Biochimica et Biophysica Acta, 684 (1982) 75–82
Elsevier Biomedical Press

BBA 71008

THE STRUCTURE OF MIXED CHOLESTEROL-PHOSPHOLIPID MONOLAYERS SPREAD AT THE AIR-WATER INTERFACE AS PROBED BY INTERACTIONS WITH BAND 3 PROTEIN FROM ERYTHROCYTE MEMBRANES

DIETER SCHUBERT and HANNELORE MARIE

Department of Cell Physiology, Max-Planck-Institut fur Biophysik, D-6000 Frankfurt am Main (F.R.G.)

(Received February 9th, 1981)
(Revised manuscript received September 11th, 1981)

Key words: Cholesterol-phospholipid monolayers; Air-water interface; Band 3 protein; (Erythrocyte membrane)

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 (Klappauf, E. and Schubert, D. (1979) Hoppe-Seyler's Z. Physiol. Chem. 360, 1225–1235). When band 3 protein, at pH 7 and an ionic strength of approx. 100 mM, is added to the subphase of mixed cholesterol-glycerophospholipid 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, $\Delta\pi(X)$ only increases with increasing X towards the high values of $\Delta\pi$ that are characteristic of cholesterol monolayers if $X > 0.67 \pm 0.04$; at lower cholesterol content, $\Delta\pi(X)$ is practically identical to the value obtained with the pure glycerophospholipid. With mixtures of coprostanol and glycerophospholipids, the break in the $\Delta\pi(X)$ curves occurs when $X = 0.33 \pm 0.03$. Cholesterol-sphingomyelin and epicoprostanol-phosphatidylethanolamine mixtures show an increase of $\Delta\pi(X)$ when $X > 0$. The data seem to support earlier claims that cholesterol can form stoichiometric complexes with glycerophospholipids, the stoichiometries revealed by the band 3-monolayer interactions being 2:1 and 1:2. They also show that cholesterol-sphingomyelin complexes, if they should exist, must be structurally different from the cholesterol-glycerophospholipid complexes.

Introduction

Since cholesterol is a main lipid component of many biological membranes, numerous studies have been performed to elucidate the structure of model membranes composed of this sterol and phospholipids and to characterize the interactions occurring in them. Despite all efforts to effectuate this, both topics are still highly disputed (for reviews, see Refs. 1 and 2).

The most controversial problem concerning mixed cholesterol-phospholipid bilayers and monolayers seems to be the existence and possible stoichiometry of sterol-phospholipid complexes. Several workers have presented putative evidence for the occurrence of complexes of fixed stoichiometry. Thus, data from a variety of experimental techniques have led to the proposal that complexes of stoichiometries 1:2, 1:1 and 2:1 (cholesterol:phospholipid) may exist in hydrated lipids [3–10], and from spectroscopic measurements the occurrence of 1:1 and 2:1 complexes was ascribed to dry lipids [8,11]. The 2:1 and 1:1 complexes have been regarded in some cases as metastable [8,9]. Stable 1:2 complexes have also been claimed to exist in monolayers adsorbed at the air-water interface [12]. On the other hand, many other authors could not find evidence in favour of the occurrence of cholesterol-phospholipid complexes of fixed stoichiometry (e.g.

0005-2736/82/0000–0000/$02.75 © 1982 Elsevier Biomedical Press

Refs. 13–18), and some of them strictly deny their existence.

During the last few years, our group has studied the interactions between lipid monolayers spread at the air-water interface and solubilized band 3 protein (the anion exchange protein) from human erythrocyte membranes. We have shown that injection of this protein into the subphase of cholesterol monolayers induces large changes $\Delta\pi$ in monolayer surface pressure whereas, at neutral or alkaline pH, $\Delta\pi$ is much smaller with phospholipid monolayers of the same initial surface pressure π_i [19,20]. Experiments on mixed monolayers of phospholipids plus cholesterol or coprostanol have now shown that, apparently, the dependency of $\Delta\pi$ on lipid composition can give an insight into the arrangement of the lipid molecules in the monomolecular film. These studies, which seem to add new arguments in favour of the existence of cholesterol-phospholipid complexes, will be described in this paper. A preliminary account of our work has appeared in abstract form [21].

