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NON-COVALENT CROSS-LINKING OF LIPID BILAYERS BY MYELIN BASIC PROTEIN

A POSSIBLE ROLE IN MYELIN FORMATION

ROSS SMITH

School of Chemistry, University of Sydney, Sydney, N.S.W. 2006 (Australia)

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Summary

Myelin basic protein associates with bilayer vesicles of pure egg phosphatidylcholine, L-α-dimyristoyl phosphatidylcholine and DL-α-dipalmitoyl phosphatidylcholine. Under optimum conditions the vesicles contain 15—18% of protein by weight. The binding to dipalmitoyl phosphatidylcholine is facilitated above its gel-to-liquid crystalline transition temperature. At low ionic strength the protein provokes a large increase in vesicle size and aggregation of these enlarged vesicles. Above a sodium chloride concentration of 0.07 M vesicle fusion is far less marked but aggregation persists. The pH- and ionic strength-dependence of this aggregation follows that of the protein alone; in both cases it occurs despite appreciable electrostatic repulsion between the associating species.

A similar interaction was observed with diacyl phosphatidylserine vesicles.

These observations, which contrast with earlier reports in the literature of a lack of binding of basic protein to phosphatidylcholine-containing lipids, demonstrate the ability of this protein to interact non-ionically with lipid bilayers. The strong cross-linking of lipid bilayers suggests a role for basic protein in myelin, raising the possibility that the protein is instrumental in collapsing the oligodendrocyte cell membrane and thus initiating myelin formation.

Introduction

Recent studies in this laboratory of the interaction of myelin basic protein with the detergent deoxycholate have shown this protein to possess extensive hydrophobic regions. Basic protein binds appreciable amounts of deoxycholate above the latter's critical micelle concentration (McDonald, B.J. and Smith, R.,

unpublished observations) and concomitantly adopts a more ordered secondary structure [1]. Neither the binding nor the structural change is appreciably affected by the addition of salt.

Further evidence for hydrophobic association with lipids is derived from monolayer studies, which show expansion by basic protein in a manner consistent with partial penetration of the polypeptide into the hydrophobic region of the monolayer [2]. This protein also increases the permeability of lipid bilayers and decreases their gel-to-liquid crystalline transition temperature and the enthalpy change of the transition [3,4]. As evidenced by the greater binding to negatively charged lipids, electrostatic attraction between protein and lipid is perhaps not unimportant but it is clearly not the sole interactive force.

As an extension of earlier studies with deoxycholate we undertook experiments to establish the structure of basic protein attached to phosphatidylserine lipid bilayer vesicles. In the course of this work we observed a dramatic and specific aggregation of lipid vesicles by the protein. This paper outlines salient features of this process and discusses their implications for the structure of myelin.

Methods

Egg L-α-diacyl phosphatidylcholine (Sigma, St. Louis, U.S.A.), DL-α-dimyristoyl phosphatidylcholine (Sigma), and L-α-diacyl phosphatidylserine (Calbiochem, San Diego, U.S.A.) were tested for purity on thin-layer silica gel plates. The solvents used were chloroform/methanol/acetic acid/water (25 : 15 : 4 : 2, v/v) and chloroform/methanol/28% ammonia (65 : 25 : 4, v/v). The lipids (100—1000 μg) were detected by charring with sulphuric acid or with a specific phospholipid stain [5]. All phosphatidylcholine samples migrated as single species with R_F = 0.36 and 0.25 respectively in the two solvent systems. Impurities were considerably less than 1% in each lipid: they were therefore used in most experiments without additional purification. However, in view of conflicting reports in the literature [6,7] concerning the binding or lack of binding of basic protein to phosphatidylcholine vesicles, for some experiments the lipid was prepared by chromatography on preparative silica gel plates (E. Merck, Darmstadt, G.F.R., without fluorescent indicator) using both of the solvent systems specified above.

Vesicles were prepared at 4 ± 1°C under nitrogen in a sonicator bath: 5—10 min sonication was usually sufficient to produce a solution in which the light scattering would not diminish significantly with further irradiation. Initially vesicles were prepared using a microtip sonicator at low power: however, as thin layer chromatography revealed some lipid decomposition these solutions were not further utilized. For some experiments the larger aggregates remaining after sonication were removed by centrifugation at 140 000 × *g* for 3 h.

Myelin basic protein was prepared from human and bovine brain by chloroform/methanol extraction of the white matter following the method of Oshiro and Eylar [8]. The protein was shown to be more than 99% pure by electrophoresis at pH 4.3, as described previously [1]. The amount of protein bound to the vesicles was initially determined in two ways: (a) by centrifuging

the aqueous solution of lipid and protein at 90 000 × *g* (average) at 20°C for 2 h in a swinging bucket rotor (Beckman, SW50.1). The protein concentration in the original solution and the unbound protein in the supernatant were measured colorimetrically [9]. (b) Approximately 0.4 ml of the sample was mixed with 5 ml of sucrose solution of density 1.20 g · ml^{-1} then centrifuged as in (a) to float the vesicles with attached protein. Protein concentrations before centrifugation and half way down the tube after centrifugation were measured in duplicate samples. In preliminary experiments both methods gave the same values within experimental error (50 ± 5% and 53 ± 5%, respectively) for the proportion of protein bound to the vesicles, indicating that dissociation of protein from the vesicles on dilution is not significant. Most subsequent binding measurements followed the second method. Where necessary, NaCl was added to the sucrose solution to maintain the ionic strength of the sample. Similar experiments performed in the absence of lipid showed that sedimentation of uncomplexed protein was negligible under all experimental conditions used.

