Biochimica et Biophysica Acta 819 (1985) 225–230
Elsevier

BBA 72759

Interaction of myelin basic protein with gangliosides and ganglioside-phospholipid mixtures

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(Received May 9th, 1985)

Key words: Myelin basic protein; Ganglioside; Fluorescence; Differential scanning calorimetry

The interaction of myelin basic protein with monosialoganglioside GM_1 was investigated. It was found that the emission maximum of the tryptophan of the protein is blue-shifted due to the interaction. In mixtures of the monosialoganglioside with phosphatidylcholine, the myelin basic protein induces phase separation of the lipids as inferred from differential scanning calorimetry experiments.

Introduction

The myelin sheath in the nervous system is a well-organized structure, but at present the role of some of its individual components is not well understood. For instance, the presence of gangliosides as minor constituents of myelin has been shown in various mammals yet their molar concentration is similar to that of myelin basic protein [1], which led Moore to imply a specific in situ association of these two components [2].

There is little information as to whether or not any functional interaction exists between these glycosphingolipids with various myelin proteins and especially with myelin basic protein, which comprises about 30% of the proteins in myelin. Such interaction between the basic protein and glycosphingolipids could be both electrostatic and hydrophobic. About one-fifth of myelin basic proteins amino acids are positively charged; however, over 50% of the amino acids in myelin are apolar or hydrophobic, permitting these types of interactions.

Very little has been reported on the interaction of myelin basic protein with gangliosides, whereas its association with phospholipids was investigated extensively [3]. It was reported that myelin basic protein causes release of glucose from liposomes containing gangliosides [4,5]. Fidelio et al. [6] showed that myelin basic protein interacts with monolayers of gangliosides. By employing NMR Littlemore and Ledeen [7] found that binding of myelin basic protein to gangliosides caused immobilization of the phenylalanine, methionine and methylarginine residues of the protein. Mendz and Moore [8] investigated circular dichroism of myelin basic protein bound to gangliosides and found an increase in the α-helical conformation of the protein. In a previous publication [9] we have shown that myelin basic protein has a strong perturbing effect on the thermotropic properties of GM_1. It was also shown by us [10] that GM_1 is completely miscible with PC. In the presently reported study we further analyzed the interaction of GM_1 with MBP by following changes in the protein intrinsic fluorescence and by employing DSC to investigate

Abbreviations: PC, phosphatidylcholine; PS, phosphatidylserine; GM_1, galactosyl-*N*-acetylgalactosaminyl(*N*-acetylneuraminyl)galactosylglucosylceramide; DSC, differential scanning calorimetry; *F*, fluorescence.

the possibility of phase separation induced by MBP in GM_1-PC mixtures.

Materials and Methods

Myelin basic protein (porcine) was kindly supplied by Dr. E.L. Grinnan, Lilly Research Laboratories. Bovine myelin basic protein was separated from myelin according to Hirschfeld et al. [11]. A molecular weight of 18000 was used for the protein. Gangliosides were isolated from bovine brain by chloroform/methanol extraction, followed by phase partition, alkaline methanolysis, DEAE-Sephadex and DEAE-Sepharose [13]. For GM_1 an M_r of 1500 was used. Phosphatidylcholine and phosphatidylserine (both grade I) were purchased from Lipid Products, South Nutfield, U.K. *N*-Methylpicolinium perchlorate was a gift from Prof. M. Shinitzky (Membrane Research Dept.).

For the fluorescence experiments the protein or GM_1 was dissolved in 0.15 M NaCl buffered to pH 7.4 with Tris-HCl (0.01 M). The concentration of stock solutions was about 0.4 mg/ml. The protein and GM_1 were mixed at different ratios indicated in the legends to figures, vortexed for 2 min and incubated for 2 h at 35°C. In the case of PC, PC + GM_1 mixtures or PS, the lipids were first mixed in organic solvents (chloroform : methanol 2 : 1 or 1 : 1), the solvents were driven off by a stream of nitrogen, dispersed in the salt buffer at a concentration of about 0.2 mg/ml and sonicated in bath sonicator (Laboratory Supplies Hicksville N.Y.) for about 30 min. *N*-Methylpicolinium or $NaClO_4$ were also dissolved in salt/buffer at concentrations of 1 M. Fluorescence measurements were performed at room temperature on a Perkin Elmer LS-5 Luminescence Spectrophotometer with 5 nm excitation and emission slits. The samples were excited at 275 nm. For the experiments in the presence of the quencher *N*-methylpicolinium perchlorate, myelin basic protein or myelin basic protein + GM_1 mixtures were incubated for 2 h at 35°C then titrated at room temperature with the concentrated solutions of *N*-methylpicolinium or $NaClO_4$. In the quenching experiments an excitation wavelength of 287 nm and an emission wavelength of 350 nm were used.

