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THE INTERACTION OF THE "FOLCH–LEES" PROTEIN WITH LIPIDS AT THE AIR–WATER INTERFACE

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SUMMARY

1. The interaction of "Folch–Lees" apoprotein with different lipids has been studied at the air–water interface. Measurements of the change in surface pressure showed a high affinity of the Folch–Lees protein for a variety of myelin lipids such as cerebroside sulphate, phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine and cholesterol. Lipids such as sphingomyelin, cerebroside and lecithin show markedly less affinity for the Folch–Lees protein.

2. The remarkable interaction of the Folch–Lees protein with cholesterol is dependent on the sterol structure. Epicholesterol, cholest-4-en-3-one, coprostanol, lanosterol and androstanol showed a reduced binding in this order.

3. The two major myelin proteins, the Folch–Lees protein and the A_1 basic protein, show a different lipid affinity. The subsequent injection of A_1 basic protein and Folch–Lees protein underneath a cholesterol monolayer shows a preferential binding of Folch–Lees protein with cholesterol and a rejection of A_1 basic protein from the interface. The subsequent injection of A_1 basic protein and Folch–Lees protein underneath a cerebroside sulphate monolayer shows that both proteins can bind to cerebroside sulphate. The A_1 basic protein shows however the highest affinity for this typical myelin lipid.

INTRODUCTION

The protein composition of myelin is a relatively simple one [1–3]. Two main protein components of central nerve myelin which make up 85% [1–4] of the membrane protein content have been recognized, purified and characterized, namely, the "Folch–Lees" protein [5–11] and the basic encephalitogenic protein [12,13], or A_1 basic protein. In our former studies we have tried to elucidate the interaction of the A_1 basic protein with myelin lipids [14–18]. For a better understanding of lipid–protein interactions in the myelin membrane a study was performed also of the interaction of the Folch–Lees protein with lipids at the air–water interface. This model system has already been proved by various investigators [17–19] to be a powerful tool to detect and compare the specific interactions between membrane

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proteins and lipids. This method is preferable to studies of the complex formation in aqueous solutions which require the use of detergents [20]. Precipitation reactions following the interaction of negatively charged lipids with positively charged protein make those experiments difficult to interpret. The use of ^{131}I-labelled Folch–Lees protein and ^{131}I-labelled A_1 basic protein enabled us to follow directly the adsorption and desorption from the interface. The results of this study are interpreted in the light of the assumed lipid and protein asymmetry of the myelin membrane [21,22].

MATERIALS AND METHODS

Sephadex LH-20 was purchased from Pharmacia fine-chemicals, Sweden, Dowex 1 X-2 acetate (100–200 mesh) was purchased from Fluka, Germany.

^{131}I, carrier-free, was purchased from New England Nuclear, U.S.A. and lactoperoxidase, Grade B from B.D.H., England.

Preparation of the proteins

The A_1 basic protein was purified from bovine spinal cords as already described [14]. The Folch–Lees apoprotein was solubilized, delipidated and rendered soluble in water according to the method of Mokrasch [10] with some modifications. The total lipid extract of bovine white matter was washed seven times with teoretical upper phase [5], and the phases were separated by centrifugation. For a better separation of lipid and protein and therefore higher yield of protein other columns dimensions were used. The Sephadex LH-20 column was 150 cm $\times$ 5 cm and the Dowex 1 X-2 column 140 cm $\times$ 3.5 cm.

The purification of the Folch–Lees protein was followed by the use of thin-layer chromatography, organic phosphorous and protein determinations. The protein was rendered soluble in water also according to the methods of Hendrickson et al. [11] and Folch-Pi and Stoffyn [8]. The surface activities and the interaction with lipid monolayers of these preparations were compared to the one prepared according to the method of Mokrasch [10]. The Folch–Lees apoprotein was kept at a temperature of 4 °C using 1 mM propionic acid as a stabilizer [10].

The purity of the proteins was determined by the use of disc electrophoresis performed according to the method of Shapiro et al. [23] in 1% sodium dodecyl sulphate (Fig. 1). The Folch–Lees protein was found to contain 0.04–0.07% phosphoros.

The approximate molecular weight determined by disc electrophoresis was found to be 23000.

The iodination of Folch–Lees and the A_1 basic proteins

The iodination of the A_1 basic protein was already described [17]. The Folch–Lees protein was iodinated using some modification of the method of Morrison et al. [24]. The iodination was performed at pH 4.0 as the Folch–Lees protein precipitates at pH 7.4 (isoelectric point pH 7.4–9.6). The iodination system was as follows: 1.5 mg Folch–Lees protein ($0.65 \cdot 10^{-7}$ moles, mol. wt 23000), dissolved in 6 ml of 0.05 M acetate buffer (pH 4.0), $1.2 \cdot 10^{-6}$ moles. K^{131}I, $3 \cdot 10^{-10}$ moles lactoperoxidase and $3 \cdot 10^{-6}$ moles H_2O_2. The reaction was stopped after 5 min by cooling to 0 °C and precipitation of the protein by bringing the pH of the solution to pH 8–9 by the

addition of diluted NH_4OH. The pellet recovered after centrifugation (4500 × *g* for 10 min at 0 °C), was dissolved in 1 ml of a 1 mM propionic acid solution in water. The iodinated protein was dialysed against cold iodide and water containing 1 mM propionic acid. The incorporation of iodine was to be 1–3 iodine atoms per molecule of Folch–Lees protein assuming a molecular weight of 23000. The specific activity was of 10^6–10^7 cpm/mg protein (1–10 μCi/mg protein). The iodinated Folch–Lees protein was hydrolysed in 6 M HCl in evacuated tubes for 24 h at 110 °C. Amino acid analysis were performed in order to determine if oxidation of cystein occurred during iodination. No difference was found for the iodinated protein compared to the none iodinated except for reducing of the number of tyrosine residues.

