Biochimica et Biophysica Acta, 693 (1982) 237–245
Elsevier Biomedical Press

BBA 71439

LATERAL SEGREGATION OF MEMBRANE LIPIDS AND FORMATION OF STABLE ROD-SHAPED MEMBRANE PROJECTIONS IN ERYTHROCYTES TREATED WITH LONG-CHAIN ALCOHOLS

M. GRUNZE *, C.W.M. HAEST and B. DEUTICKE **

Abteilung Physiologie, Medizinische Fakultät der Rheinisch-Westfälischen Technischen Hochschule, Schneebergweg 211, D-5100 Aachen (F.R.G.)

(Received June 28th, 1982)

Key words: Cell shape; Membrane lipid; Lipid segregation; Alcohol; (Erythrocyte)

Human erythrocytes, incubated with sonicated dispersions of phosphatidylcholine, cholesterol and saturated straight-chain alcohols (C_{16}–C_{18}) develop stiff, rod-shaped, hemoglobin-containing membrane projections within 120 min. The number of these 'rods' varies (1–3 per cell), they reach a length of up to 14 μm (twice the cell diameter) and a thickness of 0.3–1.0 μm. 'Rods' may be separated from 'residual cells' by shear flow and centrifugation without severe hemolysis. Lipid analyses carried out on residual cells and rods indicate lateral segregation of the phospholipids of the outer leaf of the membrane lipid bilayer (phosphatidylcholine and sphingomyelin) and of the alcohol applied. Phosphatidylcholine accumulates in the residual cells, sphingomyelin and the alcohol in the rods. No differences in membrane protein patterns were observed between rods and residual cells. The rod-shape is dependent on the presence of the alcohol, extraction of the alcohol converts rods into hemoglobin-containing spheres without lysis. The formation of rods, which is indicative of a lateral phase separation, is discussed in terms of lipid-lipid interactions and with respect to parameters determining the shape of cells.

Introduction

The peculiar biconcave resting shape of the mammalian erythrocyte and its changes induced by chemical agents [1], ATP depletion [2,3], pH changes [4] and various other treatments [5,6] have aroused the interest of hematologists and membrane biologists for a long time. The shape changes range from echinocytosis to the formation of myelin figures and exocytosis (fragmentation) on the one hand, and from stomatocytosis to invagination and endocytotic vesiculation on the other hand [1,7–10]. Some of the conditions which lead to such shape changes have been studied in our laboratory before [1,5,6].

In the course of studies on the influence of lipid modification on membrane permeability we incidentally observed a new type of striking morphological changes of erythrocytes exposed to long-chain aliphatic alcohols. The cells slowly formed long, stiff rods protruding out of the plane of the membrane. These rods could be separated from the cells by shear without hemolysis, thereby providing a system which allows direct chemical analysis of different parts of a continuous membrane. The results presented in the following indicate that lateral phase separations occur in this system giving rise to shape changes dominated by the properties of the lipids.

* Present address: Medizinische Klinik Universität Heidelberg, D-6900 Heidelberg, F.R.G.
** To whom requests for reprints should be addressed.

Materials

Human blood was obtained from the local Blood Bank and anticoagulated with heparin.

Lipids used were obtained from Sigma Chemie, Munich: Cholesterol (CH-S), egg phosphatidylcholine (P 8640 and P5763), dipalmitoylphosphatidylcholine (P 6769) and dimyristoylphosphatidylcholine (P 0888), myristyl alcohol (M 3628), cetyl alcohol (C 7882), stearyl alcohol (S 5751), oleyl alcohol (O 1125), heptadecanol (H 7760) and eicosanol (A 7760). Bovine albumin, essentially fatty acid-free was also obtained from Sigma (A 7511). All other chemicals used were reagent grade.

Methods

(a) Incubation procedures

Erythrocytes were washed 3 times in 154 mM NaCl and suspended in an incubation medium having the following constituents in addition to the lipids described below: KCl 100 mM, NaCl 50 mM, sucrose 40 mM, NaH_2PO_4/Na_2HPO_4 12.5 mM, glucose 5 mM, pH 7.35. The lipid mixtures dispersed in the incubation media had a standard composition of 2 mg/ml phospholipid, 1 mg/ml cholesterol and 0.5 mg/ml alcohol. The molar ratio of the three constituents was thus approximately 1 : 1 : 0.5. Alcohol-free lipid dispersions served for control incubations. The lipids were dissolved in chloroform, evaporated in a round-bottom flask, covered with the saline medium and swollen overnight. Prior to incubation the suspensions were sonicated for 5 min in an ice bath (Branson sonifier B 12 at 100 watt). When lipid analyses were intended, the lipid dispersion was centrifuged at $12000 \times g$ for 5 min prior to incubation. Incubation of erythrocytes in the lipid dispersions was carried out at 37°C, pH 7.35 and a hematocrit of 10%.

