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Photolabeling of myelin basic protein in lipid vesicles with the hydrophobic reagent 3-(trifluoromethyl)-3-(*m*-[^{125}I]iodophenyl)diazirine

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The hydrophobic photolabel 3-(trifluoromethyl)-3-(*m*-[^{125}I]iodophenyl)diazirine([^{125}I]TID) was used to label myelin basic protein or polylysine in aqueous solution and bound to lipid vesicles of different composition. Although myelin basic protein is a water soluble protein which binds electrostatically only to acidic lipids, unlike polylysine it has several short hydrophobic regions. Myelin basic protein was labeled to a significant extent by TID when in aqueous solution indicating that it has a hydrophobic site which can bind the reagent. However, myelin basic protein was labeled 2–4-times more when bound to the acidic lipids phosphatidylglycerol, phosphatidylserine, phosphatidic acid, and cerebroside sulfate than when bound to phosphatidylethanolamine, or when in solution in the presence of phosphatidylcholine vesicles. It was labeled 5–7-times more than polylysine bound to acidic lipids. These results suggest that when myelin basic protein is bound to acidic lipids, it is labeled from the lipid bilayer rather than from the aqueous phase. However, this conclusion is not unequivocal because of the possibility of changes in the protein conformation or degree of aggregation upon binding to lipid. Within this limitation the results are consistent with, but do not prove, the concept that some of its hydrophobic residues penetrate partway into the lipid bilayer. However, it is likely that most of the protein is on the surface of the bilayer with its basic residues bound electrostatically to the lipid head groups.

Introduction

Myelin basic protein is a water soluble protein which appears to interact with some lipids by both electrostatic and hydrophobic interactions (see Ref. 1 for a review). It binds to a significant extent only to acidic lipids and inhibits binding of divalent cations to the lipid [2] demonstrating the importance of electrostatic interactions. It also has a number of hydrophobic residues which are distributed throughout its sequence together with other neutral amino acids in several short segments. Although it is recognized that myelin basic protein is much different from an intrinsic protein, both in sequence and behavior, and that it is unlikely to be deeply embedded in the bilayer, a number of studies indicate that myelin basic protein can also interact hydrophobically with the lipid bilayer, perhaps by penetration of some of its hydrophobic amino acid side chains partway into

Abbreviations: [^{125}I]TID, 3-(trifluoromethyl)-3-(*m*-[^{125}I]iodophenyl)diazirine; DPPA, dipalmitoylphosphatidic acid; DPPG, dipalmitoylphosphatidylglycerol; DMPS, dimyristoylphosphatidylserine; DPPE, dipalmitoylphosphatidylethanolamine; DPPC, dipalmitoylphosphatidylcholine; PA, phosphatidic acid; PG, phosphatidylglycerol; PC, phosphatidylcholine; NFA-S-CBS, nonhydroxy fatty acid form of cerebroside sulfate containing stearic acid; HFA-S-CBS, hydroxy fatty acid form of cerebroside sulfate containing α-hydroxy stearic acid.

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the lipid bilayer, while its basic residues are bound to the acidic lipid head groups. These studies indicate that the protein has a perturbing effect on the lipid which differs from the effects of either intrinsic membrane proteins or of water soluble proteins, which interact only electrostatically [2–10]. Certain residues or regions of the protein appear to be sequestered in the bilayer [11–16], while some hydrophobic and apolar residues are motionally restricted by the lipid [17–23].

However, much of the evidence suggesting that myelin basic protein penetrates partway into the bilayer is indirect, and there are possible alternative explanations for many of the individual results. The concept that myelin basic protein penetrates even partway into the bilayer has been questioned recently on the basis of analysis of its hydropathic profile [24] and X-ray diffraction studies of myelin basic protein-lipid bilayers [24,26]. In the latter, no significant localization of protein within the lipid bilayer was detected, leading to the conclusion that the protein does not penetrate farther than the head-group region.

