Biochimica et Biophysica Acta, 455 (1976) 343–352

BBA 77511

LIPID-PROTEIN INTERACTIONS WITH NATIVE AND MODIFIED MYELIN BASIC PROTEIN*

A. J. STECK[a], H. P. SIEGRIST[a], P. ZAHLER[b] and N. N. HERSCHKOWITZ[a]

[a]*Department of Pediatrics, University of Bern and* [b]*Theodor Kocher Institute, University of Bern (Switzerland)*

(Received May 3rd, 1976)

SUMMARY

The basic protein of central nervous system myelin has been shown to form complexes with acidic lipids in vitro. We measured the interaction of myelin basic protein with several charged and neutral lipids in a biphasic chloroform/methanol/water system and investigated the effect of decreasing the electrical charge of the basic amino groups of the myelin basic protein by acetylation. The modified myelin basic protein, which has an average of eight acetyl residues incorporated, was characterised by gel electrophoresis and circular dichroism. Complexes formed between the acetylated myelin basic protein and acidic lipids exhibited a reduction in the amount of lipids bound, a value that could be correlated with the number of modified amino groups. The significance of these experiments with reference to protein-lipid interaction in the myelin membrane is discussed.

INTRODUCTION

Recent reviews of membrane structure have stressed the importance of types of association of lipid and protein and their contribution to the arrangement and stability of membranes [1, 2]. Although lipids in membranes may be associated with protein through both ionic and non-polar interactions, substantial experimental evidence is needed to support this assessment. Recent in vitro model studies on the specificity of myelin protein-lipid interactions have shown that the myelin basic protein seems to interact most strongly with sulfatide, whereas the proteolipid protein interacts with cerebroside and cholesterol [3, 4].

Developmental studies [5] suggest that the deposition of myelin lipids and proteins in vivo correlates with the specificity found in in vitro models. Thus during myelination there is an early appearance of myelin basic protein together with sulfatide, while the more hydrophobic components, proteolipid protein, cholesterol and cerebroside appear later. These results would favor the hypothesis that membrane

* Presented in part before the Eight Annual Meeting of the Union of Swiss Societies of Experimental Biology in April, 1976.

proteins at least partly influence the lipid composition of a given membrane [6] and in particular give further support to the concept that the myelin basic protein may represent a committing step in myelin formation.

Furthermore it has been shown that the interaction between myelin basic protein and acidic lipids results in an increased stability with respect to proteolytic enzymes [8, 9]. This fact is of particular importance in certain pathological conditions, such as demyelinating disorders, in which the myelin basic protein is susceptible to proteolytic attack, at least with cerebral acid proteinase [10].

If the myelin basic protein plays an important role in the assembly and stability of the myelin membrane it is of importance to determine the nature of the association of this protein with acidic lipids. While it has been suggested that the complexes myelin basic protein-acidic lipids are stabilised by electrostatic interactions [11], no direct experimental evidence supporting this concept has been available so far. We therefore measured the interaction of myelin basic protein with several charged and neutral lipids and investigated the effect of decreasing the electrical charge of the basic amino groups of the myelin basic protein by acetylation. From the composition of the complexes formed (as judged by the ratio of their constituents) we obtained an evaluation of the effect of charge modification of the myelin basic protein on lipid-protein interaction.

MATERIALS AND METHODS

Preparation of myelin basic protein. Myelin basic protein was prepared from whole bovine brain essentially according to the method described by Oshiro and Eylar [12], using Cellex-P column chromatography as a final step. The protein was dialysed against distilled water and stored at −20° C as a 2 mg/ml solution. The protein preparation, which showed one major single band on analytical disc gel electrophoresis described below, was used in this study.