(b) Microscopy

Microscopy of erythrocytes was carried out using a thermostabilized system to maintain a constant temperature of 37°C in a microchamber ($18 \times 18 \times 0.2$ mm) which permitted continuous observations through a Leitz Orthoplan Microscope equipped with interference optics. Photographs were taken using a Leica M 4, or for studies on the time course of morphological changes, using a Bolex H 16 camera. Samples for scanning electron microscopy were fixed on specimen studs by glutaraldehyde, washed, dehydrated and subjected to critical-point drying. After sputter-coating with gold the samples were viewed in a Leitz AMR 1000 A scanning electron microscope.

(c) Analytical procedures

After incubation of the cells and formation of the rods the cell samples were washed 3 times in saline medium and subjected to shear stress by repetitive passage through the orifice of a 10-ml glas pipette. The residual cells were then first sedimented by centrifugation at $800 \times g$, rods were concentrated at $5000 \times g$. Rods and residual cells were analysed for their lipid content after extraction with chloroform/isopropanol [11]. For quantitative gas chromatographic determinations of alcohol and cholesterol an internal standard procedure was applied using an automatic peak integrator (Minigrator, Spectra Physics). Androstane served as an internal standard. Gas-chromatography was carried out on a Becker 419 gas chromatograph equipped with a 1.5 m, 2 mm i.d. glass column filled with 2% QF1 on gas chrom Q (carrier gas N_2, temperature: programmed increase from 145°C to 225°C).

Lipid phosphorus was determined in an aliquot of the lipid extract after removal of non-lipid phosphorus [12]. Individual phospholipid classes were quantified [13] after separation by one-dimensional thin-layer chromatography on silica plates (Merck, Darmstadt, No. 5715) using chloroform/methanol/acetic acid/water (60 : 30 : 12 : 2, v/v) as a solvent. Total membrane protein was quantified by the procedure of Lowry et al. [14], protein patterns were determined by SDS-polyacrylamide gel electrophoresis [13].

Results

(a) Observations on rod growth and rod motions

Erythrocytes incubated in lipid dispersions containing stearyl alcohol develop rod-shaped, hemoglobin-containing membrane projections (Fig. 1). The development of these rods occurs in a time-dependent fashion. In a first step, multiple small

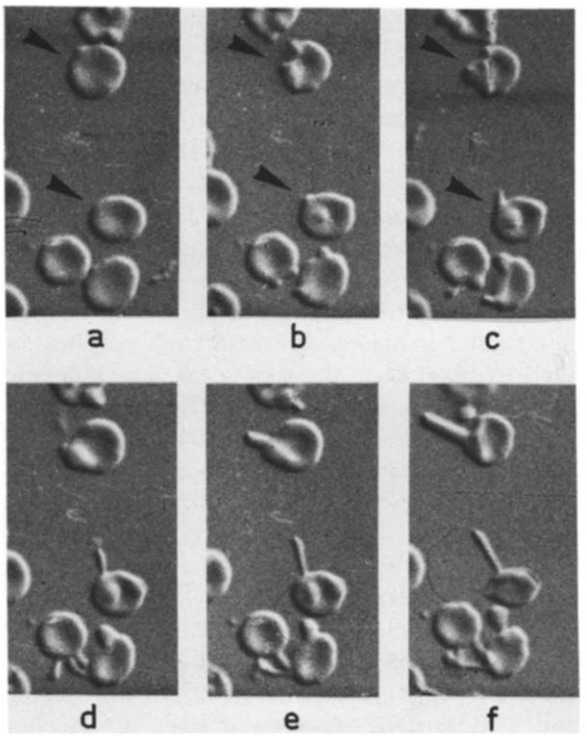
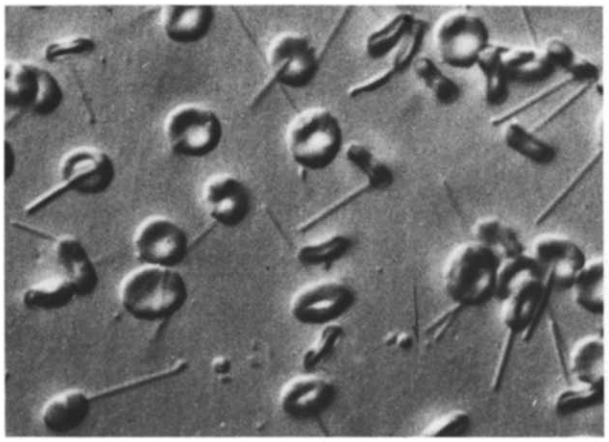


