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DIRECT MEASUREMENT OF THE ENERGETICS OF ASSOCIATION BETWEEN MYELIN BASIC PROTEIN AND PHOSPHATIDYLSERINE VESICLES

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Rekha Prabhu

December 1985

ACKNOWLEDGEMENTS

I take this opportunity to express my deep appreciation and gratitude to my advisor, Dr. Ernesto Freire, for his assistance, patience and expert guidance with my research. I would also like to thank my colleagues in the laboratory for their help and assistance. A special thanks to Cora Wortman for her encouragement.

Finally, I owe this achievement to my parents who have helped and encouraged me throughout my career.

ABSTRACT

A newly designed, high sensitivity, isothermal reaction calorimetry system has been used to investigate the thermodynamics of the association between myelin basic protein and phosphatidylserine vesicles. This instrument has allowed us to measure directly the energetics of the protein-lipid interaction under various conditions. Above the phospholipid phase transition temperature the enthalpy of association is highly exothermic amounting to -160 kcal/mole of protein. Below the phospholipid phase transition temperature the enthalpy of association is exothermic at protein/lipid ratios smaller than 1/50 and endothermic at higher protein/lipid ratios. These studies indicate that the association of myelin basic protein to phosphatidylserine vesicle consists of at least two stages involving different types of binding. The first stage, at low protein/lipid ratios, involves a strong exothermic association of the protein to the membrane and the second, at high protein/lipid ratios, a weaker association probably involving attachment of the protein to the membrane surface only. In the gel phase, the second binding stage is endothermic and appears to be correlated with the formation of large vesicle aggregates. This vesicle aggregation is a reversible process dependent upon the physical state of the membrane. The isothermal titration studies have been complemented with high sensitivity differential scanning calorimetry experiments. It is shown that the dependence of the phospholipid transition enthalpy on the protein/lipid molar ratio can be expressed in terms of the different protein-membrane association enthalpies in the gel and fluid phases of the membrane.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

MBP	Myelin Basic Protein
DMPS	Dimyristoylphosphatidylserine
Brain PS	Brain phosphatidylserine
CM 32	Carboxymethylcellulose

CHAPTER I

INTRODUCTION

Previous studies of the interaction of purified proteins with well defined phospholipid bilayers of varying composition have dealt primarily with the effects of proteins on the fluidity and organization of lipids. Studies in this area have focused mainly on interactions within the bilayer matrix and have yielded significant amounts of thermodynamic information (Freire et al., 1983; Chapman et al., 1979). Until today however, little was known regarding the energetics of protein association and insertion into membranes. The aim of this project is to thermodynamically characterize the interaction of myelin basic protein with phospholipid bilayers and thereby provide a fuller understanding of the structural role played by this protein in the myelin sheath.

Myelin basic protein is found in myelin membranes which protect and insulate nerve axons from the surrounding environment. Myelin membranes contain approximately 27% by weight protein of which 31% is Myelin basic protein (Eng et al., 1968). One proposed role for the basic protein is maintenance of the multilamellar structure of the myelin sheath which is found in the central and peripheral nervous systems (Golds et al., 1978). Myelin basic protein is rich in lysine and arginine and hence is positively charged at pH 7.4, thereby it is able to interact electrostatically with negatively charged lipids (Boggs et al, 1978). Hydrophobic interactions have also been reported to play a part in the association of myelin basic protein with lipid bilayers (Stollery et al., 1980; Boggs et al., 1981a). The role of this protein in the induction of experimental allergic encephalomyelitis and its possible

participation in neurological diseases such as multiple sclerosis, stress the need to understand more fully its mode of interaction with the phospholipids in the myelin membrane.

Various physical techniques have been used to study this interaction (Boggs et al., 1982; Keniry et al., 1979; Lampe et al., 1983; Sixl et al., 1984). For this research project a novel, highly sensitive, computer controlled isothermal microcalorimeter has been used in order to measure directly the enthalpy of association of myelin basic protein with phosphatidylserine vesicles. These studies have been complemented with high sensitivity differential calorimetric measurements in order to develop a complete thermodynamic picture of the interaction.

CHAPTER II

MATERIALS AND METHODS

Purification of Myelin Basic Protein

Carboxymethyl cellulose (CM 32) was purchased from Whatman. All other chemicals were reagent grade.

Myelin basic protein (MBP) was isolated and purified from pig brains according to the method of Eylar et al. (1974).

The procedure is described for 100g of material. White matter from fresh brains was collected, stripped of meninges and rinsed with saline (0.9% sodium chloride). Defatting of the tissue was carried out using methanol and chloroform. The tissue was homogenized with 250 ml methanol (stored at -30°C) for two minues in a Waring blender. Blending was continued for another two minutes after addition of 500 ml chloroform (-30°C). The homogenate was allowed to stand for two hours in a separatory funnel and the lower chloroform layer discarded. The remaining solution was mixed with 1 g Celite 545 and filtered through Whatman No. 4 filter paper. The residue was washed with 250 ml acetone in order to remove all residual chloroform and lipids.

The defatted residue was suspended in 500 ml distilled water and pH adjusted to 2.1 with 6N hydrochloric acid. After stirring for 12 hours, the solution was centrifuged at 15,000 g for 15 minutes in a Sorvall centrifuge and the residue discarded.

The supernatant fluid was adjusted to pH 5.5 with 15N ammonium hydroxide, allowed to stir for one hour and then filtered using Whatman No. 4 filter paper. The pH 5.5 supernatant fluid was adjusted to 50% saturation

with ammonium sulfate, pH adjusted to 6.0 and stirred for four hours. The precipitate was collected on Whatman No. 1 filter paper and washed with 200 ml 50% ammonium sulfate.

The ammonium sulfate precipitate was dissolved in 30 ml water, combined with nine volumes of acidified acetone (1 ml 12N hydrochloric acid per liter of acetone) and allowed to stand for 30 minutes.

The flocculent precipitate was collected by centrifugation at 10,000 g for 10 minutes using glass centrifuge tubes. The precipitate was then dissolved in 50 ml distilled water, dialyzed overnight against 4 L distilled water and lyophilized. Final purification was achieved using a carboxymethyl ion exchange column.

Column Chromatography

The wet precycled carboxymethyl exchanger was equilibrated with 50mM ammonium acetate pH 5, and packed into a 12 ml column after removal of fines (Whatman catalog). Flow rate was maintained at 20 ml/hour. The crude lyophilized protein was dissolved in the loading buffer (50 mM ammonium acetate + 50 mM sodium chloride pH 5) and applied to the column.

Fifty 1 ml fractions were collected with the starting elution buffer of 50 mM ammonium acetate pH 5. At tube no. 50 a linear gradient was started by feeding 1 M sodium chloride. A total of 150 1 ml fractions were collected. The elution profile (Figure 1)[1], monitored by measuring absorbance at 280 nm using an LKB spectrophotometer, shows two peaks: Peak 1 - Fractions 55-74 and Peak 2 - Fractions 75-95. Fractions 55-74 and 75-95 were pooled, pH

[1]All figures may be found in the Appendix.

adjusted to 2.1 with glacial acetic acid, dialyzed separately against 4 L 0.1 M acetic acid, and lyophilized.

About 50-60% of the total protein elutes in Peak 2 as pure MBP, thus in the usual preparation 0.4-0.45 g of homogenous MBP is obtained from 100 g fresh pig brains.

