Direct Measurement of the Energetics of Association between Myelin Basic Protein and Phosphatidylserine Vesicles†

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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/mol 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 vesicles 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.

The interaction between phospholipids and membrane proteins is a subject that has attracted considerable attention in the past few years. Most of these studies, however, have dealt with the perturbation of phospholipid properties following the association or incorporation of a membrane protein into the phospholipid bilayer matrix. Several physicochemical techniques including electron spin resonance (Jost et al., 1973; Marsh et al., 1982), nuclear magnetic resonance (Van Zoelen et al., 1978), fluorescence spectroscopy (Kimelman et al., 1979; Freire et al., 1983), and differential scanning calorimetry (Chapman et al., 1979; Freire et al., 1983) have been utilized to characterize the nature and extent of this perturbation. Today, it appears to be clear that integral membrane proteins induce only a local membrane perturbation extending primarily to the layer of lipid immediately adjacent to the protein molecule. This perturbed lipid apparently exists in a more rigid configuration than the fluid lipid and is unable to undergo a cooperative phase transition. Calorimetrically this effect is manifested as a progressive decrease in the phospholipid transition enthalpy upon increasing amounts of protein in the bilayer. Peripheral membrane proteins, on the other hand, do not show such an effect on the phospholipid transition enthalpy (Wiener et al., 1983), suggesting that the enthalpy effect arises from a direct interaction between the phospholipid acyl chains and the hydrophobic protein.

While significant amounts of thermodynamic information exist regarding the protein–lipid interaction inside the bilayer matrix, very little is known regarding the energetics of protein association and insertion into membranes. To study these processes, we have designed and built a highly sensitive computer-controlled isothermal titration microcalorimeter module capable of directly measuring the heat effects accompanying the association and insertion of proteins into membranes. In this paper we present the results obtained for the association of myelin basic protein and phosphatidylserine vesicles.

Myelin basic protein (M_r 18 500) constitutes about 31% of the myelin protein (Eng et al., 1968). The isolated protein is able to associate with different lipids especially negatively charged phospholipids (Demel et al., 1973; Stollery et al., 1980). Even though the primary interaction is electrostatic in nature, there is evidence that hydrophobic segments of the protein are able to intercalate into the hydrophobic region of the bilayer and that the interaction is dependent upon the phospholipid physical state (Boggs & Moscarello, 1978; Boggs et al., 1981a,b). Thus, the myelin basic protein association with the membrane appears to be a complex process involving interactions with both the phospholipid head groups and acyl chains. In order to elucidate the energetics of this interaction, we have measured directly the enthalpy of association of myelin basic protein to phosphatidylserine vesicles in the gel and fluid phases. All the calorimetric titration experiments were conducted isothermally at 20 °C. Bovine brain phosphatidylserine and dimyristoylphosphatidylserine were used as fluid and gel bilayer systems, respectively. These studies have been complemented with high-sensitivity differential scanning calorimetric measurements in order to develop a complete thermodynamic picture of the interaction. To the best of our knowledge, this is the first time that the energetics of the association of a protein to the membrane has been measured directly.

Materials and Methods

Preparation of Vesicles. Dimyristoylphosphatidylserine (DMPS) and bovine brain phosphatidylserine (PS) were purchased from Avanti Biochemicals (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 10

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mM Tricine buffer, pH 7.5, containing 0.02% sodium azide to give a final lipid concentration of 8–10 μmol/mL. The lipid suspensions were sonicated for 1 h at 45 °C 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 and integrity of the lipid samples was checked by thin-layer chromatography and differential scanning calorimetry before and after the titration experiments.

The above method produces a stable population of small single lamellar vesicles. The sizing of the vesicles by negative-stain electron microscopy yielded an average diameter of 600 ± 200 Å for DMPS and 300 ± 120 Å for brain PS.