Light scattering was measured by determining the absorbance at 450 nm in a 0.1 cm cell, using a double-beam spectrophometer. Solutions of protein or lipid alone were run concomitantly to provide a suitable reference. In several instances the spectrum was obtained from 600 to 360 nm to enable calculation of vesicle size following Chong and Colbow [10].

Deuterium oxide (Australian Atomic Energy Commission, Lucas Heights, Australia) was 99.75%. All other chemicals were of analytical grade.

Protein concentrations were determined colorimetrically [9] with 50 μl of 1% sodium dodecyl sulphate added, or by measurement of absorbance at 280 nm [1]. Lipid concentrations were determined by addition of buffer to lipid weighed after evaporation of the solvent (hexane) under nitrogen, or by phosphorus determination [11].

Electron micrographs were obtained on a Philips 300 electron microscope at 60 kV using 2% ammonium molybdate (at pH 8.5) as the negative stain.

Results

Phosphatidylserine-basic protein interaction

Initial experiments with diacylphosphatidylserine revealed a large, protein-induced, increase in light scattering which could be reversed by the addition of excess protein (Fig. 1). Assuming that the light scattering was caused by vesicular aggregation, it was expected that addition of excess protein would reverse this aggregation, the added protein being also able to associate with vesicle-bound protein but not itself being able to bind to the lipid. Disaggregation could, however, be explained also by a net charge reversal on the vesicles: the negatively charged vesicles would at first have their charge reduced by the bound positive protein, passing through a point of zero net charge (where light scattering would be maximal) and eventually bearing a dissociating positive charge. In this system, delineation of the factors leading to the increase in light scattering is consequently obscured by the changing intervesicular electrostatic repulsion as in the aggregation of phosphatidylserine liposomes upon binding of poly(L-lysine) [13] or cytochrome *c* [14].

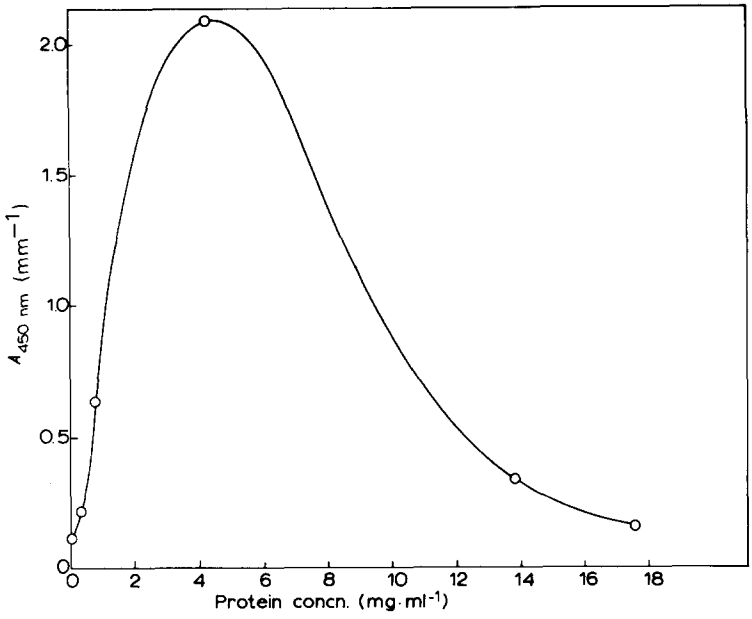

Fig. 1. Light scattering induced by the addition of human myelin basic protein to bovine L-α-phosphatidylserine vesicles. The first four points were obtained by addition of small volumes of 12.5 mg · ml^{-1} basic protein in water to 0.40 ml of 1.67 mg/ml lipid in 0.25 M NaCl. In each case the final volume and pH were the same: the pH was 6.4 ± 0.1. After the measurement at 4.2 mg · ml^{-1} protein solid protein was added to give the two higher concentrations: the pH again being adjusted to 6.4. In each experiment the final volume was 0.60 ml.

Phosphatidylcholine-containing lipids are, in contrast, zwitterionic and their vesicles possess a small net charge, if any. As basic protein is bound, the charge on the vesicles becomes increasingly positive, obviating the possibility of diminishing electrostatic repulsion causing aggregation.

Phosphatidylcholine-basic protein interaction

Diacyl phosphatidylcholine binds small amounts of basic protein almost quantitatively at pH 8.9 (Fig. 2), the increase in light scattering or turbidity following the binding of protein. Addition of a large excess of protein, up to 20 mg · ml^{-1}, does not reduce the light scattering. Up to the point where the vesicles appear to be saturated with protein (0.10 ± 0.02 g protein/g lipid) a large proportion of the protein in solution is bound to the vesicles: beyond this point added protein remains free in solution but begins to precipitate causing a continued slow rise in light scattering. At pH 8.9 in 0.1 M Tris buffer the solubility of the protein alone appears to be below 1 mg · ml^{-1}.

As the amount of protein bound to the vesicles increases, so should the charge carried by the lipid · protein complex and also the electrostatic repulsion between vesicles, but the light scattering increases, indicating more extensive aggregation and suggesting that vesicular association is not mediated by ionic bonding. This conclusion is substantiated by studies of the effect of salt on the scattering (see below).

