# Expansion of Phosphatidylcholine and Phosphatidylserine/Phosphatidylcholine Monolayers by Differently Charged Amphiphiles

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Z. Naturforsch. **56c,** 826–830 (2001); received April 11/May 25, 2001

Monolayer Technique, Nonionic Detergent, Erythrocyte Shape

The degree and time-course of expansion of palmitoyloleoylphosphatidylcholine (PC) and bovine brain phosphatidylserine (PS)/PC (75:25, mol/mol) monolayers at 32 mN/m caused by differently charged amphiphiles (detergents) added to the sub-phase buffer (pH 7.4, 22 °C) were followed. Amphiphiles were added to the sub-phase at a concentration/monolayer area corresponding to the concentration/erythrocytes surface area where sphero-echinocytic or sphero-stomatocytic shapes are induced (0.46–14.6 $\mu$M). Nonionic, cationic and anionic amphiphiles expanded the PS/PC monolayer significantly more (1.7–4.2 times) than the PC monolayer. A zwitterionic amphiphile expanded both monolayers to a similar extent. The initial rate of monolayer-expansion was higher for all amphiphiles (1.7–20.4 times) in the PS/PC monolayer than in the PC monolayer.

It is suggested that hydrophobic interactions govern the intercalation of amphiphiles into monolayers, and that monolayer packing, modulated by phospholipid head group interactions and alkyl chain saturation, strongly influence amphiphile intercalation. A possible relation between the monolayer-expanding effect of amphiphiles and their effect on erythrocyte shape is discussed.

## Introduction

Amphiphiles may induce either echinocytic (exvaginated, spiculated) or stomatocytic (invaginated) shapes in human erythrocytes (Deuticke, 1968; Fujii *et al.*, 1979). It is thought that the outward or inward membrane bending is due to an asymmetric accumulation of the amphiphiles between the bilayer leaflets, resulting in an area expansion of one leaflet relative to the other (Sheetz and Singer, 1974, 1976). It is believed that the charge of permeable cationic and anionic amphiphiles determines their asymmetrical distribution between the leaflets (and thereby the cell shape) due to a charge interaction with the net negative phosphatidylserine in the inner membrane leaflet.

This study was undertaken in order to collect information about how differently charged amphiphiles expand phospholipid monolayers. We chose monolayers having either a neutral charge and mono-unsaturated phospholipids (PC) or a negative net charge and a higher degree of unsaturated phospholipids (PS/PC, 75:25, mol/mol), regarding these as crude models of the human erythrocyte membrane outer and inner leaflet, respectively. The extent and time-course of the monolayer expansion induced by the amphiphiles were recorded, and related to the charge of the amphiphiles and to their capacity to alter the erythrocyte shape.

## Materials and Methods

Dodecyl D-maltoside (C12-maltoside) was obtained from Fluka (Buchs, Switzerland), 3-(dodecyldimethylammonio)-1-propanesulfonate (C12-zwittergent) from Calbiochem-Behring, sodium dodecyl sulfate (C12-sulfate) and chlorpromazine hydrochloride from Merck, dodecyltrimethylammonium bromide (C12-TAB), dibucaine hydrochloride, 1-decanol, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (PC) and L-alfa-phosphatidylserine from bovine brain (PS) from Sigma (St. Louis, MO, USA) and octaethyleneglycol mono n-dodecyl ether (C12E8) from Fluka. Dec-

