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Membrane potential-driven translocation of a lipid-conjugated rhodamine

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Abstract

The present study demonstrates that the permanently positively charged, lipid-conjugated rhodamine, R_{18}, can be transported from the outer to the inner leaflet of lipid bilayers in response of a transmembrane potential (negative inside). This conclusion was based on the following observations. (i) A fast decrease of the R_{18} fluorescence, when present at self-quenching concentrations in DOPC large unilamellar vesicles, was revealed upon induction of a valinomycin-induced K^+-diffusion potential. (ii) Iodide quenching experiments demonstrated that R_{18} was no longer accessible to externally added aqueous quencher after application of a transmembrane potential. (iii) ^{2}H-NMR measurements, using DOPC, specifically deuterated at the α-position of the phosphocholine head group, revealed a massive transbilayer movement of R_{18} upon induction of a membrane potential. The extent of the fluorescence changes were found to be dependent on the magnitude of the applied transmembrane potential, which opens possibilities for novel applications of R_{18} as an internal lipid-conjugated membrane potential probe.

Keywords: Octadecyl Rhodamine B; Membrane potential; Large unilamellar vesicle; Lipid transport

1. Introduction

Lipid-conjugated rhodamines are widely used in lipid mixing assays, either based on energy transfer between rhodamine and 7-nitrobenz-2-oxa-1,3-diazol-4-yl (NBD) [1], or based on the self-quenching of rhodamine [2]. The latter assay requires labeling of membranes with only one kind of fluorescent probe, most commonly octadecyl-rhodamine B (R_{18}) at self-quenching concentrations. Dilution of the probe by fusion with unlabeled target membranes allows the measurement of lipid mixing. This method has been especially applied in virus/cell fusion studies, because of the advantage that intact biological membranes can be labeled by exogenous addition. However, recently some need for caution was suggested in the literature, regarding the R_{18}-assay. The reasons for concern are based on the following observations. (i) It was demonstrated that cholesterol, or probably any membrane-condensing lipid, enhances the self-quenching of R_{18} strikingly [3], and therefore one should be carefull when R_{18} is applied in measuring fusion of membranes of different lipid compositions. (ii) The probe has been found not to be uniformly distributed in (influenza) viral membranes and the rate and extent of fusion of reconstituted virosomes measured with the R_{18} assay were lower than when measured with a pyrene-phosphatidylcholine (PC) assay [4]. (iii) Also, in PC/fatty acid mixed liposomes a non-homogeneous distribution of R_{18} in the bilayers was found [5].

The present study describes the effect of a membrane potential ($\Delta\psi$) on R_{18} fluorescence. It demonstrates the transport of R_{18} from the outer to the inner leaflet of bilayers of large unilamellar vesicles (LUVs), upon application of a membrane potential (negative inside) across the bilayers, resulting in an increased self-quenching of the probe. This makes it difficult, if not impossible, to calibrate fusion from self-quenching when R_{18} is applied in measuring fusion of membranes, across which a $\Delta\psi$ exists. The results of this study further reveal that the process of $\Delta\psi$-induced transbilayer movement of R_{18} is massive

Abbreviations: $\Delta\psi$, membrane potential; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; α-$^2H_2$-DOPC, DOPC, specifically deuterated at the α-position of choline; DOPG, 1,2-dioleoyl-*sn*-glycero-3-phosphoglycerol; LUVs, large unilamellar vesicles; NFG, *N*-formylated gramicidin; NMR, nuclear magnetic resonance; PC, phosphatidylcholine; PG, phosphatidylglycerol; R_{18}, octadecyl-rhodamine B; TX-100, Triton X-100.

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and very fast, and it depends on the magnitude of the applied membrane potential. This leads to suggestions to use R_{18} as an internal $\Delta\psi$-probe in model membrane experiments.

2. Materials and methods

2.1. Lipids

R_{18} was obtained from Molecular Probes (Eugene, OR, USA). 1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-dioleoyl-*sn*-glycero-3-phosphoglycerol (DOPG) were purchased from Avanti Polar Lipids (Birmingham, AL, USA). DOPC, specifically deuterated at the α-position of choline head group (PO_4^--C^2H_2-CH_2-$N^+(CH_3)_3$; α-$^2H_2$-DOPC) was synthesized as described [6]. R_{18} concentrations were measured by absorbance, using $\epsilon^{556} = 93 \cdot 10^3$ M^{-1} cm^{-1} in methanol (Molecular Probes catalogue), or by fluorescence in the presence of 0.05% (v/v) Triton X-100 (TX-100), using known amounts of R_{18} as standard. Phospholipid concentrations were determined according to Rouser et al. [7].

