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MYELIN BASIC PROTEIN ABILITY TO ORGANIZE LIPID BILAYERS: STRUCTURAL TRANSITION IN BILAYERS OF LISOPHOSPHATIDYLCHOLINE MICELLES

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Summary: Myelin basic protein isolated by a single step with the cationic detergent cethyltrimethylammonium bromide in a lipid-bound form is able to induce structural transition of lysophosphatydilcholine micelles into multilaminar vesicles. This finding, observed through electron microscopy, is discussed in the light of the assumed ability of the basic protein to organize myelin lipids.

Central nervous system myelin contains a considerable amount of a small basic protein (MBP), which is encephalitogenic (1,2), apparently located mainly on the inside surface of the myelin membrane (3,4) and thereby playing a central role in stabilizing myelin multilamellar structure (5,6). In agreement with this role a lipid-free preparation of the basic protein (7,8) has been shown to cross-link bilayers, thereby promoting aggregation (5,9) or fusion (10) of phospholipid vesicles. Moreover, the fact that binding to almost all myelin lipids has been established in the basic protein recently purified in a lipid-bound form (11) leads to the hypothesis

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Abbreviations: CTAB, cethyltrimethylammonium bromide; lyso-PC, lysophosphatidylcholine.

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that, besides the ability to cross-link bilayers, the basic protein could also possess a lipid-organizing capacity.

To check this point we chose a preparation in cethyltrimethylammonium bromide (CTAB) of this basic protein, which has the uncommon property of binding irreversibly to most chromatographic resins (12). By using lysophosphatidylcholine (lyso-PC) as a model system, we have observed by electron microscopy that the basic protein isolated with CTAB in the lipid-bound form is able to induce structural transition of micellar lyso-PC into bilayers with formation of mono and multilaminar vesicles.

This finding as well as one-step isolation of the lipid-binding basic protein are reported below.

MATERIALS AND METHODS: Cethyltrimethylammonium bromide (CTAB) was purchased from SERVA, hydroxyapatite from BIO-RAD Laboratories.
Myelin was prepared from bovine brain white matter according to (13) in the presence of 0.25mM phenylmethylsulfonil fluoride (PMSF).
Protein content was determined with a modified Lowry method in the presence of 0.5% sodium dodecylsulfate (SDS) (14).
Polyacrylamide gel electrophoresis in sodium dodecylsulfate was performed according to (15) as already described (11).
Phospholipid content was determined as in (16). Thin layer chromatography was carried out on 0.2 mm Merck silica gel plates. Purified MBP (50 µl, 40-50 µg) was applied in CTAB as obtained after chromatography. Lipids were detected by α-naphtol reagent and/or iodine vapours (17).
Electron microscopy: lysolecithin, myelin basic protein or the mixture of the two components were diluted 10-20 fold with 5mM Hepes, 1mM EDTA pH 7.4. A drop of the final suspension was put on a copper grid covered by formwar and carbon and washed-stained by several drops of the negative stain. This consisted of 1% uranyl acetate pH 4.5 or 2% ammonium molybdate pH 7.2 in water. The excess of stain was removed with filter paper and the specimen was left to air dry and observed in a Philips 400 T electron microscope.

RESULTS

ONE STEP PURIFICATION OF BASIC PROTEIN IN CTAB

Crucial to the isolation of the basic protein in CTAB is its selective extraction from bovine brain myelin (18). Only a single chromatographic step on hydroxyapatite is then required for final purification (fig. 1). No other chromatographic techniques could be applied because when dissolved in CTAB the basic protein irreversibly binds to the usual resins (12).