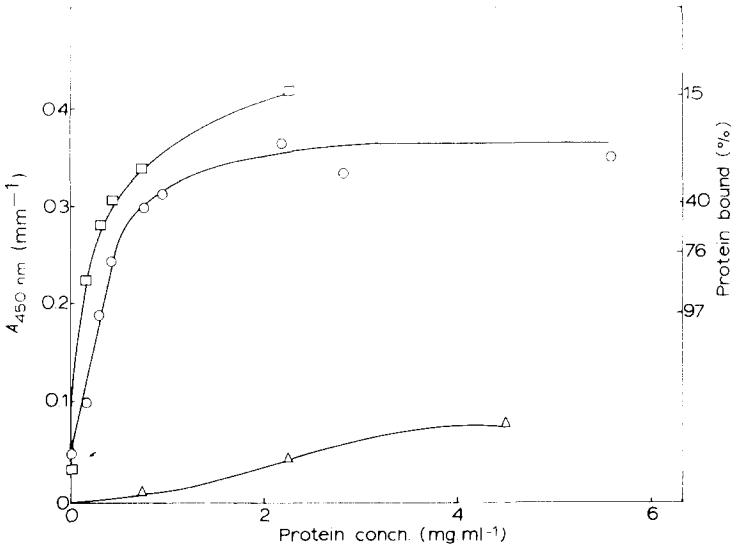

Fig. 2. Protein binding to egg phosphatidylcholine vesicles and concomitant light scattering. Top curve (□); aliquots of basic protein (12 mg · ml^{-1} in water) were added to 50 μl of 23 mg/ml L-α-diacyl phosphatidylcholine, 50 μl of 0.83 M Tris buffer and sufficient water to make the final volume 400 μl. The other curve (○) was obtained from a similar experiment with 25 mg/ml lipid and 15 mg · ml^{-1} protein solutions. The bottom line (△) was obtained under conditions identical except for the omission of lipid. In all cases the pH was finally measured as 8.92. Each point was obtained from an individual solution: All were mixed at the same time and allowed to stand overnight at 4°C before the absorption and binding were measured. The proportion of added protein which was bound to the vesicles is indicated for the top curve (□): it was measured as outlined in the Methods section.

Several groups have reported a lack of interaction between basic protein and diacyl phosphatidylcholine (e.g., refs. 6, 7) *. For this reason particular care was taken in the present work to ensure that the observed binding was not caused by either lipid or protein impurities. Although the lipid appeared to be pure on thin layer chomatograms in two quite different solvent systems it was subjected to further purification. The products behaved quantitatively in the same manner as the original lipid. Synthetic dipalmitoyl phosphatidylcholine and dimyristoyl phosphatidylcholine also showed a similar light scattering increase on addition of the protein at temperatures above the gel-to-liquid crystalline transition temperature. Binding, which is limited to about 15% protein by weight, thus does appear to be to the zwitterionic lipid.

Several preparations of both bovine and human central nervous system myelin basic protein behaved identically in their interaction with phosphatidylcholine. The protein is known to be free of other myelin proteins and lipids [1].

* In both studies cited interactions with lipids have been examined in a two-phase system which is thermodynamically quite different from liposomes dispersed in water. Demel et al. [2] using a monolayer of lipid, which is closer to the system used here, did observe interaction with phosphatidylcholine.

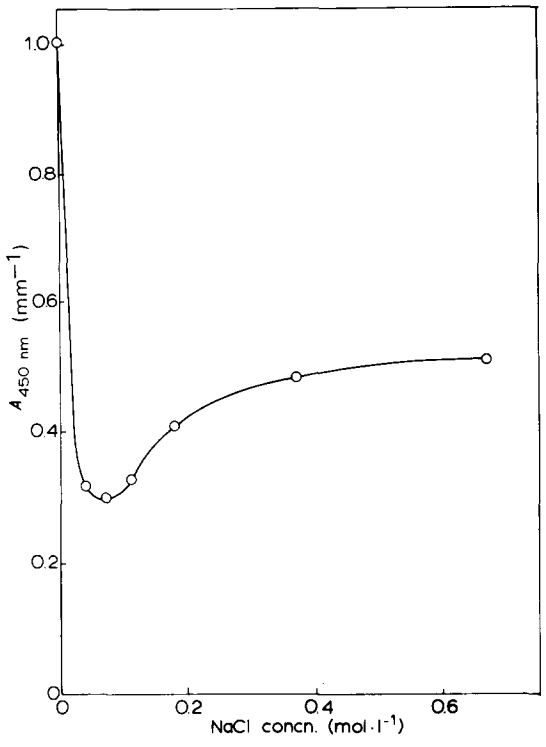

Fig. 3. Effect of ionic strength on the basic protein-induced light scattering by egg diacyl phosphatidylcholine vesicles. In these experiments 50 μl of 23 mg · ml^{-1} lipid dispersion was mixed well with 5 μl 0.083 M Tris buffer (pH 8.9), 0—50 μl 3 M NaCl and sufficient water to ensure a final volume of 405 μl. Then 15 μl of a 12 mg · ml^{-1} solution of human myelin basic protein was added to each, the solutions mixed, and allowed to stand at 4°C overnight before measurement. The scattering from the unbound protein was negligible and the contribution from the lipid was a constant 0.024 (mm^{-1}).

Effect of ionic strength on basic protein-phosphatidylcholine interaction

Except at very low salt concentrations light scattering increases with increasing salt concentration (Fig. 3). But below 0.07 M NaCl there is an inverse relationship between scattering and ionic strength, the largest light scattering increase brought about by a fixed amount of protein (enough to saturate the vesicles) occurring with complexes formed in less than 0.002 M salt. These changes with salt concentration are not a reflection of variable binding of protein to the lipid. As demonstrated in Tables I and II, binding is largely independent of NaCl concentration.

