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SPECTRIN-PHOSPHOLIPID INTERACTION

A MONOLAYER STUDY

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Summary

(1) The interaction of synthetic and natural phospholipids with spectrin, purified from human erythrocyte membranes, was studied using the monolayer technique at constant surface pressure. Spectrin penetration into the lipid monolayer was recorded as the rate of surface area increase on a two-compartment trough.

(2) High spectrin penetration rates were observed with negatively charged phospholipids while zwitterionic or neutral lipids showed only poor spectrin affinity. This penetration rate was strongly affected by the subphase pH. At pH 5.5, maximal pentration rates were observed for phosphatidylglycerol and phosphatidylserine but not for phosphatidylcholine.

(3) In comparing the penetration rates for phospholipids with a natural fatty acid composition and the dimyristoyl species of phosphatidic acid, phosphatidylglycerol, phosphatidylserine and phosphatidylcholine, the lipid fatty acid composition proved to be an important parameter. The differences are correlated with the area per lipid molecule.

(4) Other parameters affecting the area per lipid molecule such as surface pressure, pH and salt concentration also strongly influenced spectrin penetration rates for negatively charged phospholipids. Spectrin penetration into phosphatidylcholine monolayers is only slightly affected by variation of these conditions.

(5) The effect of Ca^{2+} on spectrin-lipid interactions was studied for several phosphatidylglycerol and phosphatidylserine species. Both lipids condensed upon the addition of Ca^{2+}, but only in the case of the phosphatidylserine was this accompanied by extrusion of the spectrin from the interface, which is in agreement with earlier calorimetric experiments with bilayer systems of

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Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

analogous composition (Mombers, C., Verkleij, A.J., de Gier, J. and van Deenen, L.L.M. (1979) Biochim. Biophys. Acta 551, 271–281). For this phenomenon a model is presented.

Introduction

In previous studied in which we used differential scanning calorimetric and electron-microscopic techniques, we presented evidence for the formation of a hydrophobic interaction between the spectrin-actin complex isolated from erythrocyte membranes and synthetic lipids in a bilayer model system [1,2]. These studies also showed that spectrin has a marked preference for negatively charged lipids like phosphatidylserine and phosphatidylglycerol when compared with uncharged phosphatidylcholine. This indicated that electrostatic interactions can be involved as well.

For a further characterization of the spectrin-lipid interaction the monolayer technique offers additional opportunities. After injection of the protein underneath a lipid monolayer, the interaction can be measured by the change in surface pressure at constant area or by the change in surface area when the surface pressure is kept constant [3–5]. In an earlier study, Juliano et al. [6] already noticed pressure effects of spectrin on monolayer films of phosphatidylcholine and phosphatidylserine from natural sources. In the present approach using synthetic and naturally occurring phospholipids, we have chosen to measure the increase in surface area at constant pressure. In contrast to the change in pressure, which is largely dependent on the compressibility of the film, the area increase at constant pressure is directly correlated with the amount of protein penetrating the lipid monolayer.

It is the aim of this contribution to show the influence of lipid composition, surface pressure and headgroup spacing on the spontaneous interaction of spectrin with lipid films. Furthermore, attention will be focussed on the effect of the subphase pH and the presence of monovalent and divalent cations on the spectrin-lipid interaction.

Materials and Methods

Spectrin was extracted from hypotonically prepared human erythrocyte ghosts [7] from 1-day-old blood, by a 20 min incubation at 37°C with 7 vol. of 0.1 mM EDTA (pH 8.0). After chilling in ice the extract was centrifuged at $100\,000 \times g$ for 50 min. The supernatant was concentrated by ultrafiltration (Diaflo pM 10) or precipitated with an equal volume of saturated $(NH_4)_2SO_4$ solution (0°C, pH 7.4). Subsequently, spectrin was purified according to the method of Ralston [8] using Sepharose 4B at 4°C. 1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine and 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol were synthesized as described before [2]. Natural phosphatidylcholine and phosphatidylethanolamine were isolated from hen eggs following standard procedures. Phosphatidylserine was isolated from bovine brain according to the method of Sanders [9]. Phosphatidic acid was prepared by enzymatic degradation of egg yolk phosphatidylcholine with phospholipase D. Cholesterol was obtained from