Materials and Methods

Band 3 protein from human erythrocyte membranes was isolated by zonal electrophoresis in acetic acid/H_2O/sucrose mixtures [22]. The preparation of the protein for the monolayer experiments was described earlier [20], as were the origin and purity of the phospholipids, steroids and other reagents used [20,23]. If not stated otherwise, the lipids were dissolved in chloroform at a concentration (determined by weighing or, for the phospholipids, by phosphorus analysis [24]) of 1.0–2.0 mM. Lipid mixtures were prepared by mixing stock solutions.

Surface pressure measurements of lipid monolayers spread at the air-water interface were performed in rectangular teflon troughs ($11.0 \times 6.5 \times 2.0$ cm) by the Wilhelmy plate method [19,20]. The subphase of the monolayers was 100 mM NaCl, 10 mM sodium phosphate (pH 7.1). Subphase temperature was (22.0 ± 1.0)°C. Band 3 protein was injected underneath the monolayers to a final concentration $c_p = 4\,\mu g/ml$ (corresponding to the plateau region of the $\Delta\pi(c_p)$-curves [20,23]). If possible, only a single protein preparation was used for a series of measurements (one phospholipid species, varying sterol content). However, for each protein batch appropriate controls were performed to ensure that the behaviour of the protein did not deviate from normal.

Results

Mixed monolayers of cholesterol and glycerophospholipids

Injection of band 3 protein into the subphase of cholesterol or phospholipid monolayers at the air-water interface leads to an increase in monolayer surface pressure π which is rapid during the first few minutes but afterwards becomes slower, and after 1–8 h, a constant value of $\Delta\pi$ is obtained. Monolayers prepared from different lipid classes differ, however, in respect of the magnitude of $\Delta\pi$ induced by band 3: e.g. at an initial lipid pressure $\pi_i = 10\ mN \cdot m^{-1}$, pH values between 6.5 and 10, an ionic strength of approx. 100 mM and protein concentrations $c_p > 3\,\mu g/ml$, $\Delta\pi$ for cholesterol monolayers is approx. 22 $mN \cdot m^{-1}$ whereas, for monolayers of the more abundant phospholipid classes of the erythrocyte membrane, $\Delta\pi$ is only 9–16 $mN \cdot m^{-1}$ (depending essentially on the phospholipid head group) [20,23]. With mixed monolayers of cholesterol and glycerophospholipids, the time dependence of surface pressure is similar to that observed with the pure lipids *. Surprisingly, however, at low or intermediate relative molar cholesterol content X in the monolayers, $\Delta\pi(X)$ does not assume values intermediate between those of the constituents of the mixture but is practically identical to those found for the pure phospholipids; only for cholesterol contents above 65–70% does $\Delta\pi(X)$ increase with increasing X towards the high values characteristic for cholesterol monolayers. This behaviour was observed for mixtures of cholesterol with all phosphoglycerides studied: phosphatidylcholine and phosphatidylethanolamine from egg yolk, phos-

* To study a possible influence of oxidation of the lipid during the relatively long duration of the experiments, especially with monolayers containing a high percentage of cholesterol [25,26], control experiments were performed in a N_2 atmosphere and with degassed subphase solutions. The results obtained were identical to those performed without these precautions.