Addition of up to 0.30 M salt to lipid · protein vesicles formed in 0.001 M buffer caused little alteration in light scattering, implying that the process leading to intense scattering at the lowest ionic strengths studied is not readily reversed.

Electron-microscopic examination of the protein · phosphatidylcholine vesicles

The complex ionic strength dependence of the turbidity increase on addition of protein appears to result from concomitant changes in vesicle size and extent of aggregation. The sonicated lipid dispersions contain primarily vesicles

TABLE I

EFFECT OF IONIC STRENGTH ON BASIC PROTEIN BINDING TO EGG PHOSPHATIDYLCHOLINE VESICLES

200 μl of 34.5 mg · ml^{-1} lipid, 10 μl of 0.1 M, pH 8.9, Tris buffer, 0—60 μl of 3 M sodium chloride, and water to 525 μl were mixed before the addition of 75 μl of 16.5 mg · ml^{-1} human basic protein. All solutions were left overnight at 4°C before measurement of light scattering and protein binding. Measured final pH values were 7.48 ± 0.05. Protein binding was determined after dilution into sucrose solutions (see Methods).

Sodium chloride concentration (M)	A_{450nm} (mm^{-1})	Protein bound	
		(%)	(g/g lipid)
0	1.553	32 ± 5	0.057
0.075	0.763	31 ± 4	0.056
0.30	1.290	30 ± 4	0.054

approx. 30—50 nm in diameter (Fig. 4a). Addition of basic protein at low ionic strength within seconds causes fusion to form vesicles up to 600—700 nm dimater (Fig. 4b). Complexes formed at higher ionic strength show a relatively minor increase in average vesicle size (Fig. 4c). At all ionic strengths there appears to be marked clustering of the vesicles which in many places appear flattened in the contact regions, as if tending to maximize the contact area.

The lipid · protein complexes proved too large to be amenable to size calculations [10] using the scattering spectra from 600 to 360 nm. The calculated values of the power factor, β, were generally greater than 3, indicating that the complexes were not small with respect to the wavelength of the light (450 nm) and hence were outside the range of validity of the Rayleigh-Gans approximation. The concordance of these results and the other light scattering data with the electron-microscopic observations provides

TABLE II

EFFECT OF pH ON BASIC PROTEIN BINDING TO EGG PHOSPHATIDYLCHOLINE VESICLES

In these two experiments different dispersions of lipid were used (designated A and B below). Binding of protein was measured directly (see Methods), concentrations being measured in triplicate samples. The standard deviations are noted in the table. 1.0 ml of lipid dispersion (20 mg · ml^{-1}), 0.30 ml of human basic protein (15.1 mg · ml^{-1}), 0.50 ml 3 M NaCl, 1.0 ml 0.1 M buffer and sufficient water to give 5.5 ml were mixed and left overnight at 4°C before binding measurement. In the low ionic strength samples the NaCl solution was omitted, and the buffer reduced to 0.25 ml, water being added to give the same final volume. The buffers used were acetate (pH 5.62 and 5.59), phosphate (pH 6.40) and Tris (pH 8.60—8.75). Experiments performed at pH 8.9 in the absence of lipid showed that sedimentation of the protein was negligible at the concentrations employed in these experiments.

pH	Sodium chloride concentration (M)	Protein bound (g/g lipid)	Lipid dispersion
5.52	0	0.028 ± 0.003	A
5.59	0.3	0.032 ± 0.003	B
6.40	0.3	0.025 ± 0.004	A
8.67	0	0.181 ± 0.007	B
8.60	0.3	0.150 ± 0.006	B
8.75	0.3	0.110 ± 0.006	A