Merck (F.R.G.). Unless otherwise stated, monolayer experiments were performed with an all Teflon two-compartment trough. The two compartments (each 35 ml, 5.5 × 5.9 × 1 cm) were connected by a narrow channel (0.5 × 0.3 cm). After the film was spread in one compartment on a stirred subphase and the film pressure was constant, spectrin was injected underneath the lipid monolayer through an injection hole. The surface pressure was measured by the Wilhelmy plate technique and was kept constant throughout the experiment by the movement of a Teflon barrier on the second compartment using a Tritrator (Radiometer, Copenhagen) connected to an electrobalance (Beckman LM 500). The set-up was essentially the same as the 'zero-order' trough described by Verger and de Haas [10]. The film pressure and surface area increases were recorded continuously. Expansion rates measured this way were constant for at least 15 min, unless at very high penetration rates or low protein concentrations, and gave a good reproducibility at the protein concentrations used (1.5—2.5 μg/ml). Only for dimyristoyl phosphatidylserine were penetration rates less constant. All monolayer experiments were performed at 31 ± 1.5°C. The collapse pressure of spectrin was determined by spreading of an aqueous solution at the air/buffer interface until addition of excess protein yielded no further pressure increase. Compression isotherms of the phospholipids were recorded on a 1.5 l thermostatically controlled all Teflon trough (17.3 × 31.3 cm) in Tris/NaH_2PO_4 (20 mM)/NaCl (140 mM) buffer (pH 6.0) at 30°C after spreadings of 50 nmol phospholipid.

Phospholipids (1 mM) were spread at the air/water interface from a $CHCl_3$/CH_3OH solution (9 : 1, v/v) with a glass capillary. The surface pressure was measured with a platinum Wilhelmy plate, 1.96 cm wide. Water was passed over an activated charcoal column and doubly distilled from a quartz still.

Results and Discussion

Phospholipid preference

The collapse pressure of purified spectrin at pH 7.4 determined by spreading the protein at the interface was found to be 20.5 $mN \cdot m^{-1}$ consistently for several spectrin preparations. At pH 6.0, the collapse pressure even reached 24 $mN \cdot m^{-1}$. This is of the same order of magnitude as that found for serum lipoproteins [11] and greater than that for other water-soluble proteins such as albumin and cytochrome *c* [4,5]. Thus, to exclude spontaneous protein penetration into the interface, only surface pressures above the collapse pressures were applied. At low film pressures, i.e., below the collapse pressure of the protein, very high penetration rates were found due to the protein surface activity.

The observation from previous studies, using liposomal and monolayer systems, that the spectrin-lipid interaction is favored by a negative charge of the lipid polar headgroup [1,6,12] is affirmed by our monolayer experiments. Table I shows the spectrin penetration rates, expressed as surface area increase per min, for different lipids. Anionic phospholipids such as phosphatidic acid and cardiolipin (not shown) yield very high penetration rates, while rates are small for uncharged lipids like phosphatidylcholine, phosphatidylethanolamine and cholesterol. For all charged phospholipids

TABLE I

THE RATE OF SPECTRIN PENETRATION INTO MONOLAYERS OF SYNTHETIC PHOSPHOLIPIDS OF THE DIMYRISTOYL SPECIES AND CHOLESTEROL AT SEVERAL pH VALUES

Penetration rates (expressed as film expansion rate in cm^2/min) were measured at 30°C with a protein concentration of 2.5 μg/ml and Tris/NaH_2PO_4 (20 mM), NaCl (140 mM), EDTA (0.4 mM) as the subphase. di C14:0, dimyristoyl; PA, phosphatidic acid; PG, phosphatidylglycerol; PS, phosphatidylserine; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

Lipid	Spectrin penetration rate		
	pH 6.0 (π_i = 25 mN · m^{-1})	pH 7.4 (π_i = 23 mN · m^{-1})	pH 8.6 (π_i = 23 mN · m^{-1})
di C14:0 PA	0.444	0.150	0.146
di C14:0 PG	0.385	0.224	0.133
di C14:0 PS	0.250	0.054	—
di C14:0 PC	0.013	0.025	—
di C14:0 PE	0.026	0.020	—
Cholesterol	0.022	0.028	—

the penetration rate is highly dependent on the pH. At pH 6.0, rates are high in comparison with those at pH 7.4 (Table I). Increasing the pH up to 8.6 causes a further decrease in penetration rate for the phosphatidylglycerol film but has only a little effect in the case of phosphatidic acid which may be explained by a second ionization of the phosphate (pK_2 = 8.3 [13]) of the latter phospholipid at this high pH. As will be discussed below, this is a first indication of the importance of lipid charge density and consequently the area per lipid molecule.