For the DSC experiments, gangliosides were dissolved in chloroform/methanol (1 : 1) and appropriate volumes of PC solution (in chloroform/methanol (2 : 1)) were added to give a molar fraction of gangliosides X-GM_1 = 0.3. The samples were mixed, the solvents were driven off by a stream of nitrogen and kept under high vacuum (0.1 torr) for 3 h. Subsequently, 1–1.5 mg of the GM_1-phospholipid mixture or PC were weighed (on a Cahn Model 4100 Electrobalance) directly into the aluminum pans of the instrument. Dry myelin basic protein was added to the lipid mixture, the pans were reweighed and about 10 mg of ethylene glycol/salt (0.15 M NaCl/0.01 M Tris-HCl, 1 : 1) was added, the pans were sealed, vortexed for about 2 min and incubated at 35°C for 2 h. For dispersing the lipid solution, ethylene glycol/salt had to be used to allow the scanning to start at sub-zero temperatures without interference from water melting. To evaluate the effect of the antifreeze, similar DSC measurements were carried out in the presence of salt only.

The calorimetric measurements were performed on a DuPont 990 differential scanning calorimeter equipped with cell base II. The calibrated mode was used with a scan rate of 5Cdeg/min.

Results and Discussion

Fluorescence experiments

Myelin basic protein comprises about 30% of the total myelin proteins. This protein is highly basic and interacts with negatively charged phospholipids [3]. Myelin basic protein contains one tryptophan and four tyrosine residues [13,14] per molecule. In some species the encephalitogenic determinant contains the tryptophan residue [15]. The fluorescence spectrum of myelin basic protein (Fig. 1A) is typical of tryptophan-containing proteins with a maximum at 350 nm, specific for tryptophan emission in an aqueous environment. When interacting with the ganglioside, a shift was observed in the maximum towards shorter wavelengths (Fig. 1B, C, D). The shift is a function of the ganglioside/MBP ratio up to about 10 molecules of the ganglioside per one molecule of MBP (Fig. 2). The maximal shift is about 10 nm. No change in the fluorescence intensity due to interaction was detected. A blue-shift in fluorescence emission indicates that the tryptophan moves into more hydrophobic environment when interacting

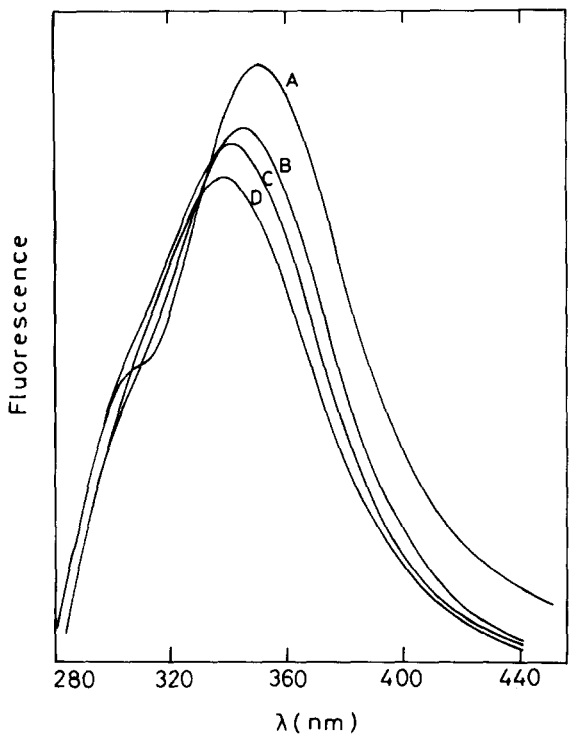

Fig. 1. Fluorescence emission spectra of myelin basic protein interacting with GM_1 at various molar ratios. A, myelin basic protein alone; B, GM_1/myelin basic protein, 8.2:1; C, GM_1/myelin basic protein, 16.3:1; D, GM_1/myelin basic protein, 32.7:1. Bovine myelin basic protein was used, with an excitation wavelength of 276 nm.