Purification of Myelin Basic Protein. Myelin basic protein was purified from pig brain according to the method of Eylar et al. (1974). White matter from pig brain was homogenized and defatted with a 1:2 $CH_3OH/CHCl_3$ mixture. Resuspension of the residue (after defatting) in water, followed by acidification to pH 2.1, extracted 90% of the myelin basic protein. The basic protein was then precipitated by using 50% saturated ammonium sulfate. The crude protein was dissolved in water, dialyzed against 5 L of water, and lyophilized. Further purification was achieved by a CM-32 cation-exchanger column using 50 mM ammonium acetate buffer at pH 5. The protein was eluted by using a gradient of 50 mM ammonium acetate plus 1 M NaCl at pH 5. Fractions containing protein were pooled, adjusted to pH 2, dialyzed overnight against 4 L of 0.1 M acetic acid, and lyophilized. Typical yields were 0.42 g of pure homogeneous protein from 100 g of pig brain tissue. The purity of the protein was established by sodium dodecyl sulfate (SDS) gel electrophoresis (Laemmli, 1970; Maizel, 1971).

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 amplifiers 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/h 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 μJ/s = 0.239 μcal/s) with a base-line noise better than 0.1 μW. The instrument accommodates up to four measuring cylinders suspended in a thermostated water bath. The water temperature is maintained within ±0.0002 °C over an 8-h 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 1. 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 direct current motor and operates at 90 rpm only while the reactant is being delivered and 30 s afterward. The efficiency of this mixing setup was evaluated

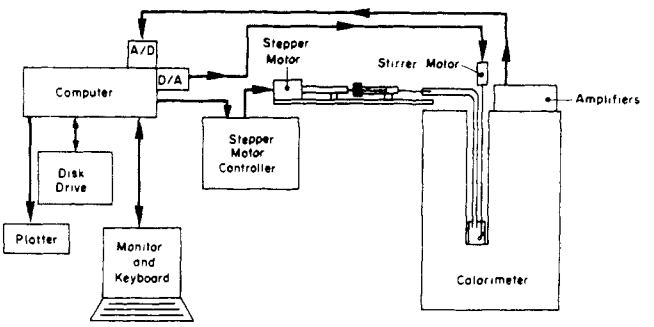

FIGURE 1: Block diagram showing configuration of computer-controlled isothermal reaction calorimetry system.

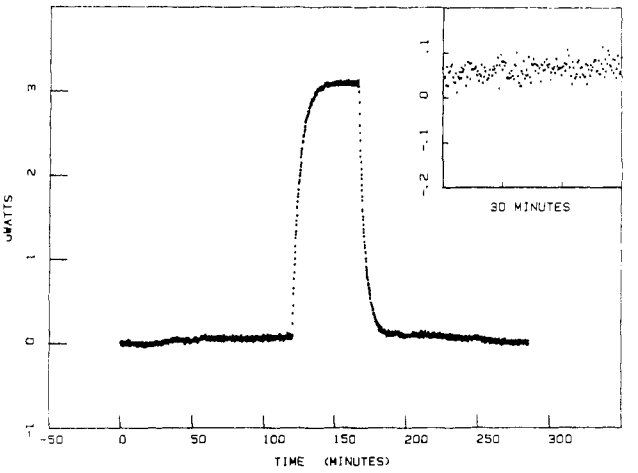

FIGURE 2: Typical electrical calibration and response of isothermal titration module. The limit of detectability is 0.15 μW with a base-line noise better than 0.05 μW without digital filtering.

spectrophotometrically by using a concentrated bromophenol blue solution. The measured half-time for mixing is 4 ± 0.5 s 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 our laboratory the titration module is operated by either 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 base line takes about 90 min. Figure 2 shows the results of an electric heat calibration experiment. As shown in the figure, the base-line 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 s.

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:

$$Q = Q_r + Q_d + Q_s \quad (1)$$

where Q_r is the heat of reaction between components A and B, usually the desired quantity, Q_d is the heat of dilution of the reactants, and Q_s is the mechanical heat associated with

