellets were taken up in micropipets (10–15 μL) and transferred to the sample pans. The reference pan contained buffer. The transition temperature, T_c, is defined as the peak of the endothermic transition.

Results

If two phospholipids are randomly mixed, the transition temperature of the mixture will be a nearly linear function of the concentration of each component (Chapman, 1975). This is generally the case for the mixtures of acidic and neutral phospholipids used in this study (PA with PC and PG with PC) as shown in Figures 6A and 6B. If they are not ideally mixed, a plot of transition temperature against composition may deviate significantly from linearity as occurs for PS–PC (Figure 6C), and if they do not mix at all, two transitions will be observed corresponding to the two pure, or nearly pure, components.

Previous studies have shown that myelin basic protein binds only to acidic lipids (Palmer and Dawson, 1969; Demel et al., 1973) and that the binding is accompanied by a decrease in both the temperature and enthalpy of the transition of the lipid (Papahadjopoulos et al., 1975). If basic protein binds preferentially to an acidic lipid in random, or nearly random mixtures of acidic and neutral phospholipids, the resulting phase separation will alter the transition temperature and appearance of the DSC scan. If it completely separates out some of the acidic lipid, two transitions will be observed: one due to the acidic lipid–basic protein complex and one due to the remaining lipid at a temperature shifted toward that of the pure PC component. The concentration of the acidic lipid–basic protein complex may not be high enough to result in an observable transition, however, so that only one peak may be observed. This peak will be shifted to a higher temperature if the acidic lipid is the lower melting component and to a lower temperature if the acidic lipid is the higher melting component. Instead of binding and segregating the pure acidic lipid, the protein may bind to a lipid mixture which has a high concentration of the acidic lipid separating it from the remaining lipid which would have a high concentration of the neutral lipid. In this case, two transitions will be observed above and below the transition temperature of the random mixture.

However, since basic protein alters the phase transition temperature of pure lipids, it may have a similar effect on a mixture of lipids without causing phase separation. Although it decreases the phase transition temperature of most acidic lipids (Papahadjopoulos et al., 1975; Boggs et al., 1977) basic protein has also been observed to increase the phase transition temperature of dilauroylphosphatidylglycerol (Verkleij et al., 1974). Therefore, either a decrease or an increase in the phase transition temperature induced by basic protein is not sufficient by itself to conclude that phase separation has occurred.

In order to allow unambiguous interpretation of the DSC results, we have studied the ability of basic protein to separate out either PA or PG from mixtures with PC using two sets of lipids for each, such that in one set the acidic lipid is the higher melting component and in the other it is the lower melting component. Thus, the following combinations of lipids were chosen: egg PA–DPPC, DMPC–DMPA, egg PG–DPPC, and DMPC–DPPG. A mixture of PS–DPPC was also used, but a higher melting PS was not available.

Incorporation of basic protein into egg PA or DMPA results in the appearance of two peaks in the DSC scan, one at the same temperature as the pure lipid and the other 8 °C lower for PA and 4.6 °C lower for DMPA (Table I, Figures 1a and 1b and Figures 2b and 2c). It has no effect on the T_c of DMPC or DPPC.

The effects of basic protein on the DSC scans of equimolar mixtures of PA–DPPC and DMPC–DMPA are shown in Figures 1 and 2, respectively. The equimolar mixtures give single broad peaks about halfway between the pure components (Figures 1d and 2d) indicating that the lipids are mixed randomly in the same bilayer. For the DSC scan shown in Figure 1, the protein was incorporated into the PA–DPPC vesicles by adding it to preformed sonicated vesicles. In the case of the DMPC–DMPA vesicles used for Figure 2, basic protein was incorporated by dialysis from 2-chloroethanol. A summary of the results obtained by different methods of incorporation is given in Table I and indicates that, when different methods were used, similar results were obtained. Incorporation of 40% basic protein into PA–DPPC vesicles shifts the T_c upward by 5 °C (Figure 1e) while, in DMPC–DMPA vesicles, basic protein at a concentration of 47% shifts T_c downward by 5.6 °C (Figure 2e). In both cases, the shift is in the direction of the

TABLE I: Phase Transition Temperatures of Lipid Vesicles with and without Basic Protein (BP).

