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THE EFFECT OF BOVINE MYELIN BASIC PROTEIN ON THE PHASE TRANSITION PROPERTIES OF SPHINGOMYELIN

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The basic protein of myelin can spontaneously associate with the synthetic phospholipid *N*-palmitoylsphingosinephosphatidylcholine. The protein alters the phase transition properties of the lipid from a single transition at 41.5°C to two overlapping transitions, one being slightly above and the other slightly below the transition temperature of the pure lipid. The effect was not seen upon the addition of poly(L-lysine) to this lipid nor does the myelin basic protein alter the phase transition properties of dimyristoylphosphatidylcholine. The results thus demonstrate that the myelin basic protein can interact with a major zwitterionic lipid component of myelin in addition to acidic phospholipids.

The basic protein constitutes about 30% of the total myelin proteins and is rich in basic amino acid residues. Of its 170 residues it contains 18–19 arginine, 12–13 lysine and 10 histidine residues [1,2]. When a few micrograms are injected with complete Freund's adjuvant, this protein induces experimental allergic encephalomyelitis. The highly basic nature of the protein allows it to interact with acidic phospholipids by electrostatic as well as by hydrophobic interactions [3]. It has also been shown that the myelin basic protein can bind to the zwitterionic lipid, phosphatidylcholine and induce aggregation of vesicles of this lipid [4]. Sphingomyelin is another major zwitterionic lipid component of the myelin membrane accounting for about 20% of the total phospholipid content of this membrane. In other studies of protein-lipid interactions, specific association of membrane proteins to sphingomyelin rather than phosphatidylcholine has been suggested (Ref. 5 and references therein). It was therefore of interest to examine the ability of the myelin basic protein to interact with sphingomyelin.

Myelin was isolated from bovine white matter and basic protein extracted form it by the method of Lowden et al. [6]. Poly(L-lysine) HBr (mol. wt. 160000) was obtained from New England Nuclear. A sample of the synthetic sphingomyelin, DL-*erythro*-*N*-palmitoylsphingosinephospatidylcholine, was generously supplied by Dr. D. Shapiro of the Weizmann Institute of Science. This lipid was twice recrystallized by the method of Estep [7], Dimyristoylphosphatidylcholine was purchased from Sigma Chemical Co.

The buffer used for the present work was 20 mM Pipes, 1 mM EDTA, 150 mM NaCl and 0.02 mg/ml NaN_3, pH 7.40.

Relative protein concentrations were determined by a modified Lowry procedure [8] and

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Abbreviation: Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid).

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phospholipid concentration by the Bartlett method of total phosphate determination [9].

Lipid films were deposited on the walls of glass test tubes by evaporation of the solvent from a chloroform-methanol (2:1, v/v) solution. Last traces of solvent were removed in a vacuum oven. The lipid was then suspended in a buffer or protein solution by vortexing above the phase transition temperature of the sample.

The phase transition properties of the lipid preparations were determined by differential scanning calorimetry (DSC) using an instrument designed by Privalov [10]. A scan rate of 0.5°C/min was used. The DSC curves were resolved into components with a computer program using an iterative procedure.

Mixtures of myelin basic protein and palmitoylsphingosinephosphatidylcholine at a molar ratio of 1:13, formed from a protein solution and lipid film, exhibited altered phase transition characteristics compared to the pure lipid suspended in buffer (Fig. 1, solid line). Computer curve fitting resolved the lipid plus basic protein curve into two components with transitions at 40.8 and 42.6°C and each having a van't Hoff enthalpy