Fig. 1. Formation of cylindrical membrane protrusions (rods) in human erythrocytes suspended in lipid dispersions containing stearyl alcohol. Primarily appearing membrane protrusions (a, b) confluate (c, d), fuse and form (e, f) a rod.

membrane protrusions appear on the cells (arrows in Figs. 1 a and b). These protrusions fuse (Figs. 1 c and d) and form a rod growing out of the plane of the membrane (Figs. 1 e and f). The size of these rods and their number per cell are variable, 1–3 rods per cell have been observed. The maximal length after 6 h of incubation was 14 μm (Figs. 2 and 3), i.e. twice the diameter of an erythrocyte. The thickness of the rods ranged from 0.2–0.3 μm to about 1–2 μm. Scanning electron microscopy allowed a closer inspection of the rod-bearing cells (Fig. 3).

Within the same incubation the size and the number of rods per cell are relatively constant (Fig. 2). Rods in situ as well as isolated rods (see below) show a bulky deformation on one side. The rod is stiff in itself but anchored in a flexible manner to the cell. Application of mild shear forces (touching the lid covering the microchamber) results in angular motions of the rod relative to the cell, the rod itself staying straight and stiff. Application of higher shear stresses results in separation of the rods from the cells without marked hemolysis. Rods and residual cells can be separated by centrifugation (Fig. 4).

Different aliphatic hydrocarbon compounds were studied with respect to their ability to induce



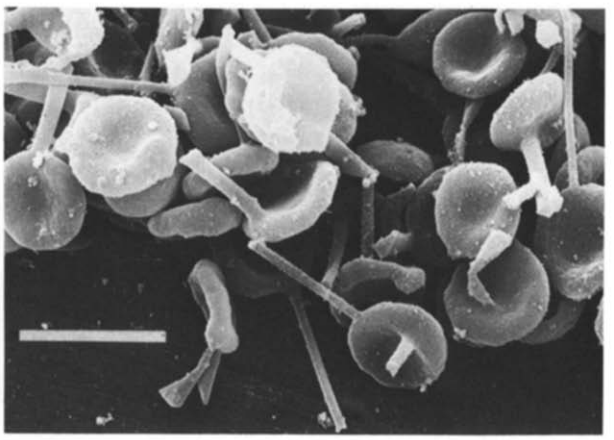


Fig. 2. Cell suspension with fully developed rods after 3 h incubation in lipid dispersions containing stearyl alcohol. Note the relative homogeneity of the rods with respect to length and diameter and the bulged distal end of the rods.

Fig. 3. Scanning electron micrograph of rod-bearing erythrocytes. The small particles adherent to the cells are lipid vesicles used for loading the cells with the alcohol. They were not removed by washing in this experiment, since the centrifugation would lead to the break-off of the rods. The bar represents 10 μm. Electron micrograph prepared by Dr. L. Busch, Abt. Anatomie, RWTH Aachen.

rods when administered to the cells by means of vesicles. Attempts to produce rods by sonicated dispersions of the alcohol alone, or by micellar suspensions of alcohols obtained by injecting alcohol dissolved in ethanol into saline solutions, ended without success. The yield of rods was highest with stearyl alcohol (octadecanol) and heptadecanol. Eicosanol and cetylalcohol (hexadecanol) were somewhat less effective. Interestingly, the effectiveness of cetyl alcohol could be enhanced by cholesterol depletion of the red cell membrane and by administration of the alcohol in cholesterol-free lipid dispersions. Oleyl alcohol, a *cis*-unsaturated analogue of stearyl alcohol, and myristyl alcohol did not induce rod formation. Other long chain alkane analogues of octadecanol (stearylamine, octadecane) and palmitic acid did not induce rod formation when applied in the same way.

The nature of the phospholipids serving as a carrier of the water-insoluble alcohols was also crucial: Attempts to induce rods with dispersions containing dipalmitoylphosphatidylcholine instead of egg phosphatidylcholine met no success. Dimyristoylphosphatidylcholine was less effective than egg phosphatidylcholine, but rods could still be induced with dispersions based on this synthetic phospholipid. A crude preparation and a highly purified preparation of egg phosphatidylcholine proved to be equally effective. The alcohols can be extracted from the isolated rods by 2% solution of bovine serum albumin and by alcohol-free phosphatidylcholine/cholesterol dispersions. According to gas chromatographic analyses 90% of the alcohol primarily present in the rods was removed by this procedure. The extraction resulted in the transformation of rods into small hemoglobin-containing spheres (Fig. 5). During this process the rods seem to retract into their bulged ends which continuously grow. The spheres have a diameter of 0.5–1 μm. Some of them show a shape resembling that of a biconcave 'mini-erythrocyte' (Fig. 5) indicative of an excess in surface area.