Establishment of purity

The purity of MBP was established using SDS gel electrophoresis (Laemmli, 1970; Maizel, 1971). A 12.5% polyacrylamide gel was loaded with appropriate molecular weight (MW) markers and different amounts of each protein from each of the 2 peaks eluted off the CM 32 column. Electrophoresis was carried out at constant voltage (200V) and the gel stained with Coomassie blue. As shown in Figure 2, MBP eluted in the second peak and migrated with a MW of 19,000 which compares favorably with previous findings (Tomasi and Kornguth, 1967).

Preparation of Vesicles

Dimyristoyl phosphatidylserine (DMPS) and Bovine Brain phosphatidylserine (Brain PS) were purchased from Avanti Biochemicals, Inc. (Birmingham, AL) and used without further purification. To prepare small unilamellar vesicles the lipid was first dried from a chloroform solution and lyophilized. The dried lipid was suspended in 10mM Tricine buffer pH 7.5 containing 0.02% sodium azide to give a final lipid concentration of 8-10 μmoles/ml. The lipid suspensions were sonicated for one hour at 45° for DMPS and at 25°C for Brain PS using a bath sonicator (Model A112 SPIG, Laboratory Supplies, Hicksville, NY). In order to prevent the oxidation of Brain PS by air, extreme care was

taken to keep the lipid under an argon atmosphere. The purity of the lipid samples was checked by thin layer chromatography.

The above method produced a stable population of small single lamellar vesicles. The sizing of the vesicles by negative stain electron microscopy yielded an average diameter of 600 $\mathring{A}$ $\pm$ 200 $\mathring{A}$ for DMPS and 300 $\mathring{A}$ $\pm$ 120 $\mathring{A}$ for Brain PS.

Differential Scanning Calorimetry

Calorimetric experiments were performed with a Microcal MC1 differential scanning calorimeter. The sensitivity and precision of the basic calorimetric unit have been improved by the use of two Keithley amplifires connected to the heat capacity and temperature outputs of the calorimeter and interfaced to an IBM PC microcomputer using a Data Translation (DT-805) A/D conversion board for automated data collection and analysis. With pure lipid dispersions, concentrations lower than 0.5 mg/ml can be used with a total sample volume of 0.7 ml. All the calorimetric scans in this paper were performed at a scanning rate of 20°C/hour except when noted otherwise.

Isothermal Reaction Calorimetry

The microcomputer controlled calorimetric titration module developed in this laboratory is used in conjunction with the LKB Bioactivity Monitor (LKB 2277). This multichannel calorimetry system based upon the original design of Suurkuusk and Wadso (1982) provides a measuring sensitivity better than 0.15 μW (1 μW = 1 μjoule/sec = .239 μcal/sec) with baseline noise better than 0.1 μW. The instrument accomodates up to four measuring cylinders suspended in a thermostated water bath. The water temperature is maintained within

$\pm$ 0.0002°C over an 8 hour period. The modular design of this instrument has allowed us to customize it to suit our requirements. A block diagram of the microcomputer controlled titration module is shown in Figure 3. Briefly, it consists of a standard cell assembly modified to accept a stirring mechanism and sample delivery system, plus the appropriate computer interfaces for control, data collection and analysis.

The stirrer is driven by a D.C. motor and operates at 90 rpm only while the reactant is being delivered and thirty seconds afterwards. The efficiency of this mixing set-up was evaluated spectrophotometrically using a concentrated bromophenol blue solution. The measured half-time for mixing is 4 $\pm$ 0.5 seconds with no detectable leak of solution between injections.

The injection assembly consists of a glass syringe with a threaded shaft. The shaft is rotated by a stepper motor connected to a stepper motor controller and interfaced to a microcomputer system by a RS-232 serial interface. In the laboratory, the titration module is operated either by a TEC-86 or an IBM-PC microcomputer. The microcomputer is programmed to automatically start the titration with preset delivery volumes and time lapse between injections. An analog/digital converter board digitizes and collects the data during the duration of the experiment.

Prior to a titration experiment, the calorimeter cell is filled with the appropriate amount of one of the reactants and the cell assembly is lowered into the calorimeter chamber for equilibration. Under these conditions equilibration of the titration cell until it reaches a constant baseline takes about 90 minutes. Figure 4 shows the results of an electric heat calibration experiment. As shown in the figure, the baseline noise is better than 0.05 μW.

Analysis of the time decay of the calibration pulse indicates a single exponential decay with a calorimeter time constant of 220 seconds.

Isothermal Calorimetry Data Analysis

When the solution containing reactant B is injected into the calorimeter cell containing reactant A, the total heat measured, Q, is the sum of three components:

$$Q = Q_r + Q_d + Q_s$$

where Q_r is the heat of reaction between components A and B, usually the desired quantity, Q_d the heat of dilution of the reactants, and Q_s, the mechanical heat associated with the injection and stirring of the solution. The heat of dilution of reactant B is obtained in a separate experiment by injecting reactant B into a buffer solution lacking reactant A. Similarly, the heat of dilution of A is obtained by injecting buffer into the calorimeter cell containing reactant A. Due to the configuration of the titration system (cell sample volume = 2-3 ml; injection volume = 25 μl, typically), the heat of dilution of A is usually negligible. Finally, the mechanical heat of stirring and injection is obtained by injecting buffer into buffer or water into water.

Association of Myelin Basic Protein and Phosphatidylserine Vesicles

For the experiments described in this thesis, 3 ml of the desired vesicle suspension at a concentration of 5 μ moles/ml were placed in the calorimetric cell. The syringe was preloaded with MBP solution (C = 1.2mM), the calorimetric cell capped and lowered into the equilibrating position. At the end of 30 minutes, the cell was lowered into the measuring position and allowed to equilibrate until a steady baseline was reached. The TEC 86 was then

programmed to automatically start the titration with a preset delivery volume of 25 μl at 120 minute intervals in order to be able to detect the existence of any slow reactions. All the isothermal experiments were conducted at 20°C.

Determination of the Amount of Bound Protein

The amount of protein bound to the phospholipid vesicle was determined using a centrifugation assay similar to the one used by Boggs et al. (1982). The protein-lipid suspensions were centrifuged at 15,000 g in an Eppendorf microfuge and the supernatant and the pellet assayed for protein and lipid. Protein concentration was determined by Lowry et al. (1951) and total phosphorus by a modified Bartlet procedure as described by Marinetti (1962).

CHAPTER III

RESULTS

Association of Myelin Basic Protein to Brain Phosphatidylserine

Figure 5 shows the complete calorimetric titration of Brain PS vesicles with MBP at 20°C. Equal amounts of the protein were injected into the vesicle suspension with a preset time interval of 120 minutes. As can be observed in the figure, the association of MBP to Brain PS, which is in the liquid crystalline state at 20°C, is a strongly exothermic process. The magnitude of the titration peaks decreases monotonically upon increasing the protein/lipid ratio even though the amount of protein injected is the same at each step. This behavior arises as a result of the saturation of the ability of the membrane to associate with MBP and/or a decreasing enthalpy of association at increasing protein/lipid ratios. The heat of the reaction Q_R was calculated using the following equation:

$$Q_R(A) = \sum_{i=0}^{A} Q_R(i) = Q_R$$

where,

$Q_R(A)$ is the sum of the heats up to the desired injection

$Q_R(i)$ is the heat of the individual reaction.

The integrated heat ($Q_R(A)$) after correction from dilution and stirring has been plotted as a function of protien/lipid ratio (Figure 6).