Lipids. Phosphatidylcholine, phosphatidylinositol, cerebroside and sulfatide were obtained from Applied Science Laboratories. Phosphatidic acid and cholesterol were from Koch-Light Laboratories. Purity of all batches was tested by thin-layer chromatography. In some batches a slight contamination of sulfatide with cerebroside was removed by thin-layer chromatography, scraping off the sulfatide spot and eluting the lipid from the silica gel with chloroform/methanol (2 : 1, v/v) containing 5 % water. Triphosphoinositol was prepared from bovine brain by the method of Dittmer and Dawson [13]. All lipids were stored in chloroform/methanol at −20 °C.

Biphasic solvent system. The interaction between the purified myelin basic protein and various lipids was studied in a biphasic system consisting of chloroform/methanol/water (8 : 4 : 3, v/v) [11]. About 250 μg of myelin basic protein in 0.3 ml of water was added to increasing amounts of dry lipids (after removal of the organic phase under N_2) and the volume made up to 1.5 ml with chloroform/methanol (2 : 1, v/v). The contents of the tube were thoroughly mixed, allowed to stand for 30 min at room temperature, the solution centrifuged and the resulting upper and lower phases were analysed for protein. The values for protein were calculated by assuming a volume of the upper phase of 0.7 ml.

Acetylation of myelin basic protein. The myelin basic protein was acetylated by the procedure of Fraenkel-Conrat [14]. Reaction was at 0 °C for 1 h. The modified

protein was separated from unwanted components of the reaction solution by dialysis against water.

Polyacrylamide gel electrophoresis. The method of Panyim and Chalkley [15] was used. The gels were composed of 15 % acrylamide, 0.1 % *N,N'*-methylenebis-acrylamide, 5.4 % glacial acetic acid, 0.5 % *N,N,N',N'*-tetramethylenediamine, 6.25 M urea and 0.125 % $(NH_4)_2S_2O_8$. Tray buffer was 0.9 M acetic acid with a pH of 3.2. The samples were run on gels 5 mm × 9 cm at 2 mA per gel for 2.5 h. Gels were stained with Coomassie blue and de-stained by diffusion.

Circular dichroism measurements. These measurements were performed with a Roussel-Jouan Model CD 185 Instrument calibrated with isoandrosterone. Molar ellipticity was calculated on the basis of a mean residue weight of 115.

Analytical procedures. Protein was measured by the method of Lowry et al. [16]. When necessary, organic solvents were first removed by evaporation. Bovine serum albumin was used as a standard. For circular dichroism measurements protein concentration was determined by measuring the absorbance at the absorption peak near 280 nm, taking for the extinction coefficient of a 0.1 % solution, with 1 cm path length, 0.589 [17]. Lipids were measured by gravimetry or by phosphate analysis [18].

Chemicals. Acrylamide and *N,N'*-methylenebisacrylamide were from Serva. *N,N,N',N'*-tetramethylenediamine was from Fluka. [1-^{14}C]Acetic anhydride (specific activity 29 Ci/mol) was from the Radiochemical Centre, Amersham. All chemicals used were analytical grade.

RESULTS

Interaction of native myelin basic protein with lipids

Fig. 1 shows the interaction between myelin basic protein and the major acidic lipids of myelin. With increasing amounts of acidic lipids the myelin basic protein