Preparation of the lipids

The different lipid fractions and pure lipids were prepared as described [14,16,17].

Lauroyl cerebroside sulphate and stearoyl cerebroside sulphate were a generous gift of Dr K. Larsson (University of Göteborg, Sweden).

Determination of surface characteristics

Pressure and surface radioactivity increase measurements of lipid monolayers after injection of the proteins were performed as already described [16,17].

RESULTS

The Folch–Lees protein showed no surface activity at pH 3.0 (Fig. 1) when rendered water soluble according to the method of Mokrash. The surface activity of this protein increased especially at pH values above 4.0. At a pH of 5.0 or 6.5 the protein demonstrated a surface pressure as high as 17 dynes/cm. At higher pH values the protein precipitated. The isoelectric point of this protein is thought to be 7.4–9.6. Folch–Lees protein rendered water soluble according to the methods of Folch [8] and Hendrickson [11] showed also at pH 3.0 surface pressure of 5–10 dynes/cm. This observation is at variance with that of Colacicco [25] who did not detect any surface activity after spreading the protein from an aqueous solution. However, no exact pH values or protein concentrations were given by the author. Differences in the surface activities of different preparations could probably be affected by differences in α-helix content and deamination of glutamine or asparagine residues because of the acidic conditions. Folch–Lees protein, prepared and rendered water soluble according to the method of Mokrash and used for the further studies described,

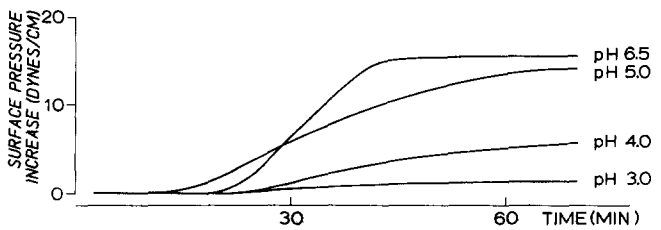


Fig. 1. The surface activity of the Folch–Lees protein at different pH values. The protein (final concn 1 μg/ml) was injected in a subphase of 10^{-2} M Tris–acetate buffer of the indicated pH.

TABLE I

Protein	Lipid added	Solvent	Maxima	θ	Helix
			(nm)		(%)
1. Folch–Lees *	—	Chloroform–methanol–acetic acid (100:100:1, by vol.)	223, 208	$31 \cdot 10^{-3}$	100
2. Folch–Lees **	—	1 mM propionic acid	220, 210	$12.3 \cdot 10^{-3}$	42
3. Iodinated Folch–Lees ***	—	1 mM propionic acid	218, 209	$11.6 \cdot 10^{-3}$	40
4. Iodinated Folch–Lees †	Phosphatidyl-choline/cholesterol (1:1)	10 mM Tris–acetate buffer (pH 5.0)	223, 210	$9.2 \cdot 10^{-3}$	100

* Crossover 204.

** 220 nm blue shifted, 210 nm red shifted indication for β form. Crossover unresolved, conformation predominantly α helixal with remaining part β form and little of random coil.

*** 218 nm blue shifted, 210 nm red shifted indicating β form. Crossover 202 indicating β form very similar to Prepn 2.

† Crossover 201. The curve fits qualitatively with 100% α-helix of phosphoglycerate. In spite of absorption flattening still a very high value for θ 223 was found.

showed a high α-helix content (Table I). This is also the case with the protein when present in chloroform–methanol (1:1, v/v). Spreading of the Folch–Lees protein from the organic phase showed a surface pressure of 17–22 dynes/cm [25]. Also at low pH values these high surface pressures were found, whereas no desorption from the interphase was apparent.

The interaction of the Folch–Lees protein with different monomolecular lipid films was primarily studied at pH 3.0 where the protein showed the lowest surface activity by itself. The effect of the initial pressure of the lipid film on the pressure increase after the injection of Folch–Lees protein underneath the lipid monolayer, is given in Fig. 2. Cholesterol, phosphatidylethanolamine and cerobroside sulphate give a high pressure increase. Even at an initial pressure of 30 dynes/cm the protein is able to penetrate these lipid monolayers. The pressure increase of a dipalmitoyl lecithin monolayer is limited at low initial pressures and absent at initial pressures above 25 dynes/cm. In most of the following studies an initial pressure of 10 dynes/cm was used. The effect of the Folch–Lees protein concentration on the interaction with a cholesterol film is given in Fig. 3. At protein concentrations of 0.7 μg/ml the maximal pressure increase is found. In the following experiments a final concentration of 1 μg/ml was used. Although the Folch–Lees protein showed different surface activities by itself at pH 3.0, 4.0 and 6.5 (Fig. 1) the pressure increase observed after injection underneath a cholesterol film was practically the same at all these pH values (Fig. 3). In contrast to the A_1 basic protein [17], the interaction of the Folch–Lees protein with most of the lipids showed to be very little effected by the subphase pH as it was judged from the pressure increase (Fig. 4). The pressure increase for cholesterol, phosphatidylserine and phosphatidylethanolamine showed little variation in the pH range 3–6.5. Cerebroside and lecithin showed a slight increase whereas cerebroside sulphate showed some decrease in surface pressure change at higher pH values. The lipid