Because of the indirect nature of earlier results suggestive of partial penetration and because the interpretation of results about lipid-protein interactions provided by different techniques is not straightforward, further studies to delineate the degree of penetration of myelin basic protein into the lipid bilayer are necessary in order to enhance our understanding of the membrane conformation and mode of interaction of myelin basic protein with the lipid bilayer. Only by correlating the results from a number of techniques on different types of proteins can we understand how to interpret them and the value of the information provided.

Although no technique at present can be considered definitive, the hydrophobic photolabel, 3-(trifluoromethyl)-3-(*m*[^{125}I]iodophenyl)diazirine ([^{125}I]TID) has proven to be useful for identifying segments of intrinsic proteins which are buried in the lipid bilayer [27–31] and for demonstrating the occurrence of hydrophobic contacts of amphipathic peptides with the lipid bilayer [32,33]. This photolabel has a high lipid/water partition coefficient and has been found to label only hydrophobic regions of peptides and not hydrophilic regions [30,32,33]. However, it does label aggregates of hydrophobic peptides in aqueous solution and hydrophobic sites on water soluble proteins in solution [32,34,35], so that appropriate controls are necessary in order to interpret any labeling which occurs.

In the present study we have used this photolabel to label myelin basic protein and polylysine in a number of different lipids and at different pH values. The results are consistent with the concept of penetration of some residues or regions of myelin basic protein partway into the bilayer.

Materials and Methods

Lipids and proteins. Dipalmitoylphosphatic acid (DPPA) and dipalmitoylphosphatidylethanolamine (DPPE) were from Fluka (Switzerland), dipalmitoylphosphatidylcholine (DPPC) was from Sigma Chemical Co. (St. Louis, MO), dimyristoylphosphatidylserine (DMPS), dipalmitoylphosphatidylglycerol (DPPG), egg phosphatidylcholine (PC) and phosphatidylglycerol (PG) and phosphatidic acid (PA), both prepared from egg PC, were purchased from Avanti (Birmingham, AL). The stearoyl and the α-hydroxystearoyl forms of cerebroside sulfate (NFA-S-CBS and HFA-S-CBS, respectively) were prepared from bovine brain cerebroside sulfate as described previously [36]. All were chromatographically pure and were stored at $-20\,^{\circ}$C. Myelin basic protein was prepared from bovine brain white matter by the method of Cheifetz and Moscarello [37]. Polylysine ($M_r > 20000$) was purchased from Miles-Yeda.

Preparation of vesicles. The lipid was dispersed in buffer at a concentration of 2 mg/0.75 ml by vortex mixing at a temperature above the lipid phase transition temperature. The buffer contained 10 mM NaCl, 30 mM glutathione, and 10 mM Mes for pH 6; 10 mM Hepes for pH 7 and 8; or 10 mM sodium borate for pH 9.5. Myelin basic protein or polylysine were dissolved in the appropriate buffer at a concentration of 2 mg/ml and the pH was checked. The desired amount of protein solution (generally 0.25 ml) was added to 0.75 ml of the lipid suspension and the sample was vortexed again and incubated at a temperature above the lipid phase transition temperature for at least 3 min. In order to label protein in the ab-

sence of lipid a similar amount of protein solution was added to 0.75 ml of the appropriate buffer. The lipid-protein samples generally contained 20 wt% protein but other concentrations from 5 to 40 wt% were also used, with similar results being obtained.

Photolabeling reaction. An ethanolic solution of [^{125}I]TID (specific activity 10 mCi/μmol) was purchased from Amersham, Canada. It was diluted with 0.4 ml ethanol. The samples to be labeled were flushed with nitrogen and 2–6 μl of [^{125}I]TID containing approx. 10^7 cpm were added to 1 ml of vesicle suspension and the sample was mixed in the dark. It was equilibrated at room temperature for 20–30 min and then irradiated for 2 min using a 100 watt high pressure Hg lamp (Photochemical Research Associates, London, Ont.). The light beam was cooled by passage through a reservoir of circulating cold water and was directed through a filter consisting of a saturated solution of $CuSO_4$.