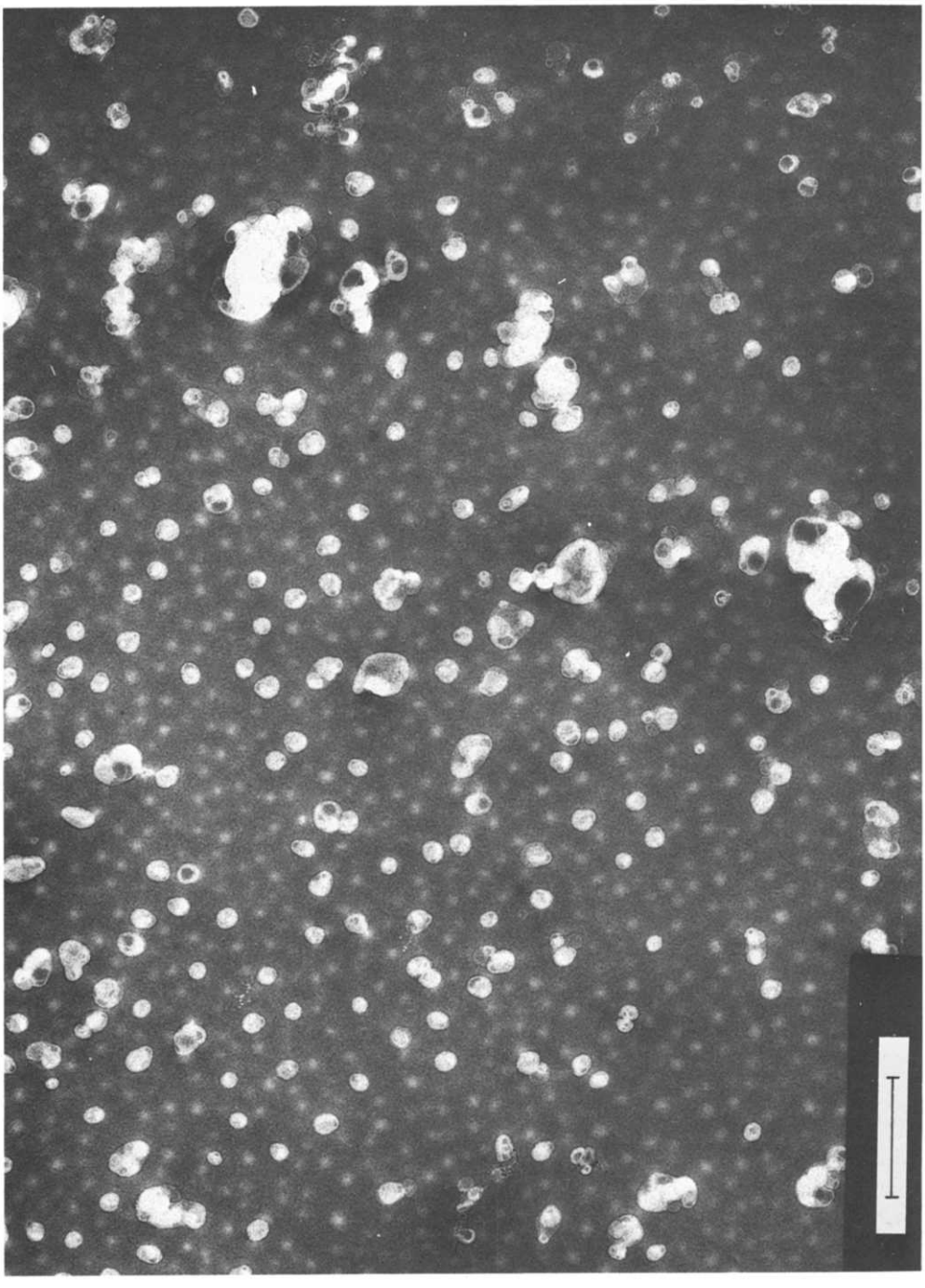

Fig. 4a. (See p. 179 for legend.)

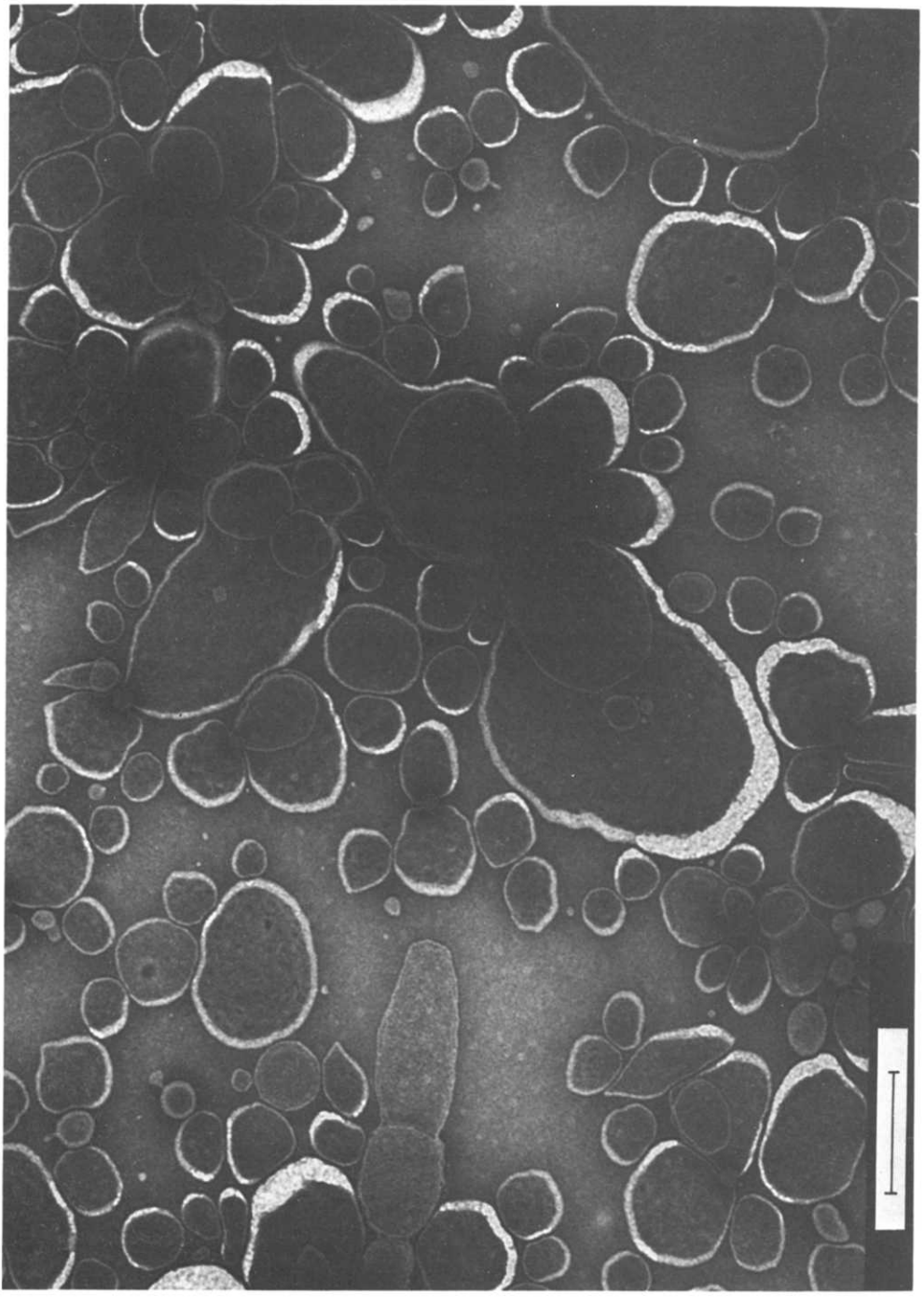

Fig. 4b. (See opposite page for legend.)