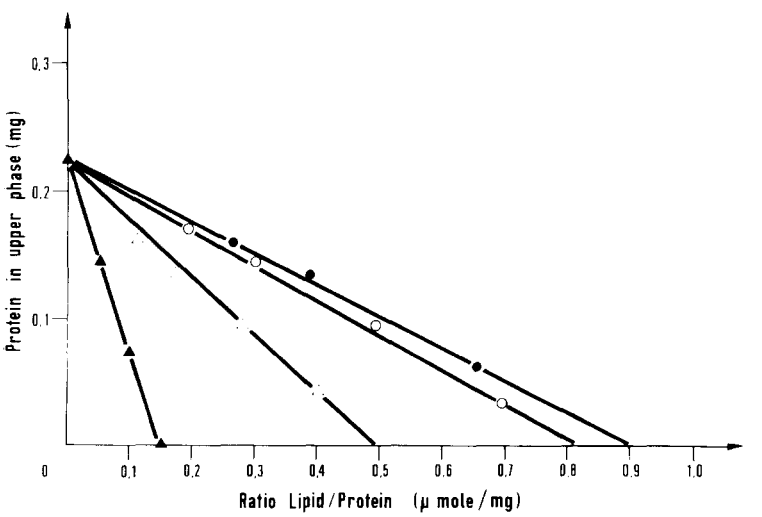


Fig. 1. Complex formation of myelin basic protein with acidic lipids in a biphasic system. ●, sulfatide; ○, phosphatidylinositol; △, phosphatidic acid; ▲, triphosphoinositol. Conditions as described in Materials and Methods.

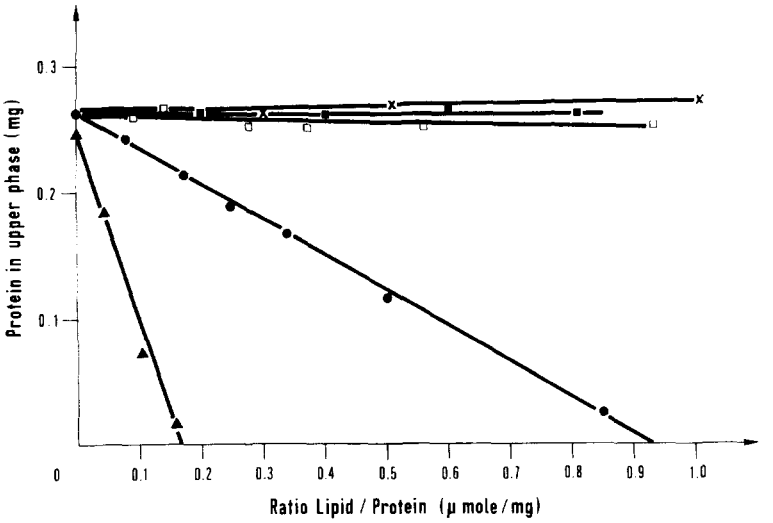


Fig. 2. Interaction of myelin basic protein with neutral and acidic lipids in a biphasic system. ■, cholesterol; □, cerebroside; ×, phosphatidylcholine; ●, sulfatide; ▲, triphosphoinositol. Conditions as described in Materials and Methods.

progressively disappears from the upper aqueous phase. The formation of the protein-acidic lipid complex exhibits a linear relationship to the amount of lipid present. As was shown initially by Palmer and Dawson [11], the concentration ratio between the lipid and the protein depends approximately on the charge available on the lipid molecule. Thus the complex formed with triphosphoinositol (a lipid with five negative

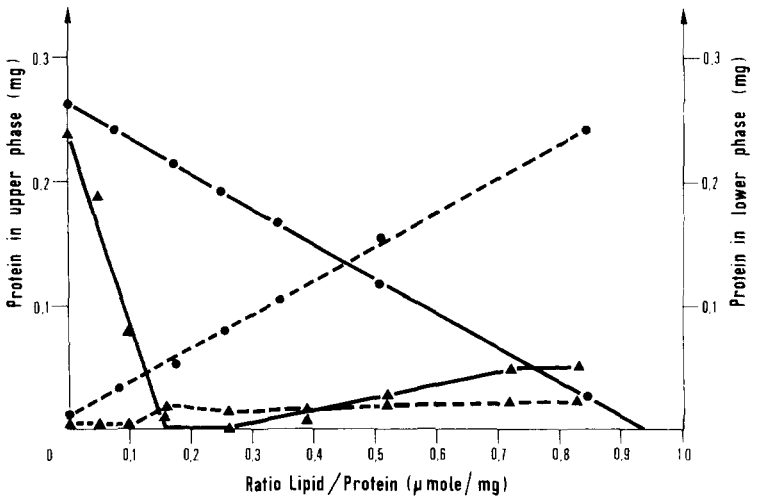


Fig. 3. Complex formation of myelin basic protein with sulfatide and triphosphoinositol in a biphasic system. —, protein in upper phase; - - -, protein in lower phase; ●, sulfatide; ▲, triphosphoinositol. Conditions as described in Materials and Methods.