2.2. Vesicle preparation

Dry mixed lipid films of DOPC, R_{18}, and, where indicated, DOPG, were hydrated by manual shaking in buffer consisting of 150 mM K_2SO_4, 20 mM Hepes (pH 7.0) and 2 mM EDTA (K^+-buffer), followed by 5 times freezing and thawing. Large unilamellar vesicles (LUVs) were prepared by the extrusion technique [8] using 100 nm (fluorescence spectroscopy) or 400 nm (NMR experiments) polycarbonate filters. When desired, to these LUVs (K^+_{in}/K^+_{out}) an ion gradient was applied by replacing the external buffer by Na^+-containing buffer (consisting of 150 mM Na_2SO_4, 20 mM Hepes (pH 7.0) and 2 mM EDTA), by passing them through 1 ml Sephadex G-50 spin columns eluted with Na^+-buffer (resulting in K^+_{in}/Na^+_{out} LUVs).

2.3. Fluorescence measurements

Fluorescence of R_{18} was measured on a Perkin Elmer L550B luminescence spectrometer, using an excitation wavelength of 560 nm and an emission wavelength of 590 nm (with 5 nm band passes). All experiments were carried out at 20° C under continuous stirring. LUVs (5 to 10 mM lipid; K^+_{in}/K^+_{out} or K^+_{in}/Na^+_{out}, as indicated) containing R_{18}, were added to 1.2 ml Na^+-buffer, K^+-buffer or a combination of Na^+-and K^+-buffer. When indicated, valinomycin (Boehringer, Mannheim, Germany; 1 μl from stock solutions of different concentrations in ethanol) was added to the LUV-solution. To dissipate the membrane potential, *N*-formylated gramicidin (NFG) [9] from a 0.5 mM solution in dimethylsulfoxide, was added to the LUVs to a 1:50 molar ratio, with respect to lipid. Triton X-100 (0.05% (v/v) final concentration) was added to relieve R_{18} self-quenching.

Quenching of R_{18} fluorescence by iodide was determined by reading the fluorescence intensity after the addition of increasing amounts of NaI from a 2 M stock solution, containing 1 mM $Na_2S_2O_3$ to prevent I_2 and I_3^- formation. The addition of NaI led to an immediate decrease in fluorescence, which was then stable, except for quenching of R_{18}, incorporated in K^+_{in}/K^+_{out} LUVs in the presence of valinomycin. In that case, the initial fast decrease was followed by a slow decrease, after which a stable fluorescence level was reached (within a few minutes). From this value the quenching was determined. The data were analyzed according to the Stern-Volmer equation for collisional quenching [10]: $F_0/F = 1 + K_{SV} \cdot [NaI]$, where K_{SV} is the quenching constant and F_0 and F are the fluorescence intensities in the absence and presence of iodide, respectively.

2.4. NMR experiments

Deuterium nuclear magnetic resonance (^{2}H-NMR) spectra were recorded at 46.1 MHz on a Bruker MSL-300 spectrometer at 5°, as described [6]. Samples of LUVs contained 50 μmol of lipid in 0.5–1.0 ml buffer. To generate a membrane potential, valinomycin was added to LUVs, exhibiting a K^+_{in}/Na^+_{out}-ion gradient, to a $1{:}10^5$ molar ratio, with respect to lipid. Dissipation of $\Delta\psi$ was achieved by keeping the sample of LUVs to which valinomycin had been added for 48 h at 4° C.