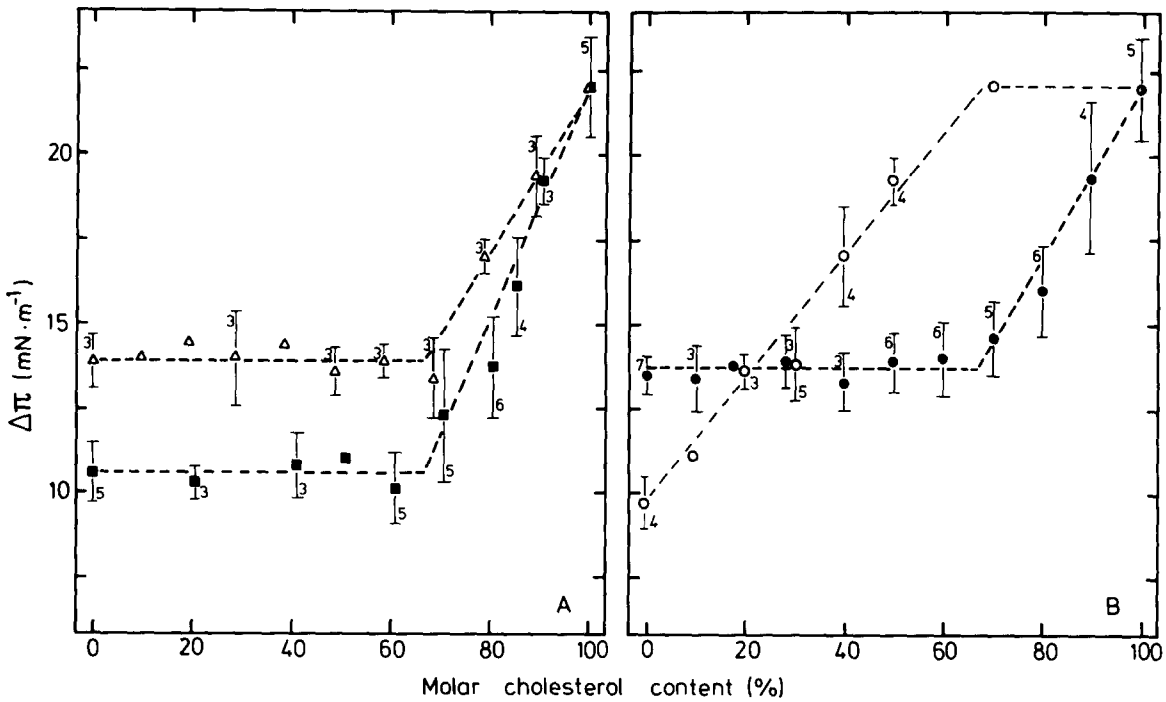

Fig. 1. Dependency of $\Delta\pi$ on molar cholesterol content for mixed monolayers of cholesterol and egg yolk phosphatidylcholine (■), bovine spinal cord phosphatidylserine (△) (part A), egg yolk phosphatidylethanolamine (●) and bovine brain sphingomyelin (○) (part B). Initial lipid pressure: $\pi_i = 10$ mN·m^{-1}. The monolayers were formed 5–30 min before injection of the protein. The bars and figures at the data points represent standard deviation and number of measurements, respectively. For the construction of the dotted lines, see text.

phatidylserine from bovine spinal cord and 1,2-distearoylphosphatidylcholine (Figs. 1, 2). Tests with the latter system have shown that the results are independent of the initial lipid pressure π_i (Fig. 2). They are also independent of the time interval between spreading of the lipid monolayer and addition of the protein ($\tau = 3$–120 min). Furthermore, $\Delta\pi$ was unaffected by the use of ethanol instead of chloroform for the spreading of the monolayer (cholesterol/phosphatidylcholine mixtures, $\pi_i = 10$ mN·m^{-1}) or by gentle magnetic stirring of the monolayer subphase. On the other hand, with monolayers containing 50–70% cholesterol, rigorous stirring in the presence of the protein led to an increase in $\Delta\pi$ *.

A thorough inspection of Figs. 1 and 2 reveals that the experimental data for all glycerophospholipids can be fitted quite well; (i) for cholesterol contents X between 0 and 66.7% by a parallel to the abscissa through the mean value of all data from this region, and (ii) for higher values of X by a straight line from the point at $X = 66.7\%$ on the former line to the $\Delta\pi$ value for cholesterol monolayers (dotted lines in Figs. 1, 2). This way of fitting the data will be further justified below. In alternative fits following this procedure, but allowing for different abscissa values of the 'break' in the fitted curves, the position of the break will not differ for more than ±4% from the former value, despite the considerable scatter of the data points. Thus, the position of this break on the X-axis corresponds closely to a molar ratio cholesterol: glycerophospholipid of 2.0.

The behaviour of cholesterol-sphingomyelin mixtures

Monolayers made of mixtures of cholesterol and sphingomyelin from bovine brain react entirely differently towards band 3 than those con-

* In all cases, if no protein was added, monolayer surface pressure did not vary for more than ±1 mN·m^{-1} for at least 5 h. Thus, artifacts due to surfactant impurities of the buffers used [27] were absent or at least negligible in our experiments.

