INTERACTION OF THE ERYTHROCYTE - MEMBRANE PROTEIN, SPECTRIN, WITH MODEL MEMBRANE SYSTEMS

by

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Summary: Spectrin causes an increased rate of solute diffusion from liposomes. The protein binds both to negatively charged and positively charged liposomes. Binding to negative liposomes appears to be insensitive to ionic strength of the medium. It appears that hydrophobic bonding of this membrane protein to lipid is the likely mode of interaction in the erythrocyte membrane.

Spectrin is a protein which can be purified from erythrocyte membranes (1-3). It has been reported to be present in large amounts in carefully prepared erythrocyte ghosts, representing up to 20% of the membrane protein. Spectrin has no known enzymatic activity but may play a structural role in the membrane.

Suspensions of phospholipids in water form vessicles (liposomes) which can serve as model biological membranes (4,5). To investigate the nature of lipid-protein interactions in biological membranes, we have conducted experiments on binding of proteins to liposomes and the resultant functional alterations, if any. We report here experiments on the nature of the association of spectrin with liposomes.

Experiments and Results. We prepared spectrin from human erythrocytes as described by Marchesi et al (3). Yields of up to 10Mg/100cc whole blood were obtained by this procedure. Examination of the protein by disc electrophoris gave results similar to those reported by Marchesi, although upon occasion, we observed two or three bands with one major species predominant.

Previously (6), we have monitored protein interactions with liposomes by measurement of the rate of diffusion of trapped solutes from liposomes in the presence and absence of protein. Figure 1 shows the effect of addition of spec-

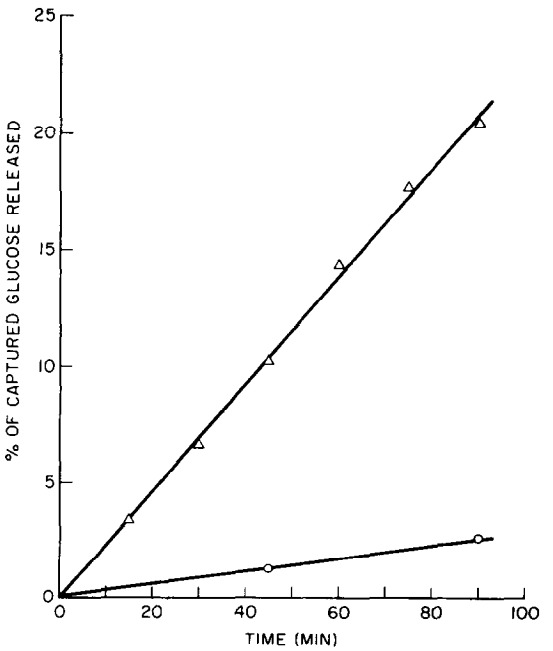

Figure 1. Liposomes (12 Mg lecithin, 2.3 Mg dicetyl phosphate, 0.9 Mg cholesterol per ml suspension) were prepared in 10^{-3} M phosphate-tartrate buffer containing 0.07 M NaCl and 0.145 M ^{14}C-glucose. Untrapped solute was removed by passage of the suspension through a Sephadex G-75 column. Diffusion was measured as described earlier (6). 1.3 Mg of spectrin was added per ml of suspension; pH 7.7; temp. 23°C; liposomes plus spectrin; 0-0 liposomes without spectrin.

trin on the rate of glucose diffusion from liposomes. Under the conditions of the experiment, this protein significantly alters the rate of glucose diffusion when compared to the basal rate.

Direct binding of protein to lipid was measured by centrifugation of the liposome-spectrin mixture at 100,000 g for 30 minutes. Protein in the supernatant was then determined and the amount bound to liposomes calculated by difference. Figure 2 shows the dependence of binding on protein concentration for both positively charged and negatively charged liposome systems. Liposomes with a positive surface charge should bind electrostatically with spectrin at pH 7.5 since the isoelectric point of the protein is