Set	Composition	Method of prep	Initial concn of BP (% w/w)	Amount BP incorp (% w/w)	T_c[a]
1.	Egg PA + BP	Dispersion	0	0	17
		Dispersion	30	34	9/17
		Dispersion	50		3.7/8
2.	DPPC				43
3.	PA-DPPC 50:50 + BP	Dispersion	0	0	32.5
		Dispersion	30	21	38.5
4.	PA-DPPC 50:50 + BP	Dispersion	0	0	31.3
		Sonicated	30	32	25.7/35.5
		Sonicated	50	40	36.2
				Prolonged heating	8.9/29.3/36.3
5.	DMPA + BP	Dispersed	0	0	52.6
		Dispersed	30	20	48/53.3
6.	DMPC	Dispersed			14.7/25.2
7.	DMPC-DMPA 50:50 + BP	Dialysis	0	0	39.9
		Dialysis	30	37	36.7
		Dialysis	50	47	34.3
				Prolonged heating	32/49.3
8.	Egg PG-DPPC 50:50 + BP	Dispersed	0	0	28.3
		Dispersed	30	15	28.9–33.3
		Dispersed	50	25	29.1/34
				Repeated heating	33.7
9.	DPPG + BP	Dispersed	0	0	37.8/42.4
		Dispersed	30	28	41.3
		Dispersed	50	40	41.4
		Dispersed	65	50	30.8/37.7
10.	DMPC-DPPG 50:50 + BP	Dispersed	0	0	23.2/34
		Dispersed	30	16	32
		Dispersed	50	26	31
				Prolonged heating	30.4/35.1
		Dialysis	30	16	32.7
		Dialysis	50	26	29.7/33
				Repeated heating	30.6
11.	PS	Dispersed			8.1
	PS-DPPC 50:50 + BP	Dispersed	0	0	36.5
		Dispersed	30	15	39.2
12.[b]	DMPC	Dispersed			12/23.9
	DPPG	Dispersed			36.6/41
	DMPC-DPPG 70:30 + BP	Dispersed	0	0	15.8/28
		Sonicated	50	26	16.9/23.8/27.1/31/41.9
					15.9/28.1
	DMPC-DPPG 70:30 + BP	Dispersed	30	16	18/27.2
		Dispersed	50	26	15.8/24/26.8/31.2/41.2

[a] T_c is defined as the temperature of the peak of the transition. Major peak is underlined. [b] Recorded at 5 °C/min. All other scans at 10 °C/min.

T_c of the pure PC component indicating that it increases the concentration of PC in the bulk lipid by separating out some PA.

After repeated and prolonged heating, up to 52 °C for PA–DPPC and 62 °C for DMPC–DMPA, additional changes were noted in the DSC scans of the samples containing over 40% protein (Figures 1f and 2f) but not for samples containing less or no basic protein. Prolonged heating results in further broadening of the peaks indicating domains of lipid mixtures of varying composition. A shoulder at 29.3 °C in PA–DPPC indicates a component enriched in PA in addition to the main peak at 36.3 °C which is due to a component-enriched in PC. In the case of DMPC–DMPA, a further shift in T_c downward occurs to make the total decrease 7.9 °C, indicating further enrichment of the bulk lipid in PC. In addition, a small peak appears at 8.9 °C in the PA–DPPC mixture and at 49.3 °C in the DMPC–DMPA mixture. These peaks occur at the same temperatures as observed for basic protein incorporated into pure PA or DMPA respectively and, thus, indicate the presence of clusters of PA and DMPA bound to basic protein and separated out from the bulk lipid. Prolonged heating may allow acidic lipid clusters to be segregated into domains of sufficient size to interact cooperatively and give a transition which can be observed.