with ganglioside. A similar blue-shift of the tryptophan fluorescence emission was reported by Mullin et al. [16] in cholera toxin interacting with GM_1. Additional information on the environment of the tryptophan residues can be deduced from their availability to fluorescence quenchers. *N*-Methylpicolinium perchlorate acts as a quencher of tryptophan from the aqueous phase [17] and this quencher was used in the present work. Fluorescent titrations of the free protein or of the interaction products with a concentrated solution of the quencher were performed. When similar titrations were performed with $NaClO_4$ no change in the fluorescence was detected. The fluorescence data obtained were treated according to two assumptions: (i) heterogeneous population of fluorophores; (ii) homogeneous population of fluorophores. In the first case the relationship between fluorescence and the quenching constant K_{q_i} is

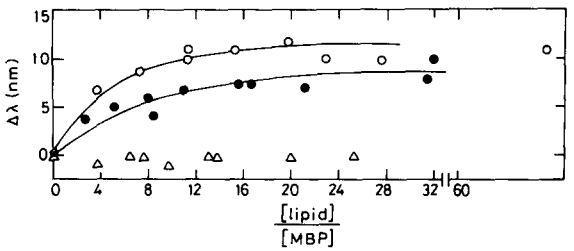

Fig. 2. The blue-shift in the λ_{max} emission of tryptophan of myelin basic protein as a function of the molar ratio [lipid]/[MBP]. △, PC-porcine myelin basic protein; ○, GM_1-porcine myelin basic protein; ●, GM_1-bovine myelin basic protein.

given by [17]:

$$\frac{F_0}{F_0 - F} = \frac{1}{\sum_i f_i K_{q_i}} \cdot \frac{1}{[Q]} + \frac{\sum K_{q_i}}{\sum_i f_i K_{q_i}} \quad (1)$$

where F_0 and F are fluorescence intensities in the absence and presence of a quencher; [Q], molar concentration of the quencher; f_i, relative contribution of group i to the fluorescence intensity in the absence of quencher; $f_i K_{q_i}$, mean quenching constant. For a homogeneous distribution the Stern Volmer equation is valid:

$$\frac{F_0}{F} = 1 + K_q[Q] \quad (2)$$

The fluorescence data are plotted in Fig. 3a according to Eqn. 1. As seen in the figure, the intercept equals 1; From Eqn. 1 the intercept is given by $\Sigma K_{q_i}/\Sigma f_i K_{q_i}$, which shows that $f_i = 1$, indicating the homogeneous distribution of tryptophan residues. Homogeneous distribution of fluorophores means that all the tryptophan residues in the free protein are available to the quencher, and in the presence of GM_1 all the tryptophan residues move into a more hydrophobic environment, hence in both cases only one population of fluorophores exists. For a homogeneous distribution Eqn. 2 is valid. The data drawn according to this equation are presented in Fig. 3b. Quenching constants calculated from Fig. 3a and b are presented in Table I. K_q values for myelin basic protein alone indicate that the tryptophan residues in the protein are completely available to the quencher, as the K_q for *N*-acetyl-L-tryptophanamide is only slightly higher [17]. However, when