(b) Alcohol uptake and lipid segregation

As stearyl alcohol treatment resulted in the most pronounced rod formation this agent was chosen for an analysis of alcohol incorporation and lipid patterns of rods and residual cells. For the analyses the preparations were carefully washed after exposure to lipid dispersions, in order to remove adherent vesicles. In Fig. 6 the extent of shape transformation is related to the time dependent increase of alcohol levels in total cells (rods and residual cells). The half time of uptake of stearyl alcohol is approx. 30 min. The formation of

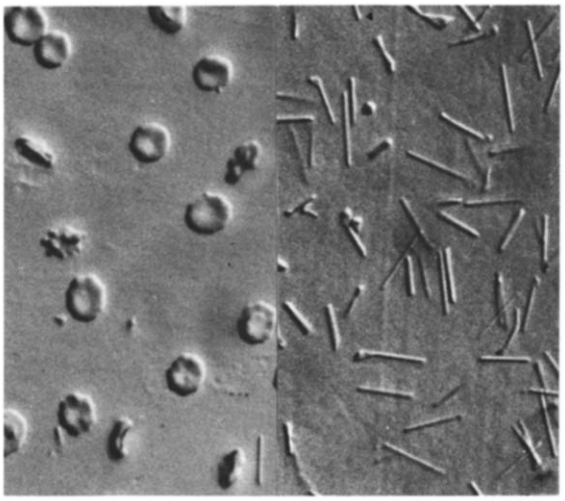


Fig. 4. Separation of rods (left) from 'residual cells' (right) by mild shear stress and subsequent centrifugation. Note that the bulged end on one side is preserved, giving a drum-stick like appearance to the rods.

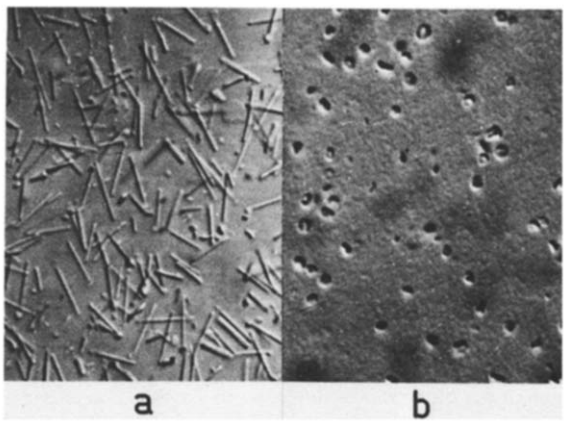


Fig. 5. Treatment of alcohol-induced rods (Panel a) with 2% serum albumin induces conversion into small spherical vesicles (Panel b), some of them resembling 'mini-erythrocytes'.