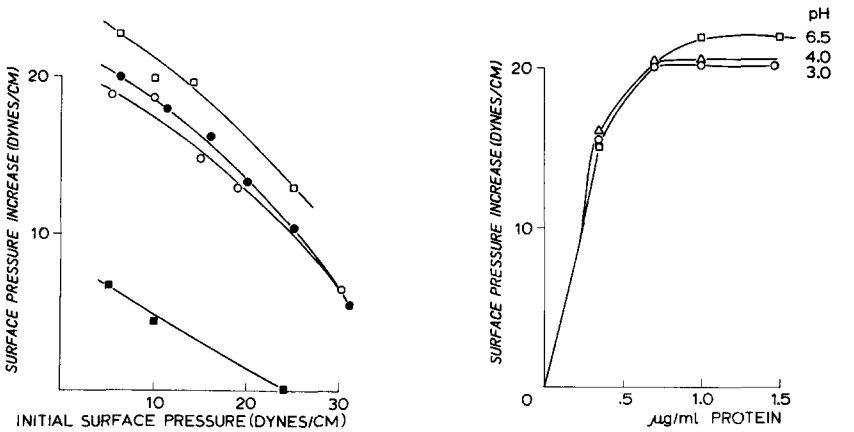


Fig. 2. The effect of the initial surface pressure on the surface pressure increase after injection of the Folch–Lees protein underneath a monolayer of cholesterol (●), phosphatidylethanolamine (○), cerebroside sulphate (□) and dipalmitoyl lecithin (■). The protein concentration was 1 μg/ml. The subphase was a 10^{-2} M Tris–acetate buffer (pH 3.0).

Fig. 3. The effect of Folch–Lees protein concentration on the surface pressure increase of cholesterol monolayers. The subphase was a 10^{-2} M Tris–acetate buffer (pH 3.0 (○), 4.0 (△), 6.5 (□)). The initial film pressure was 10 dynes/cm.

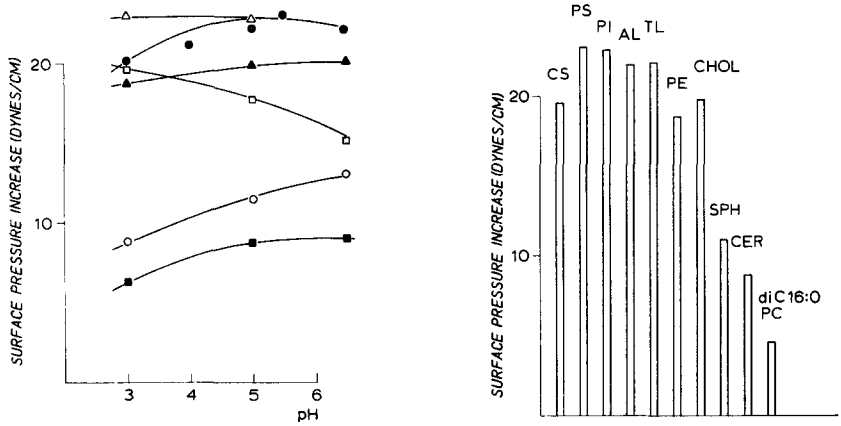


Fig. 4. Surface pressure increase after the injection of Folch–Lees protein underneath a monolayer of cholesterol (●), cerebroside sulphate (□), phosphatidylserine (△), phosphatidylethanolamine (▲), cerebrosides (○), (1-oleoyl-2-stearoyl)-3-lecithin (■), at different pH values. The protein concentration was 1 μg/ml in a subphase of 10^{-2} M Tris–acetate buffer. The initial film pressure was 10 dynes/cm.

Fig. 5. Comparison of the interaction of the Folch–Lees protein with different lipid monolayers, The following lipid films have been used: cerebroside sulphate (CS), phosphatidylserine (PS). phosphatidylinositol (PI), acidic lipid fraction from myelin (AL), total lipid fraction from myelin (TL), phosphatidylethanolamine (PE), cholesterol (CHOL), sphingomyelin (SPH), cerebroside (CER) and dipalmitoyl lecithin (di C 16:0 PC). The initial pressure was 10 dynes/cm. The final protein concentration after the injection was 1 μg/ml. The buffer was 10^{-2} M Tris–acetate (pH 3.0).

affinity of the Folch–Lees protein for different lipid monolayers is illustrated in Fig. 5. A high affinity of the Folch–Lees protein is found for a wide variety of lipids such as: cerebroside sulphate, phosphatidylserine, phosphatidylinositol, the acidic lipid fraction from central nervous system, the total lipid fraction from central nervous system, phosphatidylethanolamine and cholesterol. The affinity is decreased for sphingomyelin, cerebrosides and dipalmitoyl lecithin in this order. Thus the interaction of the Folch–Lees protein is not directed only to negatively charged lipids but also to neutral lipid monolayers. No pressure increase was found when a positively charged film of lysylphosphatidylglycerol from *Staphylococcus aureus* was applied. Essentially the same specificity pattern and pressure increases were found at pH 5.0. Most remarkable is the affinity of the Folch–Lees protein for cholesterol. The specificity of this interaction is studied by varying the sterol structure in different ways (Fig. 6). All sterols studied showed a comparable interfacial behaviour [26]. The surface pressure increase, after the injection of Folch–Lees protein, was found to decrease in the following order: 7-dehydrocholesterol (extra double band at Position 7), epicholesterol (3α isomer of cholesterol), cholest-4-en-3-one (hydroxyl group replaced by keto group and double bond at Position 4), coprostanol (A/B ring in *cis* configuration), lanosterol (2 methyl groups at Position 4 and one at Position 14, double bonds at Position 8 and 24) and androstanol (missing the 8 carbon side chain and the double bond). Especially the two latter sterols showed very little interaction with the Folch–Lees protein. These results indicate that the interaction with the

hydrophobic part of the sterol molecule is of critical importance. The significance of hydrophobic interactions in the A_1 basic protein–lipid interactions was demonstrated [17] by the effect of the fatty acid chain length of lecithins. Lecithins with a chain length shorter than 9 carbon atoms showed a significantly reduced pressure increase after A_1 basic protein injection [17]. The lecithins monolayers tested in the presence of the Folch–Lees protein varied in chain length from 8 to 16 carbon atoms whereas