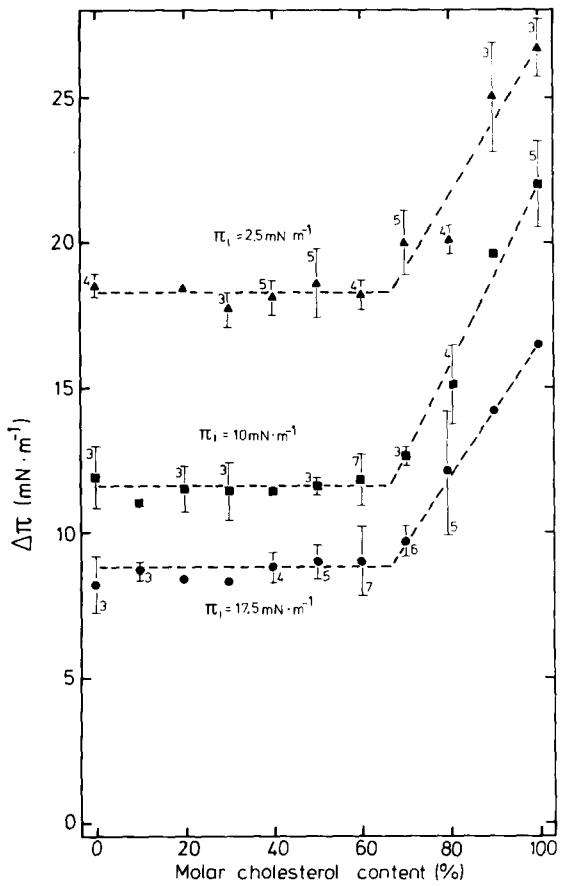

Fig. 2. Dependency of $\Delta\pi$ on molar cholesterol content for mixed monolayers of cholesterol and distearoylphosphatidylcholine. $\pi_i = 2.5$, 10.0, or 17.5 mN·m^{-1}. For further details see legend to Fig. 1. The apparent nonlinearity of the $\Delta\pi(\pi_i)$-dependency for the pure phospholipid is due to the use of different band 3 preparations [20,23].

taining phosphoglycerides: as is also shown in Fig. 1, there is a strong and linear increase of $\Delta\pi$ with increasing cholesterol content, X, even at low values of X, and at X values around 70% $\Delta\pi$ reaches the range observed with cholesterol monolayers. However, if sphingomyelin from bovine erythrocyte membranes was used, the behaviour of the system was more complicated: with some batches of band 3, the results were identical to those obtained with sphingomyelin from bovine brain, whereas with other batches the increase of $\Delta\pi$ with increasing X was much less pronounced than in the former case.

In the human erythrocyte membrane, at least 80–85% of the sphingomyelin is located in the membrane's outer monolayer, sphingomyelin making up approx. 44% of the phospholipid of this half of the membrane [28,29]. Therefore, the differences in the behaviour of glycerophospholipid-cholesterol and sphingomyelin-cholesterol mixtures towards band 3 could be highly relevant with respect to the problem of band 3-cholesterol interactions in the erythrocyte membrane. Studies on this subject are now in progress.

Mixtures of glycerophospholipids with coprostanol or epicoprostanol

The $\Delta\pi$ values induced by band 3 in coprostanol monolayers are very similar to those for cholesterol [20]. Mixed monolayers of coprostanol and the glycerophospholipids studied also behave similarly to cholesterol-glycerophospholipid mixtures as long as the molar coprostanol content X' is below approx. 35%. At higher sterol content, however, $\Delta\pi$ strongly increases with increasing X' (Fig. 3). If the data points are fitted in an analogous way as are those for cholesterol-glycerophospholipid monolayers, the 'break' in the curves is found to occur at a sterol content of (33 ± 3)%. This corresponds to a molar ratio coprostanol:glycerophospholipid of 0.50.

In mixed monolayers of phosphatidylethanolamine and epicoprostanol (which shows weaker interactions with band 3 than cholesterol or coprostanol [20]), an increase of $\Delta\pi$ towards the value for epicoprostanol is detectable at a sterol content as low as 10–20%. A good fit of the data is obtained assuming that the increase of $\Delta\pi$, as compared to the value for the pure phospholipid, is initially proportional to the sterol content of the monolayer (Fig. 3b).