Development of procedures to purify labeled proteins. In order to develop a procedure for purifying the labeled protein from the lipid, vesicles without TID were prepared as described above, the protein was extracted in various ways, and the lipid and protein content of the different fractions was determined. In order to monitor the myelin basic protein content, a trace amount of ^{125}I-labeled myelin basic protein, labeled with lactoperoxidase as described earlier [13], was added to the myelin basic protein solution to give a specific activity of 30000 cpm/0.5 mg, prior to preparation of the vesicles. The polylysine content was assayed according to the method of Kaplan and Pederson [38]. The phospholipid content was assayed by a phosphorus assay according to Bartlett [39] and the cerebroside sulfate content was assayed by a sulfate assay according to Radin [40]. The methods which gave good separation of the protein from the lipid and which were used to purify [^{125}I]TID-labeled protein are given below.

In order to more accurately determine the amount of labeled lipid remaining bound to the protein after these purification procedures, a sample of lipid vesicles without protein was labeled with [^{125}I]TID. Unlabeled myelin basic protein or polylysine was then added to the labeled lipid. The sample was vortexed at a temperature above the lipid phase transition temperature, extracted, dialyzed and assayed as described below.

Treatment of [^{125}I]TID-labeled vesicles. After photolysis, the pH of samples containing phospholipid-myelin basic protein vesicles was lowered to pH 6 and the pH of samples containing cerebroside sulfate (CBS)-myelin basic protein vesicles or polylysine-lipid vesicles was lowered to below pH 1 with 75 μl of 2 M HCl per 1 ml of vesicle suspension. 4 ml of acidified chloroform/methanol (1:1) containing 5% 0.1 M HCl were added to the sample (per 1 ml of aqueous suspension), and it was vortexed above the lipid phase transition temperature, centrifuged at 2000 rpm for 10 min, and the aqueous and organic phases separated. The aqueous phase (2.8 ml) was extracted three more times using 2.2 ml of fresh lower phase. The aqueous phase was then dialyzed against 6 changes of 4 liters distilled water for at least 48 h. After dialysis aliquots of the aqueous phase were counted and assayed for protein content. Polylysine was determined as described above. Myelin basic protein was assayed according to the method of Peterson [41]. The amount of [^{125}I]TID bound to the protein was generally between 10^5 and 10^6 cpm/mg, depending on the specific activity of the TID and the amount used. Because these varied from one experiment to another, labeling of myelin basic protein-DPPC vesicles was measured in every experiment and the specific activity values for other samples were normalized to the values for the myelin basic protein-DPPC vesicles so that results from different experiments could be averaged. Samples of myelin basic protein in solution and myelin basic protein-DPPA vesicles were also included in almost every experiment to compare to labeling of myelin basic protein in other lipids or to polylysine in various lipids Most of these experiments were repeated at least three to five times.

Solubility of TID in different lipids. The solubility of [^{125}I]TID in vesicles of DPPA, DPPC, egg PA, and egg PC, in the absence or presence of 20 wt% myelin basic protein at pH 7, was determined by adding 12000 cpm [^{125}I]TID to 1 ml of vesicle suspension containing 2 mg lipid, prepared as described above, in glass test tubes. The samples were allowed to equilibrate at room temperature for 30 min and were then centrifuged at 4000 rpm

(1900 × g) for 30 min at 5 °C in a Beckman J2-21 centrifuge. The amount of TID in the lipid pellet was determined by removing the supernatant, resuspending the pellet in 1 ml of distilled water, and taking aliquots for counting in a gamma counter and analysis of lipid content by phosphorus assay. A control with [^{125}I]TID added to 1 ml of buffer showed that only 2% of the TID could be sedimented in the absence of lipid, under these conditions.