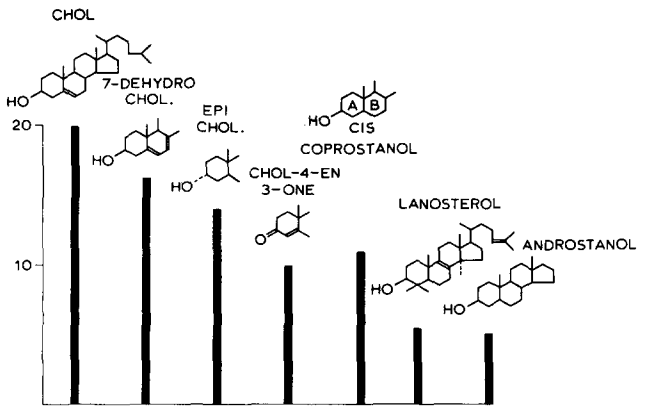
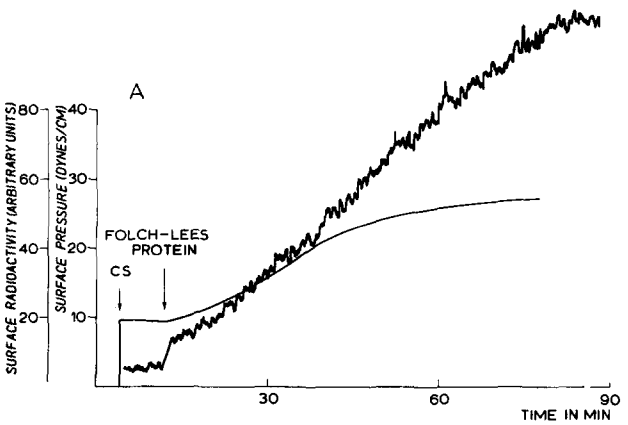


Fig. 6. Surface pressure increase after the injection of Folch–Lees protein underneath a monolayer of respectively cholesterol, 7-dehydrocholesterol (cholest-5,7-dien-3β-ol), epicholesterol (cholest-5-en-3α-ol), cholest-4-en-3-one, coprostanol (5β-cholestan-3β-ol), lanosterol (8,24,(5α)-cholest-dien-4,4 14α-trimethyl-3β-ol) and androstanol.

also mono- and diunsaturated lecithins were tested. No significant fatty acid chain dependency could be observed for the interaction of the Folch–Lees protein with lecithin monolayers. The pressure increase varied inconsistently for the different lecithins from 5.6 to 9.0 dynes/cm. Cerebroside sulphates with a fatty acid chain length of 12 or 16 carbon atoms, were found to give the same pressure increase as the cerebroside sulphate derived from central nervous system, having an average fatty acid chain length of 22 carbon atoms.

To study the interaction of the Folch–Lees protein with monomolecular lipid



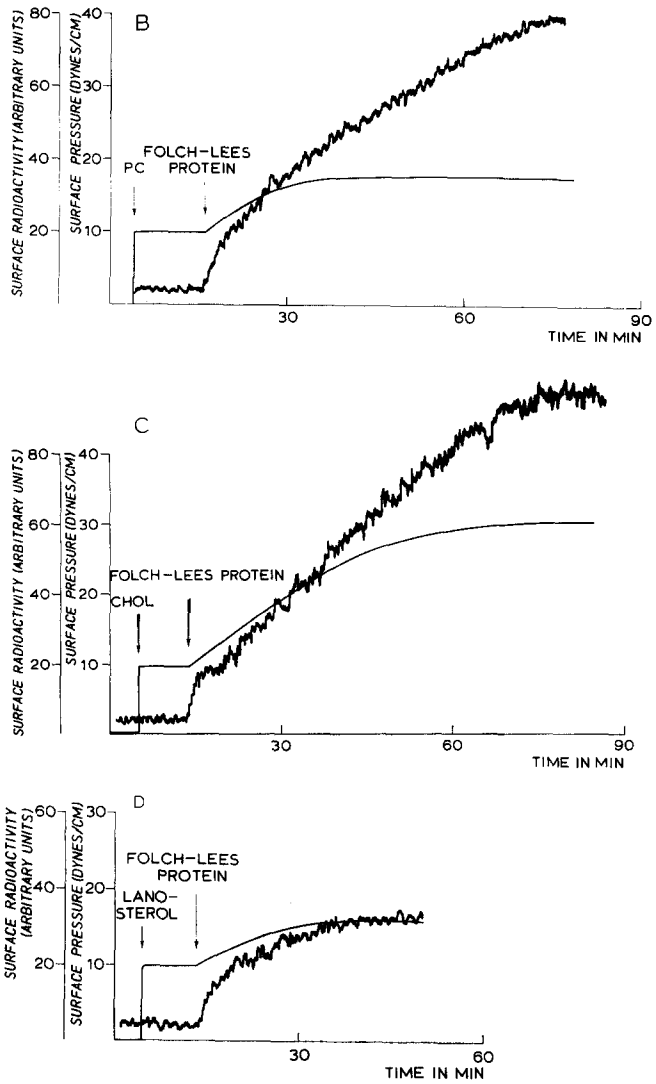


Fig. 7. Automatically recorded surface pressure increase and surface radioactivity increase after the injection of Folch–Lees protein underneath a monolayer of cerebroside sulphate (CS) (A); egg lecithin (PC) (B); cholesterol (C) and lanosterol (D). The protein concentration was 1 μg/ml. The buffer was 10^{-2} M Tris–acetate (pH 3.0).