3. Results

3.1. Fluorescence measurements

In studies on the effect of a membrane potential on certain (semi-) fusion processes, in which R_{18} was used to monitor lipid mixing, it was observed that a valinomycin-induced K^+-diffusion potential affected the fluorescence intensities of LUVs with R_{18} incorporated in the bilayers. The typical changes in fluorescence, observed in such experiments are shown in Fig. 1. DOPC LUVs, containing 5 mol% R_{18}, experiencing a K^+_{in}/Na^+_{out}-ion gradient, were diluted either in Na^+-buffer or in K^+-buffer. Upon addition of valinomycin, a K^+-diffusion potential is generated in case of dilution in Na^+-buffer. This led to a rapid decrease in fluorescence (by about 30%). When the $\Delta\psi$ was dissipated by the addition of NFG, the fluorescence increased again to almost the same level as in LUVs without $\Delta\psi$, indicating the reversibility of the observed effects. In the control situation (K^+-buffer), the additions of valinomycin and NFG did not affect the R_{18} fluorescence. Addition of TX-100 to dissolve the vesicles led to the relieve of R_{18} self-quenching.

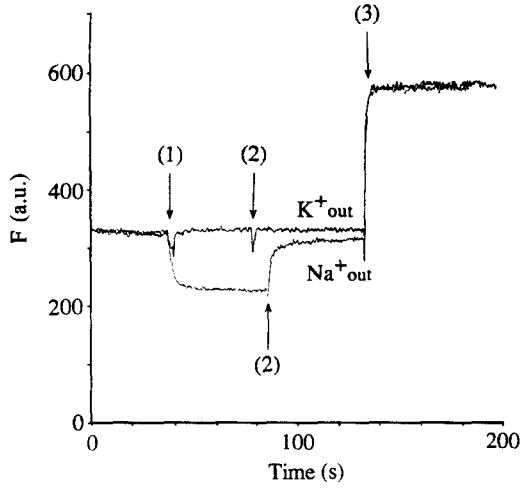

Fig. 1. Typical changes in R_{18} fluorescence upon application of a $\Delta\psi$ across R_{18}-containing DOPC bilayers. LUVs (15 nmol lipid) consisting of DOPC/R_{18} (95:5, molar ratio), experiencing a K^+_{in}/Na^+_{out} ion gradient, were diluted in either Na^+-or K^+-buffer (Na^+_{out} and K^+_{out}, respectively). The arrows indicate the subsequent additions of (1) valinomycin (1:4000, molar ratio with respect to lipid), (2) NFG, and (3) TX-100.

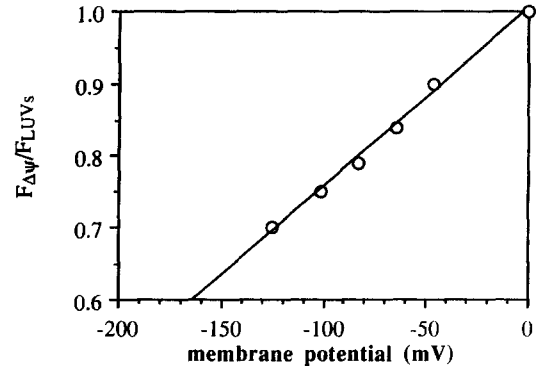

Fig. 3. The increased self-quenching of R_{18} upon induction of $\Delta\psi$ is proportional to the magnitude of $\Delta\psi$. LUVs consisting of DOPC/R_{18} (95:5, molar ratio) and experiencing a K^+_{in}/Na^+_{out} ion gradient, were diluted in buffer containing different concentrations of K^+. Fluorescence was measured before and after the addition of valinomycin (1:4000 molar ratio, with respect to lipid) to the LUV solution (F_{LUVs} and $F_{\Delta\psi}$, respectively). The value of $\Delta\psi$ was calculated according to $\Delta\psi = -59 \cdot \log([K^+]_{in}/[K^+]_{out})$ mV.