A similar experiment with egg PG–DPPC and DMPC–DPPG is shown in Figures 3 and 4, respectively. Basic protein also decreases the T_c of DPPG by 1.1 °C at low concentrations (28% w/w protein in lipid (Figure 4c)). At higher concentrations (50%), the protein induces the appearance of two new peaks at 30.8 °C and 37.3 °C, both lower than pure DPPG (Figure 4d) as reported earlier (Papahadjopoulos et al., 1975). Although basic protein was reported to produce an increase in transition temperature in dilauroylphosphatidylglycerol (Verkleij et al., 1974), this lipid does not form stable bilayers by itself. Liposomes formed of phosphatidylglycerol containing fatty acids shorter than palmitic do not trap Na^+ (Van Dijck et al., 1975). Basic protein may stabilize the bilayers of dilauroylphosphatidylglycerol by an electrostatic effect and thus raise the transition temperature while for DPPG which forms stable bilayers by itself it decreases the transition temperature. The effect on the T_c of egg PG was not investigated since it occurs at approximately −10 °C and is obscured by ice melt-

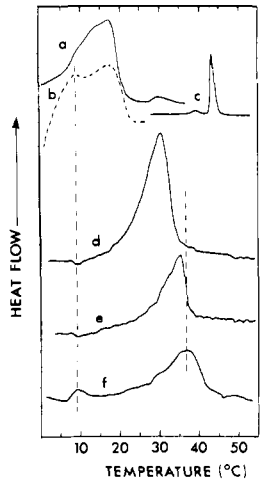

FIGURE 1: Differential scanning calorimetry thermograms for vesicles of (a) egg PA; (b) egg PA + 34% basic protein (dashed line); (c) DPPC; (d) PA/DPPC 50:50 (molecules/molecule); (e) PA/DPPC 50:50 + 40% basic protein prepared by adding basic protein in solution to sonicated preformed vesicles; (f) same as in e after prolonged heating at 52 °C. Heating rate 10 °C/min except d and e which are at 5 °C/min. Increasing heating rate to 10 °C/min raises T_c by about 1 °C. T_c of main peak for e and f are at same temperature if heating rate the same (Table I).

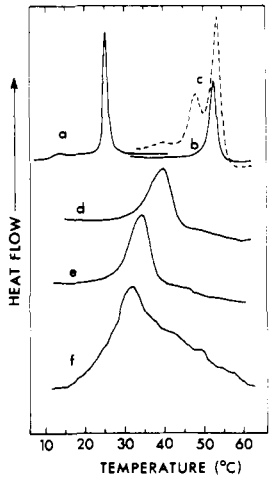

FIGURE 2: Thermograms for vesicles of (a) DMPC; (b) DMPA; (c) DMPA + 20% basic protein (dashed line); (d) DMPC/DMPA 50:50; (e) DMPC/DMPA 50:50 + 47% basic protein prepared by dialysis from 2-chloroethanol; (f) same as in e after prolonged heating at 62 °C. Heating rate, 10 °C/min.

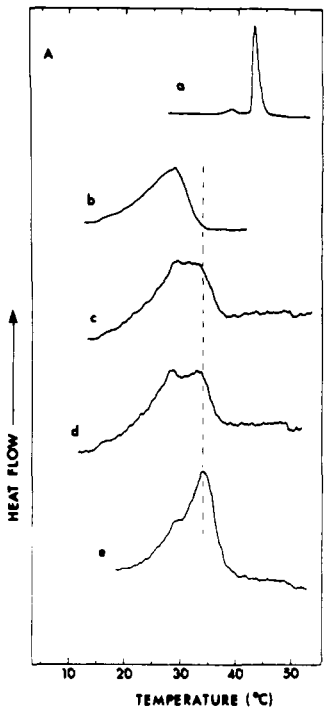

FIGURE 3: Differential scanning calorimetry thermograms for vesicles of (a) DPPC; (b) egg PG/DPPC 50:50; (c) PG/DPPC 50:50 + 15% basic protein; (d) PG/DPPC 50:50 + 25% basic protein prepared by dispersion; (e) same as in d after prolonged heating at 52 °C. Heating rate, 10 °C/min.