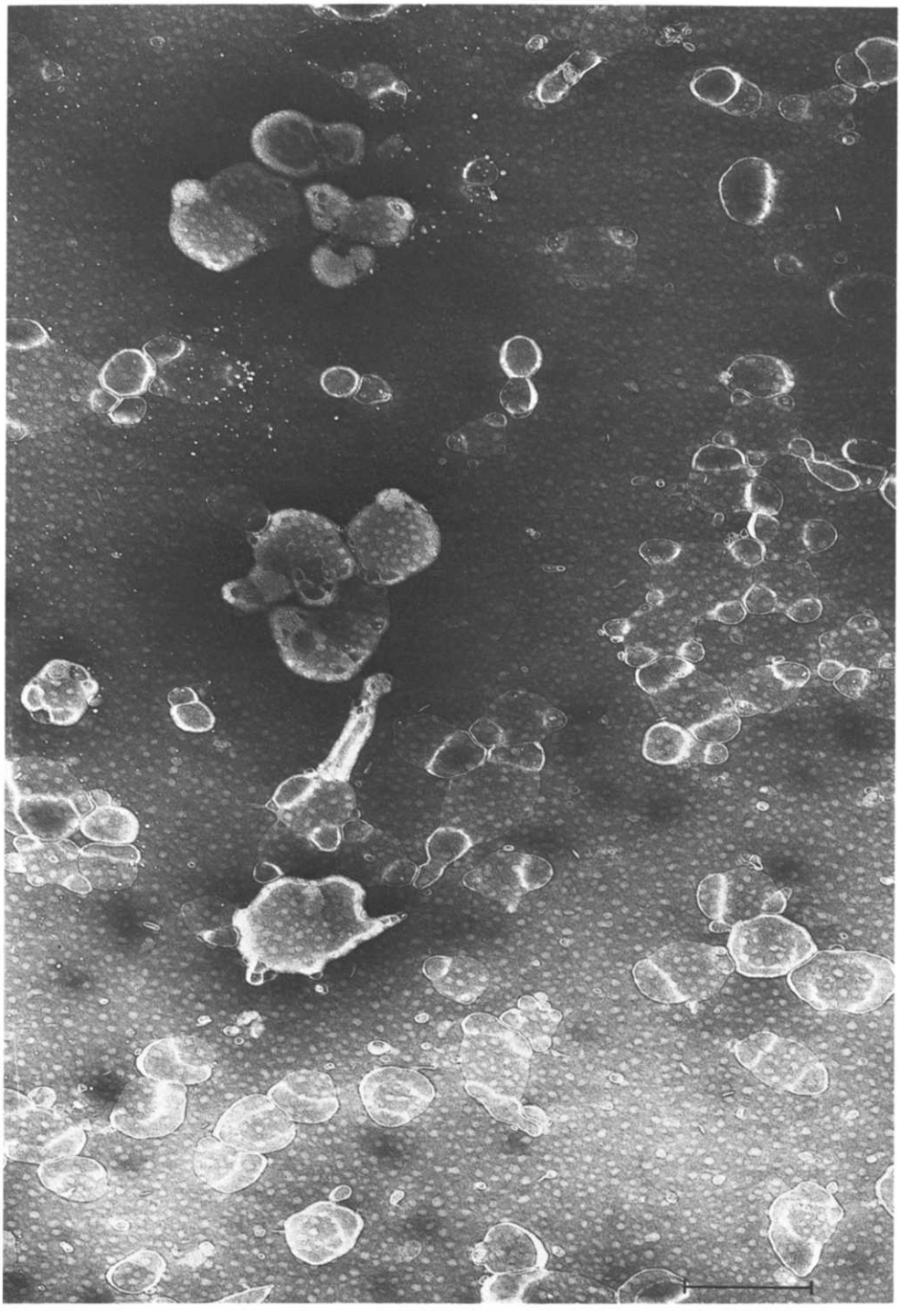

Fig. 4. Electron micrographs obtained by negative staining with 2% ammonium molybdate, pH 8.5, of (a) a sonicated dispersion of egg diacyl phosphatidylcholine (7.6 mg · ml^{-1}), (b) egg phosphatidylcholine (7.6 mg · ml^{-1}) plus myelin basic protein (1.2 mg · ml^{-1}) in 0.002 M Tris buffer pH 8.9, (c) egg phosphatidylcholine (7.6 mg · ml^{-1}) plus basic protein (1.2 mg · ml^{-1}) in 0.002 M Tris buffer, pH 8.9, containing also 0.5 M NaCl. In b and c the protein was added last. In each the magnification is the same (the bars indicating 250 nm) as also was the degree of dilution with negative stain solution. (1 volume of protein · phosphatidylcholine solution with 6 of stain).

assurance that the negative staining does not itself cause changes in the system. Ammonium molybdate solution (2%) added to a basic protein · phosphatidylcholine solution at pH 9 and high ionic strength caused no change in the light scattering other than that attributable simply to dilution.