Results

Because of its high degree of hydrophobicity, most of the TID added to membranes or lipid-protein vesicles binds to the lipid rather than the protein even when hydrophobic intrinsic proteins are present [27,31]. Therefore it is important to demonstrate that the purification procedures used are able to remove all of the labeled lipid from the proteins. Extraction of myelin basic protein-phospholipid vesicles at pH 6 with acidified chloroform/methanol left 90% of the protein in the aqueous phase and was sufficient to remove almost all of the lipid from the protein. After four extractions the recovered myelin basic protein contained only 1 wt% lipid according to phosphorus analysis. The values of phosphate found were at the lower limits of the phosphorous assay and may not have been significantly above background levels. When this procedure was used for polylysine-lipid vesicles or myelin basic protein-CBS vesicles, however, 77% of the polylysine and 29% of the myelin basic protein went into the organic phase. Furthermore, the protein which went into the aqueous phase carried significant amounts of lipid with it. Therefore, for these samples the pH was lowered to below 1 before extraction in order to dissociate the protein from the lipid. This caused 94% of the protein to go into the aqueous phase and allowed almost complete removal of the lipid into the organic phase. This procedure was also used for some myelin basic protein samples but had no effect on the results. The amount of [^{125}I]TID-labeled lipid bound to the proteins when they were added to the lipid after irradiation was very low after extraction (vide infra), also indicating that the extraction procedures were able to remove virtually all of the lipid from the protein. Thus when lipid-protein vesicles are labeled with TID, the label found in the aqueous phase with the protein is bound to the protein and not to lipid which has not been completely removed from the protein.

TABLE I

EFFECT OF GEL PHASE PHOSPHOLIPIDS AND pH ON [^{125}I]TID LABELING OF MYELIN BASIC PROTEIN

Sample [b]	Relative labeling [a]		
	pH 6	pH 8	pH 9.5
In solution	1.7 ± 0.3	1.6 ± 0.2	3.0 ± 0.6
DPPA	2.1 ± 0.4	3.9 ± 1.3	4.1 ± 1.3
DPPG	3.8 ± 1.2	3.7 ± 1.3	4.8 ± 1.3
DMPS	2.7 ± 1.4	3.4 ± 1.3	2.3 ± 1.2
DPPC	1.0 [c]	0.8 ± 0.1	1.0 ± 0.1
DPPE	1.0 ± 0.4	0.9	N.D.
Control – DPPA [d]	0.2 ± 0.2	N.D.	N.D.
Control – DPPG [d]	0.2	N.D.	N.D.

N.D., not determined.

[a] Ratio of specific activity of myelin basic protein (cpm/mg) in sample indicated to that in DPPC-myelin basic protein sample at pH 6. Data for each sample are averaged from 3–5 experiments unless no standard deviation is indicated. Most of the different samples were compared in each experiment.

[b] All samples contain 0.5 mg myelin basic protein/ml with or without 2 mg lipid.

[c] No standard deviation is indicated because all other values were normalized to this one in each experiment.

[d] For control, 0.5 mg protein was added to 2 mg (in 1 ml) of lipid after labeling with TID and photolysis.

A significant amount of [^{125}I]TID was bound to myelin basic protein in the presence of different lipids (Table I). However, a significant amount was also bound to the protein in solution. The results were similar in the presence or absence of 30 mM glutathione, added to scavenge photolabel present in the aqueous phase [42]. However, TID has been shown in several recent studies to label hydrophobic sites of water soluble proteins in the absence of lipid, in spite of the presence of glutathione [32,34,35]. More TID is available to bind to the protein in the absence of lipid than in its presence, since the lipid competes for the TID. Thus, the amount of label bound to myelin basic protein in the presence of DPPC, a lipid to which this protein does not bind to a significant degree [6], is less than the amount of label bound to the protein in solution (Table I). In order to de-

termine if myelin basic protein can be labeled with TID from the lipid bilayer of lipids to which it is bound, it is necessary to compare the results to those in the presence of DPPC rather than to the protein in solution. A similar procedure was followed in a recent study in which the smaller subunit of the water soluble protein factor Va was found to be labeled more by TID in the presence of acidic lipids than PC [43]. Thus, in Table I and subsequent tables, the specific activity values for protein in solution or bound to different lipids have been divided by the values found for myelin basic protein in the presence of DPPC vesicles.