films in greater detail ^{131}I-labelled Folch–Lees protein was used. In Fig. 7A–D the recording of the surface pressure increase and the surface radioactivity increase are given. The ^{131}I-labelled protein showed essentially the same surface activity and pressure increases as found for the unlabelled protein, expressed in a high pressure increase for cerebroside sulphate and cholesterol and a small pressure increase for lecithin and lanosterol. However, the surface radioactivity increases were found to be practically the same for cerebroside sulphate, cholesterol, and egg lecithin and to corres-

pond with the surface radioactivity of the pure protein (Fig. 8). Whereas the protein showed a higer surface activity at pH 5.0 than at pH 3.0, the surface radioactivity was practically the same in both cases (Fig. 8). In the case of the poorly interacting lanosterol only a limited radioactivity increase was measured (Fig. 7D).

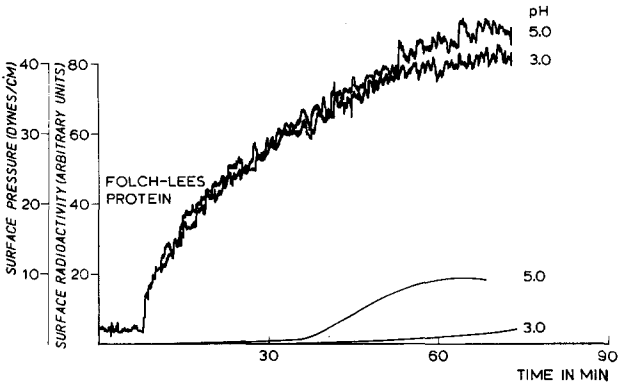


Fig. 8. Automatically recorded surface radioactivity increase after the injection of Folch–Lees protein in a subphase of 10^{-2} M Tris–acetate buffer (pH 3.0 and 5.0). The protein concentration was 1 μg/ml.

It is suggested that the protein and/or lipids are asymmetrically distributed in the myelin membrane [21,22]. A comparison of the results of this study on the Folch–Lees protein and preceding studies on the A_1 basic protein [14,18] showed that these two major myelin proteins show a different lipid specificity spectrum (Fig. 9). The Folch–Lees protein shows a high affinity for myelin such as: cerebroside sulphate, phosphatidylserine, phosphatidylethanolamine and cholesterol and little

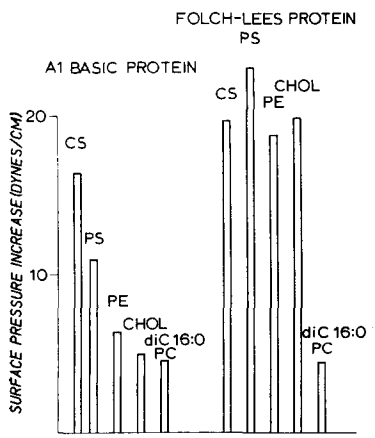


Fig. 9. Comparison of the affinity of the two major myelin proteins, the A_1 basic protein and the Folch–Lees protein, for different lipid monolayers. The films as indicated in Fig. 5 have been used. The initial pressure of the lipid film was 10 dynes/cm. The final protein concentration after injection was 1 μg/ml. The buffer was 10^{-2} M Tris–acetate (pH 3.0).

affinity for lecithin. The A_1 basic protein favours the interaction with cerebroside sulphate and to a lesser extent phosphatidylserine. Little interaction is found with phosphatidylethanolamine, cholesterol and lecithin. The preferential lipid binding of the Folch–Lees protein and the A_1 basic protein is studied further by submitting subsequently both proteins to the same lipid interface. For this purpose one of the proteins was ^{131}I-labelled whereas the other protein was unlabelled. Fig. 10A des-

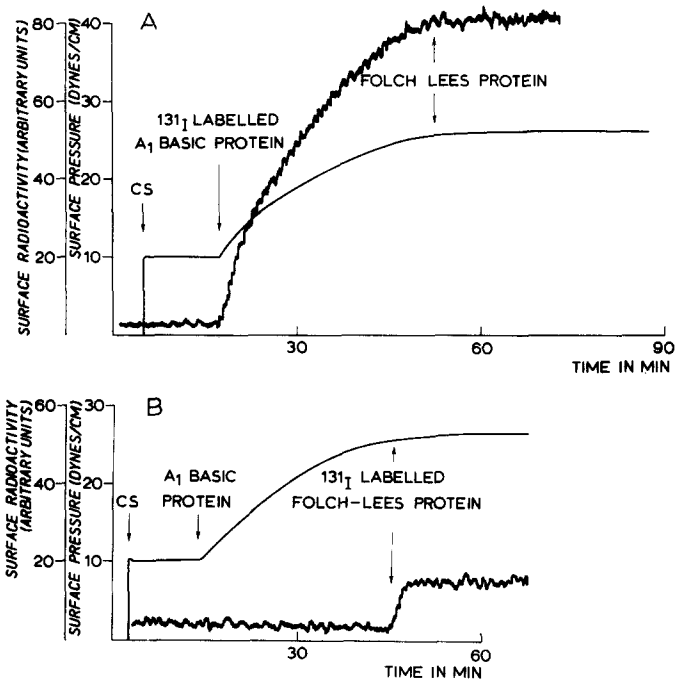


Fig. 10. Automatically recorded surface pressure increase and surface radioactivity increase after the subsequent injection of A_1 basic protein and Folch–Lees protein underneath a cerebroside sulphate monolayer. (A), the A_1 basic protein is ^{131}I labelled; (B), the Folch–Lees protein is ^{131}I labelled. The protein concentrations were 1 μg/ml. The buffer was 10^{-2} M Tris–acetate (pH 3.0).