Discussion

Band 3 protein from human erythrocyte membranes shows strong and highly specific interactions with cholesterol monolayers. In our opinion, this effect is due to the existence of a sterol binding niche in the surface of the band 3, the shape of which closely follows the shape of the cholesterol molecule. The bonds responsible for the formation and stability of the band 3-cholesterol complex would, in this model, be a

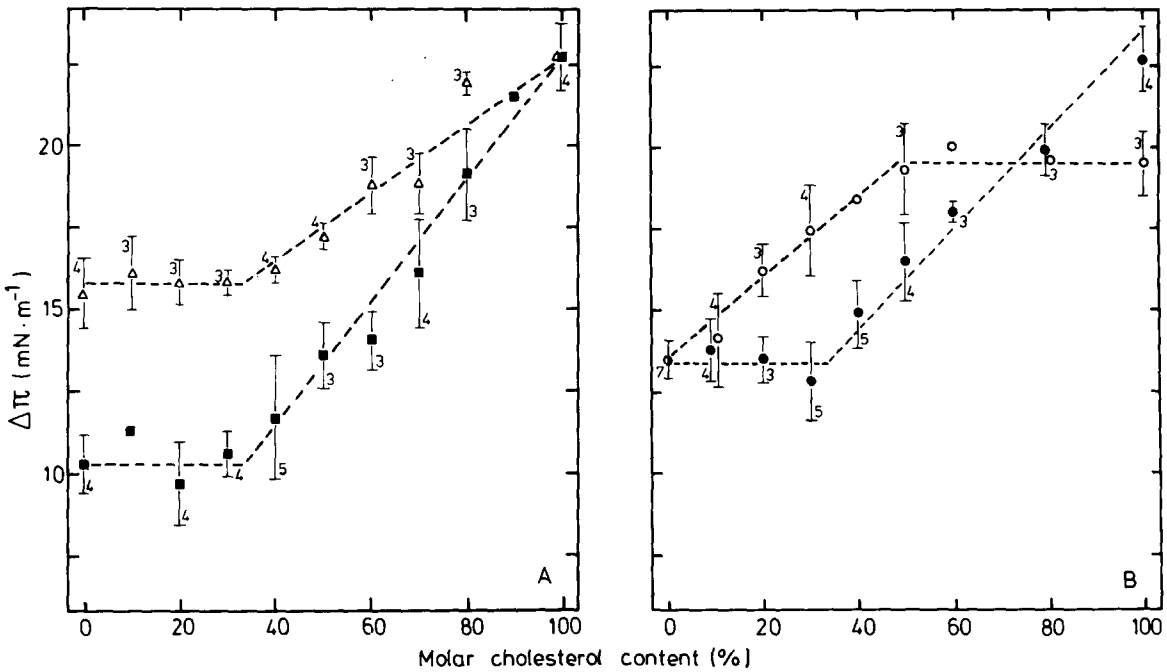

Fig. 3. Dependency of $\Delta\pi$ on molar sterol content for mixed monolayers of coprostanol and phosphatidylcholine (■) or phosphatidylserine (△) (part A), coprostanol and phosphatidylethanolamine (●) and epicoprostanol and phosphatidylethanolamine (○) (part B). $\pi_i = 10$ mN·m^{-1}. For further details see legend to Fig. 1. The differences in $\Delta\pi$ for phosphatidylserine between Figs. 1 and 3 are due to the use of different band 3 preparations.

hydrogen bond involving the 3β-OH group of cholesterol and a combination of hydrophobic and van der Waals bonds along the apolar region of the sterol molecule [20].

When band 3 is allowed to penetrate mixed cholesterol-glycerophospholipid monolayers of molar cholesterol contents below 67%, the protein molecules apparently do not interact with the sterol, the $\Delta\pi$ values induced by them in the monolayer being identical to those induced in monolayers of the pure phospholipids. However, above a sterol content of 67%, cholesterol molecules become available for interactions with band 3; their number increases with increasing sterol concentration *. Thus, in mixed cholesterol-glycerophospholipid monolayers of sterol contents below 67%, cholesterol seems to exist in a state or number of states where it is prevented from interaction with its high-affinity binding site on band 3 by strong sterol-glycerophospholipid interactions (the alternative interpretation that this effect is due to blockage of the binding site by band 3-phospholipid interactions is ruled out by the results obtained with coprostanol or epicoprostanol). On the other hand, at a higher cholesterol content an additional state seems to appear which allows for high-affinity band 3-cholesterol interactions. The position of the 'transition' on the *X*-axis corresponds to a molar ratio cholesterol:phospholipid of 2.0. This ratio strongly suggests that the molecular basis of the occurrence of the transition could be the formation of stoichiometric complexes between two cholesterol molecules and one