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anol and dibucaine were dissolved in ethanol. Phospholipids, used without further purification, were dissolved in n-hexane/isopropanol (3:2, v/v) and stored in dark at −20 °C. Area expansion kinetics induced by amphiphiles in pure PC or mixed PS/PC monolayers (32 mN/m, 22 °C) prepared at the air/buffer interface were followed in a computer controlled KSV 3000 (Wilhelmy-plate) surface barostat (KSV Instruments, Helsinki). Data were collected by software from KSV Instruments. The phospholipid solution was added with a Hamilton syringe on the subphase buffer staying in a rectangular teflon trough (10160 $mm^2$). This through was connected to the circular teflon reaction through (2825 $mm^2$/~30 ml) via a teflon bridge. The subphase buffer contained 145 mM NaCl, 5 mM KCl, 4 mM $Na_2HPO_4$, 1 mM $NaH_2PO_4$ (pH 7.4). No pH shift was observed during the experiments. A nominally $Ca^{2+}$-free buffer was used, since $Ca^{2+}$ may induce a lateral phase separation in PS/PC mixtures (Onishi and Ito, 1974). To mimic normal membrane conditions the monolayers were compressed to 32 mN/m (Seelig, 1987), and constant lateral pressure was maintained during experiments by computer controlled compensatory barrier movements. The monolayer area at 32 mN/m was about 7905 $mm^2$. The experiment was started ten minutes after attaining a stable surface pressure by introducing amphiphile suspension by a Hamilton syringe into the magnetically stirred sub-phase in the reaction chamber. The amphiphile concentrations used (Table I) were chosen in such a way that the amount of amphiphile/monolayer area was equivalent to the amount of amphiphile/erythrocyte plasma membrane area at which the amphiphile induces a pronounced shape alteration (sphero-echinocytes or sphero-stomatocytes), but no hemolysis (Isomaa *et al.*, 1987; Hägerstrand and Isomaa, 1991). In case of decanol, which does not induce typical echinocytic or stomatocytic shapes, a concentration being 75% of that where lysis starts was considered. The monolayer expansion in time was plotted in graphs like that shown in Figure 1. Usually three experiments were performed with each amphiphile. In experiments with mixed PS/PC monolayers, phospholipids were pooled before use. The purity of phospholipids was confirmed by determining the collapse pressure of monolayers composed of the phospholipids.

## Results

The degree and time-course of amphiphile-induced monolayer expansions are shown in Table I. We used amphiphiles at a concentration/monolayer area equivalent to the sublytic concentration/erythrocyte surface area where the amphiphiles induce sphero-echinocytic or sphero-stomatocytic shapes (Isomaa *et al.*, 1987; Hägerstrand and Isomaa, 1991). The molar concentrations are given in Table I.

Table I. Characteristics of amphiphile-induced monolayer expansion.

| Amphiphile, conc. [μM] | | PC monolayer | | | | PS/PC monolayer | | | | Ratio of max. area expansion; PS/PC versus PC | Shape induced in erythrocytes */ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Maximum area expansion (A): $mm^2$ | min | 50% area expansion (B): min | $mm^2$/min | A $mm^2$ | min | B min | $mm^2$/min | | |
| Decanol, 1.5 | N | 26± 8 | 0.6 | 0.3 | 43 | 96±11 | 0.5 | 0.2 | 240 | 3.7 | D |
| C12-maltoside, 0.48 | N | 33±10 | 2.0 | 0.3 | 55 | 69±15 | 0.5 | 0.2 | 173 | 2.1 | E |
| C12E8, 0.54 | N | 49± 3 | 2.4 | 0.4 | 61 | 106± 2 | 1.6 | 0.2 | 265 | 2.2 | S |
| Dibucaine, 14.6 | C | 66± 9 | 0.8 | 0.2 | 165 | 247±18 | 1.0 | 0.3 | 412 | 3.7 | S |
| Chlorpromazine, 2.4 | C | 77± 3 | 3.1 | 0.4 | 96 | 133± 3 | 1.4 | 0.4 | 166 | 1.7 | S |
| C12-TAB, 3.6 | C | 73± 6 | 3.0 | 0.4 | 91 | 240± 9 | 2.1 | 0.5 | 240 | 3.3 | E→S |
| C12-sulfate, 0.46 | A | 71±11 | 3.9 | 1.0 | 36 | 286±33 | 3.5 | 0.4 | 358 | 4.0 | E |
| C12-zwittergent, 3.0 | Z | 59±12 | 8.3 | 3.6 | 8 | 65±18 | 0.8 | 0.2 | 163 | 1.1 | E |

D = discocyte (no shape change), E = echinocyte, S = stomatocyte.
N = nonionic, C = cationic, A = anionic, Z = zwitterionic, at pH 7.4.
*/ Adapted from Isomaa *et al.* (1987) and Hägerstrand and Isomaa (1991).

Comparing the amphiphile-induced expansion of PC and PS/PC monolayers; nonionic, cationic and anionic amphiphiles expanded the PS/PC monolayer area significantly more (1.7–3.7 times) than the PC monolayer area, and the initial expansion rate of the PS/PC monolayer was also faster (1.7–20.4 times) than that of the PC monolayer. The zwittergent also expanded the PS/PC monolayer faster than the PC one, but both monolayers to a similar extent.