The most straight-forward explanation for the observed changes in fluorescence is the transport of the positively charged R_{18} from the outer to the inner leaflet upon application of a $\Delta\psi$ (negative inside). This would lead to an increased concentration of R_{18} in the inner leaflet, which in turn would result in an increased self-quenching (decreased fluorescence). In order to test this hypothesis, the accessibility of R_{18} from the external medium was investigated. For this purpose, fluorescence quenching experiments were done, using the aqueous quencher iodide. In Fig. 2, the results are shown in a Stern-Volmer plot (see Materials and methods). R_{18}, symmetrically incorporated in the two leaflets of the bilayer of DOPC LUVs, is quenched efficiently with a Stern-Volmer constant (K_{SV}; slope of the line) of 21 M^{-1}. When K^+_{in}/K^+_{out} LUVs are present in K^+-buffer and valinomycin is added to these LUVs, the quenching efficiency of iodide is increased by almost a factor of 2 ($K_{SV} = 38\ M^{-1}$) compared to quenching in the absence of valinomycin. It seems as if iodide is able to co-translocate with the valinomycin/K^+-complex, resulting in quenching at both sides of the bilayer. This was also suggested by the fact that the initial fast quenching was followed by a slow phase in this case (see Materials and methods). When a valinomycin-induced K^+-diffusion potential was induced, almost no quenching of R_{18} fluorescence could be detected ($K_{SV} = 2\ M^{-1}$), indicating that hardly any R_{18} molecules are accessible from the external medium. Co-translocation of iodide with the valinomycin/K^+-complex is unlikely in this case, because of the presence of $\Delta\psi$ (negative inside) across the bilayer. When the valinomycin-induced $\Delta\psi$ is collapsed with NFG, the R_{18} molecules become accessible again, and are as efficiently quenched as was the case with K^+_{in}/K^+_{out} LUVs in the presence of valinomycin.

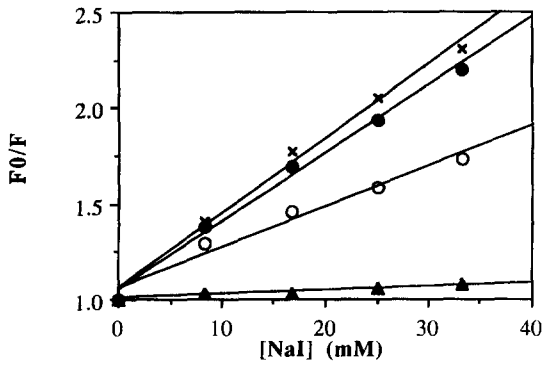

Fig. 2. Stern-Volmer plots of quenching of R_{18} fluorescence by iodide. LUVs were made of DOPC/R_{18} (95:5, molar ratio) in K^+-buffer (K^+_{in}). Valinomycin was added to a 1:4000 molar ratio with respect to lipid. (○), K^+_{out}/no valinomycin; (●), K^+_{out}/valinomycin; (▲), Na^+_{out}/valinomycin; (×), Na^+_{out}/valinomycin/NFG. F_0 and F are fluorescence intensities in the absence and presence of quencher, respectively.

In order to quantify the $\Delta\psi$-induced increased self-quenching of R_{18}, the value of $\Delta\psi$ was varied. Therefore, PC/R_{18} (95/5 molar ratio) LUVs, experiencing a K^+_{in}/Na^+_{out}-ion gradient, were diluted in buffer containing different concentrations of K^+, and the Nernst potential was calculated. Fig. 3 shows that the $\Delta\psi$-induced increased self-quenching of R_{18} linearly increased with the magnitude of the membrane potential. This is probably due to less R_{18} molecules being transported in response to the smaller $\Delta\psi$. To confirm that, iodide quenching experiments were done after induction of K^+-diffusion potentials of different values across R_{18}-containing bilayers of PC LUVs. The resulting Stern-Volmer plots are shown in Fig. 4. With smaller values of the Nernst potential, the quenching by iodide increased, as is obvious from the steeper Stern-Volmer lines, indeed indicating that more R_{18}

molecules are still present in the outer leaflet and accessible to the externally added quencher.

To investigate whether the presence of a negative surface charge could influence the $\Delta\psi$-induced transport of the positively charged R_{18} molecules, different amounts of the negatively charged phospholipid DOPG were incorporated in LUVs, containing 5 mol% R_{18}. The extent of the $\Delta\psi$-induced decrease in fluorescence of R_{18}-containing LUVs was independent on the PG content (0–50 mol%) in the membranes (data not shown). Moreover, the disappearance of R_{18} from the outer leaflet upon induction of $\Delta\psi$ could be demonstrated using iodide, although the quenching efficiency of this anion was decreased by the negative surface charge (not shown). The half-time of R_{18} translocation, upon addition of valinomycin to a 1:4000 molar ratio with respect to lipid, was approx. 1.5 s and independent on the concentration of PG in the membranes (Fig. 5). Addition of higher amounts of valinomycin did not change the half-time of R_{18} translocation. However, with a 10-fold decreased amount of valinomycin added to the LUVs, the rate of R_{18} translocation was dependent on the PG content (Fig. 5). With decreasing PG content, the half-time of the process increased from 1.6 to 6.2 s. A likely explanation for this is that the rate of potential-driven R_{18}-translocation is limited by the rate with which the K^+-diffusion potential is generated by valinomycin. This would mean that the $\Delta\psi$-driven R_{18} transport is faster than the translocation of valinomycin/K^+-complexes across the membrane. Moreover, the translocation of the valinomycin/K^+-complex across the bilayer, and not that of R_{18}, would then be dependent on the PG-content in the membrane.