* It could be argued that one of the curves of Fig. 1 and two of Fig. 2 could also be fitted drawing a gradual transition from one of the straight lines drawn by us to the other. This could mean that, at cholesterol concentrations of approx. 50%, part of the sterol is already available for interaction with band 3 protein but that extensive protein-cholesterol interaction cannot occur until significant 'microphases' of cholesterol are present. However, this is ruled out by the data on the mixtures sphingomyelin-cholesterol (Fig. 1) and phosphatidylethanolamine-epicoprostanol (Fig. 3). These data demonstrate that, as soon as 'accessible' sterol is present, $\Delta\pi$ increases strongly with increasing sterol content. In addition, the obvious linearity of this increase fully justifies our procedure of fitting the data for the other lipid mixtures studied by us.

glycerophospholipid molecule. The position of the corresponding transition for coprostanol-glycerophospholipid mixtures at a molar ratio of 0.50 could indicate that, in addition to the 2:1 complexes, complexes consisting of one sterol and two glycerophospholipid molecules could also exist.

As has been described in the introduction, the occurrence of cholesterol-phospholipid complexes of fixed stoichiometry has been suggested earlier [3–12]. According to the picture derived from some of these studies, there is a close connection between the existence of these complexes and the phase behaviour of the lipid mixtures: below a molar ratio X of cholesterol and phospholipid of 1:2, a phase of pure phospholipid coexists with a phase of 1:2 complex; at $0.33 < X < 0.67$, either complexes of ratios 1:2 and 2:1 coexist as separate phases, or a phase of 1:2 complex and a phase of pure cholesterol coexist (depending on the conditions), and at $X \geq 0.67$ coexistence of either a phase of 1:2 or 2:1 complexes and a cholesterol phase is assumed to occur [9,12]. At least under some conditions, the 2:1 complexes and thus the corresponding phases are thought to be unstable [8,9,12]. The same holds for the 1:1 complex [9], the existence of which was inferred from a variety of experiments [4,5,7–10], though its relation to the simple phase diagram described is not clear. However, many other authors not only arrived at more complex phase diagrams for the cholesterol-phospholipid mixtures but also could not find evidence for the existence of stoichiometric complexes between the two kinds of molecules (e.g. Refs. 13–18).

It is obvious that the results presented in this paper are fully consistent with the assumption that cholesterol-glycerophospholipid complexes with a stoichiometry of 1:2 and 2:1 do exist (though our data do not seem to prove or disprove the occurrence of separate phases). These complexes may, however, be short-lived. Within the frame of this assumption, the data indicate that the putative sterol binding site on the surface of band 3 protein has to compete with glycerophospholipid molecules for cholesterol, and that the glycerophospholipid-cholesterol interaction is stronger than the band 3-cholesterol interaction (as long as the glycerophospholipid is not complexed to two cholesterol molecules). Since the interactions between phospholipids and sterols decrease in the order cholesterol-coprostanol-epicoprostanol [1,30], the absence of breaks in the $\Delta\pi(X)$-curves at $X = 0.67$ for coprostanol and $X = 0.33$ and 0.67 for epicoprostanol fits into this picture and will mean either that the corresponding complexes do not exist or that the interactions between these sterols and glycerophospholipids are weaker than the corresponding band 3-sterol interactions. Of course, our data do not argue against the occurrence of complexes different from the 2:1 and 1:2 complexes or of additional phase boundaries (e.g. a 1:1 complex or a phase boundary at $X = 0.20$ [31,32]) but could simply mean that band 3 is not a suitable probe for their detection *. On the other hand, we were unable to find a reasonable interpretation of our data, the basis of which is not the formation of cholesterol-glycerophospholipid complexes of the stoichiometries described, and we therefore regard our findings as a strong argument in favour of their existence. In addition, the present data represent the first evidence for the occurrence of the 2:1 complex in mixed monolayers at the air-water interface. It may be added that all these considerations are independent of whether or not the band 3 preparations used by us are partly or even completely denatured.