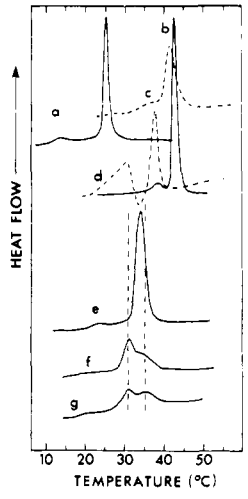

FIGURE 4: Thermograms for vesicles of (a) DMPC; (b) DPPG; (c) DPPG + 28% basic protein (dashed line); (d) DPPG + 50% basic protein (dashed line); (e) DMPC/DPPG 50:50; (f) DMPC/DPPG 50:50 + 26% basic protein; (g) same as in f after prolonged heating. All prepared by dispersion. Heating rate 10 °C/min.

ing. DSC scans of equimolar mixtures of egg PG-DPPC and DMPC-DPPG are shown in Figures 3b and 4e, respectively. The mixture of egg PG-DPPC gives a single, although broad, peak indicating that these two lipids are fairly randomly mixed. The mixture of DMPC-DPPG shows a fairly sharp peak halfway between those of the two pure lipids, indicating very random mixing of the lipids. However, it also has a second small peak at 23.2 °C which is different from the T_c and premelt temperatures of either of the two pure lipids (Table I and Figures 4a and 4b). Therefore, it must be due to a unique premelt transition of this particular mixture of lipids. A premelt also occurs for a 70:30 mixture of DMPC-DPPG as shown in Figure 5. It is interesting that these two lipids which differ in polar head group, net charge and by two carbons in fatty acid chain length can pack together in the gel state in a random mixture which melts nearly as cooperatively as the individual lipids and undergoes a premelt transition similar to that which occurs in the pure lipids. The mixture of DMPA and DMPC which also differs in head group and net charge but not in fatty acid chain length did not have its own premelt transition and was considerably broader than the DMPC-DPPG mixture indicating less cooperativity. However, the DMPA used did not have a premelt transition of its own under the conditions used here (Figure 2b).

The effects of 15% and 25% basic protein on the transition of egg PG-DPPC are shown in Figures 3c and 3d, respectively. In both cases, the protein was incorporated by dispersion. With 15% protein, a broad peak at higher temperatures than the lipid mixture is observed. With 25% protein on the first heating scan, two peaks are observed 0.8 °C above and 5.7 °C above that of the mixture. On the second heating scan, a single peak is ob-

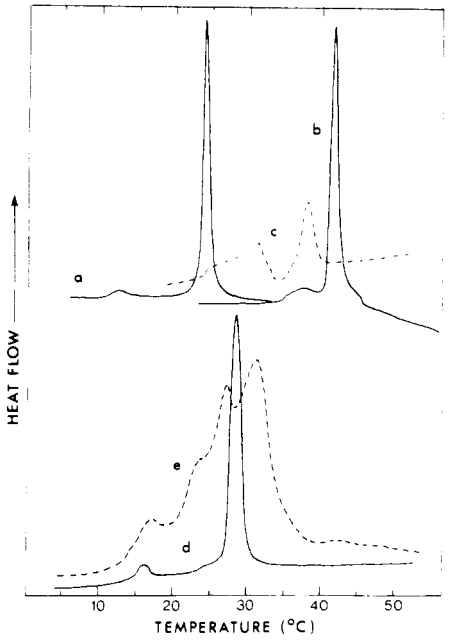

FIGURE 5: Differential scanning calorimetry thermograms for vesicles of (a) DMPC; (b) DPPG; (c) DPPG + 50% basic protein; (d) DMPC/DPPG 70:30; (e) DMPC/DPPG 70:30 + 26% basic protein prepared by adding basic protein in solution to sonicated preformed visicles. All heated at 5 °C/min except c which was at 10 °C/min.

