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Phospholipid–protein interactions: Membrane permeability correlated with monolayer "penetration"

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SUMMARY

The effectiveness of various soluble proteins in increasing the permeability of phospholipid membranes was found to be directly correlated with their ability to "penetrate" monomolecular films of the same phospholipids. We propose that the increase in permeability depends on hydrophobic associations of the protein with the phospholipid which are facilitated by initial electrostatic binding.

It was originally shown by Schulman and colleagues[1,2] that the increase in surface pressure (Π) of phospholipid monolayers at an air/water interface following the injection of proteins below the surface depended on electrostatic interactions. We have recently observed that soluble basic proteins such as cytochrome *c* and lysozyme can cause increases of 10^2-10^3-fold in the $^{22}Na^+$ permeability of sonicated phosphatidylserine vesicles, but not phosphatidylcholine vesicles, changing the permeability coefficient from $1 \cdot 10^{-12}$ cm/sec to a maximum of about $1 \cdot 10^{-9}$ cm/sec[3]. This communication describes experiments indicating that the effects of proteins on the permeability of phospholipid vesicles are correlated with the ability of these proteins to "penetrate" phospholipid monolayers.

Pure phosphatidylserine from beef brain was prepared as described previously[4]. Sonicated, unilamellar, phospholipid vesicles containing $^{22}Na^+$ were prepared under nitrogen[5], and diffusion of the $^{22}Na^+$ out of the vesicles was measured by dialysis[6,7]. Surface pressure measurements on phospholipid monolayers were made at constant area with a platinum wire dipping into the surface[8]. Protein concentrations were measured by biuret,

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and phospholipid by phosphorus assays. Egg white lysozyme and poly-L-lysine (mol.wt. 17 000) were obtained from Mann Research Laboratories; cytochrome *c* (type VI) from Sigma; beef pancras ribonuclease from Worthington (type RAF); and crystallized human serum albumin (Pentex) from Miles Laboratories.

TABLE I

EFFECTS OF PROTEINS ON $^{22}Na^+$ PERMEABILITY OF PHOSPHATIDYLSERINE VESICLES

$^{22}Na^+$ permeability measured in 10 mM NaCl, 2 mM histidine, 2 mM *N*-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid, 0.1 mM EDTA, pH 7.4 at 24°. Protein was added inside the dialysis bags, each containing 1 μmole of phospholipid in 1 ml final volume. The equivalents of $^{22}Na^+$ diffusing per h per μmole phospholipid were calculated on the basis of % total $^{22}Na^+$ per h and the μmoles Na^+ captured per μmole phospholipid (=0.13). The permeability coefficient in cm/sec was then calculated on the basis of an area of 1800 cm^2/μmole lipid[7] and a $^{22}Na^+$ concentration difference of 10^{-5} moles/cm^3.

Protein	*Protein/phospholipid (molar ratio)*	*$^{22}Na^+$ diffusion rate (% total per h)*	*$^{22}Na^+$ permeability coefficient (10^{-12} x cm/sec)*
None		0.05	1
Lysozyme	0.26	40	800
Cytochrome *c*	1.80	28	560
Poly-L-lysine	0.03	8	160
Ribonuclease	0.57	0.15	3
Ribonuclease (unfolded)	0.40	1.0	20
Albumin (pH 4.5)	0.005	75	1500
Albumin (pH 7.4)	0.005	2.5	50
Albumin (pH 7.4)	0.80	15	300

Soluble basic proteins such as lysozyme, cytochrome *c* and ribonuclease were found to neutralize or reverse the zeta potential of phosphatidylserine vesicles at neutral pH in a similar manner, but they had very different effects on the $^{22}Na^+$ permeability of these vesicles[3]. In Table I, we summarize the effects of various proteins on the $^{22}Na^+$ permeability of phosphatidylserine vesicles. In contrast to lysozyme, cytochrome *c*, and poly-L-lysine, ribonuclease has a minimal effect. Irreversibly unfolding ribonuclease by reducing its –S–S– bonds and reacting them with iodoacetamide[9], however, causes a 10-fold increase in its effect on permeability. The effect of albumin is much more marked at acid pH. We also see, however, an effect at neutral pH when both vesicles and protein are negatively charged. This effect is seen more clearly at higher concentrations of albumin.

Fig. 1 shows the effect of some of these proteins on the surface pressure of phosphatidylserine monolayers. Each point is for the final increase in Π (ΔΠ), after the protein has been injected into the subphase below a phosphatidylserine monolayer. The values plotted are for separate monolayers of different initial pressures, obtained by the addition of different amounts of phosphatidylserine to the surface of the trough. As can be seen, the proteins which cause large permeability changes (lysozyme, cytochrome *c* and albumin at acid pH) fall into one group which shows appreciable positive ΔΠ up to very high initial pressures of approx. 40 dynes/cm. Ribonuclease, and albumin at neutral