When incorporated into DPPA, DPPG, or DMPS vesicles, myelin basic protein was labeled by TID 2–4-times more than in the presence of DPPC or when bound to DPPE (Table I). At the concentration of myelin basic protein used, 20 wt%, almost all of the protein added is bound to all of these lipids except DPPC [6]. The amount of [^{125}I]TID picked up by the protein when it was added to [^{125}I]TID-labeled DPPA or DPPG after irradiation was less than 10% of the amount bound when the protein was added to the lipid before irradiation indicating that, for the latter, most of the TID present after extraction is bound directly to the protein and not to the unextracted lipid. Myelin basic protein showed some tendency to bind more TID with increase in pH, particularly for DPPA, and at pH 9.5 for the protein in solution and when bound to DPPG. However, there was no increase in the presence of DPPC or DPPE.

The amount of TID bound to polylysine in solution or in DPPA or egg PA vesicles was only 10–20% of that of myelin basic protein in DPPA vesicles (Table II). In contrast to myelin basic protein, the amount of TID labeling of polylysine bound to DPPA was not significantly greater than in the presence of DPPC vesicles, a lipid to which it does not bind. Polylysine in solution bound much less label (almost 4-times less in this set of experiments) than myelin basic protein in solution.

Myelin basic protein bound to NFA-S-CBS vesicles at pH 7 was labeled by TID a little more than when bound to DPPA as shown in Table III. However, the labeling in HFA-S-CBS was comparable to that in DPPA. Labeling of myelin basic

TABLE II

COMPARISON OF [^{125}I]TID LABELING OF MYELIN BASIC PROTEIN AND POLYLYSINE

At pH 6. All samples contain 0.5 mg protein/ml with or without 2 mg lipid.

Sample	Relative labeling [a]
Myelin basic protein	
In solution	1.5 ±0.4
DPPA	2.1 ±0.1
DPPC	1.0 [b]
Polylysine	
In solution	0.40±0.01
DPPA	0.30±0.3
Egg PA	0.20±0.06
DPPC	0.20±0.06
Control – DPPA [c]	0.12±0.04

[a] See footnote a of Table I.
[b] See footnote c of Table I.
[c] See footnote d of Table I.

TABLE III

COMPARISON OF [^{125}I]TID LABELING OF MYELIN BASIC PROTEIN AND POLYLYSINE IN PHOSPHOLIPIDS AND CEREBROSIDE SULFATE

At pH 7. 0.5 mg protein/ml with or without 2 mg lipid.

Sample	Relative labeling [a]
Myelin basic protein	
In solution	0.9±0.3
DPPA	2.1±0.2
DPPC	1.0 [b]
NFA-S-CBS	2.7±0.5
HFA-S-CBS	2.2±0.6
(DPPA/DPPC 1:1)	2.1
(NFA-S-CBS/DPPC 1:)	2.1
Control – NFA-S-CBS [c]	0.18±0.03
Polylysine	
In solution	0.6
DPPA	0.7
DPPC	0.5
NFA-S-CBS	0.5
HFA-S-CBS	0.5
Control – NFA-S-CBS [c]	0.3

[a] See footnote a of Table I.
[b] See footnote c of Table I.
[c] See footnote d of Table I.

protein bound to equimolar mixtures of DPPA/DPPC or NFA-S-CBS/DPPC was comparable to that in the pure acidic lipids. The labeling of polylysine in this set of experiments, done at pH 7, was greater than in the set shown in Table II, done at pH 6. Whether this is due to the increase in pH is not clear, since the effect of pH on labeling of polylysine was not studied directly. However, the degree of labeling of polylysine at pH 7 was still considerably less than that of myelin basic protein bound to DPPA and CBS. Polylysine was not labeled more in CBS than when bound to DPPA, in solution, or in the presence of DPPC. The amount of TID bound to myelin basic protein and polylysine was low when these proteins were added to labeled NFA-S-CBS after irradiation, indicating that most of the labeled CBS could be removed from the proteins as found for the phospholipids.

Since all of the lipids used above are in the gel phase at room temperature, the degree of labeling of myelin basic protein in egg PA and PG vesicles was determined and compared to that in egg PC, DPPC and DPPA vesicles, in order to determine the labeling of the protein when bound to liquid crystalline phase lipid. Surprisingly, the protein was labeled much less when bound to egg PA and PG than when bound to DPPA (Table IV). It was labeled less even than in the presence of DPPC. However, when bound to egg PA and egg PG, it was labeled 2–3-times more than in the presence of egg PC vesicles. Thus when compared to egg PC the degree of labeling of myelin basic protein in egg PA and egg PG is similar to that in DPPA and DPPG when compared to DPPC. It was also labeled twice as much as polylysine bound to egg PA (Table II).