Analysis of the first portion of the calorimetric titration curve indicates that the initial association of MBP molecules to the membrane produce an enthalpy change of -160 kcal/mol of protein. This enthalpy change, however,

is not the same at all protein/lipid molar ratios. Calculation of the amount of MBP bound to the Brain PS vesicles at the end of the calorimetric titration revealed that only 41.9% (W/W) of the protein added was associated with the lipid giving a final effective protein/lipid molar ratio of 1/125. Normalization of the total enthalpy of association with respect to the amount of protein bound under saturation conditions yields a mean ΔH of association of -72 kcal/mol of protein. This overall mean enthalpy is smaller in magnitude than the one obtained at the early stages of binding. These results indicate that there are at least two different stages in the association process: a first stage at low protein/lipid molar ratios involving a larger enthalpy change and a second stage at protein/lipid molar ratios larger than 1/300 involving a smaller enthalpy change. It must be noted, however, that the association of MBP to Brain PS in the fluid phase is exothermic at all protein/lipid molar ratios studied.

Association of Myelin Basic Protein to Dimyristoyl Phosphatidylserine

At 20°C DMPS vesicles are below their phase transition temperature both before and after the calorimetric titration. Figure 7 shows the results of the calorimetric titration of DMPS vesicles with MBP. The titration pattern of DMPS with MBP is more complicated than that of Brain PS. At low protein/lipid ratios the titration peaks are exothermic as in the case of Brain PS; however, at protein/lipid ratios greater than 1/150 a second faster process appears to be taking place as indicated by the appearance of the endothermic peak. As the protein/lipid ratio increases, the endothermic component increases in magnitude and the overall enthalpy becomes positive. Plotting the integrated heats as a function of protein/lipid ratio yields a curve shown in Figure 8. At low protein/lipid ratios, the enthalpy of association is negative and

monotically increases in magnitude as the amount of protein bound increases. However, at protein/lipid ratios greater than 1/100, the enthalpy of association becomes increasingly positive as a result of the appearance of the endothermic component in the calorimetric titration peaks. Finally, at protein/lipid ratios greater than 1/40, the overall integrated enthalpy becomes positive.

These experiments clearly indicate that the association of MBP to DMPS in the gel phase is a complex phenomenon consisting of at least two different and separate processes. At low protein/lipid ratios, the association process is exothermic indicating that the protein molecules form strong bonds with the phospholipid molecules. This process is similar to the one observed with Brain PS in the fluid phase even though it is characterized by a smaller enthalpy change (ΔH = -98 kcal/mol of protein). The first type of association appears to reach saturation at protein/lipid molar ratios similar to those found for Brain PS even though an exact estimate is difficult due to the appearance of the endothermic component. At higher protein/lipid molar ratios a second type of association is observed. This association is characterized by an endothermic enthalpy change and occurs in a faster time scale than the exothermic association as can be observed in the titration peaks for which the two components are present.

The endothermic component in the titration peaks appears to be correlated with the formation of large aggregates in the MBP/vesicle suspensions. These aggregates are not visible in the fluid phase and in the gel phase their presence is correlated with the amount of protein added. The DMPS vesicle suspensions in the gel phase become immediately turbid after the addition of myelin basic protein at a protein/lipid ratio of 1/150 or higher. Heating the vesicles to a

temperature above the phase transition makes them immediately clear, and cooling below Tm makes them turbid again indicating that the aggregation process is reversible. Examination of the vesicles by negative staining electron microscopy reveals that the aggregation-disaggregation process induced by heating and cooling cycles does not lead to vesicle fusion under the conditions of these experiments.

High Sensitivity Differential Scanning Calorimetry of Myelin Basic Protein-Dimyristoyl Phosphatidylserine Reconstitutions

In order to complete the thermodynamic characterization of the association of MBP to PS vesicles, high sensitivity differential scanning calorimetric experiments were performed as a function of the protein/lipid molar ratio. The results for MBP/DMPS vesicles are shown in Figure 9. In the absence of myelin basic protein the DMPS vesicles show a typical gel-liquid crystalline transition centered at 37.4°C and characterized by an enthalpy change of 5.5 kcal/mol of lipid. Brain PS vesicles, on the other hand, undergo a gel-liquid crystalline transition centered at 13.5°C and characterized by an enthalpy change of 5.2 kcal/mol of lipid (data not shown). The addition of MBP to DMPS vesicles up to a protein/lipid ratio of 1/150 causes a broadening of the transition peak and a slight decrease in the transition temperature with no measurable difference in the transition enthalpy. Increasing the protein/lipid ratio up to 1/50 causes the appearance of two well defined peaks in the capacity profile, the first one centered at 32°C and the second at 36°C. Each peak contains approximately half of the total enthalpy change for the transition. At this protein/lipid ratio the total enthalpy change increases slightly (ΔH=5.8 kcal/mol). Increasing the protein/lipid ratio up to 1/25 has a dramatic effect

on the thermotropic behavior of the system. Under these conditions the transition becomes very broad centered around 29.8°C and with a total enthalpy change of only 3.9 kcal/mol. Under all the conditions studied, the calorimetric profiles were identical after repeated scans of the same samples indicating that the observed processes were reversible.

CHAPTER IV

DISCUSSION

The calorimetric results presented in this paper indicate that the association of MBP to PS vesicles is a complex process dependent upon the physical state of the phospholipid bilayer. In the fluid phase the association is exothermic at all protein/lipid ratios, however at low protein/lipid ratios, the enthalpy of association is larger than at higher protein/lipid ratios. This effect may arise as a result of the existence of two or more stages in the binding process, the first of which is characterized by the formation of stronger bonds between the protein and the lipid molecules. This stage reaches saturation at a protein/lipid ratio of ~ 1/300. The second stage is characterized by a lower enthalpy indicating a weaker association process. Recently, Sedzik et al (1984) have concluded, on the basis of X-ray diffraction studies, that the binding of MBP to myelin lipids involves a strong initial association process followed by a weaker process once the primary binding sites are saturated.

The association of MBP to DMPS vesicles in the gel phase involves two clearly distinguishable processes. At low protein/lipid ratios, the association is exothermic and not unlike that found in the fluid phase except that it is characterized by a smaller enthalpy change. The larger enthalpy observed in the fluid phase can be attributed to additional exothermic contributions arising form the bilayer rigidification induced by MBP (Boggs et al., 1981b). This contribution is absent when the association occurs in the gel phase. If the observed enthalpy difference is normalized with respect to the number of lipid molecules perturbed per protein molecule, we obtain a perturbation enthalpy

of -2.3 kcal/mol of lipid, using the average value of 27 perturbed lipids per protein molecule (Boggs et al., 1981a; Lampe et al., 1983; Sixl et al., 1984). These observations suggest that the phospholipid molecules perturbed by MBP are in a somewhat intermediate state between that of the fluid and gel configurations.

The second stage in the association of MBP to DMPS vesicles in the gel phase is endothermic. This process is correlated with the formation of large aggregates in the vesicle suspensions and probably involves vesicle-vesicle assocations mediated by MBP molecules sticking to the surfaces of two adjacent bilayers (Figure 10). The origin of the net positive enthalpy observed for this binding stage is still unknown but most likely involves the release of water molecules from adjacent bilayers. It must be noted that for the protein/lipid ratios studied, the aggregation process is reversible upon heating and cooling through the lipid phase transition temperature. Under these conditions, the enthalpy change associated with the phospholipid phase transition at any protein/lipid ratio can be expressed in terms of the contributions due to phospholipid melting and protein association:

$$\Delta H = H\ (\text{fluid}) - H\ (\text{gel}) = \Delta H^{o} + \Delta Hb\ (\text{fluid}) - \Delta Hb\ (\text{gel})$$

where ΔH^{o} is the transition enthalpy change in the absence of protein and ΔHb (fluid), ΔHb (gel) the protein binding enthalpies in the fluid and gel phases normalized with respect to the lipid concentration. Figure 11 shows the expected dependence of the phospholipid transition enthalpy on the protein/lipid molar ratio as well as the experimental values obtained by differential scanning calorimetry. The expected values were calculated using the experimental enthalpies of binding and the experimental transition enthalpy

in the absence of protein. As shown in the figure, the calculated dependence predicts a slight decrease in ΔH at very low protein/lipid ratios, and then an increase in ΔH up to a protein/lipid molar ratio of ~1/50 followed by a monotonic decrease at higher protein/lipid ratios. This pattern closely resembles the one obtained experimentally.