cribes an experiment where ^{131}I-labelled A_1 basic protein is injected first underneath a cerebroside sulphate film. The surface pressure increases and simultaneously the surface radioactivity increases due to the penetration of this protein into the cerebroside sulphate monolayer. The subsequent injection of Folch–Lees protein shows no change in surface pressure or surface radioactivity. In Fig. 10B the A_1 basic protein is unlabelled, the pressure increase is identical as with ^{131}I-labelled A_1 basic protein. Subsequent injection of ^{131}I-labelled Folch–Lees protein shows no pressure increase and no significant increase in surface radioactivity .The increase is due to background counting of the high specific labelled protein in the subface. From the experiments in Fig. 10A and B it can be concluded that after interaction of A_1 basic protein with a cerebroside sulphate monolayer the Folch–Lees protein is not able to interact with this lipid or to reject the A_1 basic protein from the interphase. The order of injection

of the two proteins was reversed, first was injected the Folch–Lees protein and then the A_1 basic protein. Also the Folch–Lees protein is able to interact with the cerebroside sulphate monolayer when submitted first (Fig. 11A, cf. also Fig. 9), since surface pressure and surface radioactivity increased significantly after injection. When the A_1 basic protein is injected thereafter, there is still some further pressure increase which could indicate that some A_1 basic protein is still able to interact with the cerebroside sulphate monolayer. However no Folch–Lees protein is rejected since no decrease in surface radioactivity is observed. In Fig. 11B a similar experiment is done where the A_1 basic protein is labelled. It can be seen from the increase in surface radioactivity that indeed some A_1 basic protein is still able to interact with cerebroside sulphate even in the presence of the Folch–Lees protein.

It is clear from Figs 5, 6 and 7C that only the Folch–Lees protein is able to

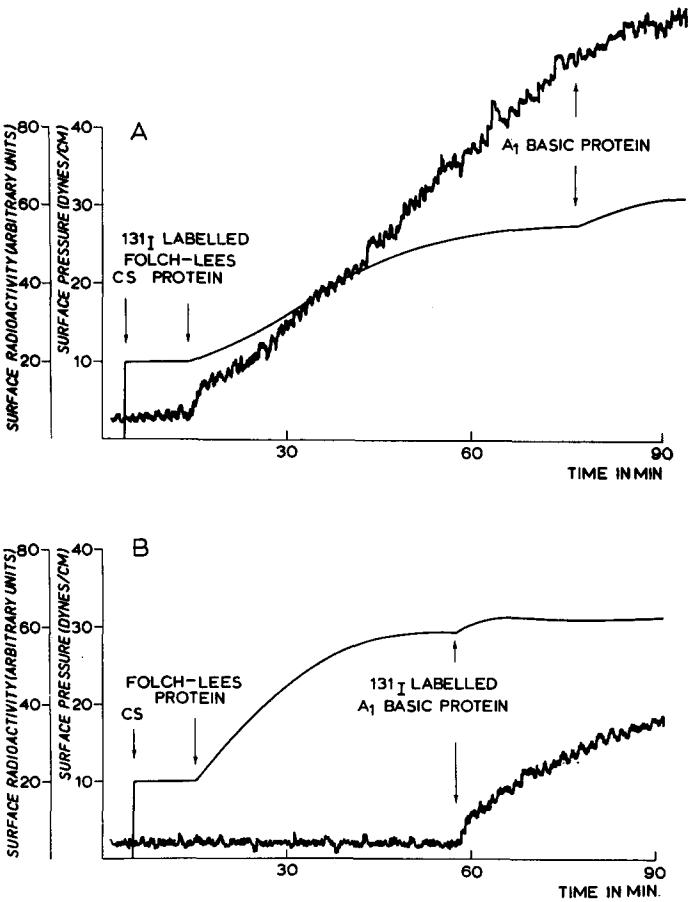


Fig. 11. Automatically recorded surface pressure increase and surface radioactivity increase after the subsequent injection of Folch–Lees protein and A_1 basic protein underneath a cerebroside sulphate monolayer. (A), the Folch–Lees protein is ^{131}I labelled; (B), the A_1 basic protein is ^{131}I labelled. The protein concentrations were 1 μg/ml. The buffer was 10^{-2} M Tris-acetate (pH 3.0).

interact with cholesterol. To see if this specificity of the Folch–Lees protein holds also in the presence of A_1 basic protein, the following experiments were carried out. When ^{131}I-labelled A_1 basic protein is injected underneath a cholesterol monolayer a relatively small pressure and radioactivity increase is found (Fig. 12A). Injection of the Folch–Lees protein shows a further surface pressure increase and displacement of A_1 basic protein. This means indeed that the affinity of the Folch–Lees protein for cholesterol is stronger than of the A_1 basic protein. Fig. 12B shows that the pressure increase caused by the subsequent injection of labelled Folch–Lees protein injection is also accompanied by an increase in surface radioactivity. When the Folch–Lees protein is submitted first to the cholesterol monolayer a high pressure and surface radioactivity increase is found (Fig. 13A). The subsequent addition of A_1 basic protein shows that this protein is no more able to interact with the sterol monolayer to any extent. This can also be concluded from Fig. 13B where ^{131}I-labelled A_1 basic protein was used, no surface radioactivity increase above the background value can be observed.