3.2. NMR experiments

For an alternative approach to test the hypothesis of $\Delta\psi$-driven R_{18}-translocation from the outer to the inner leaflet, ^{2}H-NMR experiments were done. For this purpose, DOPC was specifically labeled at the α-position of its choline head group. The quadrupolar splitting ($\Delta\nu_q$) of this deuterated lipid has been demonstrated to be sensitive to the conformation of the head group [11]. The head group conformation in turn is dependent on the surface charge density of the bilayers, according to the so-called electrometer concept [12], and not to a transmembrane potential [6]. Furthermore, ^{2}H-NMR of head group-labeled DOPC has been shown to provide a method to monitor the individual behavior of the two lipid interfaces in large unilamellar vesicles [6]. This allows for the detection of the expected redistribution of the positively charged R_{18}, induced by the $\Delta\psi$. First, the effects of R_{18}-induced positive surface charge on the $\Delta\nu_q$ of the α-$^2H_2$-DOPC LUV system was confirmed. With 20 mol% R_{18} incorporated in the membrane, the change in $\Delta\nu_q$ was 2 kHz (not shown). Next, the effect of a membrane potential on the R_{18} localization in the two leaflets of the bilayer was studied. For this experiment, LUVs consisting of α-$^2H_2$-DOPC/DOPG/R_{18} (5:3:2 molar ratio) were used. DOPG was included in the membrane, because it will increase the spectral window to observe the expected asymmetrical effects on the bilayer, as was demonstrated earlier [6]. The resulting NMR spectra are shown in Fig. 6. Spectrum A depicts the spectrum of LUVs with a K^+_{in}/K^+_{out} ion distribution, with a $\Delta\nu_q$ (distance between the two peaks) of 7.6 kHz. After changing the external buffer for Na^+-buffer, the spectrum remains identical (not shown). Upon addition of valinomycin to the LUVs, experiencing a K^+_{in}/Na^+_{out} ion gradient, the spectrum did change drastically (spectrum B). Now two doublets are observed, reflecting indeed the asymmetrical distribution of the R_{18} molecules, with an increased positive surface charge of the inner leaflet ($\Delta\nu_q$ decreased with approx. 2 kHz) and a less positively charged outer leaflet resulting in an increased $\Delta\nu_q$ (also by approx. 2 kHz). The fact that the $\Delta\nu_q$ changes with 2 kHz in either

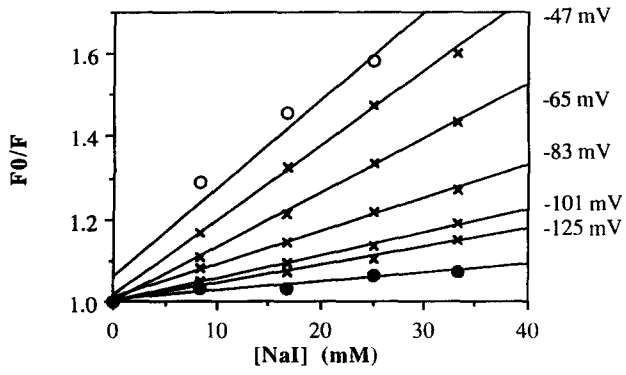

Fig. 4. Stern-Volmer plots of quenching of R_{18} fluorescence by iodide after application of K^+-diffusion potentials of the indicated values across the bilayers. LUVs were made of DOPC/R_{18} (95:5, molar ratio). The quenching was determined after induction of $\Delta\psi$ of different values, as described in the legend of Fig. 3. (○), K^+-buffer/no valinomycin ($\Delta\psi = 0$); (●), Na^+-buffer/valinomycin ($\Delta\psi = -\infty$). F_0 and F are fluorescence intensities in the absence and presence of quencher, respectively.