When comparing the effects of amphiphiles on the absolute monolayer area expansion, as below, it should be kept in mind that the concentrations used where chosen according to their physiological effect on the erythrocyte membrane and that the concentrations of amphiphiles in the monolayers are not known.

The amphiphiles expanded the PC monolayer area in the order cationic ≈ anionic ≥ zwittergent > nonionic amphiphiles. The PC monolayer area increase was ~0.3–0.9%. The initial rate of PC monolayer expansion, i.e. the rate up to 50% of total expansion, was induced by the amphiphiles in the order cationic > nonionic > anionic > zwitterionic amphiphiles. The expansion rate with the zwittergent was low.

The PS/PC monolayer area was expanded by the amphiphiles in the order anionic ≥ cationic > nonionic ≥ zwitterionic amphiphiles. The monolayer area increase was ~0.8–3.6%. There were no clear differences in the initial rates whereby groups of differently charged amphiphiles expanded the PS/PC monolayer. The cationic dibucaine and the anionic C12-sulfate showed the highest expansion rates, while the lowest rate was observed with the zwittergent.

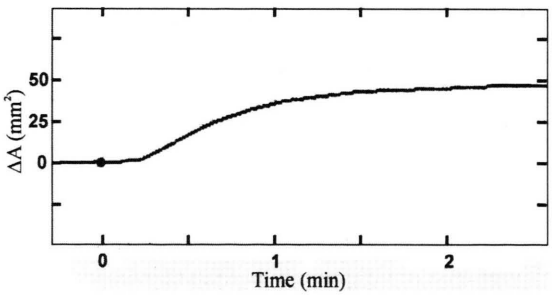


Fig. 1. Expansion ($\Delta A$) of a PC monolayer in time (min) induced by C12E8 (0.54 μM).

## Discussion

The results (Table I) show that, at the relative concentrations used, the nonionic amphiphiles expanded the PC and PS/PC monolayers less than the cationic and anionic amphiphiles.

In order to compare, per molecule, the expansive effect of amphiphiles intercalated into the monolayer, their partition should be known. We are not aware of such monolayer studies. Few comparable data on the partition of amphiphiles into bilayers were found from literature. Kragh-Hansen *et al.* (1998) reported partition coefficients of C12-maltoside = 28000, C12E8 = 28000 and C12-sulfate = 6000, using dioleoylphophatidylcholine unilamellar liposomes. Similar results they obtained with sarcoplasmic reticulum lipid vesicles. When considering these partition coefficients and the monolayer expansions induced by the amphiphiles (Table I) a qualitatively similar conclusion as above at a molecular level is obtained (Table II), i.e. the area expansion per molecule is much smaller for the nonionic amphiphiles than for the anionic one, especially in the PS/PC monolayer.

It may not be surprising that nonionic amphiphiles show a relatively weak capacity to expand monolayers, because these amphiphiles should not increase charge repulsion in the monolayers. The same may concern the zwittergent due to its net neutral charge. The strong expansion induced by C12-sulfate in the net negative PS/PC monolayer is what could be expected on electrostatic grounds. Surprisingly, dibucaine showed very high expansion rates in both monolayers. Possibly, counteracting effects of partition and effective membrane area

Table II. Relative monolayer expansion per amphiphile molecule.

| Amphiphile | PC-monolayer 10 (−3) | PS/PC-monolayer 10 (−3) |
|---|---|---|
| C12-maltoside | 2.5 | 5.1 |
| C12E8 | 3.2 | 7.0 |
| C12-sulfate | 25 | 104 |

The relative monolayer expansion per amphiphile molecule ($mm^2$ monolayer expansion/ subphase concentration × partition coefficient) is shown. The monolayer expansions and the amphiphile concentrations are given in Table I, while the partition coefficients, taken from (Kragh-Hansen *et al.*, 1998), are given in the text .

expansion may explain the similar effects of anionic and cationic amphiphiles in both monolayers.