pH effect

Fig. 1 shows in more detail the effect of pH on spectrin penetration into dimyristoyl phospholipid monolayers. A striking effect is the difference in the penetration rate profile of negatively charged and zwitterionic phospholipid films. It can be seen that the anionic phospholipids have a sharp maximum at pH 5.5, while such an optimum is not observed for phosphatidylcholine. The maximum coincides with the isoelectric point of the spectrin which is in the region of 5.2 [14] to 5.6 [15]. This means that at a higher pH, charge repulsion between the negatively charged lipids and the net negatively charged protein inhibits protein-lipid association, which is also observed. However, at pH values below the isoelectric point, electrostatic interaction of the positively charged protein with the still negatively charged lipid is expected to favor spectrin penetration; but this is not observed. This may indicate that at pH values below the isoelectric point, the interaction of the lipids with positively charged spectrin is purely electrostatic without penetration of hydrophobic residues into the interface. This type of interaction could resemble the effect of polylysine and ribonuclease, two basic water-soluble proteins, on negatively charged bilayers, where there is electrostatic binding but no penetration into the interface [16].

In an earlier study by Juliano et al. [6], a large surface pressure increase was observed for bovine brain phosphatidylserine films at pH 3.5. As shown

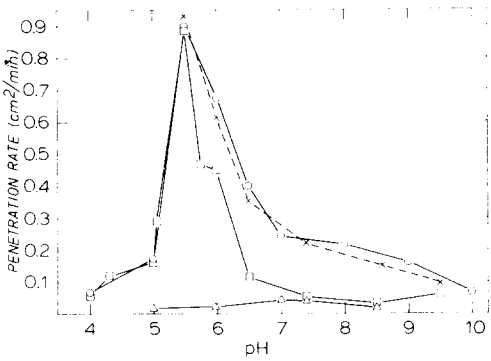

Fig. 1. Effect of pH on spectrin penetration rate (2.5 μg/ml spectrin). ○———○, dimyristoyl phosphatidylglycerol; △———△, dimyristoyl phosphatidylcholine; □———□, dimyristoyl phosphatidylserine; X———X, dioleoyl phosphatidylserine. π_i = 23 mN · m^{-1}. Conditions: Tris/NaH_2PO_4 (20 mM), NaCl (140 mM), 30°C.

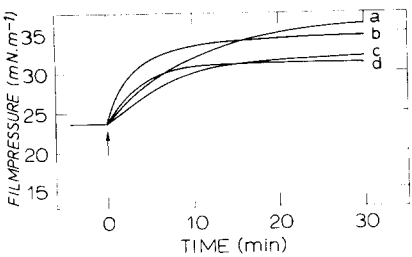

Fig. 2. Spectrin penetration at constant surface area for bovine brain phosphatidylserine at pH 3.5 (a) and pH 5.5 (b) and dimyristoyl phosphatidylserine at pH 3.5 (c) and pH 5.5 (d). Trough, 35 ml (5.5 X 5.9 cm). Conditions: citric acid (3 mM), NaCl (150 mM), EDTA (0.5 mM), 30°C. The arrow indicates when spectrin (2.5 μg/ml) was injected into the subphase.

in Fig. 2, we obtained similar results in experiments at constant surface area for this phosphatidylserine at pH 3.5 and 5.5. For synthetic dimyristoyl phosphatidylserine the final surface pressures were somewhat lower (Fig. 2). At first sight, the large surface pressure increase at pH 3.5 seems contradictory to the low penetration rate found for phosphatidylserine at low pH (compare Fig. 1). The surface pressure increase, however, cannot be identified with the amount of protein penetrating into the interface. At pH 3.5, as a result of the protonation of the carboxylic group of the phosphatidylserine (pK_c = 4.4 [13]), the packing density of the lipid is increased in comparison with that at pH 5.5 and penetration of a relatively small number of protein molecules into the lipid film will result in a large increase in the surface pressure.