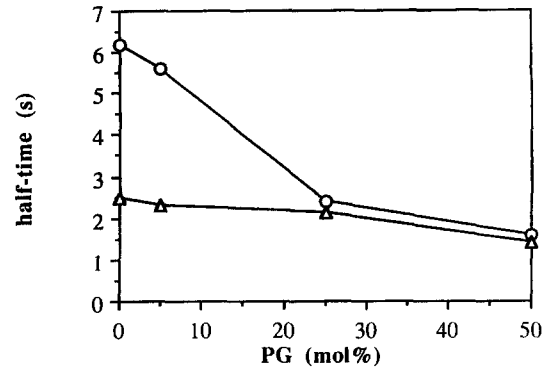

Fig. 5. Half-times of the $\Delta\psi$-induced enhancement of R_{18} self-quenching as a function of the PG content in the LUVs. Vesicles (K^+_{in}/K^+_{out}) consisting of DOPC/DOPG (different molar ratios), containing 5 mol% R_{18}, were diluted 240-fold in Na^+-buffer. Valinomycin was added to a 1:4000 (triangles) or a 1:40000 (circles) molar ratio, with respect to lipid.

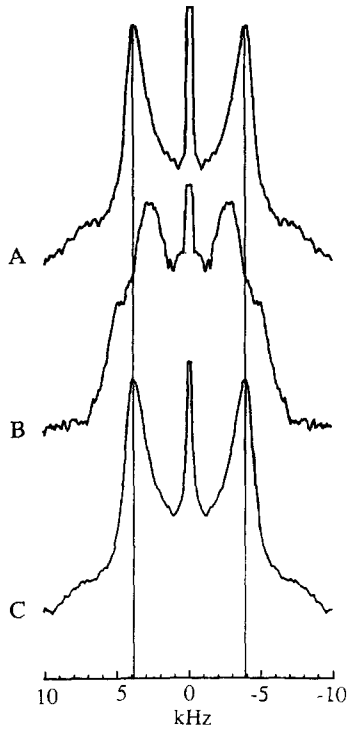

Fig. 6. Effect of $\Delta\psi$ on R_{18} distribution between the inner and outer leaflet of the bilayers of LUVs. ^{2}H-NMR spectra of LUVs consisting of α-$^2H_2$-DOPC/DOPG/R_{18} (5:3:2, molar ratio) with K^+_{in}/K^+_{out} (A), or K^+_{in}/Na^+_{out} after the addition of valinomycin (B), so that a membrane potential is generated; and after the dissipation of the membrane potential (C).

direction, indicates that almost all R_{18} molecules (20 mol%) are translocated from the outer to the inner leaflet upon induction of $\Delta\psi$. After dissipation of the $\Delta\psi$, the R_{18} redistributes again symmetrically across the bilayer as revealed by the single doublet in the ^{2}H-NMR spectrum (C), demonstrating the reversibility of the R_{18} translocation.

4. Discussion

The present study demonstrates the translocation of endogenous lipid-conjugated rhodamine, R_{18}, from the outer to the inner leaflet of the bilayer, upon induction of a transmembrane potential. This result may seem not too surprising given the fact that rhodamine dyes have been revealed to respond to $\Delta\psi$ [13,14], but it is to our knowledge for the first time that it is described for a lipid to move from one leaflet of the bilayer to the other as a result of a membrane potential. Apparently, $\Delta\psi$ can drive the accumulation of rhodamine even if it is lipid-conjugated.

The time scale with which both the forward (flip) and the backward (flop) movement of R_{18} across the bilayer take place, as revealed by the fluorescence measurements (see Fig. 1), appeared to be very short. Both processes seem to take place in the seconds time-scale and for the flip-process, the half-time was determined to be 1.5 s. The observation that the rate of the flop-process is comparable to that of the flip-process (according to Fig. 1), can be explained in different ways. One possibility is that accumulation of R_{18} in the inner leaflet of the bilayer leads to increased packing density, which might result in an unfavorable balance of forces over both leaflets in the membrane. This imbalance can exist as long as the driving force ($\Delta\psi$) for it is present, but as soon as the $\Delta\psi$ is collapsed the R_{18} molecules will redistribute. Alternatively, the spontaneous transbilayer movement of R_{18} might also be fast, so that if no $\Delta\psi$ is present the equilibrium is rapidly restored. This would then mean that the spontaneous R_{18}-translocation process would also display half-times on the order of seconds. However, according to Hoekstra et al. [2], the insertion of R_{18} into Sendai virus membranes resulted in the predominant localization of the probe in the outer leaflet of the bilayer.