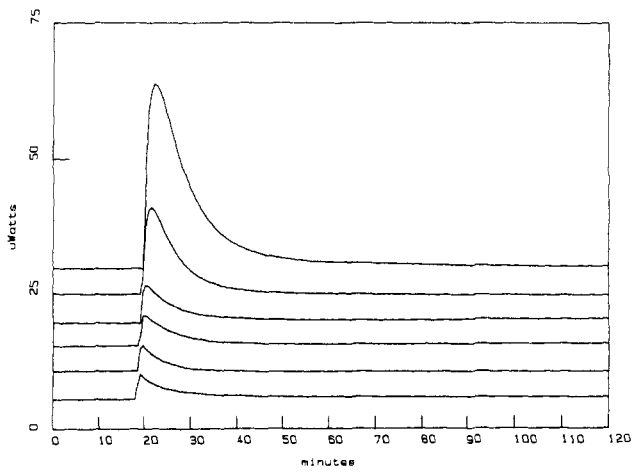

FIGURE 3: Isothermal titration of brain PS with myelin basic protein at 20 °C. The calorimetric peaks (microwatts vs. time) represent the heat evolved after successive injections of myelin basic protein to the same vesicle suspension. From top to bottom the resulting protein/lipid molar ratios are 1/500, 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.

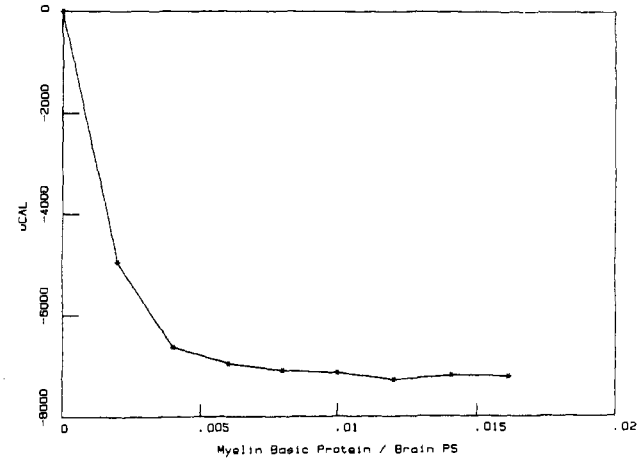

FIGURE 4: Heat of association of myelin basic protein to brain PS vesicles at 20 °C as a function of the protein/lipid molar ratio. The values plotted are the cumulative areas of the titration peaks after subtracting the mechanical heats of stirring and injection.

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. Similarily, 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 presented in this paper 3 mL of the desired vesicle suspensions, at a concentration of 5 μmol/mL, was placed in the calorimeter cell. After equilibration the titration experiments were started by injecting 25 μL of the protein solution (C = 1.2 mM) for each titration step. Injections were made at constant time intervals of 120 min in order to be able to detect the existence of any slow reactions. All the isothermal experiments in this paper were conducted at 20 °C. The amount of protein bound to the phospholipid vesicles was determined independently by using a centrifugation assay similar to that proposed by Boggs et al. (1982). Briefly, the vesicle suspensions were centrifuged at 15000g for 5 and 15 min and the supernatant and pelleted material assayed for protein and phosphate. Protein concentration was determined as described by Lowry et al. (1951), and total lipid phosphorus was assayed by using a modified Bartlett procedure as described by Marinetti (1962).

RESULTS

Association of Myelin Basic Protein to Brain Phosphatidylserine Vesicles. Figure 3 shows the results of a complete calorimetric titration of brain phosphatidylserine vesicles with myelin basic protein. The injections were performed at 120-min intervals, each one delivering the same amount of myelin basic protein into the vesicle suspension as indicated in the figure legend. At the temperature of these experiments brain PS is in the liquid-crystalline state. As can be observed in the figure the association of myelin basic protein to brain PS is a strongly exothermic process under the conditions of these experiments. The magnitude of the titration peaks decreases monotonically upon increasing the protein/lipid molar ratio even though the amount of protein injected is the same at each titration step. For a calorimetric titration the origin of this effect may be twofold: the saturation of the ability of the membrane to associate with myelin basic protein and/or a decreasing enthalpy of association at increasing protein/lipid molar ratios. This effect is better illustrated in Figure 4 in which the integrated heat after correction from dilution and stirring effects has been plotted as a function of the protein/lipid molar ratio.

Analysis of the first portion of the calorimetric titration curve indicates that the initial association of myelin basic protein molecules to the membrane produces 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 myelin basic protein 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 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 myelin basic protein to brain PS in the fluid phase is exothermic at all protein/lipid molar ratios studied.