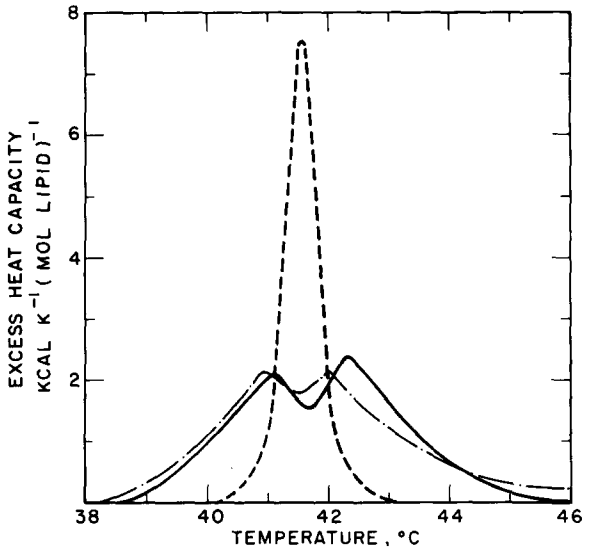

Fig. 1. DSC of the transition region of *N*-palmitoylsphingosinephosphatidylcholine in the presence and absence of myelin basic protein. 20 mM Pipes, 1 mM EDTA, 150 mM NaCl and 0.02 mg/ml NaN_3, pH 7.40. 1000 μM lipid alone (- - - - -); 75 μM basic protein with 1000 μM lipid (———); 20 μM basic protein with 660 μM lipid (· – · – ·).

of approx. 350 kcal/mol and a cooperative unit of 50. At a higher lipid to protein molar ratio of 33:1, the increase caused by the protein in the transition temperature of the higher melting component was somewhat less (Fig. 1). A complete study of varying lipid to protein molar ratios is required to obtain a complete phase diagram for this system. The protein did not cause a detectable change in the total enthalpy of the transition of 7 kcal/mol at any of the protein to lipid ratios studied. The appearance of the DSC curve was unaltered by sequential cycles of heating and cooling. No transition of a basic protein solution in the absence of lipid could be observed in this temperature range. Mixing a solution of the basic protein with an aqueous suspension of palmitoylsphingosinephosphatidylcholine (rather than a lipid film) resulted in a similar perturbation of the phase transition characteristics of the lipid. Therefore alterations in phase transitions were independent of the method of preparation of the vesicles. At room temperature (below the phase transition) the basic protein was not tightly bound to the lipid as it could be recovered in the supernate by centrifugation for 2 min in an Eppendorf centrifuge followed by a wash of the precipitate with an equal volume of buffer. Of the original lipid, 85% was recovered in the precipitate which had the same phase transition characteristics as untreated lipid in the absence of protein. The effects of the protein are therefore reversible.

The effects on the phase transition were not the result of nonspecific interactions of a basic protein with lipid. Adding a solution of poly(L-lysine) to a lipid film of this synthetic sphingomyelin did not result in any alteration of the phase transition at similar weight ratios of lipid to peptide (approximately equal weights).

Addition of a solution of myelin basic protein to a lipid film of dimyristoylphosphatidylcholine, in contrast to the results with sphingomyelin, did not lead to an alteration of the phase transition properties of the lipid. In a separate experiment, taurodeoxycholate was used to assure intimate mixing of the lipid and protein. Thus equal weights of dimyristoylphosphatidylcholine, myelin basic protein and taurodeoxycholate were dissolved in Pipes buffer. The taurodeoxycholate was removed by dialysis against Pipes buffer at room tempera-

ture for 4 days with several changes of buffer. The phase transition of resulting turbid retentate was only slightly affected. The premelt transition disappeared and the main transition was slightly broadened compared with an untreated sample of lipid but the melting temperature was lowered by less than 0.2°C. Similar changes were observed for a lipid control prepared from taurodeoxycholate solution in the absence of protein. These small effects may be due to residual traces of taurodeoxycholate or lipid degradation products produced during the long dialysis procedure.

The apparent stronger effect of the basic protein on sphingomyelin phase transitions compared with lecithin phase transitions led us to test the effects of adding a solution of the basic protein to a lipid film composed of equimolar amounts of palmitoylsphingosinephosphatidylcholine and dimyristoylphosphatidylcholine. The phase transition properties of this lipid mixture is already broad but no further broadening or separation into components could be observed in the presence of the basic protein.

These results further confirm that the myelin basic protein can interact with zwitterionic lipids as well as acidic phospholipids. The ability of this protein to alter the phase transition properties of a sphingomyelin is greater than its ability to effect the phase transition properties of a phosphatidylcholine suggesting a stronger interaction with the former class of lipids.

The sphingomyelin has a complex phase transition behaviour in the presence of myelin basic protein. A number of factors can give rise to the effects including changes in the lipid hydration, as may occur with metal ions and zwitterionic lipids, leading to an increased melting temperature [11]. In addition penetration into the hydrophobic matrix and certain electrostatic interactions can lead to a lower melting component [3].

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