Solubilization of myelin was carried out at low CTAB/protein ratio (0.6-0.8mg CTAB/mg protein) in Tris-HCl, pH 8.5. After 30 min incubation in

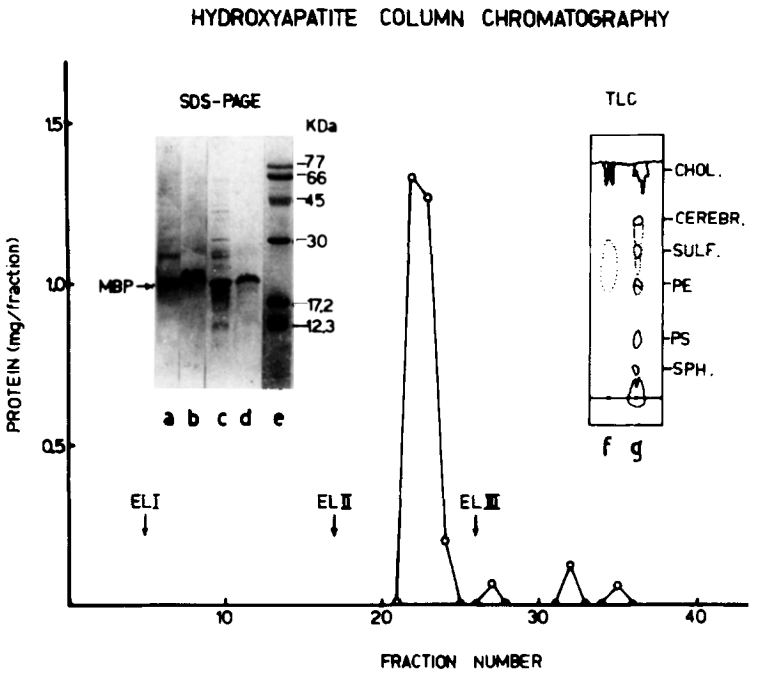


Fig. 1. Hydroxyapatite column chromatography of the CTAB-extract (7% of myelin proteins) at 4°C. 5mg protein in 14.8ml were applied to the 1.6x8.5 cm column. 2.9ml fractions were collected at a flow rate of 34ml/h. El I: 0.1% CTAB, 20mM Tris-HCl pH 8.5. El II: 0.1% CTAB, 0.5M NaCl, 20mM Tris-HCl pH 8.5. EL III: 0.1% CTAB, 400mM NaPi, 20mM Tris-HCl pH 8.5.
Inset on the left: SDS-Polyacrylamide gel electrophoresis (SDS-PAGE). From the left to the right the samples are: (a,c) CTAB-extracts, 23 μg and 16 μg; (b,d) purified basic protein, 15 μg and 10 μg; (e) standards proteins: ovotransferrin, BSA, ovoalbumin, carbonic anhydrase, myoglobin, cytochrome c (from top to bottom). The gel of lanes (a), (b) was fixed with 20% methanol, 2.1% perchloric acid before staining.
Inset on the right: thin layer chromatography (TLC) of lipids bound to the purified basic protein. Eluting system: chloroform:acetone:methanol:acetic acid:water (50:20:10:10:5, v/v). Flow time: 180 min. Spots were detected by spraying the plate with α-naphtol reagent and heating at 100°C for approx. 5 min. Lane f: CTAB (0.73mg, yellow); lane g: myelin basic protein (45 ug) purified in CTAB. From top to bottom the lipids are: cholesterol (red); cerebrosides (bluish purple); sulfatides (bluish purple); phosphatidyl-ethanolamine (PE) (yellow brown); phosphatidylserine (PS) (yellow brown) and sphyngomyeline (Sph) (yellow brown).

ice the suspension was centrifuged for 40 min at 40000 rpm in the 60 Ti Beckman rotor. The extract, containing mostly basic protein (7-22% of myelin proteins) (inset in fig. 1; lanes a, c), was applied to a hydroxy-apatite column (about 0.3mg protein/ml hydroxyapatite) equilibrated with low ionic strenght eluant 0.1% CTAB, 20mM Tris-HCl, at pH 8.5. After washing with 1.5 volumes of the same medium, the basic protein (35-60% of

applied proteins) was eluted by addition of 0.5M NaCl to the first eluant (fig. 1), as expected for a cationic protein-detergent complex (19).

The recovered protein fraction, 4-8% of myelin proteins, was electrophoretically pure (inset in fig. 1; lanes b, d).