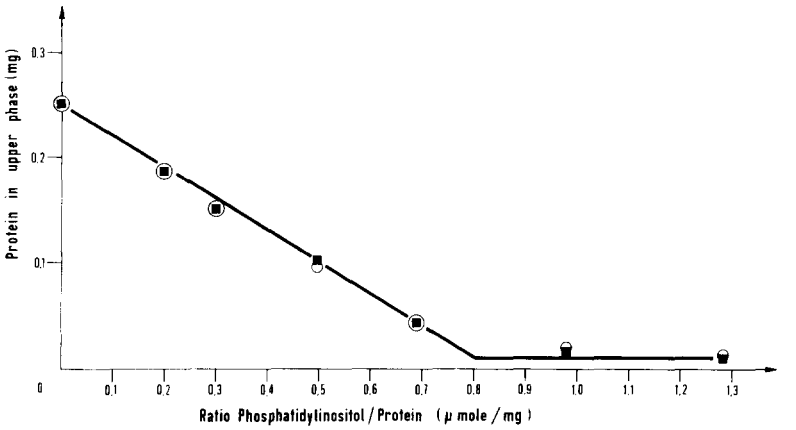


Fig. 4. Effect of cholesterol on complex formation between phosphatidylinositol and myelin basic protein in a biphasic system. ○, phosphatidylinositol alone; ■, phosphatidylinositol with an excess of cholesterol (1.75 μmol cholesterol per mg myelin basic protein) added to the system. Conditions as described in Materials and Methods.

charges) has a much lower lipid protein ratio than the complex formed with phosphatidylinositol (a lipid with one negative charge).

In this system there was no evidence for complex formation between myelin basic protein and non-ionic lipids like cholesterol, cerebroside or phosphatidylcholine (Fig. 2). Here the myelin basic protein remained in the upper phase.

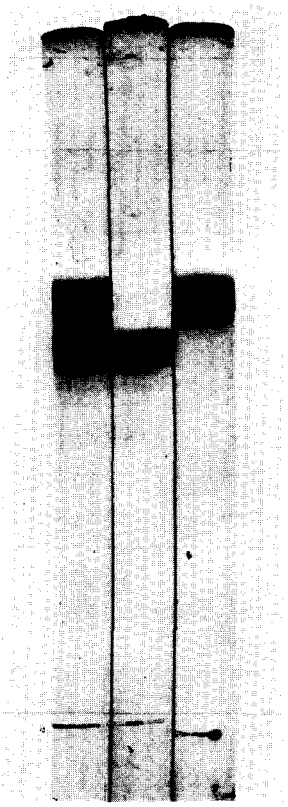
The complex between sulfatide and myelin basic protein is soluble in the lower phase (Fig. 3). With increasing amounts of added lipid the myelin basic protein progressively disappears from the upper phase to be recovered almost quantitatively in the lower phase. However, the complex formed between triphosphoinositol and myelin basic protein does not pass into the lower phase, that is, the complex is not soluble in the lower phase.

In one experiment (Fig. 4) we studied the effect of adding a neutral lipid to different ratios of the phosphatidylinositol-myelin basic protein complex. The added cholesterol had no effect on the phospholipid-myelin basic protein complex formation, indicating that in this system a neutral lipid could not compete for binding sites with an acidic lipid. In separate experiments we also found that an increasing amount of cholesterol added to a constant ratio of phosphatidylinositol-myelin basic protein had no effect on the complex formation.