TABLE IV

COMPARISON OF [^{125}I]TID LABELING OF MYELIN BASIC PROTEIN IN DPPA AND LIQUID CRYSTALLINE PHASE NATURAL PHOSPHOLIPIDS

Each sample contained 0.5 mg myelin basic protein/ml with or without 2 mg lipid.

Sample	Relative labeling [a]	
	pH 6	pH 8
DPPA	1.9 ±0.1	3.8 ±1.4
DPPC	1.0 [b]	
Egg PA	0.5 ±0.1	0.4 ±0.2
Egg PG	0.4 ±0.2	0.3 ±0.1
Egg PC	0.15±0.08	0.17±0.07

[a] See footnote a of Table I.
[b] See footnote c of Table I.

TABLE V

COMPARISON OF SOLUBILITY OF [^{125}I]TID IN NATURAL UNSATURATED AND SYNTHETIC SATURATED PHOSPHOLIPIDS

In the absence or presence of 20 wt% myelin basic protein.

	cpm [^{125}I]TID/ μmol lipid	Ratio [a] natural/ synthetic
DPPA	483	
Egg PA	3052	6.3
DPPC	1240	
Egg PC	3505	2.8
DPPA-myelin basic protein	931	
Egg PA-myelin basic protein	2600	2.8
DPPC-myelin basic protein	1300	
Egg PC-myelin basic protein	3512	2.7

[a] Ratio of specific activity of TID in natural lipid to that in synthetic lipid for each set.

In order to determine if the difference in labeling of myelin basic protein in gel phase and liquid crystalline phase lipid is due to greater competition of liquid crystalline phase lipid for the TID, the solubility of TID in different lipids was measured (Table V). It was found to be 2.7–2.8 times more soluble in the natural unsaturated lipids, egg PA and egg PC, than the synthetic saturated lipids DPPA and DPPC, in the presence of myelin basic protein. In the absence of myelin basic protein, which has a disordering effect on DPPA, the difference between DPPA and egg PA was even greater due primarily to a decrease in the solubility of TID in DPPA. The presence of myelin basic protein had little effect on the solubility of TID in the other lipids. Since not all of the lipid could be pelleted under the conditions used, no attempt was made to determine the partition coefficient of TID between the lipid and the aqueous phase. However, a control in which a similar amount of TID was added to buffer and centrifuged showed that this amount of TID is soluble in the aqueous phase.

Discussion

The results from this study show that (1) the hydrophobic photolabel TID labels myelin basic protein more when it is bound to the acidic lipids DPPA, DPPG, DMPS and CBS than in the presence of DPPC, to which this protein does not bind appreciably; and (2) it labels myelin basic protein bound to these lipids considerably more than polylysine, which can bind only electrostatically to the polar head groups. These results suggest that the increase in labeling of myelin basic protein bound to acidic lipids is a result of labeling from the lipid bilayer rather than from the aqueous phase. Since the carbene generated from TID is capable of reacting with most if not all of the amino acid side chains, including lysine [29], they suggest further that some residues of myelin basic protein must penetrate farther into the bilayer than the lysyl residues of polylysine. A similar conclusion was reached for the small water soluble subunit of factor Va [43].