This shows another aspect of the spectrin-lipid interaction at low pH; not only the total charge of the protein, but also the charge and the packing density of the lipid are important. As the final values of the surface pressure of bovine brain phosphatidylserine and dimyristoyl phosphatidylserine are different (Fig. 2), the composition of the polar headgroup cannot be the only parameter, thus the lipid packing density must also be involved.

Effect of lipid packing and surface pressure

The packing of the lipid molecules often plays an important role in protein-lipid interactions [3,5] and the monolayer technique offers the opportunity to study this parameter in detail. Fig. 3 shows compression isotherms for the dimyristoyl species of phosphatidylcholine, phosphatidylglycerol and phosphatidylserine. Whereas phosphatidylserine has a lower area per molecule at all pressures, the molecular areas of phosphatidylcholine and phosphatidylglycerol are quite similar at all pressures. Thus, the difference in the penetration rate between phosphatidylcholine and phosphatidylglycerol as observed in the experiment of Fig. 1 is not a consequence of the difference in lipid

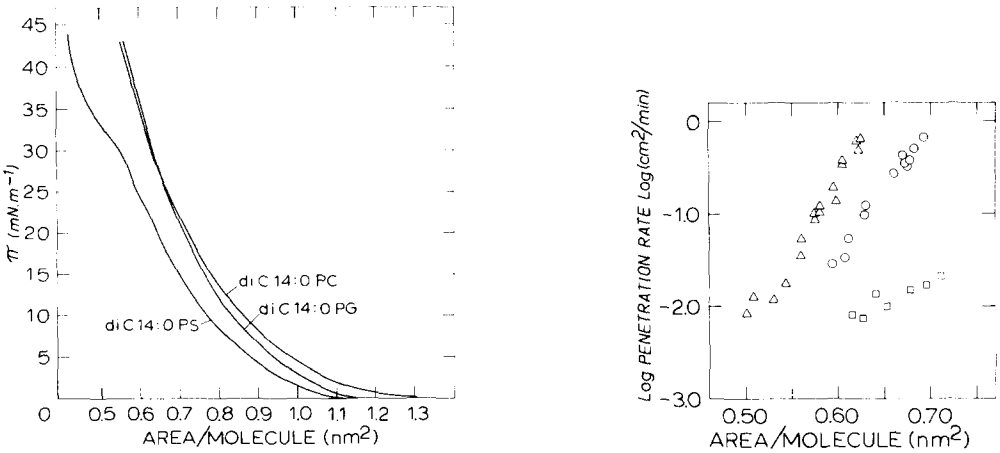

Fig. 3. Compression isotherms of dimyristoyl phosphatidylcholine (diC 14 : 0 PC), dimyristoyl phosphatidylglycerol (diC 14 : 0 PG) and dimyristoyl phosphatidylserine (diC 14 : 0 PS) monolayers at 30°C on Tris/NaH_2PO_4 (20 mM), NaCl (140 mM), pH 6.0.

Fig. 4. Semi-logarithmic plot of the spectrin penetration rate at different areas per molecule for dimyristoyl phosphatidylserine (△), dimyristoyl phosphatidylglycerol (○) and dimyristoyl phosphatidylcholine (□) at pH 6.0; Tris/NaH_2PO_4 (20 mM), NaCl (140 mM), 30°C. Spectrin penetration rate was measured at varying surface pressures. This penetration rate was plotted logarithmically as a function of the corresponding area per molecule which was obtained from the π-A curves of Fig. 3.

packing but is due to the difference in the composition of the polar headgroups. In contrast it can be seen that at equal pressures phosphatidylserine is more closely packed. Therefore, the difference in penetration rate for phosphatidylglycerol and phosphatidylserine films may be due to a difference in the area per lipid molecule. This is confirmed by the increased penetration rates for dioleoyl phosphatidylserine when compared with dimyristoyl phosphatidylserine (Fig. 1). The unsaturated phosphatidylserine has a more expanded type of film due to a higher molecular area of the fatty acyl chains. In fact, not only the penetration rates for dimyristoyl phosphatidylglycerol and dioleoyl phosphatidylserine are similar but also their compression isotherms are almost the same (not shown).

In monolayer experiments, there are three different ways to adjust the lipid packing density: (1) by changing the surface pressure, (2) by varying the fatty acid chains and (3) by varying the charge of the lipid polar headgroup.