Characterisation of the acetylated myelin basic protein

Fig. 5 illustrates the electrophoretic behaviour of the acetylated myelin basic protein in polyacrylamide urea gel at acid pH. The modified protein migrates more slowly than the native protein, indicating conversion to a less positive form. By this technique the modified protein appears essentially homogeneous although it runs as a slightly larger band.

We used [1-^{14}C]acetic anhydride to measure the extent of acetylation. The [1-^{14}C]acetyl-labelled myelin basic protein obtained after extensive dialysis contained 420 nmol [1-^{14}C]acetyl per mg of protein. Assuming a molecular weight of 18 400



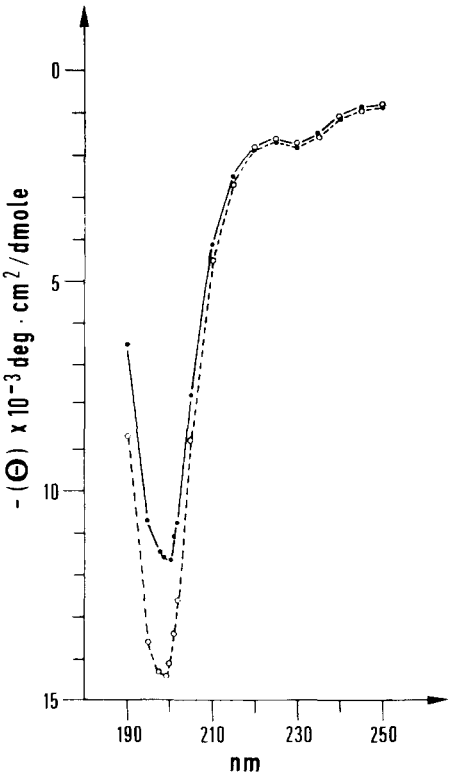


Fig. 5. Analytical disc gel electrophoresis of the acetylated and native myelin basic protein. About 10 μg of each protein was subjected to electrophoresis. The protein migrated from top to bottom. Right, acetylated myelin basic protein; center, native myelin basic protein; left, combination of both.

Fig. 6. Circular dichroism curves for the native and acetylated myelin basic protein in diluted HCl at pH 4.0. ○– –○, native myelin basic protein; —, acetylated myelin basic protein. Conditions as described in Materials and Methods.

for the myelin basic protein [19], the modified myelin basic protein contains a total of eight acetyl residues per molecule. Under the conditions used the acetylation is relatively specific for the ε-amino group of lysine. We have thus modified eight of the 13 lysine residues reported to be present in the myelin basic protein [19].

Obviously modification of the individual charged groups of a protein may also have an effect on its conformation. We examined the circular dichroism of the native and the acetylated myelin basic protein (Fig. 6). Spectra quite similar to those previously reported for myelin basic protein and exhibiting a typical curve of random coil with a trough at 199–200 nm were found [20, 21]. In the acetylated myelin basic protein the molar ellipticity at 199–200 nm decreased from about $14.5 \cdot 10^{-3}$ degrees · cm^2 · dmol^{-1} to about $11.5 \cdot 10^{-3}$ degrees · cm^2 · dmol^{-1} at pH 4.0. At pH 7.0 no difference in circular dichroism curves between the native and acetylated myelin basic protein was found.

Interaction of acetylated myelin basic protein with lipids

The ability of the acetylated myelin basic protein to form complexes with lipids is shown in Figs. 7 and 8. We tested two acidic lipids with similar charge, sulfatide and phosphatidylinositol. The acetylated protein formed complexes with both lipids as shown by its disappearance from the upper phase. However, only about half