However, myelin basic protein in solution is also labeled to a significant extent, particularly if there is no non-interacting lipid, such as DPPC, available for the TID to bind to. Significant labeling by TID of aggregates of hydrophobic peptides in water [32] and hydrophobic sites of several other water soluble proteins has been reported [34,35,43]. Although myelin basic protein forms hexamers in solution at concentrations of 6 mg/ml or greater, at the concentrations used in this study, 0.5 mg/ml, it is primarily in the monomer form [44]. Thus for the solution form of myelin basic protein, TID is probably bound to a hydrophobic site or pocket on the monomer. Randall and Zand [45] also concluded that myelin basic protein in solution has a hydrophobic region which can bind a fluorescent probe. However, myelin basic protein does cause vesicle aggregation. This might be caused by binding of a monomer to two vesicles simultaneously or by dimerization of protein molecules on two separate vesicles. Thus it cannot be entirely ruled out that the increased labeling of myelin basic protein when bound to acidic lipids, compared to DPPC, is a result of binding of TID to hydrophobic protein-protein contacts at the surface between aggregated vesicles. It is also possible that binding of myelin basic protein to the surface of the lipid bilayer induces a conformation which contains a larger or greater number of hydrophobic sites, thus binding more TID. However, the fact that myelin basic protein is labeled less in DPPE than in the other acidic lipids supports the conclusion that the protein is labeled from within the bilayer and not at hydrophobic sites on the surface. Myelin basic protein also binds to egg PE and DPPE and aggregates vesicles of these lipids [46]. However, it has little perturbing effect on the lipid bilayer suggesting that it does not interact hydrophobically with PE [1], consistent with the low labeling by TID.

Thus although the results are not entirely unequivocal, they are consistent with the model of penetration of some regions or residues of myelin basic protein partway into bilayers of DPPG, DPPA, DMPS, and CBS, but not DPPE (reviewed in Refs. 1,47,48). The greater labeling of myelin basic protein bound to egg PA and egg PG compared to that in the presence of egg PC, or that of polylysine bound to egg PA, also indicates that a similar interaction occurs with these liquid crystalline phase lipids. However, the much lower degree of labeling in these lipids compared with the saturated gel phase lipids is, at first sight, puzzling. It is very unlikely that the degree of penetration is greater in the gel phase than in the liquid crystalline phase. In fact all the evidence suggests the opposite [1,19]. The most likely explanation is that the liquid crystalline phase lipids compete with myelin basic protein for TID more effectively than the gel phase lipids because of the greater solubility of TID in the liquid crystalline phase. This is supported by the result that the labeling of myelin basic protein in the presence of egg PC is much less than that in the presence of DPPC even though the protein does not bind to either of these lipids. It is also possible that TID is located deeper in the bilayer in the liquid crystalline phase and farther away from the protein hydrophobic sites located closer to the bilayer surface. These factors may make it difficult to compare the degree of labeling of myelin basic protein in different lipids since the lipid fluidity and location of TID in these lipids may vary. A further implication is that in natural membranes and natural lipids, TID may label those parts of intrinsic proteins which are located in the center of the

bilayer disproportionately more than parts which are less deeply embedded.

Although the results suggest that myelin basic protein bound to acidic lipids is labeled from the lipid bilayer and not from the aqueous phase, and therefore support the model that it penetrates partway into the bilayer, they do not delineate how deeply the protein penetrates. The fact that the labeling is much greater for gel phase lipids, where the TID may be located closer to the headgroup region, than for liquid crystalline phase lipids, and the fact that myelin basic protein is labeled much less by hydrophobic photolabels than the intrinsic protein lipophilin in intact myelin [49,31] suggests that it may not penetrate very deeply. We can conclude only that it penetrates farther than polylysine. It seems that this must be deeper than the polar head group region but possibly not much deeper than the ester linkage of the fatty acid chain. Similar conclusions were reached earlier from consideration of the sequence of myelin basic protein and studies indicating that its behavior is much different from that of intrinsic proteins [4,1,47]. This conclusion is not inconsistent with X-ray diffraction results from other laboratories [25,26] since the electron density of hydrophobic amino acid side chains is probably not sufficiently different from that of the lipid acyl chains to detect their presence in this region of the bilayer by X-ray diffraction.

Although the degree of hydrophobic interaction of myelin basic protein with the bilayer is clearly much less than that of intrinsic proteins, it could nevertheless have significant consequences for the protein function and myelin structure. Therefore it is important to develop techniques for measuring the extent of this interaction with different lipids under various conditions. Photolabel groups which are bound to the acyl chains of phospholipids close to the ester linkage may be more useful for this purpose than TID.

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