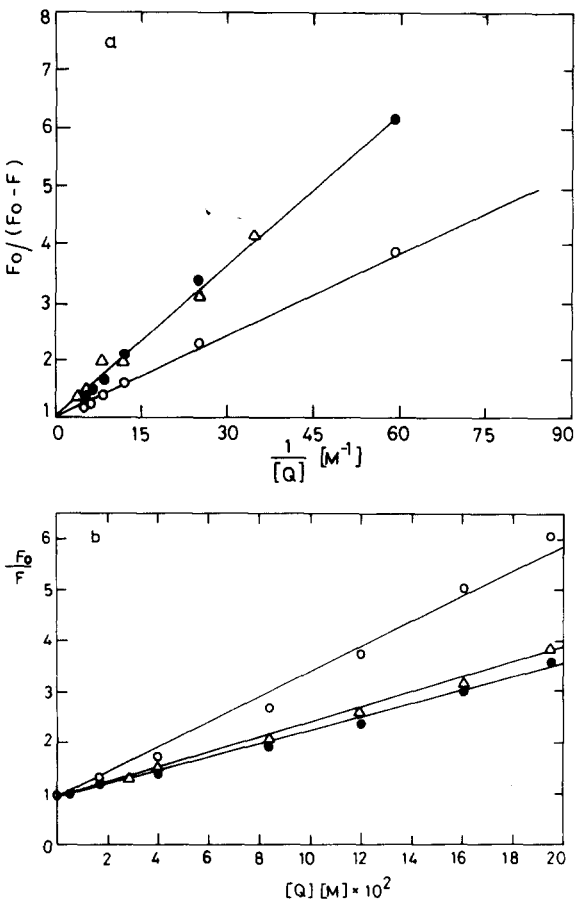

Fig. 3. Quenching of tryptophan fluorescence by *N*-methylpicolinium perchlorate (excitation 287 nm, emission 350 nm) for bovine myelin basic protein alone and at different $[GM_1]/[MBP]$ ratios. (a) Data drawn according to Eqn. 1. ○, myelin basic protein alone; GM_1/myelin basic protein at (△) 16.3 : 1 and (●) 32.1 : 1. (b) Data drawn according to Eqn. 2. ○, myelin basic protein alone; GM_1/myelin basic protein at (△) 16.3 : 1 and (●) 32.1 : 1.

interacting with GM_1 the availability decreases significantly as indicated by the much lower quenching constants. The values of K_q given in Table I compare well with those for membrane proteins (Eqn. 17) when one compares the ratio of K_q for MBP alone to K_q for MBP-GM_1 with $f_i K_i$ for disrupted biological membranes (full exposure of the tryptophan residues) to $f_i K_{q_i}$ in intact biological membranes.

TABLE I

QUENCHING CONSTANTS OF MYELIN BASIC PROTEIN (MBP) ALONE AND IN THE PRESENCE OF GM_1

$[GM_1]/[MBP]$	$f_i K_{q_i}$ (Eqn. 1) (M^{-1})	K_q (Eqn. 2) (M^{-1})
0	21.4	24
12.6	11.4	14.4
25.2	11.4	12.8

In an attempt to determine whether the interaction of GM_1 with myelin basic protein, as indicated by the fluorescent measurements, is specific for gangliosides and to discover which are the forces governing that interaction, we have investigated the association of myelin basic protein with phospholipids by employing fluorescence techniques. When myelin basic protein interacts with the zwitterionic lipid-PC no blue-shift in the tryptophan emission is detected (Fig. 2). As very low concentrations of PC were employed no turbidity induced by myelin basic protein was apparent, in agreement with the data of Epand et al. [18], ruling out any interference with fluorescence measurements.

The lack of blue-shift shows that the tryptophan residues do not move into more hydrophobic environment, indicating that the negative charge of GM_1 is a prerequisite for this interaction.

We have tried to follow the interaction of myelin basic protein with another negatively charged lipid, PS, also by fluorescence measurements. However, in lipid excess, even at very low lipid concentrations (0.1 mg/ml), it was impossible to measure fluorescence as precipitation occurred upon mixing of the two components. The appearance of precipitation due to the interaction of acidic phospholipids with myelin basic protein was reported previously [19]. We have also investigated the interaction of mixtures of PC + GM_1 (at molar fractions of GM_1 of 0.05, 0.10, 0.26, 0.53) with myelin basic protein by measuring the fluorescence spectrum. In the case of low molar fractions of GM_1 addition of myelin basic protein to the lipid mixture (at a great lipid excess) caused the appearance of turbidity, probably by crosslinking of the vesicles, preventing valid fluorescence measurements. At GM_1 molar fraction of 0.53 the turbidity was low, permitting measurements to be made. A blue-shift of 3–7 nm was detected depending on the lipid-to-protein ratio.

It is concluded from these measurements that the interaction of GM_1 and myelin basic protein is

both electrostatic and hydrophobic, inducing a partial penetration of the protein into the more hydrophobic environment. There is some specificity with respect to GM_1, as GM_1-myelin basic protein complexes (at least at low concentration) do not precipitate, in contrast with those of myelin basic protein with negatively charged phospholipids. This specificity might be structural, as GM_1 forms micelles in aqueous solutions [20] and has a very large polar head group, as compared to phospholipids that form bilayers and have much smaller head groups.