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APPENDIX

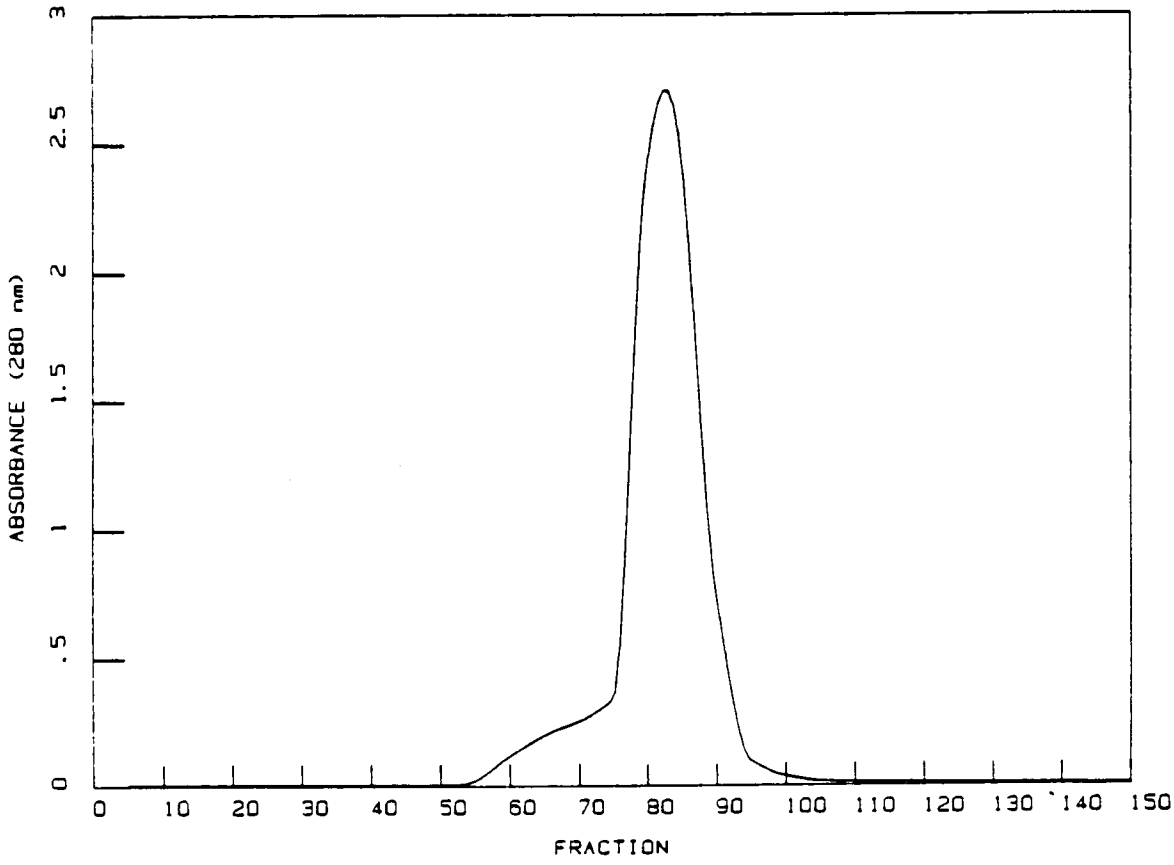

Figure 1. Elution profile off CM-32 column. Fifty, 1 ml fractions were collected with starting elution buffer of 50 mM ammonium acetate pH 5. One molar NaCl was fed at tube # 50. MBP elutes in the second peak comprising fractions 75-95.

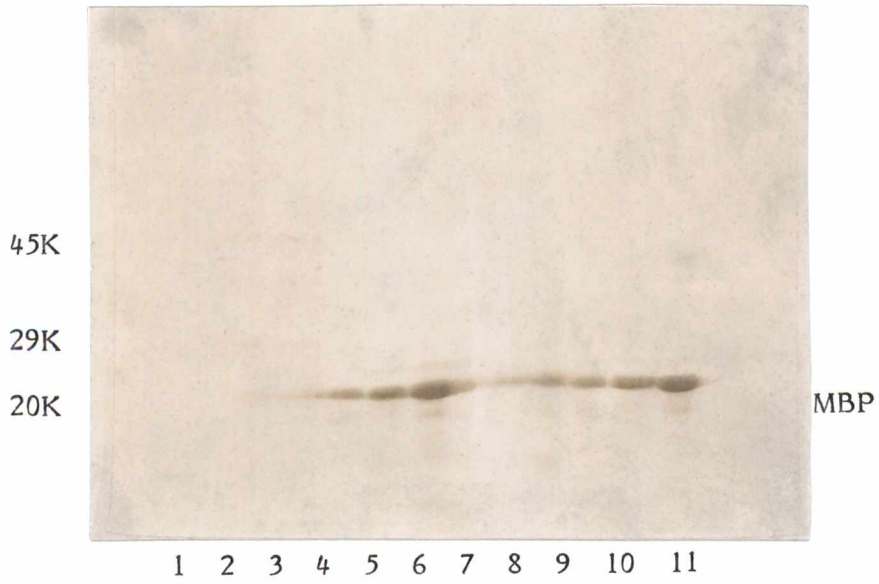

Figure 2. SDS polyacrylamide gel stained with Coomassie blue. 10, 20, 40 µg protein of crude MBP (Lanes 3, 4, 5), pure MBP (Lanes 6, 7, 8), Peak 1 fraction (Lanes 9, 10, 11).

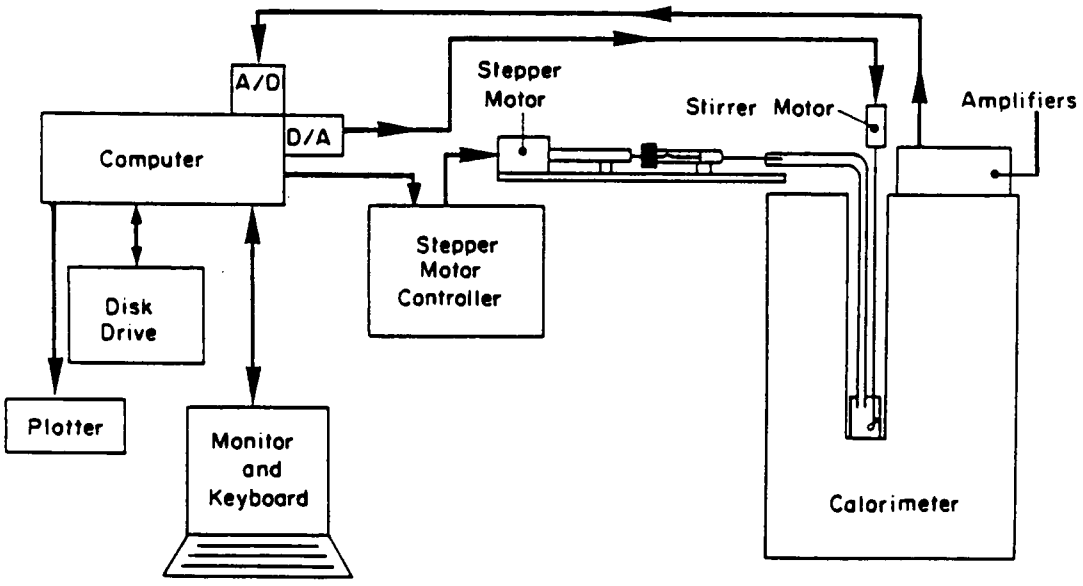

Figure 3. Block diagram showing configuration of computer controlled isothermal reaction calorimetry system.