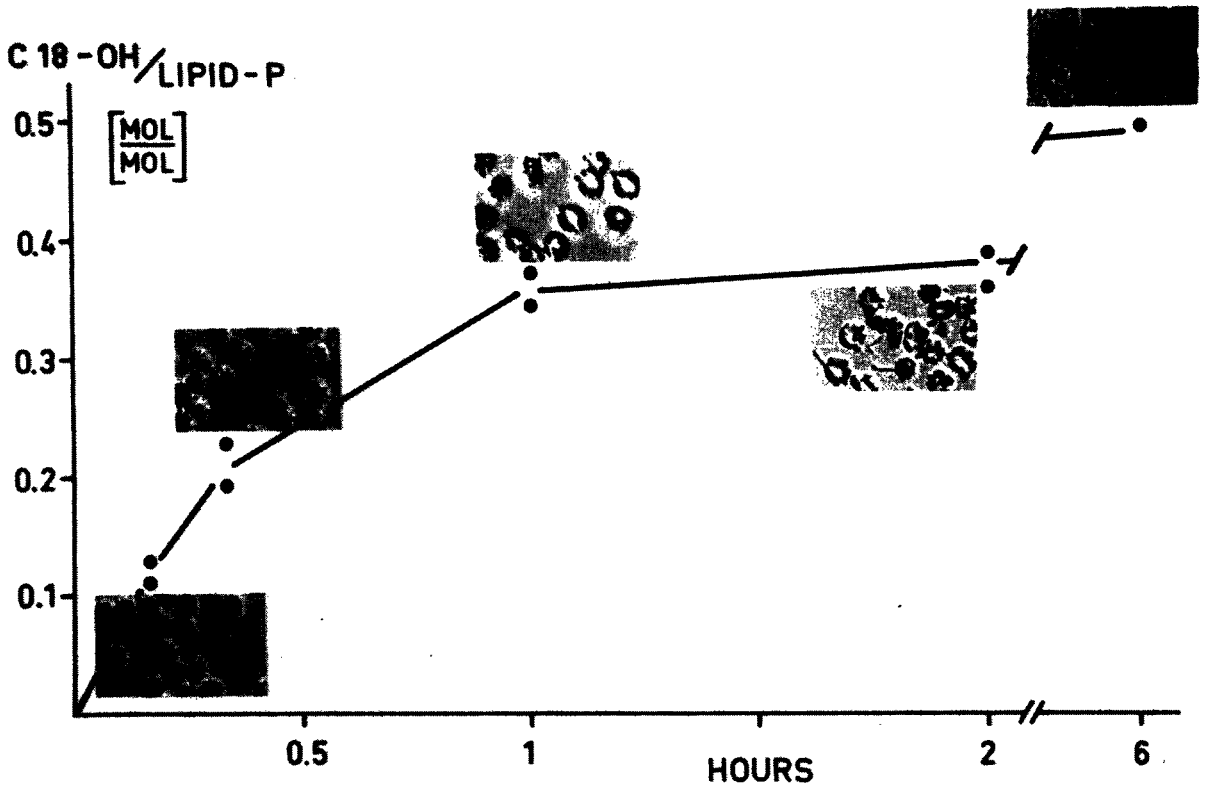


Fig. 6. Time-dependence of the incorporation of stearyl alcohol (C18-OH) into human erythrocytes and its relationship to rod formation. Alcohol content of the cells determined as described in Methods. At a molar ratio C18-OH/lipid phosphorus of 0.5 the cells contain about 2–2.5 μmol C18-OH per ml of cells.

rods occurs with a delay relative to the time course of alcohol uptake. The level of the alcohol in the membrane reaches values up to 0.5 mol per mol phospholipid, a ratio which is near to that in the lipid vesicles. This suggests some sort of equilibration of the alcohol between the lipid domain of the

TABLE I

LIPID PATTERNS OF RODS AND RESIDUAL CELLS

Analyses as described in Methods. Mean values ± S.D.

	Fresh cells	Residual cells ($n = 4$)	Rods ($n = 4$)
Lipid ratios (mol/mol)			
Cholesterol/phospholipid	0.65	0.68 ± 0.12	0.69 ± 0.39
Octadecanol/phospholipid	0	0.32 ± 0.08	1.50 ± 0.86
Phospholipid pattern (% of the sum of phospholipids)			
Sphingomyelin	25.2 ± 2.9	21.0 ± 4.3	33.5 ± 4.5
Phosphatidylcholine	29.1 ± 0.9	32.3 ± 4.8	20.9 ± 4.0
Phosphatidylethanolamine	30.0 ± 1.4	33.9 ± 5.1	30.5 ± 2.8
Phosphatidylserine	14.6 ± 3.4	11.0 ± 4.0	13.9 ± 1.89

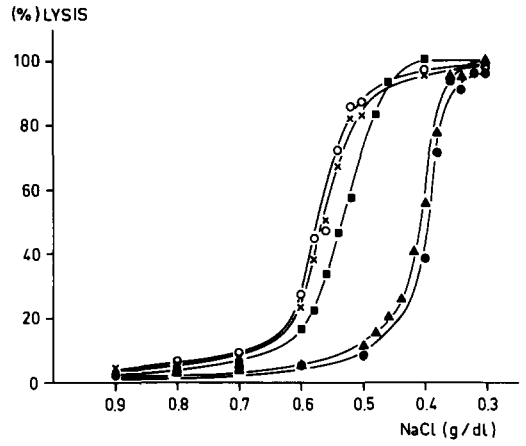


Fig. 7. Decrease of the osmotic fragility of erythrocytes after incorporation of long-chain aliphatic alcohols. For technical reasons cholesterol-depleted erythrocytes (−41%) were used for this study. Cells were exposed to different alcohols under standard conditions (see Methods) for 30 min, washed and suspended in hypotonic NaCl solution for determining osmotic fragility as described elsewhere [16]. Alcohol content of the cells was determined as described in Methods. ○, controls; ●, myristyl alcohol 0.7 mol/mol lipid P; ▲, cetyl alcohol 0.8 mol/mol lipid P; ■, stearyl alcohol 0.3 mol/mol lipid P; ×, eicosanol (< 0.05 mol/mol lipid P).

membrane and the surrounding lipid vesicles serving as a source of the alcohol.