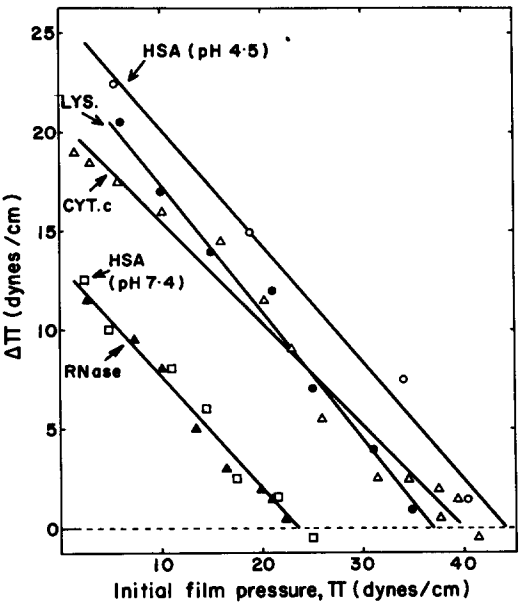

Fig. 1. Effects of proteins on surface pressure of phosphatidylserine monolayers. 10 mM NaCl, 0.1 mM EDTA used throughout, and 2 mM histidine, 2 mM *N*-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid at pH 7.4 and 4 mM sodium acetate at pH 4.5. Temp. 24°. Volume of trough was 100 ml and 250 μg of protein was added in each case. ▲, ribonuclease (RNase); △, cytochrome *c* (CYT.c); ●, lysozyme (LYS.); □, human serum albumin (pH7.4) (HSA); ○, human serum albumin (pH4.5).

pH, however, only "penetrate" up to an initial monolayer pressure of approx. 23 dynes/cm. At higher concentrations, albumin at neutral pH shows increased "penetration". The unfolded ribonuclease also shows increased "penetration". At an initial pressure of 23 dynes/cm when native ribonuclease shows no $\Delta\,\Pi$ effect, the unfolded ribonuclease gives a $\Delta\,\Pi$ of 4 dynes/cm.

One of the obvious differences between pure phospholipid membranes and biological membranes is that the former show very low permeabilities toward monovalent cations, of the order of $10^{-12}-10^{-13}$ cm/sec [5,10]. This is 10^2-10^3 times less than the passive diffusion of Na^+ in erythrocytes[11]. As we have shown, the addition of soluble basic proteins raises these diffusion rates to the levels found for biological membranes. Since this is done with no decrease in the slope of the Arrhenius plot, which gives a value of 27–30 kcal/mole, it would appear that this effect cannot be attributed to lysis of the vesicles[3]. As suggested recently[10] for a similar effect involving valinomycin, a large increase in entropy would account for the results, implying an increase in the disorder of the whole system. This could involve not only the packing of acyl chains and the protein conformation, but also breaking of the water structure around the membrane. We have recently reported data suggesting[3] that although the initial interaction of basic proteins with phosphatidylserine vesicles is electrostatic, further interactions including hydrophobic associations occur which lead to the observed permeability changes. A similar suggestion has also been put forward to explain the action of

albumin[12] and the lytic properties of the polypeptide melittin[13]. The biological relevance of such interactions is illustrated by the recent finding of Zwaal and Van Deenen[14] that a similar sequence of interactions is involved in the recombination of proteins and lipids from erythrocy membranes.

The results reported here substantiate the above hypothesis by showing a direct correlation between effects on phospholipid membrane permeability and the ability to increase the surface pressure of phospholipid monolayers. Only those proteins showing permeability effects penetrate the monolayer at surface pressures greater than 23 dynes/cm, well above the collapse pressures of the proteins themselves (approx. 15 dynes/cm). The value of 23 dynes/cm may be significant since it has been calculated that each phospholipid molecule in an aqueous dispersion occupies an area of 68 Å[15] corresponding to a pressure of 20–25 dynes/cm for a monolayer at an air/water interface[8].

An increase in Π above the collapse pressure of the protein has usually been considere to involve penetration of some part of the protein into the hydrocarbon portion of the monolayer, although at low pressures simple adsorption of the protein itself may occur at the interface[1,2,16,17]. In Fig. 2 we illustrate two possible explanations for the increase in Π, both involving initial electrostatic interactions followed by conformational changes in the protein. Fig. 2A shows a soluble, globular protein with a hydrophobic interior and a polar exterior of net positive charge in the aqueous subphase below a negatively charged phospholipid monolayer. Fig. 2B shows, as a result of electrostatic attraction, changes in the conformation of the protein whereby a hydrophobic portion of the protein penetrates into the hydrocarbon region of the phospholipid monolayer. Similar "binding-mediated penetration" has also been described by Colacicco[16] for the interaction of proteins with cholesterol monolayers. By contrast, in Fig. 2C the conformational change exposes a hydrophobic surface of the protein at the interface. The fatty acyl chains being in a liquid, mobile state[15] are able to interact with this region producing an area of low dielectric closer to the interface. We term the former case "penetration" and the latter "deformation" of the lipid monolayer. Of course any actual interaction may involve elements of both "penetration" and "deformation". Both involve perturbations of the hydrocarbon

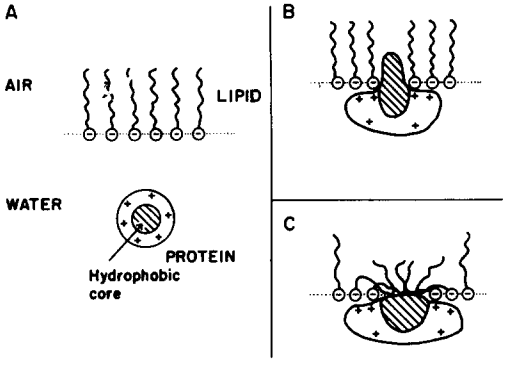

Fig. 2. Postulated interactions of proteins and a phosphatidylserine monolayer at an air–water interface.

region which we postulate are responsible for the increases in the cation permeability of phospholipid bilayer membranes.

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