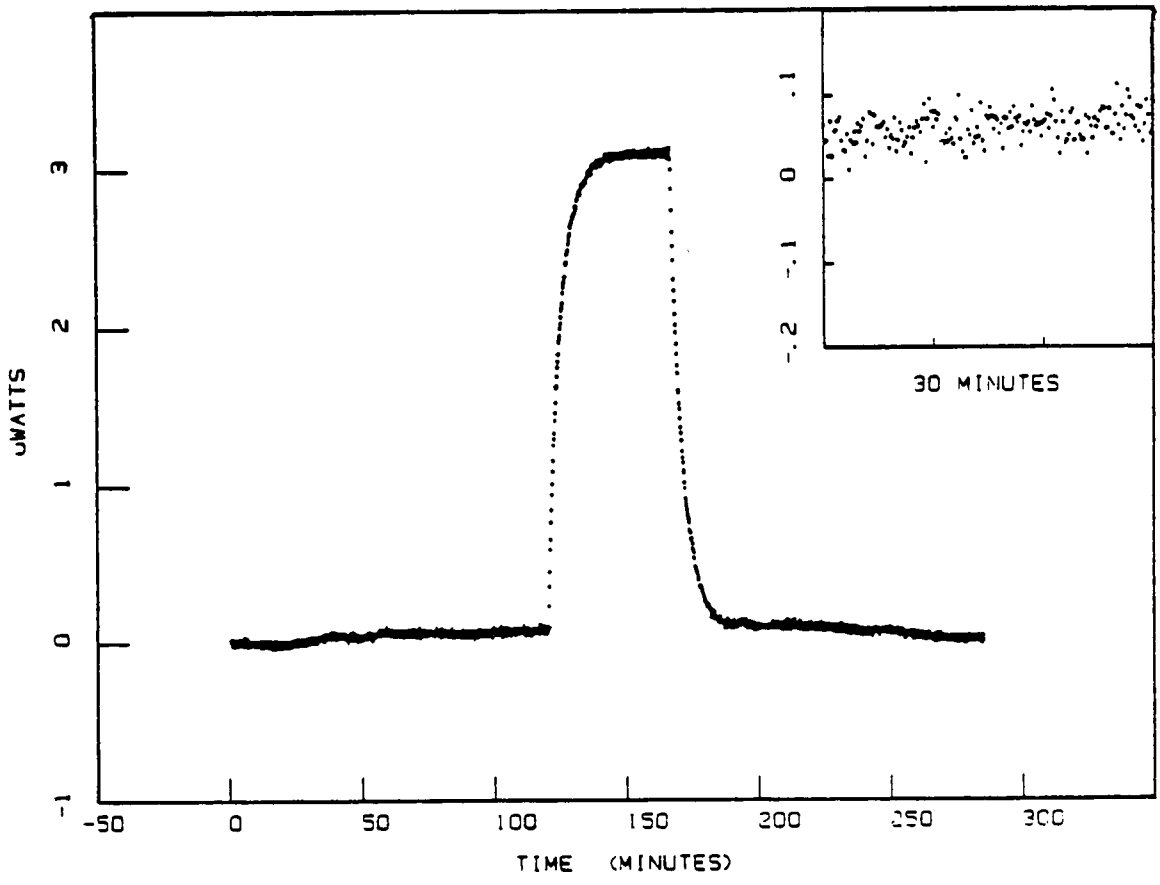

Figure 4. Typical electrical calibration and response of isothermal titration module. The limit of detectability is 0.15 μW with a baseline noise better than 0.05 μW without digital filtering.

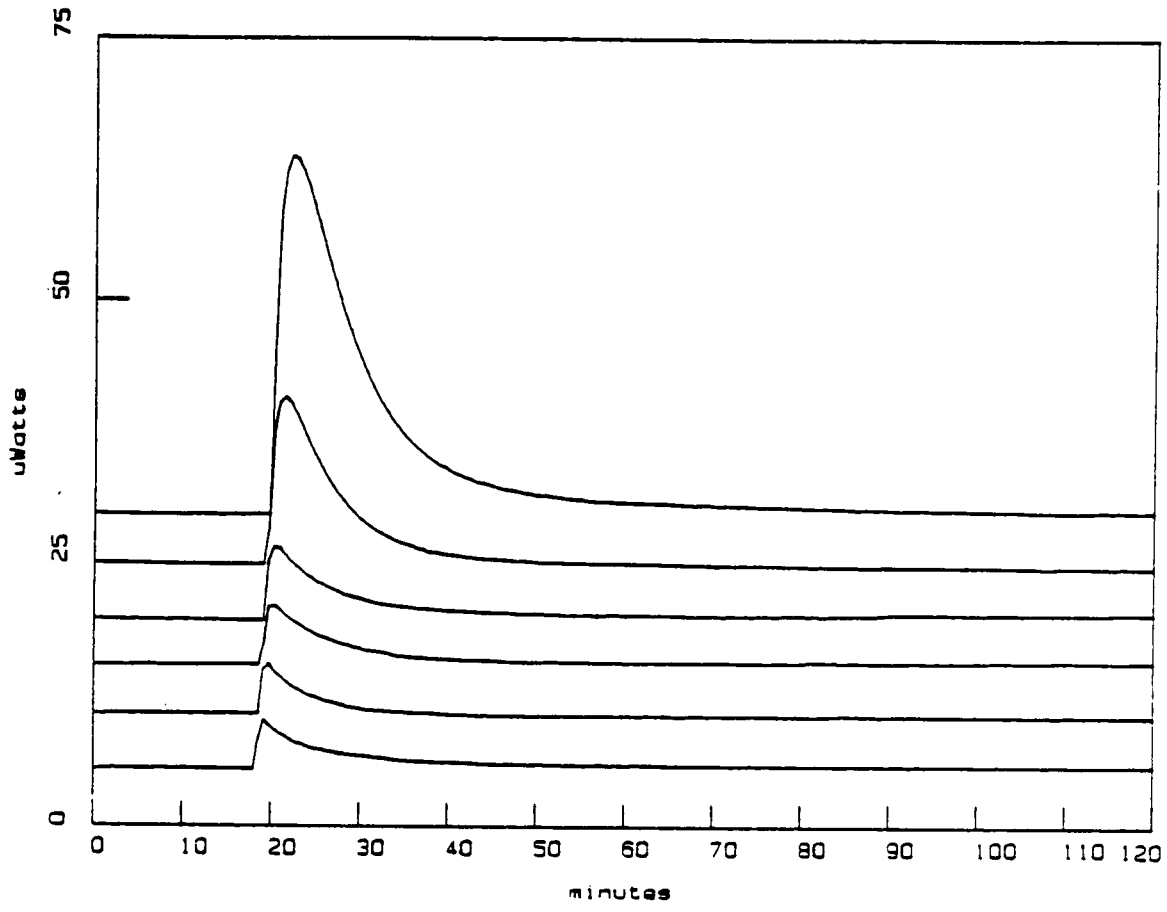

Figure 5. Isothermal titration of brain PS with myelin basic protein at 20°C. The calorimetric peaks (μ Watts vs time) represent the heat evolved after successive injections of myelin basic protein to the same vesicle suspension. From top to bottom, the resulting protein/lipid molar ratios are: 1/500, 1/250, 1/166, 1/125, 1/100, and 1/83. The area under each curve is proportional to the heat released at each titration step (see text for details). The curves have been displaced in the Y axis for presentation purposes.