The alcohol as well as other lipid constituents of the erythrocyte membrane distribute unevenly between the rods and the residual cells (Table I). Based on alcohol/phospholipid ratios, the alcohol accumulates in the rods, while the cholesterol/phospholipid ratio is the same in both, rods and residual cells. In terms of mole fractions of the four major phospholipids, sphingomyelin accumulates in the rods, lecithin in the residual cells, while the aminophospholipids, phosphatidylethanolamine and phosphatidylserine, do not segregate.

In view of this inhomogeneous distribution of the lipids it was of interest to investigate the membrane proteins in rods and residual cells. A precise evaluation of rod membrane proteins was compromised by the fact that the lysis required for hemoglobin removal was difficult in erythrocytes treated with alcohol. Rods could not be lysed completely even in the presence of a mild detergent, saponin. Nevertheless, SDS-polyacrylamide gels of the rods and of the membranes of the residual cells did not reveal striking differences in the peptide patterns. The total membrane protein of the rods was not determined in view of large contaminations of hemoglobin due to incomplete hemolysis.

(c) Surface expansion by alcohol

Incorporation of lipids and amphiphilic drugs into the membrane has been shown to decrease the osmotic fragility of erythrocytes [15–17]. The effect can be accounted for by an increase of the surface area due to the incorporation of additional material. In a series of experiments it was attempted to measure the surface expansion by the alcohol. As shown in Fig. 7, the alcohols reduce the osmotic fragility of cholesterol-depleted erythrocytes (which have an increased fragility) in approximate correlation to the amount of alcohol taken up. This effect is indicative of an increase of the surface area to volume ratio. The effects are more pronounced than those in previous studies of Raz and Livne [18], in which the alcohol was added to the cells in micellar form just prior to the hypotonic incubation.

Discussion

Saturated long-chain alcohols taken up by the erythrocyte membrane induce morphological changes of the cell as well as structural reorganizations in the membrane. There can be little doubt that the alcohol is actually incorporated into the lipid bilayer in view of its expanding effect on the membrane surface area, indicated by the increase of osmotic resistance (Fig. 7). This is true for rod-inducing alcohols as well as for alcohols not inducing rods. In the case of eicosanol a very slow uptake of the alcohol may explain the lack of effect on osmotic fragility. The technique of applying the alcohols by means of lipid suspensions precludes the uncertainties with respect to the partitioning of amphiphilic compounds between aqueous media and cell membranes recently brought up by Conrad and Singer [19]. The alcohol obviously determines and stabilizes the shape of the rods since its extraction converts the rods into spheres.

During the process of rod formation endogenous lipids of the erythrocyte membrane segregate

TABLE II

CALCULATION OF FRACTIONAL MOLAR CONTENTS (IN PERCENT) OF INDIVIDUAL LIPID CLASSES IN FRESH CELLS, RESIDUAL CELLS AND RODS

'Expected' refers to the fractional content to be expected for a simple dilution effect of alcohol incorporation. 'Found' refers to fractional molar content experimentally observed as a result of dilution by alcohol and lateral lipid segregation. Recalculated from the data in Table I.

	Fresh cells	Residual cells		Rods	
Phospholipid	60	46		28	
Cholesterol	40	21		17	
Alcohol	0	23		55	
	Found	Expected	Found	Expected	Found
Sphingomyelin	15.0	11.5	9.2	7.0	9.0
Phosphatidylcholine	17.4 } 32.4	13.3 } 24.8	15.4 } 24.6	8.1 } 15.1	5.9 } 14.9
Phosphatidylethanolamine	18.0	13.8	14.0	8.4	9.5
Phosphatidylserine	8.8 } 26.8	6.7 } 20.5	6.3 } 20.3	3.9 } 12.3	3.1 } 12.6
Sphingomyelin/ phosphatidylcholine	0.86		0.60		1.53

laterally to some extent. The alcohol as well as sphingomyelin accumulate in the rods, phosphatidylcholine in the residual cells. This is demonstrable by the octadecanol to phospholipid ratios and the relative phospholipid fractions in rods and residual cells (Table I). To exclude that in the case of the individual phospholipid classes a decrease of one component pretends an increase of another component, a calculation on the basis of the fractional contents of all lipid constituents has been carried out. As evident from Table II, in the group of phospholipids phosphatidylethanolamine and phosphatidylserine behave as if they were only diluted by the incorporated alcohol, while segregation of sphingomyelin and phosphatidylcholine, i.e. the phospholipids of the outer membrane layer [20], is clearly evident. Table II also reveals that the three major classes of constituents, phospholipids, cholesterol and alcohol are present at different fractional molar contents in rods and residual cells.