DSC experiments of PC-GM_1 mixtures interacting with myelin basic protein

We have reported recently that GM_1 is completely miscible with egg phosphatidylcholine as judged by the appearance of one peak only in DSC profiles [10]. By employing this technique we have also shown that myelin basic protein has a strong perturbing effect on the GM_1 structure [9]. It was therefore of interest to determine whether the higher affinity of myelin basic protein towards GM_1 than to PC leads to phase separation in homogeneous mixtures of the lipids. The technique of DSC is especially suitable for investigation of phase separation (reviewed in Ref. 21) insofar as due to phase separation the mixture will be enriched with the non-reacting lipid, resulting in a shift towards its melting temperature. The experiments were performed with GM_1-PC mixtures with a GM_1 molar fraction of 0.3 as a model mixture for investigation of myelin basic protein-induced phase separation. A preceding and prerequisite step of this study is a demonstration that the affinity of myelin basic protein for PC is indeed low as judged by thermotropic criteria. As shown in Fig. 4 the midpoint melting temperature (t_m) of PC alone (C) or interacting with myelin basic protein (D) is similar. Also no change in the enthalpy of melting was detected. These experiments were performed at various PC/myelin basic protein molar ratios, the lowest being 17 : 1. The lack of effect of myelin basic protein on the thermotropic properties of lecithins is in agreement with the results of Epand and Moscarello [22], who showed that myelin basic protein does not alter the phase-transition temperature of dimyristoylphosphatidylcholine. However, when myelin basic protein is incubated with a mixture of GM_1-PC, the midpoint melting temperature is shifted downward by about 5 Cdeg (Fig. 4B). This shows that myelin basic protein induces phase separation in the homogeneous mixture of lipids, enriching the mixture with the lower-melting-point component PC.

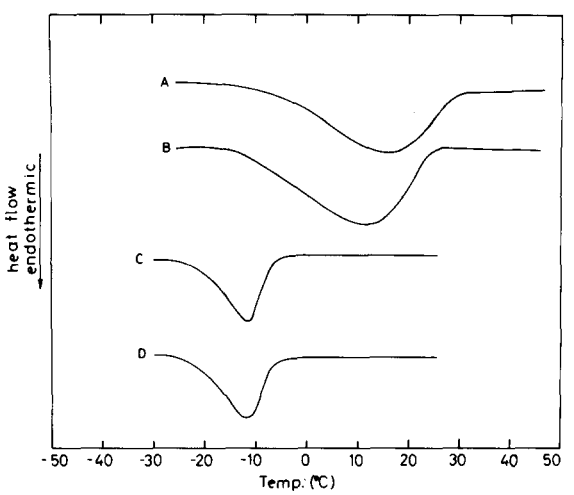

Fig. 4. Thermograms of GM_1 + PC and PC alone or interacting with myelin basic protein. A, GM_1 + PC mixture alone; B, GM_1 + PC mixture + myelin basic protein, [GM_1]/[MPB] = 23.1 : 1; [total lipid]/[MBP] = 72 : 1. C, PC alone; D, PC + myelin basic protein, [PC]/[MBP] = 43.7 : 1. Scan rate, 5Cdeg/min; sensitivity, 0.02 mcal/s·inch^{-1} (A, B); 0.04 mcal/s·inch^{-1} (C, D).

By employing DSC Boggs et al. [23] showed that myelin basic protein induces phase separation in mixtures of acidic phospholipids with PC. Following treatment according to Boggs et al. [23], the number of GM_1 molecules bound to a myelin basic protein molecule in the enriched phase was calculated and was found to be about 6. This number compares well with the number of molecules producing a maximal shift in the tryptophan fluorescence, taking into account the limits inherent in this calculation.

To conclude, myelin basic protein separates GM_1 from homogeneous mixtures with zwitterionic phospholipids. The data presented might have some relevance to the postulated interaction of gangliosides and myelin basic protein in myelin.

Acknowledgements

We would like to thank Professor M. Shinitzky for the *N*-methylpicolinium perchlorate and for his help in interpretation of the quenching data, and Dr. E.L. Grinnan, Lilly Research Laboratory for the donation of the porcine myelin basic protein. B.S. was supported by the Israeli Multiple Sclerosis Society.

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