The Structure of Mixed Cholesterol/Phospholipid Monolayers as Probed by Interactions with Band 3 Protein from Erythrocyte Membranes

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Solubilized band 3 protein from human erythrocyte membranes (the anion transport protein) interacts strongly and specifically with monolayers of cholesterol spread at the air-water interface whereas, at pH 7 - 10, it shows only moderate interactions with phospholipid monolayers [1,2].

When band 3 protein, at pH 7 and an ionic strength of approximately 100 mM, is added to the subphase of mixed phosphatidylcholine/cholesterol monolayers, the changes $\Delta\pi$ in monolayer surface pressure induced by the protein depend on the mole fraction X of sterol in the mixture. However, $\Delta\pi(X)$ increases with increasing X towards the high values of $\Delta\pi$ characteristic of cholesterol monolayers only if $X > 0.67 \pm 0.04$; at lower cholesterol content, $\Delta\pi(X)$ is practically identical to the value obtained with the pure phospholipid. Analogous results are obtained with cholesterol/phosphatidylserine and cholesterol/phosphatidylethanolamine mixtures. With mixtures of phosphatidylethanolamine and coprostanol (which also interacts strongly with band 3 [2]), the break in the $\Delta\pi(X)$-curves occurs already at $X = 0.33 \pm 0.03$.

The following model is consistent with the data described: In mixed monolayers with phospholipids, cholesterol is complexed to the phospholipid up to X = 0.67 (2 cholesterol molecules per phospholipid). At higher values of X, domains of pure cholesterol coexist with domains of complex. Besides complexes with stoichiometry of 2:1, also those of stoichiometry 1:2 may exist. In the mixed monolayers, band 3 can undergo its specific interaction with cholesterol molecules only if the latter are not complexed to phospholipid.

Our model is, at least in part, similar to earlier suggestions of some authors but contrasts sharply with those of others.

1 Klappauf, E. and Schubert, D. (1977) FEBS Lett. 80, 423-425.
2 Klappauf, E. and Schubert, D. (1979) Hoppe-Seyler's Z. Physiol. Chem. 360, 1225-1235.