served shifted upward by 5.4 °C. Thus, the protein increases the PC content of the bulk lipid by separating out PG. Incorporation of 26% basic protein shifts the T_c of DMPC-DPPG downward by 3 °C with a shoulder at higher temperatures (Figure 4f). Thus, the PC content of the bulk lipid is also increased in this mixture. After prolonged heating at 52 °C, two peaks are observed, one 3.6 °C lower than the lipid mixture and one 1.1 °C above it. The lower melting peak is probably a component enriched in PC while the higher one could be a component enriched in PG.

Basic protein had a more pronounced effect on vesicles containing DMPC-DPPG in a mole ratio of 70:30. The DSC scan of this mixture is shown in Figure 5. It gives a sharp peak with a T_c of 28 °C. Again it has its own premelt transition at 15.8 °C which is higher than the premelt of DMPC. In the presence of 26% basic protein incorporated by adding the protein to sonicated vesicles, four distinct peaks can be seen. The two major ones occur on either side of that of the initial mixture, one decreased by 0.9 °C representing a component rich in DMPC and one increased by 3 °C representing a component rich in DPPG as observed for the equimolar mixture. There is also a large shoulder at 23.8 °C which may represent nearly pure DMPC. A smaller peak at 16.9 °C is also seen which does not correspond to any transition of the pure lipids or the mixture without basic protein. It may be a new premelt transition of one of the major components present, perhaps that enriched in DPPG giving rise to the highest temperature peak. However, since it is so broad and larger than expected for a premelt, it may be a composite of two premelts for two different components present. A small peak at 41.9 °C is also apparent which may be a cluster of DPPG. Similar results were observed when the vesicles were prepared by dispersion. Repeated heating did not alter the DSC scan.

A mixture of PS-DPPC also gave a single broad peak. However, a plot of transition temperature against composition shown in Figure 6c or its complete phase diagram (Boggs et al., 1977) indicates that it is not completely randomly mixed. However, incorporation of basic protein by dispersion shifts the transition temperature upward by 2.7 °C (Table I) indicating that it probably increases the phase separation in this mixture as well. A higher melting PS was not available for confirmation of this conclusion.

Shifts in the phase transition temperature produced by two concentrations of basic protein for nearly every lipid mixture are presented in Table I. A progressive increase or decrease in the transition temperature is produced with increasing concentration of basic protein in all the equimolar mixtures while for the 70:30 mixture of DMPC-DPPG it is clear that a higher concentration (26%) of basic protein results in greater phase separation since more transitions occur than with 16% basic protein.

Similar effects were observed regardless of which method was used to prepare the vesicles as indicated in Table I, although the magnitude of the effect produced by similar concentrations of protein in vesicles prepared by different methods is not always identical. Thus, basic protein can separate out the acidic lipid when it is present initially during formation of the vesicles by dialysis from 2-chloroethanol or by dispersion of the dry material in buffer, and also when it is added to preformed vesicles containing randomly mixed lipids. Since very little of the lipid could be sedimented in the absence of protein when vesicles were prepared by dialysis, the ratio of the two lipids in the samples with and without protein was measured for PA-DPPC vesicles and was found to be identical in both cases with the starting ratio in the initial solution.

From a plot of the T_c of the major peak against composition of the mixture of lipids in the presence and absence of basic protein (Figure 6), the composition of the bulk lipid enriched in PC for the lipid protein vesicles can be estimated. From the concentration of basic protein incorporated in the vesicles and assuming that only the acidic lipid binds to the protein, the amount of acidic lipid bound to basic protein can be estimated.