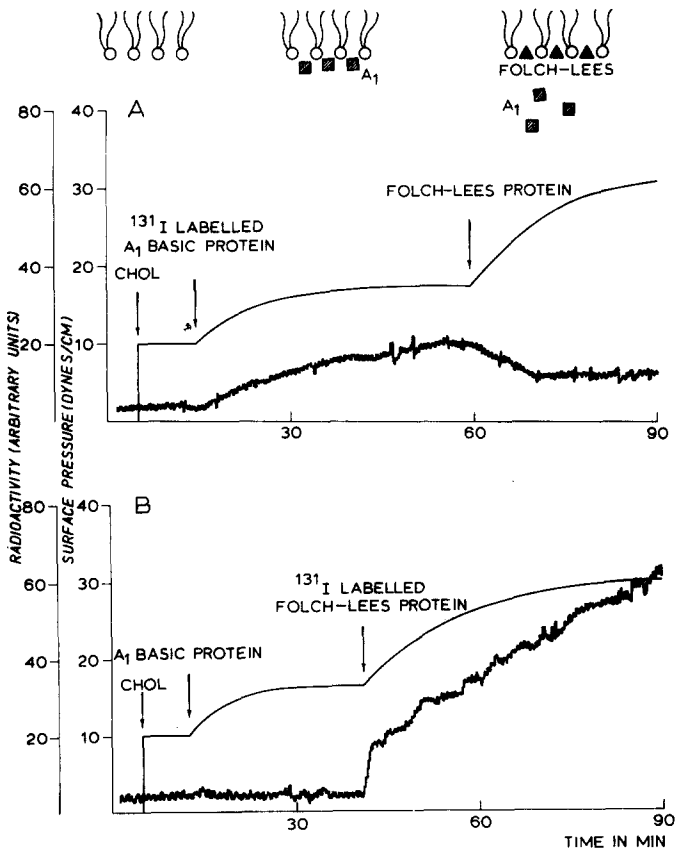


Fig. 12. Automatically recorded surface pressure increase and surface radioactivity increase after the subsequent injection of A_1 basic protein and Folch–Lees proteins underneath a cholesterol monolayer. (A), the A_1 basic protein is ^{131}I labelled; (B), the Folch–Lees protein is ^{131}I labelled. The protein concentrations were 1 μg/ml. The buffer was 10^{-2} M Tris-acetate (pH 3.0).

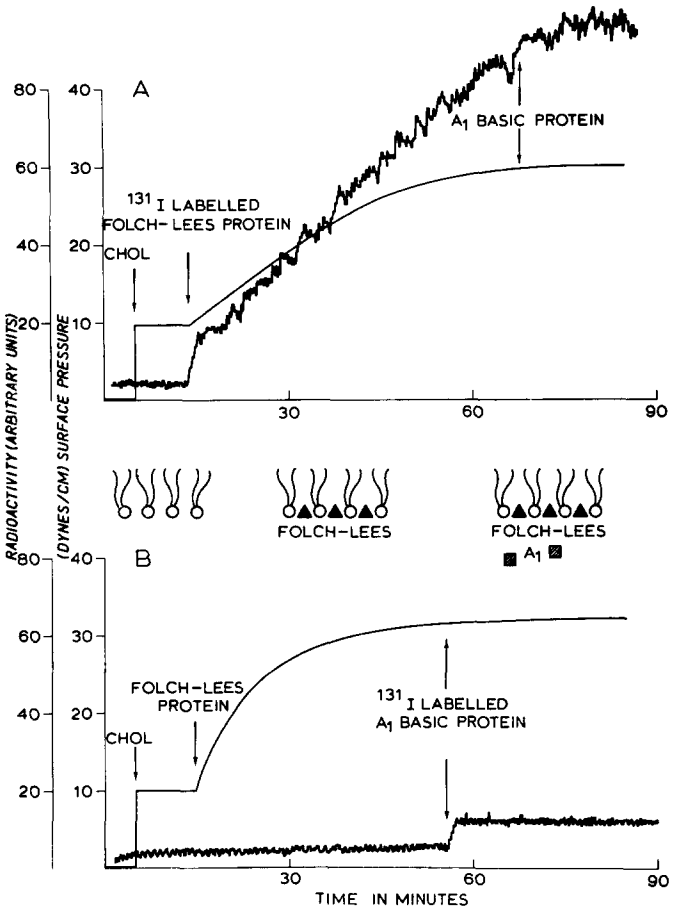


Fig. 13. Automatically recorded surface pressure increase and surface radioactivity increase after the subsequent injection of Folch–Lees protein and A_1 basic protein underneath a cholesterol monolayer. (A), the Folch–Lees protein is ^{131}I labelled; (B), the A_1 basic protein is ^{131}I labelled. The protein concentrations were 1 μg/ml. The buffer was 10^{-2} M Tris–acetate (pH 3.0).

DISCUSSION

The conformation of the Folch–Lees protein at the air–water interface is probably different at various pH values. The pH dependency of the surface activity can also be explained by taking into account the net charge of the protein molecule. At pH 3.0 the protein is positively charged and strong repulsion forces wil reduce the probability of aggregation at the interface. At pH 6.5 however, the repulsion forces are reduced because the protein is close to its isoelectric point, and aggregation of protein molecules is enhanced.

The surface activity of the protein can be the result of reversible conformational changes of the Folch–Lees protein, which take place in solvents of different polarities [8]. In water the protein showed to have 42% α helix content while in chloroform–methanol (1:1, v/v) the α helix content (Table I) is close to 100%. The contact between

the protein molecules and the hydrophobic non-specific gaseous phase at the air–water interface in the absence of lipids will lead to a more hydrophobic conformation which will be accompanied by increase in α-helical content. The behaviour of the protein under a lipid film spread at the air–water interface is different. There was little effect of the pH on the interaction of the Folch–Lees protein with cholesterol, phosphatidylethanolamine and phosphatidylserine and phosphatidylserine and lecithin.