Our observation that the behaviour of cholesterol-sphingomyelin mixtures towards band 3 differs greatly from that of cholesterol-glycerophospholipid systems seems to be surprising since the affinity of sphingomyelin towards cholesterol was found to exceed or at least to be equal to that of phosphatidylcholine and other glycerophospolipids [35–39]. However, our data do not contradict these results. Rather, in the light of these findings, our observations show that the structure of mixed cholesterol-sphingomyelin monolayers must be different from that of cholesterol-glycerophospholipid monolayers and that cholesterol-sphingomyelin complexes, if they

* Colacicco and co-workers, studying the penetration of rabbit γ-globulin into mixed cholesterol-phosphatidylcholine monolayers, observed a 'break' in the $\Delta\pi(X)$ curves at $X = 0.50$. The position of the break was, however, shifted to lower values of X by vigorous stirring of the monolayer subphase [33,34]. This may indicate the occurrence of a 1:1 complex of low stability.

should exist, must be structurally quite different from those between the sterol and glycerophospholipids. Similar conclusions have been derived from X-ray diffraction studies on oriented lipid multilayers [40].

The large number of publications on the subject of this paper cannot be discussed here, but two of them, dealing with mixed cholesterol-phosphatidylcholine monolayers, seem to deserve special attention. Tajima and Gershfeld [12] have studied the phase relations in both the aqueous dispersions and the equilibrium surface films of cholesterol-phosphatidylcholine systems, by measurements of the equilibrium spreading pressure π^e of the dispersions. According to their interpretation, clear evidence was obtained for the existence of the 1:2 complex both in the bulk phase and in the adsorbed monolayers (for $X > 0.33$). Furthermore, these authors deduced from their data that, at $\pi < \pi^e$, the monolayers would consist of separate phases of phosphatidylcholine and 1:2 complex (cholesterol:phosphatidylcholine) when $X < 0.33$ whereas at $X > 0.33$, phases of 1:2 complex and of pure cholesterol would coexist. However, they admit that spread films, due to their metastable nature, may also contain 2:1 complexes as non-equilibrium states. Clearly, these conclusions are fully consistent with our observations and interpretation, especially since the metastable state of the putative 2:1 complex in our experiments is indicated by the influence of stirring on the $\Delta\pi(X)$ curves. On the other hand, in a thorough study on the condensing effect of cholesterol on phospholipids by measurement of the pressure/area relationship of spread cholesterol-dipalmitoylphosphatidylcholine monolayers, Müller-Landau and Cadenhead [17] could find no evidence for the occurrence of either stoichiometric cholesterol-phospholipid complexes or the corresponding phase boundaries. However, neither of the two events will necessarily have a distinct influence on the pressure/area curves, especially if the contribution of complex formation to the condensing effect of cholesterol should be relatively small (as has been claimed by Tajima and Gershfeld [12]). Thus, the data of Müller-Landau and Cadenhead do not seriously disagree with our view, though they certainly do not support it.

In summary, the unavailability of cholesterol arranged in mixed cholesterol-glycerophospholipid monolayers for interaction with band 3 protein at molar sterol contents below 67% seems to indicate that cholesterol can be complexed to glycerophospholipid molecules up to a maximum stoichiometry of 2:1 (cholesterol:phospholipid). The corresponding accentuation of a sterol content of 33% in coprostanol-glycerophospholipid monolayers suggests that, besides the 2:1 complexes, complexes of stoichiometry 1:2 will also exist.

Acknowledgements

We are grateful to Professor H. Passow for stimulating discussions, to Dr. E. Grell for critical comments on the manuscript and to Dr. E. Klappauf and Mrs. B. Domning for help during the initial phase of our experiments. This work was supported by the Deutsche Forschungsgemeinschaft (Fa 48/22).