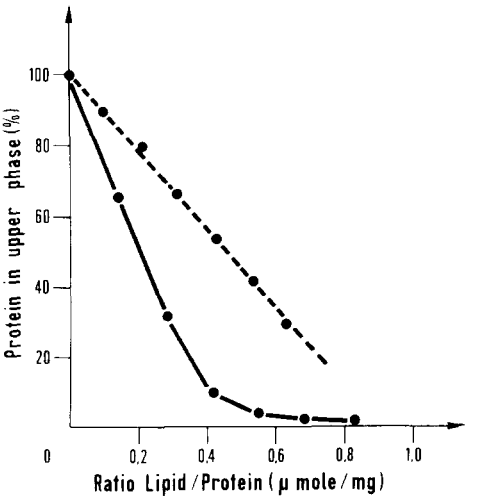


Fig. 7. Effect of acetylation on lipid-protein interaction. ●, sulfatide; – – –, native myelin basic protein; —, acetylated myelin basic protein. Conditions as described in Materials and Methods. Data are percent of protein disappearing from the upper phase. For each experiment the amount of protein in the upper phase without lipid was measured separately and taken as 100 %.

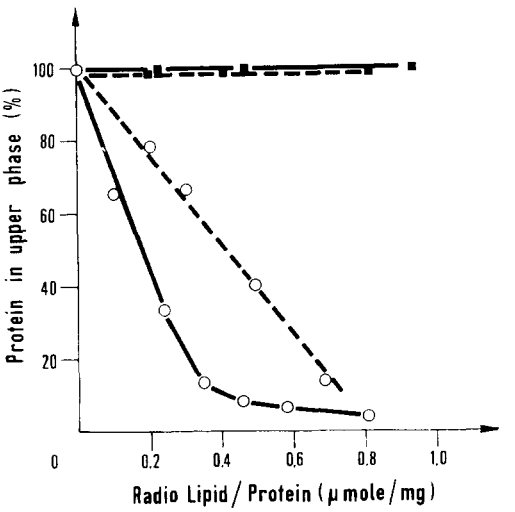


Fig. 8. Effect of acetylation on lipid-protein interaction. ■, cholesterol; ○, phosphatidylinositol; – – –, native myelin basic protein; —, acetylated myelin basic protein. Conditions as described in Materials and Methods and Fig. 7.

of the lipid was necessary to cause the same loss of protein from the upper phase when acetylated myelin basic protein was used. On a molar basis* the complex formed with native myelin basic protein and sulfatide (Fig. 7) contains an average of 17 mol lipid per mol myelin basic protein, while the corresponding complex with acetylated myelin basic protein contains only 8 mol lipid per mol myelin basic protein. Similar results were obtained with phosphatidylinositol (Fig. 8) where the complex formed with native myelin basic protein contains an average of 15 mol lipid per mol myelin basic protein while the corresponding complex with acetylated myelin basic protein contains 6 mol lipid per mol myelin basic protein. Cholesterol, a neutral lipid, did not interact with either the native or the acetylated myelin basic protein since the protein remained in the upper phase (Fig. 8).

DISCUSSION

Our experiments demonstrate that in a biphasic system only anionic lipids such as those found in myelin form complexes with the myelin basic protein (Figs. 1 and 2). The ratio of lipid to protein in the complex depends essentially on the charge available on the lipid molecule and not on the type of lipid used. In this respect it is of interest to compare two lipids with similar negative charge but of different structure like sulfatide, a galactosphingolipid and phosphatidylinositol, a glycerophospholipid (Fig. 1). Both form complexes with only slight differences in the concentration ratio of lipid to protein. While the significance of this difference remains to be verified, steric or solubility factors might be involved, since we are dealing with two different types of lipid molecules.

While all acidic lipids tested formed complexes with the myelin basic protein, the hydrophobicity of the complexes varied considerably. Thus sulfatide leads to the formation of a complex having enough hydrophobic fatty acid residues to be soluble in the lower phase, while the complex formed with triphosphoinositol, which has a much lower lipid to protein ratio, remained at the interphase (Fig. 3). This may be due to the more amphipatic character of this complex, which shows low solubilities in the aqueous as well as in the organic phase. The addition of an excess of triphosphoinositol caused some resolubilisation of the complex, probably by a detergent-like action.