The protein injected under a lanosterol film showed to give only a small pressure increase and surface radioactivity increase. The surface radioactivity was much lower than the value measured at the same pH in the absence of the lipid film. The protein molecules, probably have no affinity for lanosterol and cannot "see" the hydrophobic non-specific gaseous phase. As a result the Folch–Lees protein will not be able to take an hydrophobic conformation and will stay in the subphase.

The hydrophobic conformation of the Folch–Lees protein shows a high content of α-helix. The protein showed also a high helical content after the interaction with lipids (Table I). It was shown already by Zand [27] and Sherman and Folch-Pi [28] that the effective solvents for the Folch–Lees protein possess hydrogen bonding groups. Presumably the helix in organic solvents has a preponderance of hydrocarbon groups exposed [27]. The results of the study on the affinity of the Folch–Lees protein for different lipids at the air–water interface point towards a specificity of the protein for the polar headgroup of the lipid and probably the orientation of this group at the air–water interface (the different sterols) as well as penetration and hydrophobic interaction (different sterols) and charge interaction (cerebroside sulphate). We would like to assume that by hydrogen bonding of the Folch–Lees protein and the phospholipids the protein will pass a conformational change to one with a higher helical content. In this conformation the hydrocarbon groups are exposed to give hydrophobic interaction with the alkyl chains of the fatty acids, which are homologous to the organic solvent. This assumed mechanism explains the broad spectrum of lipid interaction of the Folch–Lees protein, with charged and non charged lipids. Phosphatidylcholine can only function as an acceptor in hydrogen bonding [29]. Consequently the protein has to function as a donor in the protein–lipid complex. This restricts the amino acid residues involved in the hydrogen bonding. Sphingomyelin, phosphatidylethanolamine, and phosphatidylserine are hydrogen donors and acceptors [29] and therefore will show higher interaction with the Folch–Lees protein. The difference in degree of interaction is an expression of the difference in the ester groups of the phospholipids and the different specific groups such as choline, ethanolamine, and serine of the phospholipids, the hydroxy and amide functions of sphingomyelin, the sulphate, hydroxy and amide function of cerebroside sulphate and the hydroxyl group of the cholesterol. The presence and orientation of the hydroxyl group in cholesterol is of importance for the interaction between the lipid and the protein. The presence of the hydrocarbon chain of the cholesterol was shown to be of importance which points the importance of hydrophobic interaction in this specific case. It cannot be excluded that the very small amounts of lipid still bound to the Folch–Lees protein can play also some role in the interaction with lipids at the interphase.

It is interesting now to compare the lipid interactions of the two main proteins of the central nervous system, namely the Folch–Lees protein and the A_1 basic protein.

In previous studies [15–18] we have shown that the A_1 basic protein representing 30% of the myelin protein content, interacts preferentially with negatively charged lipids. However it was demonstrated also that hydrophobic interactions between the A_1 basic protein and lipid exist [17]. The Folch–Lees protein, representing 55% of the myelin content, dit not show a clear preference for charged lipids but interacted also with some neutral lipids.

The A_1 basic protein is a protein with an isoelectric point of 11.0 (having 35 positively charged amino acid residues below pH 6.0). The ionic interaction of this protein is shown by the expelling of Ca^{2+} from the interface after the addition of A_1 basic protein [17]. Although the Folch–Lees protein is a basic protein too with an isoelectric point of 7.4–9.6 [20] it is clear that ionic interactions play a less significant role. The interaction of this protein with cerebroside sulphate was not influenced by 10^{-3} M $CaCl_2$.

In the case of A_1 basic protein several results lead to the conclusion that hydrophobic interactions are involved. It was found that a chain length of more than 8 carbon atoms is required and that at constant pressure the area is increased 200%. The experiments on the protection of the A_1 basic protein against proteolytic degradation show that such regions are protected which are rich in non polar amino acids. With the Folch–Lees protein no significant fatty acid chain length dependence could be demonstrated. However the dependence of the sterol structure on the interaction and the increase in surface area at constant surface pressure show the involvement of hydrophobic interactions.

The different properties of the two myelin proteins are most clearly demonstrated when both proteins are subsequently injected underneath a lipid monolayer. The Folch–Lees protein is able to interact with a cholesterol monolayer even in the presence of A_1 basic protein by expelling this protein from the interface. Both the Folch–Lees protein and the A_1 basic protein show an affinity for cerebroside sulphate. The A_1 basic protein shows even some additional penetration of the monolayer after the complexation of the Folch–Lees protein with cerebroside sulphate.

The A_1 basic protein was shown by us to have a high affinity for negatively charged lipids and more specifically for cerebroside sulphate [15–18]. The Folch–Lees protein showed a much broader lipid affinity pattern and a remarkable affinity for cholesterol. The A_1 basic protein and the Folch–Lees protein which make up 85% of the total myelin proteins [4] were reported to be in the membrane in a molar ratio of 1:1 [3]. Dickinson et al. [22] suggested that the only protein present in the cytoplasmatic side (intra period line) of the myelin membrane is the basic protein. The low-angle X-ray studies of Kirschner and Casper [21] indicated a cholesterol and protein asymmetry of the myelin membrane. The cholesterol is found mainly in the external membrane (the major dense line) [21]. Our present results demonstrate the different lipid specificities of these two myelin proteins and strongly support the view of lipid and protein asymmetry of the myelin membrane.

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