It remains unclear whether the generally stronger amphiphile-induced expansion of the PS/PC monolayer, compared to that of the PC monolayer, is due to properties of the hydrophobic (e.g. saturation) or the hydrophilic (e.g. charge) part of the monolayers. Both a higher partition of amphiphiles into the PS/PC monolayer and a stronger expansive effect of amphiphiles in this monolayer are plausible explanations. The zwitterionic PC has one saturated and one unsaturated chain, while bovine brain PS is a negatively charged natural product with mixed chains of variable length and degree of saturation. The net negative charge of PS and its high degree of alkyl chain unsaturation should make the PS/PC monolayer more loosely packed than the PC monolayer. This could increase the amphiphile partition into it. Interestingly, it has been shown that monolayer-expansion induced by human erythrocyte spectrin (net negatively charged) is mostly pronounced in monolayers composed of phospholipids with a negatively charged head group and a natural alkyl chain composition, like bovine brain phosphatitdylserine (Mombers *et al.*, 1980). The increase in spectrin-induced monolayer expansion correlated with an increase in area per molecule occupied by he different phospholipids. Results showing that liposomes composed of bovine brain phosphatidylserine mix with each other (lipid-mixing occur), while liposomes composed of a palmitoyloleoylphosphatidylserine do not (Walter and Siegel, 1993), demonstrate the influence of alkyl chain packing on phospholipid vesicle properties.

A comparison of amphiphile effects on monolayer expansion and erythrocyte shape (Table I) indicates that the monolayer results cannot explain the shape alterations in line with the bilayer couple hypothesis. The nonionic, cationic and anionic amphiphiles all expanded the PS/PC monolayer more than the PC monolayer.

Although the bilayer couple hypothesis (see introduction) successfully explains shape alterations induced by a variety of ionic (charged) amphiphiles (Deuticke, 1968; Fujii *et al.*, 1979), it has been thought that it cannot account for the stomatocytic effect of nonionic amphiphiles like C12E8 and triton X-100 for which there have been no a priori reason to assume an asymmetrical membrane insertion on electrostatic grounds (Isomaa *et al.*, 1987; Deuticke *et al.*, 1990). Consequently, alternative explanations have been looked for. In compatibility with our monolayer data and reports indicating that amphiphiles expand the inner membrane leaflet of the human erythrocyte more effectively than the outer leaflet (Mohandas *et al.*, 1978; Tamura *et al.*, 1987), it could be speculated that a combination of the transmembrane amphiphile distribution (which may be symmetric or asymmetric) and a stronger amphiphile-induced expansion of the inner leaflet determines the membrane bending (cell shape) of permeable amphiphiles like C12E8. Nonetheless, a recent report stress that stomatocytosis induced by polyoxyethylene detergents like $C_{12}E_8$ and triton X-100 can be very well explained by the bilayer couple hypothesis when taking into account the ability of polyoxyethylene detergents to complex cations and thereby attain a positive charge character (Hägerstrand *et al.*, 2001).

Our results indicate that C12E8 has a slightly more expanding effect on monolayers than C12-maltoside (Tables I and II). This is in line with studies reporting that the polyoxyethylene head group of C12E8 may complex cations (Hägerstrand *et al.*, 2001) and attain a coiled, monolayer expanding, formation (Lantzsch *et al.*, 1996). While C12E8 is stomatocytogenic, C12-maltoside is echinocytogenic (Isomaa *et al.*, 1987). C12-maltoside is probably echinocytogenic because it is, due to the large relatively hydrophilic head group (Shinoda *et al.*, 1996), trapped in the outer membrane leaflet. The hydrophobic character of the head group of C12E8 (Shinoda *et al.*, 1996) apparently allows a rapid flip-flop of C12E8 in phospholipid bilayers (Kragh-Hansen *et al.*, 1998).

It can be noted that the amphiphiles, at the concentrations used, expanded the PC monolayer ~0.3–1.3%, i.e. largely the expansion of the outer leaflet of the erythrocyte membrane needed to induce a marked shape alteration (echinocytes) (Beck, 1978; Lange and Slayton, 1982; Iglic *et al.*, 1998). This may be taken to indicate that amphiphile partition and the expansive effect of amphiphiles are largely similar in the PC monolayer and in the outer leaflet of erythrocyte membrane.

*Acknowledgement*

We are indebted to Professor Peter Slotte, Department of Biochemistry and Pharmacy, Åbo Akademi University for letting us use his equipment.

K. B. was supported by the Centre for International Mobility (CIMO), Finland.

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