The formation of rods can be tentatively explained on the basis of the properties of mixed phases of phospholipids and long-chain alcohols. There is evidence [21,22] that long chain alcohols may only be miscible with certain phospholipids up to mole fractions of about 30–35%. From Fig. 6 it becomes evident, that rod formation in erythrocytes starts when the fractional alcohol content exceeds 25 mol%. It may be speculated that alcohol in excess of this mole fraction segregates into clusters containing native membrane lipids at lower concentrations. The morphological equivalents of these clusters may be the small coarse protrusions formed prior to the growth of the rods (Fig. 1). Rod growth probably starts in domains where a number of such clusters coalesces into a larger patch. The residual cells contain the alcohol at a mole fraction (23%) miscible with the other lipids. This interpretation can also account for the ineffectiveness of compounds such as palmitic acid to promote rod formation (in contrast to palmityl (cetyl) alcohol, which is a rod inducer). Palmitic acid is miscible with phospholipid at least up to 67 mol% [21].

Lipid phases rich in long-chain alcohols are presumably very rigid due to the high degree of order in the parallel alignment of saturated hydrocarbon chains, and to the increased spacing of charged headgroups, which may relieve repulsive electrostatic forces between these groups [23–25]. In addition, long-chain alcohols may resemble cholesterol in enforcing the 'hydrogen belt' [26] of the membrane, i.e. the region intermediate between the hydrophobic core and the polar headgroups.

Enrichment of sphingomyelin relative to phosphatidylcholine in such segregated alcohol phases

may be explained by preferential interaction of the long, saturated hydrocarbon chains of the ceramide moiety with the saturated alcohol, but also from sphingomyelin's tendency to from H-bonds in the hydrogen belt [26,27].

The rods are an interesting system from the point of view of bilayer structure. They contain some 70% of nonionic lipid material (cholesterol + alcohol) and only 30% of charged phospholipids. Whether the phospholipids are distributed homogeneously in the plane of the rod membrane is an open question. They might be concentrated to some extent in the bulged end of the rods, which is maintained during alcohol extraction by albumin.

Provided that the transverse distribution of phospholipids is maintained in the rods, i.e. that lecithin and sphingomyelin are dominant in the outer, phosphatidylethanolamine and phosphatidylserine in the inner leaflet of the bilayer [20], it can be claimed that the alcohol equilibrates between the two leaflets after its primary insertion into the outer one: Assuming an area per molecule of 80 $Å^2$ for the cholesterol-phospholipid complex, 60 $Å^2$ for a phospholipid, and 20 $Å^2$ for an alcohol [16,28] in the erythrocyte membrane, it can be calculated that an exclusive incorporation of the alcohol into the outer leaflet would lead to a ratio of the areas outer leaflet: inner leaflet of 65 : 35. This is extremely unlikely in a rod with a diameter of about 0.5–1 μm, i.e. a ratio of radius to membrane thickness of about 100 : 1. Long-chain alcohols thus seem to be capable of rapid transverse reorientation (flip-flop) in the membrane, a reasonable assumption in view of their similarity (with respect to polarity) to cholesterol, which has been shown to flip rapidly [29].

The shape, and the osmotic and mechanical stability of the rods are further intriguing properties. The formation of a thin rod of membrane material is certainly an effective way to dispose of cell surface with a minimal loss of cell volume. This, however, is not a mechanistic explanation and does not account for the fact, that the insertion of other long-chain alkyl derivatives, e.g. fatty acids, alkylammonium salts [1], lysophospholipids [17], polyoxyethylene-alcohols and -sorbitol esters (Piontek, M. and Deuticke, B. unpublished data) induces formation of echinocytes or stomatocytes, but not of rods. Moreover, all these other compounds usually produce lysis when their content in the erythrocyte membrane exceeds 25 mol% of the total lipid material, while stearyl alcohol produces rods containing 55 mol% alcohol which are completely stable and have an extremely high osmotic resistance.

The high bending stiffnes of the rods must also result from their high content in alcohol. It has been shown in a number of studies that long-chain alcohols convey an increased rigidity to phospholipid bilayers [23,24] and to micelles of others amphiphiles [25]. The very low lateral compressibility of a bilayer abundant in saturated chains [30] may form the link between high rigidity and high bending resistance of the rods.