Further evidence for the electrostatic type of the binding of acidic lipids to myelin basic protein is the observation that complex formation is unaffected by the presence of a neutral lipid (Fig. 4). The high content of basic amino acids, characteristic of the myelin basic protein, would provide the cationic groups for the binding of anionic lipids. Selective acetylation of the ε-amino group of lysine reduces strongly the nucleophilic character of the nitrogen atom producing the elimination of one positive charge unit per each modified group.

Our results (Figs. 7 and 8) clearly show that significantly less lipid is needed to cause the same disappearance of protein from the upper phase when acetylated myelin

* Values for the molar lipid : protein ratio were obtained by extrapolation of the linear part of the curve to the abscissa. An approximate numerical calculation can be made by assuming that at equilibrium the protein is either fully combined in the complex or completely freee [11]. It should be noted that this calculation does not take in account a possible heterogeneity of the complexes formed.

basic protein was used as compared to native myelin basic protein. It is possible that modification of the myelin basic protein, while still permitting recombination with lipids, may give complexes that are more soluble in the lower phase than complexes with native myelin basic protein. Another possibility is that the removal of positive charges results in the loss of binding sites for acidic lipids in the myelin basic protein. Although a direct assessment of these two interpretations is difficult, the following arguments in favor of the latter hypothesis can be derived from our results. On a molar basis the complex between acetylated myelin basic protein and an acidic lipid with one negative charge exhibits an average reduction of 9 mol lipid per mol protein. This figure is consonant with our results with [1-^{14}C]acetic anhydride demonstrating an average of eight acetyl residues per mol of modified myelin basic protein. From the 31 basic groups (lysine and arginine) reported to be present in the myelin basic protein [19], an average of 16 are acting in the binding of acidic lipids (Figs. 7 and 8) assuming a one to one correspondance. Therefore, after blocking eight of these groups, there still are cationic groups available to account for the residual binding activity observed with acetylated myelin basic protein. Furthermore, if a limited amount of lipid is added to an excess amount of acetylated myelin basic protein, the same behaviour of complex formation observed with native myelin basic protein, that is a linear relationship to the amount of lipid present, is found. This suggests a similar mechanism of complex formation for both, native and acetylated myelin basic protein.

Of particular significance for the interpretation of our results are the circular dichroism spectra measurements. Following acetylation there was no indication of conformational changes from random coil to more ordered structures in the myelin basic protein (Fig. 6). It seems reasonable to assume that the accessibility of the cationic groups would be comparable in the modified and in the native myelin basic protein. Therefore the observed effect of acetylation of the myelin basic protein on lipid-protein interaction most likely results from modification of the primary and not tertiary structure of the protein.

These experiments demonstrate the importance of free amino groups in the myelin basic protein for lipid-protein interaction and supports the concept that the acidic lipids interact electrostatically with these groups. Studies of Braun and Radin [23] have shown that succinylation (introduction of a negative charge) of the lysine amino group of the proteolipid protein of myelin almost completely abolishes the capacity of the protein to associate with lipids. This effect was interpreted as resulting from a drastic alteration in the three-dimensional structure of the protein. In view of our results an alternative explanation for the total inability of the modified protein to bind acidic lipids would be that succinylation suppressed the net positive charge of the protein. In comparing Braun's results with ours one should, however, keep in mind the difference in the physicochemical characteristics of the systems used. We measured lipid-protein interaction in an organic milieu (lower phase) where ionic interactions between molecules are favored, while, in the aqueous system Braun was using, the charge-charge interactions, critically important for the formation of precipitable complexes, are considerably weakened.