Association of Myelin Basic Protein to Dimyristoylphosphatidylserine. Figure 5 shows the results of the calorimetric titration of dimyristoylphosphatidylserine (DMPS) vesicles with myelin basic protein. At the temperature of these experiments (20 °C) the DMPS vesicles are below their transition temperature both before and after completion of the calorimetric titration (see Figure 7). As can be observed in Figure 5 the titration pattern of the DMPS vesicles is more complicated than that of brain PS. At low protein/lipid molar ratios the titration peaks are exothermic as in the case of brain PS; however, at protein/lipid molar ratios larger than 1/150 a distinct endothermic component becomes distinguishable in the calorimetric traces in addition to the exothermic component. It should be noted that the endothermic component

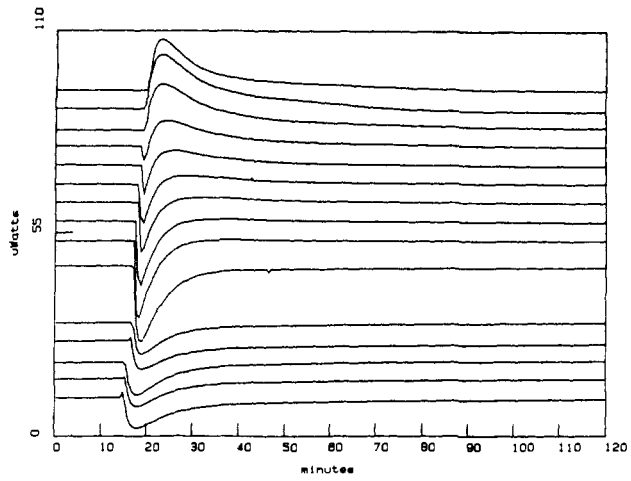

FIGURE 5: Isothermal titration of DMPS with myelin basic protein at 20 °C. The calorimetric peaks (microwatts vs. time) represent the heat evolved after successive injections of myelin basic protein to the same vesicle suspension. From top to bottom the resulting protein/lipid molar ratios are 1/500, 1/250, 1/166, 1/125, 1/100, 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.

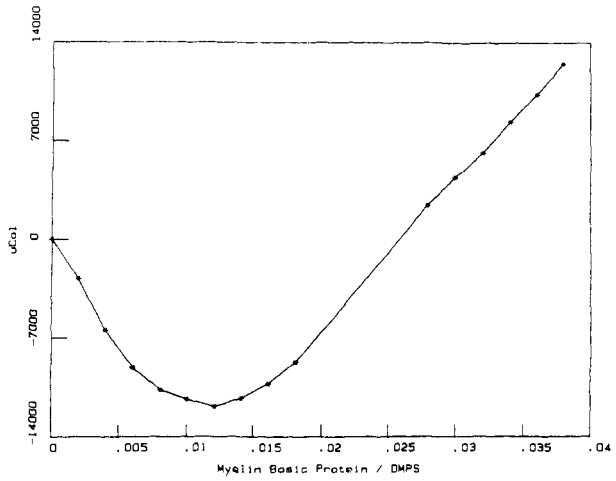

FIGURE 6: Heat of association of myelin basic protein to DMPS at 20 °C as a function of the protein/lipid molar ratio. The values plotted are the cumulative areas of the titration peaks after subtracting the mechanical heats of stirring and injection.

appears earlier than the exothermic, indicating that it arises from a faster process. As the protein/lipid molar ratio increases, the endothermic component increases in magnitude and the overall enthalpy of association becomes positive. This is illustrated in Figure 6 in which the total heat of association has been plotted as a function of the protein/lipid molar ratio. This figure clearly illustrates that at low protein/lipid molar ratios the enthalpy of association is negative and monotonically increases in magnitude as the amount of protein bound increases. At protein/lipid molar ratios greater than 1/100 the dependence of the enthalpy change on protein concentration changes sign as a consequence of the appearance of the endothermic component in the calorimetric titration peaks. Finally, at protein/lipid molar ratios greater than 1/40 the overall integrated enthalpy change of association becomes positive.