References

1 Demel, R.A. and De Kruyff, B. (1976) Biochim. Biophys. Acta 457, 109–132
2 Green, C. (1977) Int. Rev. Biochem. 14, 101–152
3 Horwitz, C., Krut, L. and Kaminsky, L. (1971) Biochim. Biophys. Acta 239, 329–336
4 Darke, A., Finer, E.G., Flook, A.G. and Phillips, M.C. (1971) FEBS Lett. 18, 326–330
5 Darke, A., Finer, E.G., Flook, A.G. and Phillips, M.C. (1972) J. Mol. Biol. 63, 265–279
6 Green, J.R. and Green, C. (1973) Biochem. Soc. Trans. 1, 365–368
7 Phillips, M.C. and Finer, E.G. (1974) Biochim. Biophys. Acta 356, 199–206
8 Freeman, R. and Finean, J.B. (1975) Chem. Phys. Lipids 14, 313–320
9 Gershfeld, N.L. (1978) Biophys. J. 22, 469–488
10 Reiber, H. (1978) Biochim. Biophys. Acta 512, 72–83
11 Zull, J.E., Greanoff, S. and Adam, H.K. (1968) Biochemistry 7, 4172–4176
12 Tajima, K. and Gershfeld, N.L. (1978) Biophys. J. 22, 489–500
13 Marsh, D. (1974) Biochim. Biophys. Acta 363, 373–386
14 Godici, P.E. and Landsberger, F.R. (1975) Biochemistry 14, 3927–3933
15 Jacobs, R. and Oldfield, E. (1979) Biochemistry 18, 3280–3285
16 Rogers, J., Lee, A.G. and Wilton, D.C. (1979) Biochim. Biophys. Acta 552, 23–37
17 Müller-Landau, F. and Cadenhead, D.A. (1979) Chem. Phys. Lipids 25, 315–328
18 Yeagle, P.L. (1981) Biochim. Biophys. Acta 640, 263–273

19 Klappauf, E. and Schubert, D. (1977) FEBS Lett. 80, 423–425
20 Klappauf, E. and Schubert, D. (1979) Hoppe-Seyler's Z. Physiol. Chem. 360, 1225–1235
21 Schubert, D. and Marie, H. (1980) Biophys. Struct. Mech. 6 (Suppl.) 109
22 Schubert, D. and Domning, B. (1978) Hoppe-Seyler's Z. Physiol. Chem. 359, 507–515
23 Schubert, D. and Klappauf, E. (1980) Hoppe-Seyler's Z. Physiol. Chem. 361, 1171–1176
24 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468
25 Kamel, A.M., Weiner, N.D. and Felmeister, A. (1971) J. Colloid Interface Sci. 35, 163–166
26 Cadenhead, D.A., Kellner, B.M.J. and Phillips, M.C. (1976) J. Colloid Interface Sci. 57, 224–227
27 Gacesa, P., Hill, M.W. and Bangham, A.D. (1973) Biochim. Biophys. Acta 307, 467–470
28 Zwaal, R.F.A., Roelofsen, B. and Colley, C.M. (1973) Biochim. Biophys. Acta 300, 159–182
29 Zwaal, R.F.A., Comfurius, P. and Van Deenen, L.L.M. (1977) Nature 268, 358–360
30 Demel, R.A., Bruckdorfer, K.R. and Van Deenen, L.L.M. (1972) Biochim. Biophys. Acta 255, 311–320
31 Shimshick, E.J. and McConnell, H.M. (1973) Biochem. Biophys. Res. Commun. 53, 446–451
32 Lentz, B.R., Barrow, D.A. and Hoechli, M. (1980) Biochemistry 19, 1943–1954
33 Colacicco, G. and Rapport, M.M. (1968) Advan. Chem. Series 84, 157–168
34 Colacicco, G. (1973) in Biological Horizons in Surface Science (Prince, L.M. and Sears, D.F., eds.), pp. 247–288, Academic Press, New York
35 Demel, R.A., Jansen, J.W.C.M., Van Dijck, P.W.M. and Van Deenen, L.L.M. (1977) Biochim. Biophys. Acta 465, 1–10
36 Van Dijck, P.W.M. (1979) Biochim. Biophys. Acta 555, 89–101
37 Nakagawa, Y., Inoue, K. and Nojima, S. (1979) Biochim. Biophys. Acta 553, 307–319
38 Lange, Y., D'Alessandro, J.S. and Small, D.M. (1979) Biochim. Biophys. Acta 556, 388–398
39 Calhoun, W.I. and Shipley, G.G. (1979) Biochemistry 18, 1717–1722
40 Khare, R.S. and Worthington, C.R. (1977) Mol. Cryst. Liq. Cryst. 38, 195–206