Using the data of Fig. 3, penetration rates measured at different surface pressures can be plotted as a function of the area per lipid molecule. This shows (Fig. 4) that an increase in molecular area is accompanied by an increase in penetration rate, but that this effect is much more pronounced for the negatively charged lipids than for dimyristoyl phosphatidylcholine. In this figure, the penetration rate is much larger for phosphatidylserine than for the corresponding phosphatidylglycerol species. However, it should be pointed out that at constant area per molecule, the surface pressure is much lower in phosphatidylserine than in phosphatidylglycerol films (Fig. 3).

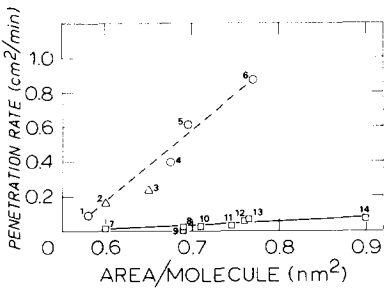

Fig. 5. Dependence of the spectrin penetration rate on the area per lipid molecule at constant surface pressure. The area per lipid molecule was varied by substitution of the fatty acyl chains. For the phosphatidylglycerol and phosphatidylserine species, the surface pressure was kept at 24.8 mN · m^{-1}, for the phosphatidylcholine species at 21.9 mN · m^{-1}. ○, phosphatidylglycerol; (1) dipalmitoyl, (4), dimyristoyl, (5) dilauroyl, (6) dipalmitoleoyl, △, phosphatidylserine; (2) dimyristoyl, (3) dielaidoyl, □, phosphatidylcholine; (7) dipalmitoyl, (8) dimyristoyl, (9) dielaidoyl, (10) dilauroyl, (11) didocosenoyl (22 : 1_c), (12) dioleoyl, (13) dipalmitoleoyl, (14) dilinoleoyl. Conditions: Tris/NaH_2PO_4 (20 mM), NaCl (140 mM), pH 6.0; spectrin concentration 2.5 μg/ml.

By changing the fatty acid composition the area per molecule is variable at constant pressure. Fig. 5 shows the penetration rates for films of several molecular species of phosphatidylglycerol, phosphatidylcholine and phosphatidylserine. Also, in this case, the molecular areas were obtained from π/A curves of all these phospholipids recorded under identical conditions. Again it can be concluded that at least for the negative phospholipids the penetration rate increases with increasing area per molecule. However, the magnitude of this effect is considerably smaller than in the experiment of Fig. 4 (note the difference in scale) from which can be concluded that next to the area per molecule, surface pressure is an important parameter. In contrast to Fig. 4, the data obtained for the phosphatidylserines are now very much in line with those obtained for phosphatidylglycerols.

A third way to control lipid packing density is by changing the charge of the lipid polar headgroups. This can be achieved by adjusting the pH or the salt concentration of the subphase, as demonstrated by Tocanne et al. [17] and Sacré and Tocanne [18] for several phosphatidylglycerol species. Low pH and low salt concentration both enhance the protonation of the phosphate of the polar headgroup which leads to a decrease in charge repulsion and a condensation of the lipid film. These conditions may not only affect the lipid but also the charge and the conformation of the protein [8]. Therefore, a correct interpretation of the effects of low NaCl concentrations on spectrin penetration in relationship to lipid packing is rather difficult. On the other hand, measurement at NaCl concentrations between 100 and 350 mM at pH 7.4, where the protein will have its native conformation, showed once more (like the pH effect) that charge shielding by salt could stimulate spectrin-lipid interaction in phosphatidylglycerol monolayers. At pH 6.0, where the protein has only little charge left, the addition of NaCl did not give a further increase in the spectrin penetration rate.

The effect of divalent cations

In previous studies, we observed that phosphatidylglycerol vesicles in interaction with spectrin are protected against the action of Ca^{2+} [1]. When Ca^{2+}