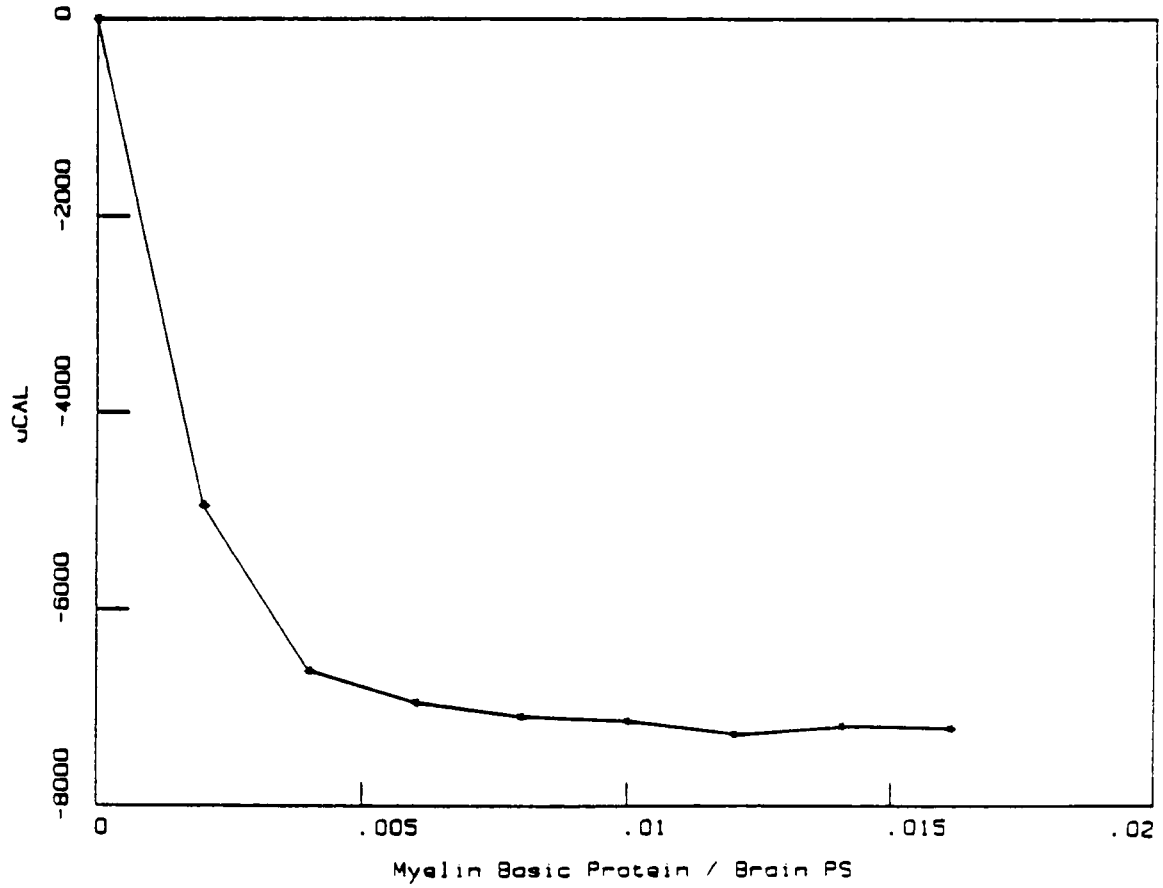

Figure 6. Heat of association of myelin basic protein to Brain PS vesicles at 20°C as a function of the protein/lipid molar ratio. The values plotted are the cumulative areas of the titration peaks after subtracting the mechanical heats of stirring and injection.

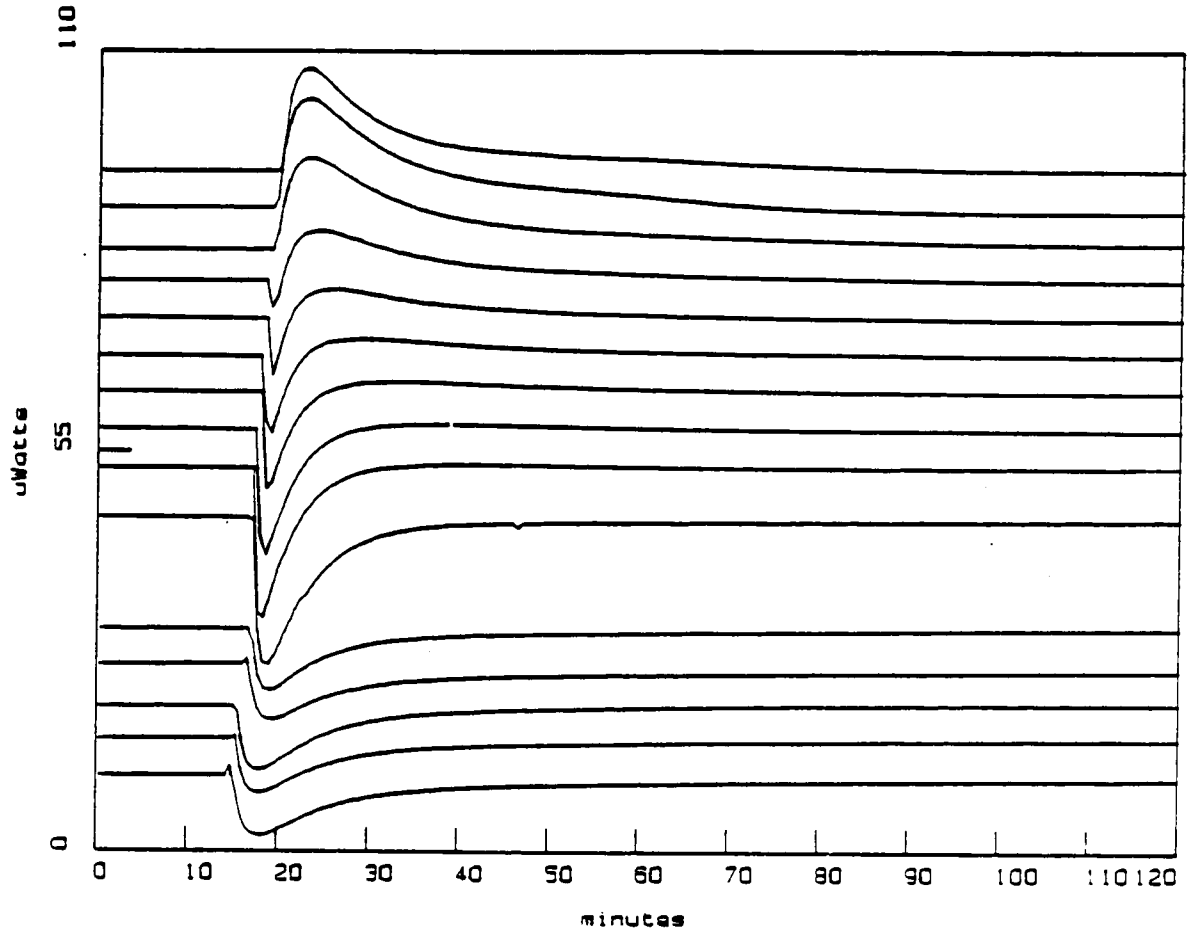

Figure 7. Isothermal titration of DMPS with myelin basic protein at 20°C. The calorimetric peaks (μWatts vs time) represents the heat evolved after successive injections of myelin basic protein to the same vesicle suspension. From top to bottom, the resulting protein/lipid molar ratios are: 1/500, 1/250, 1/166, 1/125, 1/100, 1/83, 1/71, 1/62, 1/55, 1/36, 1/33, 1/31, 1/29, 1/27, and 1/26. Note that at low protein/lipid ratios, the peaks are exothermic and that at high protein/lipid molar ratios, they become endothermic.