Effect of pH on light scattering and binding of basic protein

Fusion and aggregation effects are seen also in studies of the effect of pH (Fig. 5) which show little increase in light scattering on protein addition to the lipid dispersion at acid pH, but a rapid rise in the pH range 6—7. Titration of pH 9 samples back to low pH does not result in a return to values expected from samples prepared and maintained at low pH. It was possible to slowly titrate the vesicles back and forth following the path taken in the intial titration from pH 9 to 4. A portion of the scattering (up to 80% in some experiments) is thus readily reversed and is more likely to be associated with vesicular aggregation than with fusion, as the latter is difficult to reverse.

The pH dependence of the aggregation of the lipid · protein complexes parallels that of the protein alone, both showing a change near pH 6—7 (Figs. 5 and 6 and ref. 12). Addition of salt also appears to diminish the solubility of the protein (in the pH range 5—9) as well as promoting vesicular aggregation presumably by decreasing the electrostatic repulsion between the associating

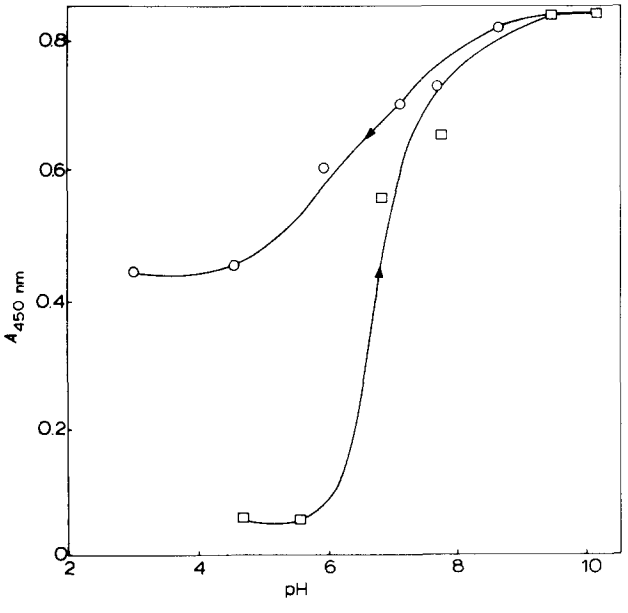

Fig. 5. Effect of pH on the light scattering of basic protein · egg diacyl phosphatidylcholine complexes. The scattering of the lipid vesicles alone, which increases slightly at low pH, has been subtracted as has the scattering due to the protein alone (allowing also for protein binding), which increases above pH 6 (Fig. 6). The solutions were prepared by mixing 0.5 ml of 24 mg · ml^{-1} egg phosphatidylcholine, 0.4 ml 3 M NaCl, 0.5 ml of 0.1 M buffer (Tris, pH 9, or acetate at pH 4.2) and 1.25 ml water. 0.35 ml of 16.5 mg · ml^{-1} human basic protein was then added and the solution stored overnight at 4°C. After the first light scattering measurement the pH values were readjusted with 2 M NaOH or HCl and the solutions kept at room temperature (approx. 25°C) for 8 h before measurement of the absorbance.

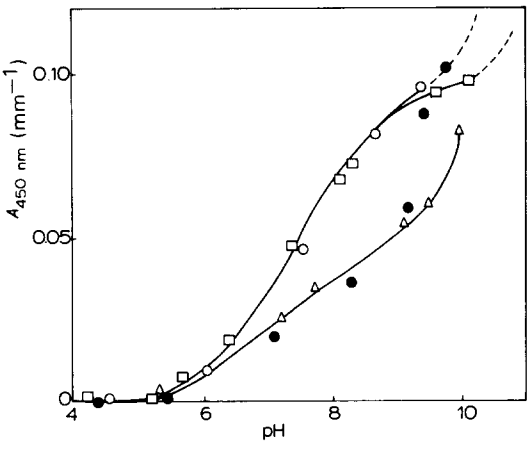

Fig. 6. Light scattering by human myelin basic protein as a function of pH. Solutions of 2.35 mg · ml^{-1} (□,○) and 2.06 mg · ml^{-1} (△,●) in water (○,●) and in 0.3 M sodium chloride (□,△) were titrated with 0.5 M NaOH. The volume of added base was negligible. Above pH 9.5 the scattering in the two solvents diverged sharply. At pH 10.9 the absorbance at 450 nm of the 2.35 mg · ml^{-1} solution in water was 0.42: at the same pH the corresponding value for the solution in 0.3 M NaCl was 0.13. For clarity, lines have been drawn to show the trend in the sodium chloride solutions only.

species. But aggregation of the vesicles does persist at low ionic strength where this repulsion is appreciable.

Electron microscopy of samples titrated from pH 9 to pH 4 should on the basis of the above results reveal disaggregation of the vesicle clusters and an increase in size of individual vesicles (over their original size), but addition of negative stain at pH 3 causes immediate precipitation in these solutions.

The effect of pH on basic protein binding to the vesicles is indicated in Table II. In other experiments binding was also observed at pH 4.9. For example 0.036 ± 0.006 g protein/g lipid was bound in a solution containing 13.1 mg · ml^{-1} phosphatidylcholine, 2.36 mg · ml^{-1} protein, and 0.3 M NaCl in 0.05 M pH 4.90 acetate buffer. Protein concentrations in this experiment were determined colorimetrically after tenfold dilution with sucrose solution, 0.20, 0.30, and 0.40 ml samples being taken before and after centrifugation. No substantial deviations in protein binding were observed in the pH range 5.52—6.40 (Table II) although the light scattering changes appreciably in this region (Fig. 5), suggesting that protein binding alone may not be sufficient to bring about vesicle fusion or aggregation.