The question arises whether the modification examined in the present investigation has any relevance to the myelin membrane. While the myelin basic protein from human and bovine brain has an acetylated N-terminal alanine [22], acetylation of the amino groups of the basic amino acids within the polypeptide chain has not

been reported. Myelin basic protein isolated from central nervous system tissue exists, however, in several forms which differ in charge, as judged by their behaviour during alkaline pH gel electrophoresis [24]. These different forms have been isolated and shown to have essentially the same amino acid composition [25]. Amino acid modification through methylation [26], deamidation [27] and phosphorylation [27] has been implicated in the microheterogeneity of this protein. Regardless, however, of what mechanism is actually involved in the production of the multiple forms of myelin basic protein, our results would suggest that there may be differences in the way the different forms of myelin basic protein interact with myelin lipids.

ACKNOWLEDGEMENTS

We thank Mrs. Marianne Farah for expert technical assistance. This investigation was supported by grant 3.660-0.75 of the Swiss National Science Foundation.

REFERENCES

1 Singer, S. J. and Nicolson, G. L. (1972) Science 175, 720–731
2 Bangham, A. D. (1972) Annu. Rev. Biochem. 41, 753–776
3 Demel, R. A., London, Y., Geurts van Kessel, W. S. M., Vossenberg, F. G. A. and van Deenen, L. L. M. (1973) Biochim. Biophys. Acta 311, 507–519
4 London, Y., Demel, R. A., Geurts van Kessel, W. S. M., Zahler, P. and van Deenen, L. L. M. (1974) Biochim. Biophys. Acta 332, 69–84
5 Detering, N. K. and Wells, M. A. (1976) J. Neurochem 26, 253–257
6 Kramer, R., Schlatter, Ch. and Zahler, P. (1972) Biochim. Biophys. Acta 282, 146–156
7 Wood, J. G. (1973) J. Neurochem. 20, 423–430
8 London, Y., Demel, R. A., Geurts van Kessel, W. S. M., Vossenberg, F. G. A and van Deenen, L. L. M. (1973) Biochim. Biophys. Acta 311, 520–530
9 Schäfer, R. and Franklin, R. M. (1975) FEBS Lett. 58, 265–268
10 Einstein, E. R., Csejtey, J., Dalal, K. B., Adams, C. W. M., Bayliss, O. B. and Hallpike, J. F. (1972) J. Neurochem. 19, 653–662
11 Palmer, F. B. and Dawson, R. M. C. (1969) Biochem. J. 111, 637–646
12 Oshiro, Y. and Eylar, E. H. (1970) Arch. Biocem. Biophys. 138, 392–396
13 Dittmer, J. C. and Dawson, R. M. C. (1961) Biochem. J. 81, 535–539
14 Fraenkel-Conrat, H. (1957) Methods Enzymol. 4, 247–269
15 Panyim, S. and Chalkley, R. (1969) Arch. Biochem. Biophys. 130, 337–346
16 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
17 Eylar, E. H. and Thompson, M. (1969) Arch. Biochem. Biophys. 129, 468–479
18 Chen, P. S., Toribara, T. Y. and Warner, H. (1956) Anal. Chem. 28, 1756–1758
19 Eylar, E. H. (1973) in Proteins of the Nervous System, pp. 27–44, Raven Press, New York
20 Anthony, J. S. and Moscarello, M. A. (1971) Biochim. Biophys. Acta 243, 429–433
21 Liebes, L. F., Zand, R. and Phillips, W. D. (1975) Biochim. Biophys. Acta 405, 27–39
22 Carnegie, P. R. and Dunkley P. R. (1975) in Advances in Neurochemistry, pp. 95–135, Plenum Press, New York
23 Braun, P. E. and Radin, N. S. (1969) Biochemistry 8, 4310–4318
24 Martenson, R. E. and Gaitonde, M. K. (1969) J. Neurochem. 16, 889–898
25 Deibler, G. E. and Martenson, R. E. (1973) J. Biol. Chem. 2392–2396
26 Carnegie, P. R. (1971) Nature 229, 25–28
27 Deibler, G. E., Martenson, R. E. Kramer, A. J., Kies, M. W. and Miyamoto, E. (1975) J. Biol. Chem. 250, 7931–7938