For example, an equimolar mixture of PA-DPPC melts at 32.5 °C. Basic protein at a concentration of 21% by weight shifts T_c to 38.5 (Figure 6A). This corresponds to 93 molecules of lipid per 1 molecule of basic protein, using a molecular weight of 18 000 for basic protein and PA_I and PC_I, the initial amounts of PA and PC present per molecule of basic protein are each 46.

By extrapolating to the lipid curve for PA-DPPC (Figure 6A), it can be determined that 38.5 °C is characteristic of a mixture of PA-DPPC in a 22:78 mole ratio and PA_F, the concentration of PA in this mixture, is 22/78 times that of DPPC. Thus assuming that only PA binds to basic protein, $PA_F = 22/78 \times PC_F = 0.28 \times PC_I = 13$, and the number of molecules of PA bound to basic protein, PA_B, can be obtained from $PA_B = PA_I - PA_F = 33$.

Similar plots for the other lipid mixtures are also shown in Figure 6. The amount of acidic lipid bound to basic protein for each lipid mixture used is shown in Table II and varies from 10 to a maximum of 34. Those samples which gave low values of bound acidic lipid had high protein concentrations and the total acidic lipid was less than or close to 30 molecules per molecule of basic protein. Therefore it appears that additional protein above the ratio of $1/30$ can be bound by lipid binding to fewer sites on each protein molecule. This may not result in separation of the acidic lipid from the remainder to as great a degree since complete phase separation was never achieved. At lower protein concentrations where much more than 30 molecules of acidic lipid per molecule of protein are available, the results indicate that basic protein can separate out 27-34 molecules of acidic lipid per molecule of protein from a random mixture of lipids.

TABLE II: Composition of PC Enriched Phase after Binding of Basic Protein and Estimated Amount of Bound Acidic Lipid.

Initial composition (molecules/molecule)	% BP bound (w/w)	Total lipid/BP (molecules/molecule)	Final composition of PC enriched phase (molecules/molecule)	Acidic lipid bound to basic protein (molecules/molecule)
Egg PA-DPPC 50:50	21	93	22:78	33
	32	53	31:68	14
DMPA-DMPC 50:50	47	47	37:63	10
	47	31	22:78	11
Egg PG-DPPC 50:50	15	133	34:66	32
DMPG-DMPC 50:50	16	131	37:63	27
	26	71	28:72	21
PS-DPPC 50:50	15	127	31:69	34

Discussion

The basic protein induces a shift in the phase transition temperature of a mixture of an acidic lipid and phosphatidylcholine in the direction of the melting temperature of the PC regardless of whether the PC is the lower or higher melting component. This indicates that the protein binds preferentially to the acidic lipid resulting in phase separation and enrichment of the PC content of the remaining lipid. It does not simply bind the lower melting component. The composition of the PC enriched lipid can be estimated from its transition temperature and thus the amount of acidic lipid bound to basic protein can be estimated. The results in Table II show that if enough acidic lipid is available, 27–34 molecules of acidic lipid are bound to and separated out by each molecule of basic protein. At pH 7.4, human CNS basic protein has 31 residues (lysine and arginine) as well as 7 asparagines and glutamines which could participate in binding of acidic lipids. Thus most of the available basic sites are used to bind acidic lipids in a mixture of lipids. However, if the amount of acidic lipid is low, the amount of acidic lipid bound per molecule of basic protein decreases and additional molecules of basic protein can be bound to the mixed vesicles. Palmer and Dawson (1969) reported that basic protein could bind acidic lipids with a ratio of 20–23 negative charges per molecule of basic protein in a biphasic chloroform/methanol/water system resulting in solubilization of the protein in the organic phase. However, more sites on the protein may be available for lipid binding when it is incorporated into lipid vesicles.