Attempts were made to delineate more closely the nature of the forces leading to aggregation of the protein-containing vesicles. Ionic attraction is unlikely given that aggregation occurs under conditions where the vesicles possess a large positive charge * (see Discussion) and that light scattering is increased at high ionic strength. No direct evidence for hydrophobic association was obtained. Use of 75% (v/v) deuterium oxide rather than water caused no change in scattering, although hydrophobic interactions are stronger in this

* Each vesicle contains of the order of several thousand lipid molecules and hence optimally about 10—30 protein molecules, each of which bears a net positive charge of approx. +20 at pH 7—8.

solvent [30]. Similarly, although chaotropic salts such as lithium chloride often diminish hydrophobic interactions this salt did not differ from the same concentration of NaCl in its effect on aggregation: it did, however, slow the increase in light scattering after the addition of basic protein. Higher temperatures also promote hydrophobic association, but variation of the temperature from 25 to 55°C caused only very minor changes in the light scattering of a sample in pH 8.9 Tris buffer.

Recently Morrisett et al. [15] have reported differences in the interaction of a lipoprotein apoprotein with single bilayer and multilayer vesicles. In the present studies centrifuged lipid solutions (which are reported to contain primarily single bilayer vesicles [14]) and uncentrifuged solutions yielded very similar light scattering increases (at pH 8.9 in 0.3 M NaCl) and similar diminutions on titrating to pH 3.5. Detailed attempts to detect possible differences have not been made.

Discussion

Although several proteins are known to interact with phosphatidylcholine vesicles they appear to cause either no change or a slight decrease in the light scattering of dispersions of phosphatidylcholine [14,21]. Several proteins tested in this work, including lysozyme and bovine serum albumin, caused no significant change in light scattering of phosphatidylcholine at protein concentrations well above those at which basic protein is effective.

Serum very low- and high-density lipoprotein apoproteins have been observed to lead to stacking of phosphatidylcholine vesicle discs as seen in electronmicrographs [16—19] but there is evidence that this is an artifact of negative staining [16,20]. Limited aggregation (probably dimerization) of dimyristoyl phosphatidylcholine by high-density apoprotein has been revealed by Stoke's radii measurements at low ionic strength, but this is eliminated in 0.1 M NaCl [16]. The very low density apoprotein (apo-Lp-Ala) at high ionic strength causes no change in the light scattering of dipalmitoyl phosphatidylcholine and distearoyl phosphatidylcholine and a decrease in the scattering of dimyristoyl phosphatidylcholine [21]. An increase in turbidity on adding poly(L-glutamic acid) to phosphatidylcholine in water has been noted [22], but in this study NaCl also caused a turbidity increase in the absence of the polyamino acid.

Thus the action of basic protein in causing a dramatic increase in turbidity over a wide ionic strength range is uncommon. Phosphatidylcholine vesicles in the absence of protein undergo only very slow spontaneous fusion; little change in scattering over several days was observed in these studies. This rate of fusion is greatly increased by basic protein. Solutions of basic-protein · phosphatidylcholine vesicles formed at low ionic strength and examined in the electron microscope within 10—15 min of adding the protein to the phosphatidylcholine had already increased their vesicle diameter several-fold.

Aggregation is expected to be a prerequisite for fusion, but the latter does appear limited in that after 0.5—1 day when there is little further change in light scattering, the electronmicroscopic evidence, the partial reversibility of light scattering on addition of acid and the complex ionic strength dependence of scattering all indicate that (at high ionic strength in pH 8.9 buffer) the solu-

tions contain aggregates of slightly enlarged vesicles. The close similarity in the ionic strength- and pH-dependence of the basic protein · phosphatidylcholine turbidity and the protein solubility suggests that the same intermolecular forces may govern both phenomena and favours the explanation of bilayer cross-linking by linking of protein molecules attached to different vesicles rather than by single protein molecules attaching to adjacent bilayers.

The observation that binding of basic-protein to phosphatidylcholine vesicles is unaffected by variation of ionic strength over a wide range belies the commonly accepted notion that the protein is held at the surface of lipid bilayers solely by ionic attraction, and supports more recent observations [2—4] suggesting some hydrophobic interaction. With other lipids ionic interactions appear to play a significant part, for example, the extent of binding of basic protein to phosphatidylserine vesicles is several times greater than to phosphatidylcholine vesicles, but this association is relatively insensitive to variations in pH or ionic strength (Keniry, M.A. and Smith, R., unpublished observations) so that the correct explanation for the difference in binding may reside in other (e.g. steric) factors.

The attractive forces leading to basic-protein · phosphatidylcholine vesicle aggregation have not been elucidated; they should be delineated more clearly by an examination of the aggregation of the protein alone. But the fact that such forces exist could have far-reaching biological implications. In forming myelin the oligodendrocyte cell membrane collapses to yield the long process which encircles the nerve axon. For this to occur there must presumably be some attractive force which will link the inside surfaces of the cell membrane and maintain them at the unusually small separation observed in myelin [23]. Basic protein, which has been shown to reside primarily at this inside surface [24] could, on the basis of its ability to link bilayers, provide the necessary cross link. After completion of this work, it was brought to my attention that P.E. Braun (at a meeting of the American Neurochemical Society) had suggested dimerization of basic protein at the cytoplasmic apposition as one of the possible ways in which this protein functions.

It is generally accepted that proteolytic digestion of nerves or purified myelin results in breakdown of the basic protein [25—27] and is often (see ref. 27, however) accompanied by morphological changes including lamellar separation of the major dense line (the intracellular apposition) [25,28,29]. But changes in other myelin components are also induced by proteolytic digestion and it is not possible at present to unequivocally ascribe this lamellar separation to basic protein degradation.

Acknowledgements

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