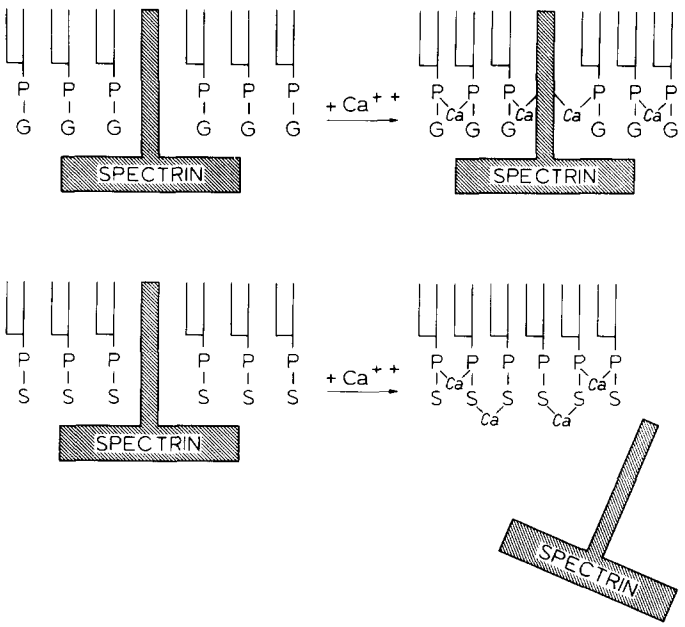

Fig. 6. Schematic model of the effect of Ca^{2+} addition on phosphatidylglycerol (P-G) and phosphatidylserine (P-S) films interacting with spectrin.

is added to spectrin-free vesicles, a spontaneous fusion process destroys the vesicular structure and stacked lamellae or large cylindrical (cochleated) structures are formed. Spectrin is able to protect vesicles against such extensive fusion processes in the case of phosphatidylglycerol but not when in interaction with phosphatidylserine vesicles [1,2]. To explain the difference the following model can be proposed (Fig. 6). When Ca^{2+} is added to a spectrin-phosphatidylglycerol recombination, cross-bridges are formed between phosphatidylglycerol molecules and between spectrin and the lipid. On the other hand, addition of Ca^{2+} to a spectrin-phosphatidylserine recombination results in the formation of a condensed phosphatidylserine-Ca^{2+} phase from which the spectrin is released.

In order to gain further support for this hypothesis the following monolayer experiment was designed. Spectrin was injected into a subphase of a phosphatidylglycerol or phosphatidylserine monolayer at 22 mN · m^{-1} and allowed to penetrate for 1 h. After this period, the film was compressed to 35 mN · m^{-1} to approach a surface pressure probably existing in bilayer systems [19]. Subsequently, Ca^{2+} was added to the subphase (final concentration 6 mM) and the film was compressed immediately to keep the surface pressure at 35 mN · m^{-1}. The decrease in the surface area was measured and compared with the area decrease for a pure lipid film under the same conditions. The results in Table II show that the area decrease for phosphatidylglycerol films is practically independent of the presence or absence of spectrin. On the other hand, for phosphatidylserine films, the area reduction is much larger when spectrin is incorporated in the lipid monolayer. This is in agreement with the idea that in phosphatidylserine films spectrin is released when Ca^{2+} is interacting.

In dimyristoyl phosphatidylcholine films spectrin penetration was not

TABLE II

THE EFFECT OF SPECTRIN PENETRATION ON PHOSPHATIDYLGLYCEROL AND PHOSPHATIDYLSERINE MONOLAYER CONDENSATION AFTER Ca^{2+} ADDITION

Spectrin was injected into the subphase to give a concentration of 2 μg/ml. During spectrin penetration, surface pressure was maintained at 22 mN · m^{-1}. After this incubation the surface pressure was increased by compression to 35 mN · m^{-1}. $CaCl_2$ was added as a 2 M solution to give a final concentration of 6 mM. The film was compressed immediately until a constant film pressure of 35 mN · m^{-1} was restored. The surface area decrease was read from the change of the barrier position. All experiments were performed at pH 7.4 (Hepes (3 mM), NaCl (150 mM), EDTA (0.1 mM)) with a rectangular all Teflon one-compartment trough (5.0 × 15.1 cm, 65 ml) at 30°C. The initial surface area was 50 cm^2. PG, phosphatidylglycerol; PS, phosphatidylserine; di C14:0, dimyristoyl; di C16:1_c, dipalmitoleoyl; di C18:1_c, dioleoyl.

Lipid	Spectrin penetration time (min)	Surface area decrease (%)
di C14:0 PG	0	100
	62	106
di C16:1_c PG	0	100
	57	94
di C14:0 PS	0	100
	68	170
di C18:1_c PS	0	100
	54	154

affected by the presence of Ca^{2+} (0—10 mM). This means that even if Ca^{2+} causes a conformation change of the protein, as suggested by Brauer et al. [20], spectrin-lipid interactions are still possible when Ca^{2+} is bound to the protein.