An x-ray diffraction study (Mateu et al., 1973) has shown that basic protein bound to an acidic lipid extract from myelin could cause a unique arrangement with two separate symmetric bilayers, one of sulfatides and the other consisting of the remaining lipids in the acidic lipid extract. The protein bound the two bilayers together. However, it is not clear whether this effect was due to a phase separation induced by the protein or was partially due to the temperature at which the sample was prepared since sulfatides melt at 40–50 °C and would separate out unless the temperature of mixing is above this. The fact that no phase separation was seen in the presence of 20 mol % cholesterol also suggests that phase separation due to temperature may have occurred since cholesterol might fluidize the lipid and allow mixing at the temperature used for preparation of the complex (Tinker et al., 1976).

We cannot determine at present if the phase separation observed in our study results in segregation of the lipids into separate bilayers, an asymmetric bilayer or simply domains of acidic lipids in each monolayer of every bilayer. However, it is unlikely that it results in segregation into distinct bilayers since two distinct peaks are not observed in the DSC scan and since the shifts in temperature observed when the protein is added to preformed sonicated vesicles containing the lipids randomly mixed are similar to those observed when the protein is present during formation of the vesicles. It remains to be determined whether the bilayers are asymmetric.

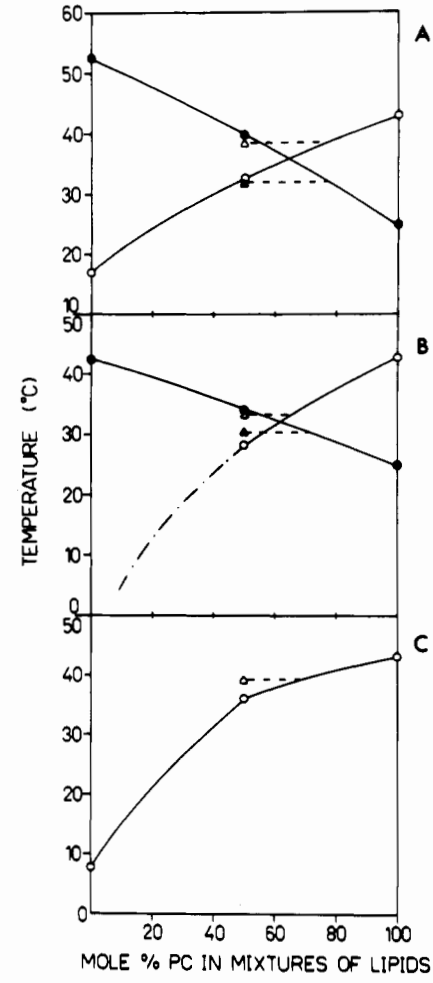

FIGURE 6: Variation of T_c with composition of lipid mixture with and without basic protein for mixtures of (A) PA/DPPC (○), PA/DPPC + 21% basic protein (△), DMPC/DMPA (●), and DMPC/DMPA + 47% basic protein (▲); (B) PG/DPPC (○), PG/DPPC + 15% basic protein (△), DMPC/DPPG (●), and DMPC/DPPG + 16% basic protein (▲). (C) PS/DPPC (○) and PS/DPPC + 15% basic protein (△).

Phase separation induced by Ca^{2+} has been observed in a mixture of PS and PC using spin labels (Ohnishi and Ito, 1973, 1974; Ito et al., 1975) and by DSC (Papahadjopoulos et al., 1974; Jacobson and Papahadjopoulos, 1975). By using a spin-labeled phosphatidylcholine with phosphatidic acid (Galla and Sackmann, 1975) and a spin-labeled steroid with cardiolipin (Birrell and Griffith, 1976), it has been shown that two other water-soluble basic proteins, poly(L-lysine) and cytochrome *c*, respectively, can result in phase separation.

Such protein or cation induced phase separation may induce

and maintain asymmetry in the bilayer. We have shown that both major proteins of myelin, basic protein and lipophilin from the proteolipid, can cause phase separation of acidic lipids and thus may be responsible for the asymmetric bilayer of myelin. Binding of a protein to acidic lipid domains in two adjacent bilayers may also be involved in causing the plasma membrane of the oligodendroglial cell to wrap around the nerve axon resulting in the unique multilayered structure of myelin.

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