These experiments clearly indicate that the association of myelin basic protein 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

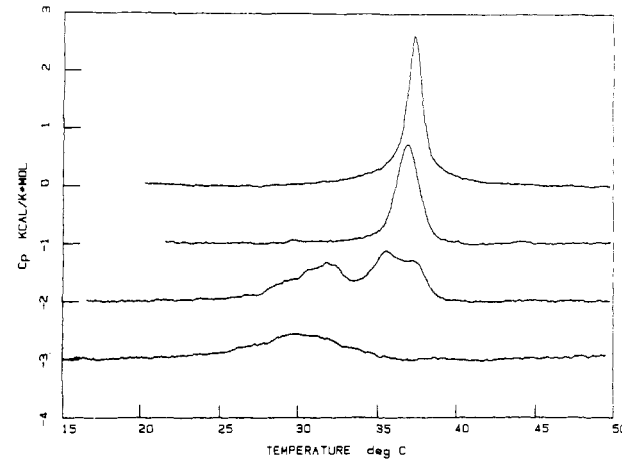

FIGURE 7: Excess heat capacity function vs. temperature for dimyristoylphosphatidylserine vesicles at increasing myelin basic protein/phospholipid molar ratios. From top to bottom the protein/lipid ratios are 0, 1/150, 1/50, and 1/25. The curves have been displaced in the y axis for presentation purposes.

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 ($\Delta H = -98$ 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 myelin basic protein/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 T_m 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–Dimyristoylphosphatidylserine Reconstitutions. In order to complete the thermodynamic characterization of the association of myelin basic protein to phosphatidylserine vesicles, high-sensitivity differential scanning calorimetric experiments were performed as a function of the protein/lipid molar ratio. The results for myelin basic protein–dimiristoylphosphatidylserine vesicles are shown in Figure 7. 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 myelin basic protein 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 and that the samples were structurally and chemically stable during the time frame of these experiments.

Discussion

The calorimetric results presented in this paper indicate that the association of myelin basic protein to phosphatidylserine 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 $\sim$1/300. The second stage is characterized by a low enthalpy, indicating a weaker association process. Recently, Sedzik et al. (1984) have also concluded, on the basis of X-ray diffraction studies, that the binding of myelin basic protein to myelin lipids involves a strong initial association process followed by a weaker process once the primary binding sites are saturated.

The association of myelin basic protein 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 from the bilayer rigidification induced by myelin basic protein (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 myelin basic protein are in a somewhat intermediate state between that of the fluid and gel configurations.

The second stage in the association of myelin basic protein 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 associations mediated by myelin basic protein molecules sticking to the surfaces of two adjacent bilayers. 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:

$$\Delta H = H(\text{fluid}) - H(\text{gel}) = \Delta H^0 + \Delta H_b(\text{fluid}) - \Delta H_b(\text{gel}) \quad (2)$$

where ΔH^0 is the transition enthalpy change in the absence of protein and ΔH_b(fluid) and ΔH_b(gel) are the protein binding enthalpies in the fluid and gel phases normalized with respect to the lipid concentration. Equation 2 allows calculation of the expected enthalpy change for the phospholipid gel–liquid-crystalline transition at any protein/lipid ratio. By use of the experimental enthalpies of association obtained in this paper and the experimental transition enthalpy obtained in the absence of protein, equation 2 predicts only a slight variation in the transition enthalpy at protein/lipid ratios < 1/50 (a small decrease at protein/lipid ratios < 1/200 followed by a small increase of 0.5 kcal/mol) and then a monotonic decrease at higher protein/lipid ratios. This pattern closely resembles the one observed experimentally for DMPS at different myelin basic protein concentrations (see Figure 7).

The experiments presented in this paper have revealed that the association of a membrane protein to a phospholipid membrane is accompanied by rather large heat effects. Additional experiments with other membrane proteins such as cytochrome b_5 (unpublished results) suggest that the association and insertion of proteins into fluid membranes are highly exothermic processes. To stress the magnitude of these enthalpy changes we would like to point out that the observed heat effects are comparable in magnitude to those found for the complete folding–unfolding transitions of globular proteins like myoglobin, chymotrypsin, cytochrome *c*, ribonuclease, and lyzosyme to name a few (Privalov & Khechinashvili, 1974). Currently we are investigating whether the large amounts of energy released during protein insertion are able to affect other membrane-related processes.