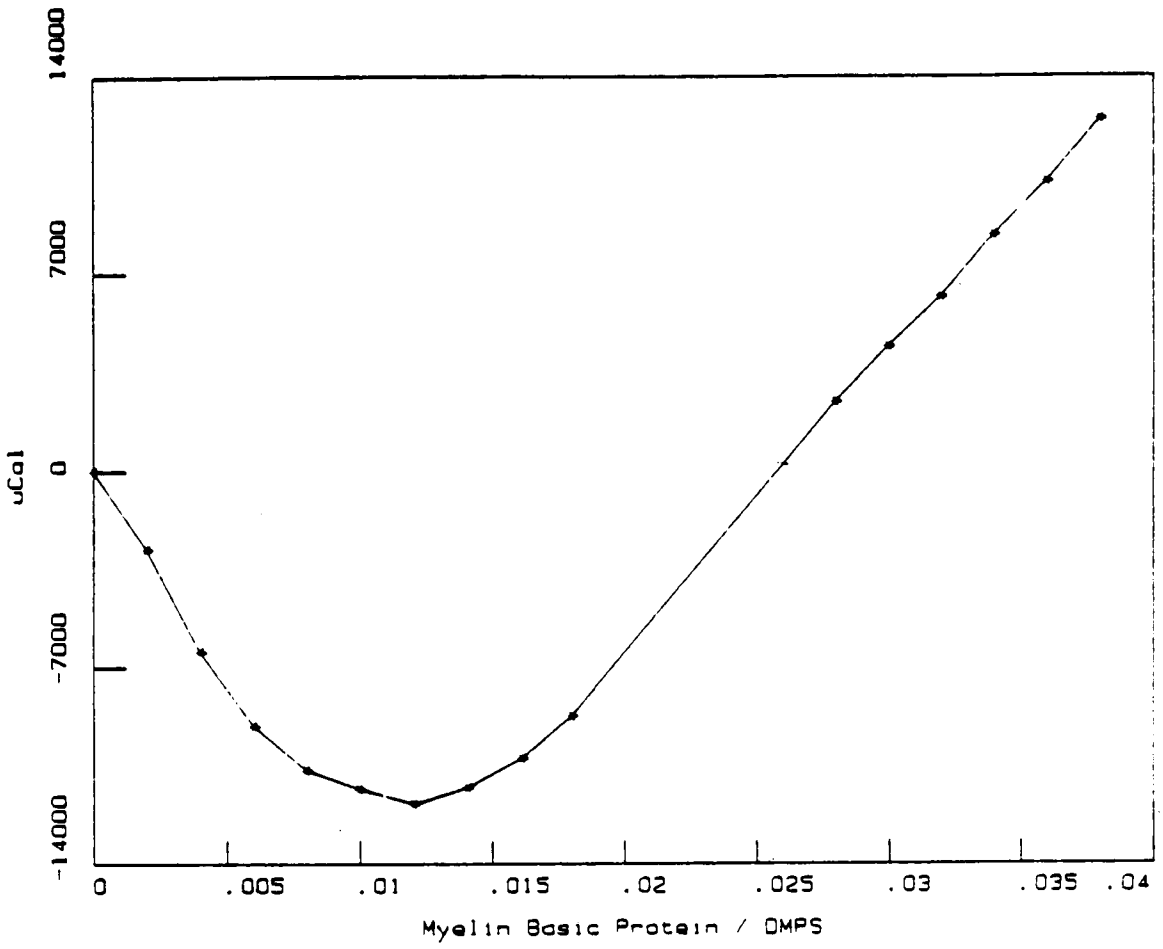

Figure 8. Heat of association of myelin basic protein to DMPS at 20°C as a function of the protein/lipid molar ratio. The values plotted are the cumulative areas of the titration peaks after subtracting the mechanical heats of stirring and injection.

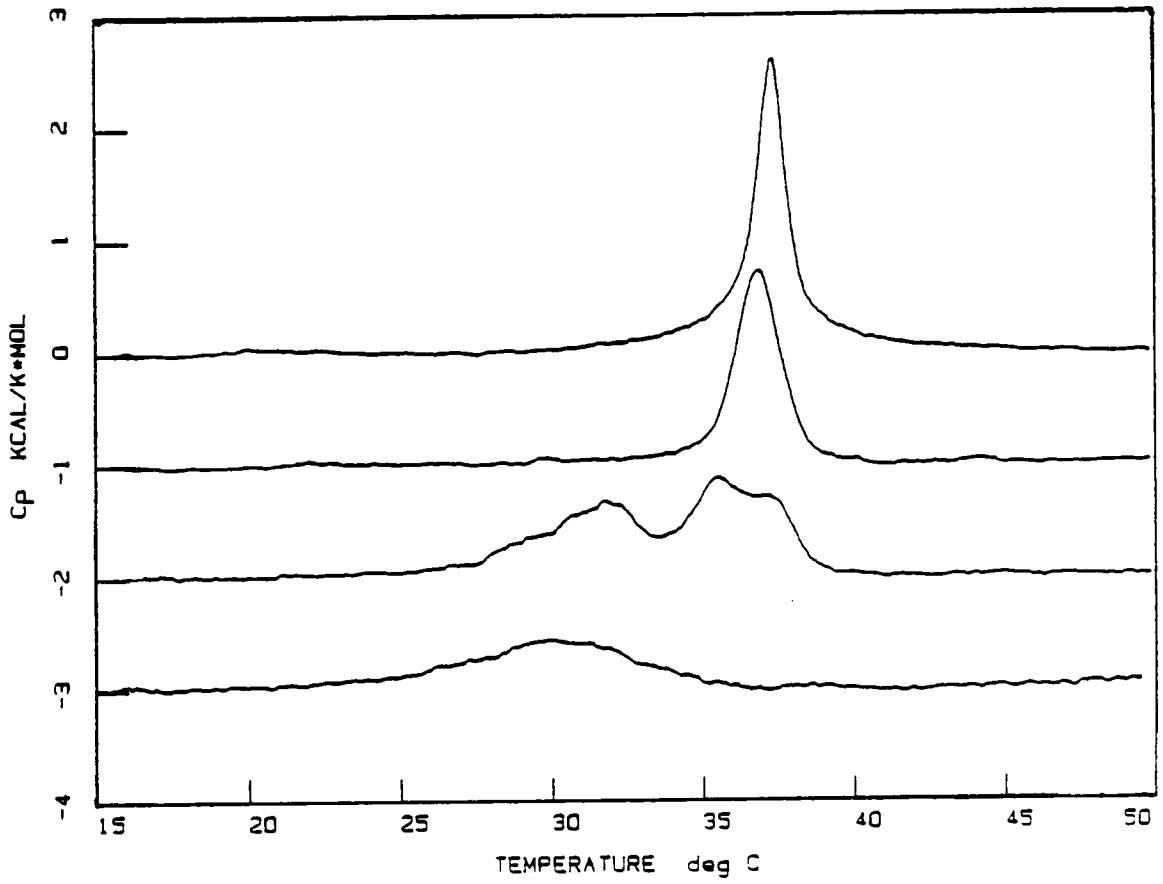

Figure 9. Excess heat capacity function versus temperature for dimyristoyl phosphatidylserine vesicles at increasing myelin basic protein/phospholipid molar ratios. From top to bottom, the protein/lipid ratios are 0, 1/150, 1/50, and 1/25. The curves have been displaced in the Y axis for presentation purposes.