Effects of protein conformation

Apart from packing and charge effects on phospholipids, protein conformation is an important factor in protein-lipid interactions as demonstrated by spectrin denaturation. Denaturation for 5 min at 100°C reduced the penetration rate for a phosphatidylglycerol monolayer (pH 7.4, 150 M NaCl) to 29%; denaturation for 10 min at 55°C gave a reduction to 57% of the value for non-denaturated spectrin. Reduction or oxidation of disulfide bridges by thiol reagents such as 2-mercaptoethanol, glutathione or tetrathionate (5 mM), as performed by Haest et al. [21] in spectrin modification studies, during the measurements did not affect spectrin penetration. Also, no major difference was found between the action of a crude ghost extract or the purified dimers or tetramers, indicating that the presence of 2-mercaptoethanol during purification was not harmful in this respect. The suggestion made by Sheetz et al. [22] that spectrin could undergo a conformation change during hypotonic extraction, inhibiting lipid interaction, could not be confirmed by spectrin extraction an purification from isoionically prepared ghosts [23] and subsequent cholate extraction [24] and purification [8].

General Discussion

When comparing, for example, spectrin penetration rates for dimyristoyl phosphatidylglycerol and dimyristoyl phosphatidylcholine at pH 7, 100 mM

NaCl, at 25 $mN \cdot m^{-1}$, it is remarkable that the penetration rate is higher for phosphatidylglycerol than for phosphatidylcholine, as the former will show a charge repulsion of the net negatively charged protein while the latter lipid film bears no net charge. Under these conditions, the area per lipid molecule will be about the same for both phospholipids so that there is no difference between the lipids in this respect. Taking also into consideration the pH effect and the effect of NaCl concentration, this indicates that electrostatic interaction of positive groups locally present on the large protein molecule with the negatively charged phospholipid is the primary event in spectrin-lipid interactions. Charge neutralization, and a local packing defect in the lipid bilayer, may consequently yield the opportunity for hydrophobic spectrin-lipid interactions.

An increase in the surface pressure resulted in a rapid decrease in the spectrin penetration rate, as can also be deduced from Figs. 3 and 4. Especially for the negatively charged phospholipids, the penetration rate strongly depended on the area per molecule, which suggests that the charge density of the lipid monolayer is important for protein penetration. In accordance with this, also at constant surface pressure the penetration rate was higher and depended much more on the molecular area for charged phospholipids than for phosphatidylcholines. A comparison of Figs. 4 and 5, however, shows that the effect of surface pressure is not just confined to changes in the area per lipid molecule as there is a notable difference in the magnitude of the effect. In our experiments, significant spectrin penetration could be detected for both phosphatidylserine and phosphatidylglycerol films up to a pressure of 36 $mN \cdot m^{-1}$ at pH 6.0 and a somewhat lower value at pH 7.4 (33 $mN \cdot m^{-1}$). This means that significant penetration into a planar lipid bilayer with an estimated surface pressure of about 35 $mN \cdot m^{-1}$ [19] will occur only under specific conditions allowing decreased lipid packing density or local packing defects. This corresponds with the observation that highly curved bilayers like sonicated vesicles interact readily with spectrin [1,2,25] while reports on spectrin binding to unsaturated bilayers are less conclusive in this respect [26,27]. In recent studies using unilamellar vesicles of various lipid composition, only small amounts of spectrin were found to be bound to the bilayer (unpublished results).

In this light it is interesting to speculate about the significance of these spectrin-lipid interaction for the in vivo spectrin-membrane binding. Both spectrin and the negatively charged phosphatidylserine are located exclusively at the cytoplasmic side of the membrane and modification of spectrin by SH-oxidizing agents as described by Haest et al. [21] resulted in the availability of phosphatidylserine to phospholipase degradation at the outside of the membrane. If only protein-protein interactions, as shown by Bennett and Branton [28], were present in the erythrocyte membrane, this would not explain the effect of spectrin on membrane permeability [6,12,29] and lipid distribution [6,12,29] and lipid distribution [21]. For this reason, a further examination of the effect of spectrin on phospholipid distribution in natural and model systems will give a better insight in the biological role of spectrin-lipid interactions.

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