Although incorporation of long-chain alcohols into biomembranes is a somewhat artificial situation we think that the resulting systems may teach us something about natural membranes, too:

1. Lateral asymmetry of lipids within a membrane and lipid segregation are probably possible on the basis of simple lipid interactions.

2. Depending on the strength of such interactions membrane lipids may convey mechanically stable non-spherical shapes to biological structures, i.e. cell organelles etc.

3. Modified biomembranes composed of only little phospholipid but large amounts of non-ionic constituents such as cholesterol and alcohol may be a promising object for the study of protein-dependent functional membrane properties such as enzyme and transport activities.

Acknowledgements

Supported by the Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 160/C 3). The authors wish to thank Dr. L. Busch, Ab. Anatomie, RWTH Aachen, for the electron microscopy study of the material and are indebted to Mrs. B. Forst for the technical assistance. The secretarial work of Mrs. H. Thomas and Mrs. R. Schäfer as well as the help of Mr. F.J. Kaiser in preparing the illustrations are gratefully acknowledged.

References

1 Deuticke, B. (1968) Biochim. Biophys. Acta 163, 494–500
2 Nakao, M., Nakao, T. and Yanazoe, S. (1960) Nature 187, 954–955

3 Feo, C. and Mohandas, N. (1977) Nature 265, 166–168
4 Rand, R.P., Burton, A.C. and Canham, P. (1965) Nature 205, 977–978
5 Haest, C.W.M., Fischer, T.M., Plasa, G. and Deuticke, B. (1980) Blood Cells 6, 539–553
6 Haest, C.W.M., Plasa, G. and Deuticke, B. (1981) Biochim. Biophys. Acta 649, 701–708
7 Chailley, B., Weed, R.I., Leblond, P.F. and Maigné, J. (1973) Nouv. Rev. Fr. Hematol. 13, 71–88
8 Lessin, L.S. and Bessis, M. (1977) in Hematology (Williams, W.D., Beutler, E., Erslev, A.J. and Rundles, R.W., eds.), pp. 103–134, McGraw-Hill Book Co., New York
9 Lutz, H.U., Liu, S.C. and Palek, J. (1977) J. Cell. Biol. 73, 548–560
10 Birchmeier, W., Lanz, J.H., Winterhalter, K.H. and Conrad, M.J. (1979) J. Biol. Chem. 254, 9298–9304
11 Rose, H.G. and Oklander, M. (1965) J. Lipid. Res. 6, 428–431
12 Grunze, M., Forst, B. and Deuticke, B. (1980) Biochim. Biophys. Acta 600, 860–869
13 Haest, C.W.M., Plasa, G., Kamp, D. and Deuticke, B. (1978) Biochim. Biophys. Acta 509, 21–32
14 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
15 Seeman, Ph.M. (1966) Int. Rev. Neurobiol. 9, 146–221
16 Deuticke, B. and Ruska, C. (1976) Biochim. Biophys. Acta 433, 638–653
17 Lichtman, M.A., Marinetti, G.V. and Gordesky, S.E. (1974) Nouv. Rev. Fr. Hematol. 14, 5–24
18 Raz, A. and Livne, A. (1973) Biochim. Biophys. Acta 311, 222–229
19 Conrad, M.J. and Singer, S.J. (1981) Biochemistry 20, 808–818
20 Verkleij, A.J., Zwaal, R.F.A., Roelofsen, B., Comfurius, P., Kastelijn, D. and Van Deenen, L.L.M. (1973) Biochim. Biophys. Acta 323, 178–193
21 Mabrey, S. and Sturtevant, J.M. (1977) Biochim. Biophys. Acta 486, 444–450
22 Eliasz, A.W., Chapman, D. and Ewing, D.F. (1976) Biochim. Biophys. Acta 448, 220–230
23 Jain, M.K. and Wu, N.M. (1977) J. Membrane Biol. 34, 157–201
24 Pringle, M.J. and Miller, K.W. (1979) Biochemistry 18, 3314–3320
25 Shinitzky, M., Dianoux, A.-C., Gitler, C. and Weber, G. (1971) Biochemistry 10, 2106–2113
26 Brockerhoff, P.A. (1974) Lipids 9, 645–650
27 Barenholz, Y. and Thompson, T.E. (1980) Biochim. Biophys. Acta 604, 129–158
28 Kavanau, J.L. (1965) Biological Membranes, Vol. I, p. 85, Holden-Day, New York
29 Lange, Y., Dole, J. and Steck, T.L. (1981) J. Biol. Chem. 256, 5321–5323
30 Lis, L.J., McAlister, M., Fuller, N., Rand, R.P. and Parsegian, V.A. (1982) Biophys. J. 37, 667–672