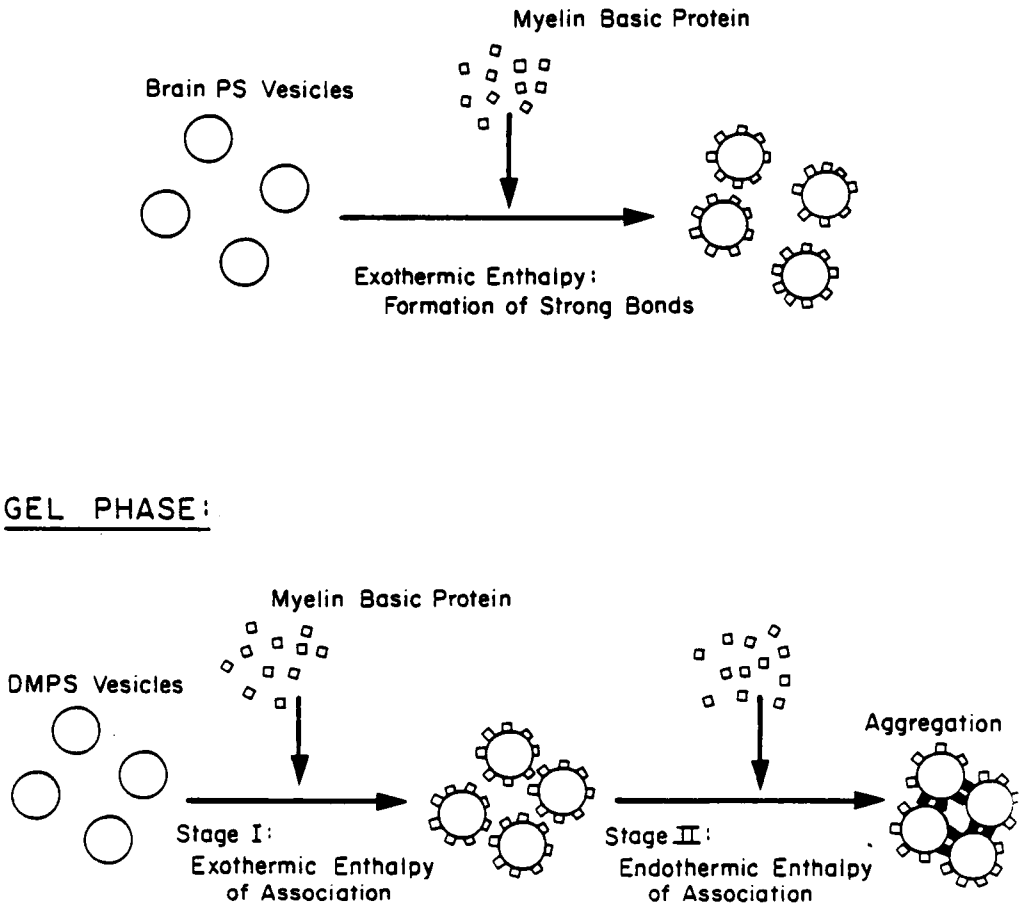

Figure 10. Schematic representation of the association of myelin basic protein to phosphatidylserine in the gel and fluid phases.

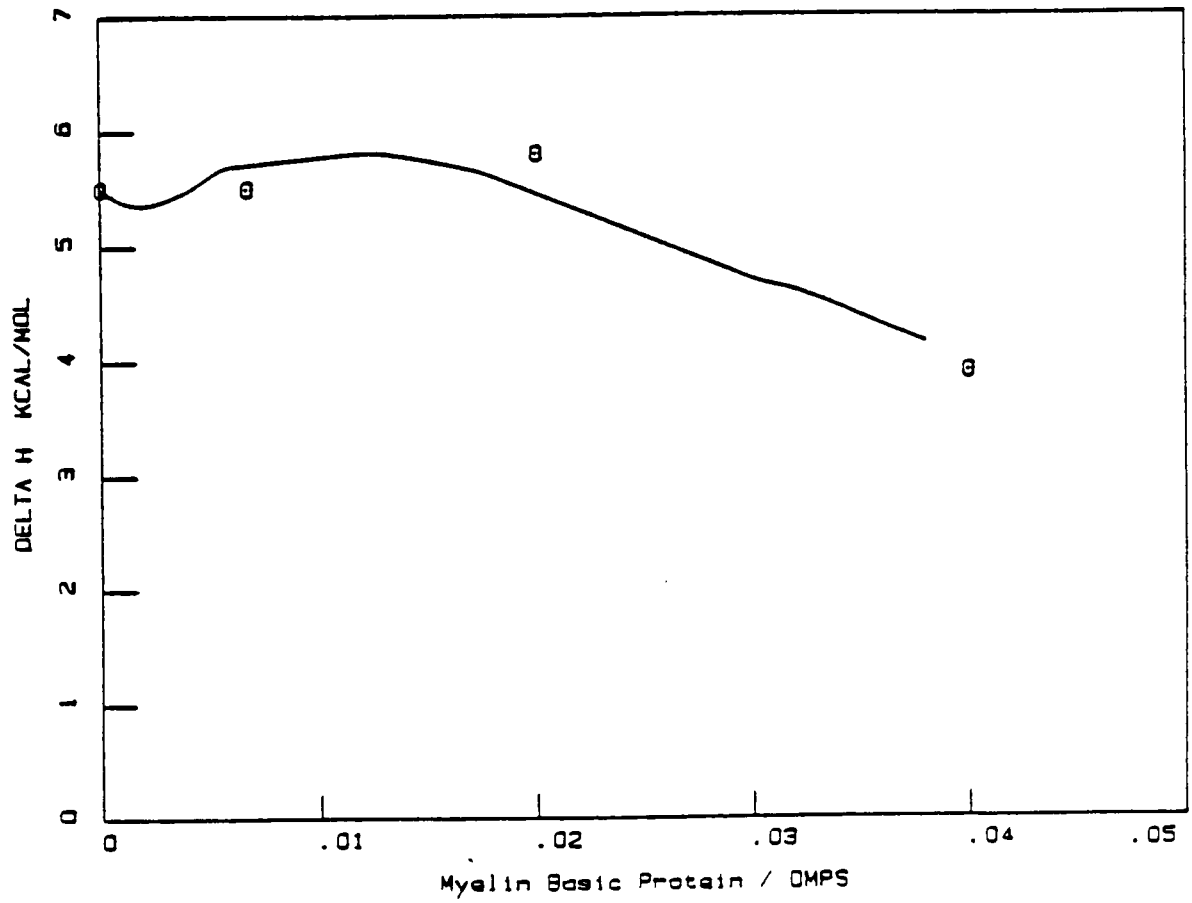

Figure 11. Calculated (solid line) and experimental dependence of the enthalpy change associated with the DMPS gel-liquid crystalline transition on the myelin basic protein/phospholipid molar ratio.

VITA

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