Registry No. DMPS, 64023-32-1.

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An Electrical and Structural Characterization of H^+/OH^- Currents in Phospholipid Vesicles†

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ABSTRACT: Paramagnetic amphiphiles have been utilized to measure and characterize electrogenic H^+/OH^- ion transport in a series of model membrane systems. Membrane conductivity to H^+/OH^- ions varies with the method of vesicle preparation and with the level of saturation of the membrane phospholipid. Small sonicated vesicles have the lowest conductivities by approximately an order of magnitude compared to reverse-phase or ether-injection vesicle systems. This conductivity is particularly sensitive to the presence of polyunsaturated lipids in the vesicle membrane. The current-ΔpH dependence of the H^+/OH^- conductivity shows a nonideal behavior and renders the phenomenological membrane permeability dependent upon the experimental value of ΔpH that is chosen. These factors can account for much, if not all, of the variability in the published values for the H^+/OH^- permeability of model membranes. A procedure has been developed to establish and estimate changes in the dipole potential of vesicle bilayers. Using this method, we demonstrate that H^+/OH^- currents are insensitive to alterations in the membrane dipole field, a result that suggests that these currents are not rate limited by diffusion over simple electrostatic barriers in the membrane interior. In addition, conduction in D_2O has been examined, and we find that there is little difference in the magnitudes of D^+/OD^- currents compared to H^+/OH^- currents in vesicle systems.

Model membrane systems that are formed entirely of membrane phospholipids are generally exceedingly impermeable to small inorganic ions such as Na^+ and K^+. In small vesicles formed from egg phosphatidylcholine, these permeabilities typically range from 10^{-12} to 10^{-14} cm/s; as a consequence, gradients of these ions can be maintained for many weeks in these model membrane systems (Hauser et al., 1972; Johnson & Bangham, 1969; Pike et al., 1982; Mimms et al., 1981). Surprisingly, the apparent permeability determined for protons is many orders of magnitude larger than that for other monovalent ions. This observation has generated considerable interest, and a number of groups have reported both values and characteristics of this proton permeability [see, for example; Nichols et al. (1980), Nichols & Deamer (1980), Biegel & Gould (1981), Clement & Gould (1981), Nozaki & Tanford (1981), Gutknecht & Walter (1981), Rossignol et al. (1982), Deamer & Nichols (1983), Elamrani & Blume (1983), Cafiso & Hubbell (1983), Krishnamoorthy & Hinkle (1984), and Gutknecht (1984)]. These measurements have been carried out with a wide range of systems and techniques that are not necessarily directly comparable. While several features of this proton (or hydroxyl) flux are generally agreed upon (for example, the relative independence of the proton current on the size of the proton gradient, $[H^+]_{in} - [H^+]_{out}$), the values for the reported permeabilities vary by at least 4 orders of magnitude. The molecular basis for this conductivity is presently unknown.

In this paper, we have utilized a novel magnetic resonance approach, employing paramagnetic amphiphiles, to further characterize this net proton flux in a number of model membrane systems. The flux that we measure is electrogenic and is measured under conditions where no neutral flow of H^+/OH^- ions is detectable (Cafiso & Hubbell, 1983). The methodology we employ makes use of membrane-permeable phosphoniums, that we will refer to as I and II. These probes, which are easily monitored with EPR[1] spectroscopy, can be utilized to estimate H^+/OH^- ion currents in phospholipid vesicle systems. Using this methodology, we compare the proton permeability of both large and small unilamellar vesicles

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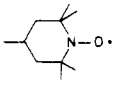
[1] Abbreviations: EPR, electron paramagnetic resonance; egg PC, egg phosphatidylcholine; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; OPPC, 1-oleoyl-2-palmitoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; DAPC, diarachidonoylphosphatidylcholine; PAPC, 1-palmitoyl-2-arachidonoylphosphatidylcholine; DLPC, dilinoleoylphosphatidylcholine; SUV, sonicated unilamellar vesicle; LUV, large unilamellar vesicle; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; Ph_4B^-, tetraphenylboron anion; MES, 2-(*N*-morpholino)ethanesulfonic acid; MOPS, 3-(*N*-morpholino)propanesulfonic acid; tempoyl radical



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