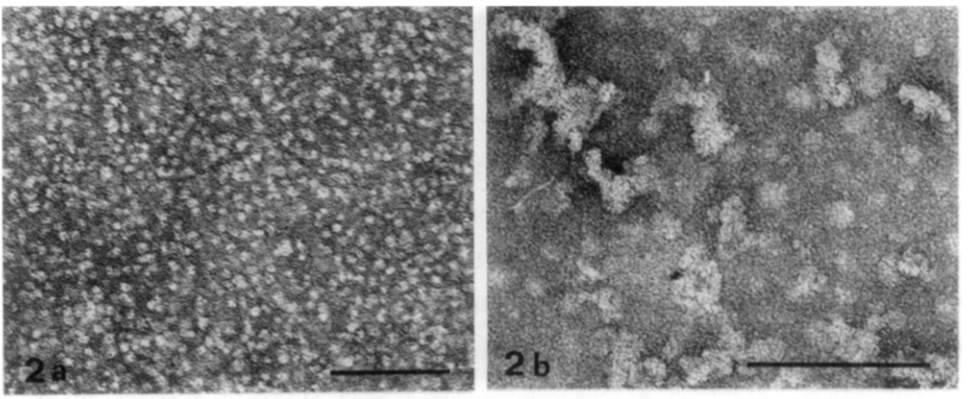
Purified basic protein was found to be in the lipid-bound form. Thin layer chromatography of the purified protein showed the presence of most myelin lipids (inset in fig. 1; lane g). Phospholipids content was as high as 16:1 (mol/mol), or 0.87 μmol Pi/mg protein.

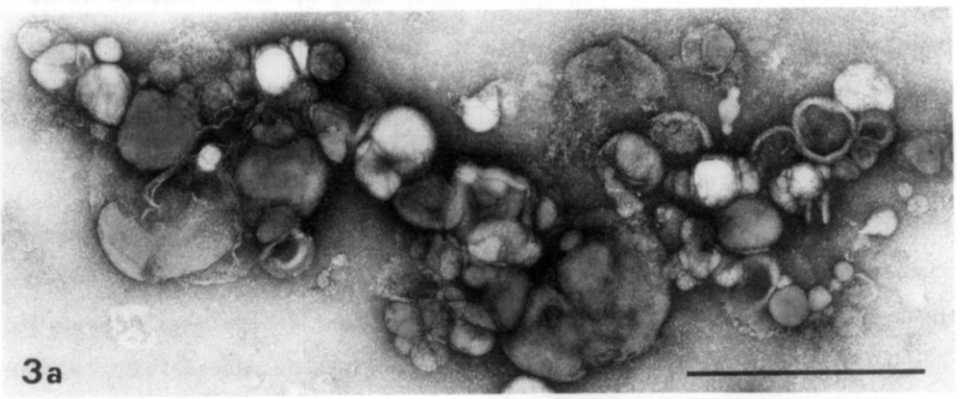
INTERACTION OF THE BASIC PROTEIN WITH LYSOPHOSPHATIDYLCHOLINE

Purified basic protein (0.9-2mg/ml), was diluted twice with 5mM Hepes, 1mM EDTA at pH 7.4 (Hepes-EDTA buffer). Lyso-PC (15-20 mg/ml) was then added so that the final lipid to protein ratio was 10-17 (w/w), i.e. 385-644 (mol/mol). After 48 hours dialysis at 4°C vs Hepes-EDTA buffer, the samples were taken for electron microscopy.

Fig. 2a shows lysolecithin dispersion in the Hepes-EDTA buffer when negatively stained with 1% uranyl acetate in water. The lipid molecules aggregated in form of homogeneus roundish particles, 7-8 nm in diameter, as already described (20,21). Purified myelin basic protein after dialysis vs Hepes-EDTA buffer appeared as in fig. 2b when observed with identical procedures. It consisted of polymorphous aggregates without any peculiar organization.