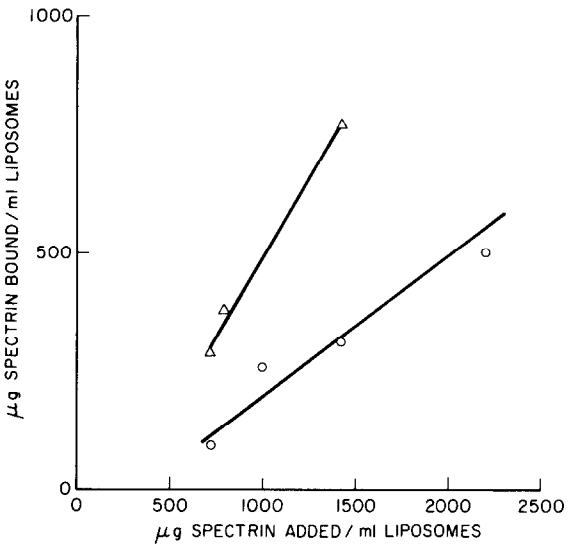

Figure 2. Negatively charged liposomes were prepared as in Figure 1. Positively charge liposomes were prepared by substitutions of 1.15 Mg stearyl amine for dicetyl phosphate. Lipid-protein mixtures were incubated at 22°C for 2 hours and then centrifuged at 100,000 g for 10 minutes. Protein concentration in the supernatant was determined by the Folin method. Bound-protein concentration shown represent the difference between a control containing no liposomes but centrifuged as above, and the sample; △- △positively charged liposomes; 0-0 negatively charged liposomes.

approximately 5.3 (3). This is verified in Figure 2. However, if electrostatic forces were the total basis for the lipid-protein interaction, negatively charge liposomes should not bind spectrin. Figure 2 shows that this criterion is not met, and since spectrin is bound to negative membranes, suggests that other phenomena such as hydrogen bonding or hydrophobic bonding must be present in the negative liposome system.

Data which are consistent with this interpretation are shown in Table I. It can be seen that although an increase in ionic strength completely displaces spectrin bound to positively charged membranes, it has little effect on the lipid-protein interaction present with the negative membrane.

With these observations in hand it became of interest to determine the relative effects of spectrin on solute diffusion in positive and negative membranes. Table II indicates

Table I. Dependence of Spectrin Binding on NaCl Concentration.

FINAL NaCl conc.	Mg Spectrin Bound to Negative Liposomes	Mg Spectrin Bound to Positive Liposomes
0.07 M	90	300
0.58 M	80	0
1.10 M	80	0

The amount of spectrin bound was determined as in Figure 2 except that after incubation at 22 C for 2 hours enough solid NaCl was added to give the desired molarity.

Table II. Diffusion Activation of Liposomes by Spectrin.

Membrane System	Diffusion Rate For Negative Membrane	Diffusion Rate For Positive Membrane
Liposomes	2-3%/Hr.	5-8%/Hr.
Liposomes + Spectrin	12-14%/Hr.	12-14%/Hr.
% Increase due to Spectrin	400-500	90-110

The rate of glucose diffusion from liposome particles was determined as in Figure 1. Diffusion rates are expressed as the percent of trapped glucose released per hour. In the case of positive membranes, 1.15 Mg of stearyl amine replaced the dicetyl phosphate in the original suspension. For spectrin activation, 1.3 Mg of protein were added to the diffusion cell for each ml of original suspension. pH 7.7, temp.= 23°C

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that while the protein activates diffusion in both systems, its effect on the negative membrane relative to its control is considerably greater than its effect on the positive membranes. This is the case even though much less protein is bound in the negatively charged system.

Discussion. The data presented indicate that the membrane related protein, spectrin, binds to lipid in the membrane conformation. This association can occur under conditions where the electrostatic charge on the lipid and protein are the same, thus eliminating electrostatic bonding as the basis for binding of lipid to protein. Further, the existence of this "non-electrostatic" bonding is clearly of greater

significance in the permeability properties of the membrane system than is the purely electrostatic bonding.

Since the question of electrostatic, versus hydrophobic bonding of protein to lipid is a central one in membrane biochemistry, it is clearly of interest that a structurally implicated protein appears to associate hydrophobically with lipid. That the lipid-protein complex exists as a membrane has not been demonstrated unequivocally, although the kinetics of diffusion suggest that this is so. There is apparently no sudden release of solute from the liposome which would be expected if a major disruption of the membranes were the cause for the increased rate of diffusion. Further, the linear nature of the diffusion suggests the continued existence of a series of lamellae buffering out the charge in gradient across the outer lamellae and thus producing this apparent deviation from Fick's law. Further evidence regarding this point will be published shortly.

The significance of these observations to membrane structure in general remains to be seen, but it is of interest that liposomes have been shown to be an excellent model for the erythrocyte membrane and its interactions with complement (7,8). In addition, it is known that the net charge on the erythrocyte membrane is negative at pH 7. Thus, from its isoelectric point alone (5.3) one would predict that spectrin does not bond electrostatically to the membrane.

Finally, since spectrin has been implicated in formation of the fibrous network attached to the inner surface of erythrocyte ghosts (2) it will be of interest to determine the appearance of the liposome-spectrin complex by electron microscopy.

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