Lysolecithin suspension mixed with basic protein gave rise to a heterogeneous population of particles, most of which consisted of apparently normal micelles and polymorphous spheroidal bodies. However, a significant amount of material appeared to be organized as liposomes and empty vesicles with a marked tendency to collapse (fig. 3a). The internal organization of these structures could be better appreciated after staining with ammonium molybdate (fig. 3b). The stain penetrated the proteolipid membranes revealing the multilayered organization of some liposomes and showing that small vesicles were enclosed in larger ones. The appearance of such vesicles was conditioned by certain aspects of their internal organization, such as number of membrane layers and whether or not small vesicles were present inside. The thickness of the membrane layers, measured by optical diffraction on multilamellar liposomes, was 5.14 ± 0.34 nm.





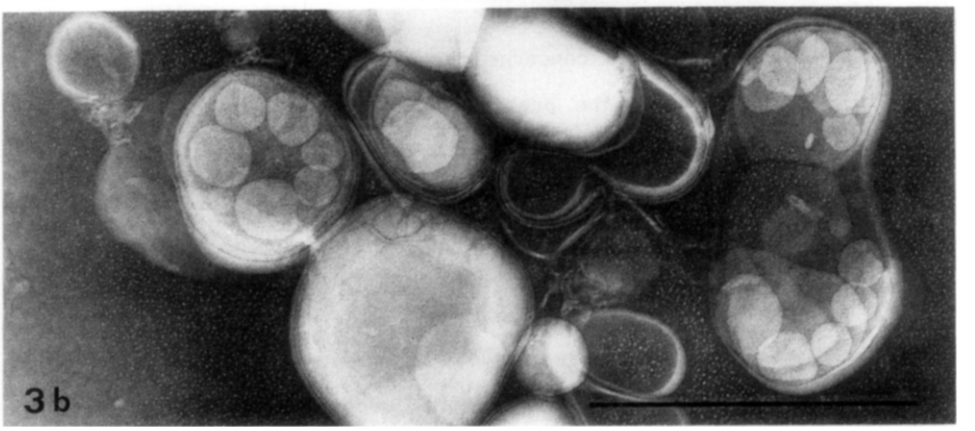


Fig. 2. (a): Lysolecithin suspension negatively stained with 1% uranyl acetate on the grid. The population was fairly homogeneous and consisted of 7-8 nm wide micelles.

(b): Purified basic protein negatively stained on the grid with 1% uranyl acetate. It was always in form of heterogeneous aggregates. Bar 0.1 μm.

Fig. 3. Lysolecithin-myelin basic protein mixture after incubation at 4°C for 48 hours. The specimens were stained on the grid with 1% uranyl acetate (a) or 2% ammonium molybdate (b). Bar 1 μm.

On the other hand, lipid-free basic protein prepared according to (8) and normally used as the isolated form of the MBP failed to give similar

structures, and in the EM inspection resulted in structures similar to those shown in fig. 2a.

DISCUSSION

Lyso-PC is a detergent-like zwitterionic lipid which does not adopt a bilayer structure in water but rather organize itself into micelles (22,23). Its action on membranes is to destabilize bilayers (22). Previous studies have shown that the lipid-free basic protein self aggregates in the presence of lyso-PC (24) without crosslinking its micelles (25).

In our study, electron microscopy produced good evidence that at least a certain amount of myelin basic protein interacts with lyso-PC molecules leading to complexes organized as membrane vesicles. No attempts were made to isolate these membrane structures from micelles or from other polymorphous aggregates still present in our experimental conditions.

Since the lipid-bound basic protein purified in the slightly polydisperse n-octyl pentaoxyethylene (octyl-POE) (11) has an equal capability to form lyso-PC vesicles (results not shown), the observed effect seems likely to be due to a special property of the basic protein when associated to myelin lipids.

As far as lipid binding is concerned, purification of the basic protein in a lipid-bound form in the presence of an often denaturing detergent such as CTAB (23), should confirm this property.

Thus, both lipid binding and the ability to form bilayers could indicate that, besides stabilizing myelin structure, the basic protein might also have a role in organizing it.

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