In comparison with other known lipid transport processes, the $\Delta\psi$-induced R_{18} translocation process is very fast. Transmembrane movement of phospholipids in lipid vesicles display half-times on the order of several days, which can be speeded up by intrinsic membrane proteins or by perturbing the bilayer to half-times on the order of minutes [15]. For example, translocation of palmitoyllysoPC over the membrane of DOPC vesicles displays a half-time of several days [16]. In glycophorin-containing vesicles, this process takes place with an estimated half-time of about 1.5 h at 4° C [17]. Translocation of non-charged species is much more rapidly. For instance, diacylglycerol derivatives translocate with half-times of less than 15 s [18]. Also, the ΔpH-driven translocation of the neutral forms of different acids or bases, like stearylamine, sphingosine, fatty acids [19], phosphatidic acid or PG [20] is very rapid. For example, for the neutral form of phosphatidic acid the half-time was approx. 25 s at 45° C [21]. The present study demonstrates that the membrane potential is able to cause a very fast translocation of a permanently charged lipid.

The presence of up to 50 mol% PG in the membrane did not affect the rate and extent of membrane potential driven R_{18} translocation. Apparently, electrostatic interactions between the positively charged R_{18} and the negatively charged PG do not stably anchor the R_{18}-molecule in the interface and thereby slow down the translocation process.

The extent of the increased fluorescence self-quenching of R_{18} upon induction of $\Delta\psi$ is somewhat lower than expected. If, as a consequence of $\Delta\psi$, R_{18} would redistribute according to the Nernst equation, it could be expected that the $[R_{18}]_{in}/[R_{18}]_{out}$ would be 100, upon induction of a $\Delta\psi$ of -120 mV. With 5 mol% R_{18}, symmetrically incorporated in the bilayer, this would result in 0.1 and 9.9 mol% in the outer and inner leaflet, respectively, after induction of $\Delta\psi$. In that case, the self-quenching, which is linearly dependent on the R_{18}-content in the

membrane [2,3], would increase so that the R_{18}-concentration upon induction of $\Delta\psi$ would be approx. 2-fold increased. In case of the measurements, as shown in Fig. 1, the self-quenching is enhanced so that it corresponds to approximately a 1.5-fold increase in R_{18}-concentration in the membrane. Therefore, it must be concluded that either R_{18} does not redistribute according to the Nernst equation, or other factors affect the fluorescence changes. The first explanation is not likely, considering the iodide quenching data and the NMR experiments. The iodide quenching measurements revealed a decrease in quenching efficiency upon induction of $\Delta\psi$ by a factor of 10, indicating that the R_{18} concentration is at least 10 times decreased in the outer leaflet. The changes in $\Delta\nu_q$ in the ^{2}H-NMR experiments indicated a $\Delta\psi$-induced transbilayer movement of almost all R_{18} molecules. Therefore, it must be concluded that from the fluorescence self-quenching measurements no quantitative analysis can be made about the amount of R_{18} molecules that are translocated. However, after calibration of the system, the linear relationship between the $\Delta\psi$-induced enhancement of R_{18} self-quenching and the magnitude of the membrane potential could be used in the application of R_{18} as a quantitative membrane potential sensitive dye. For example, from the data in Fig. 3, it could be deduced that the value of $\Delta\psi$ when diluting the LUVs in Na^+-buffer without K^+-buffer, so that the calculated Nernst potential is $-\infty$, the magnitude of $\Delta\psi$ was about -150 mV. ($F_{\Delta\psi}/F_{LUV} = 0.65$). The advantage of such a probe could be that it can be introduced in LUVs as an internal probe in model membrane experiments. After calibration of the system, the membrane potential can be measured relatively easy, yet accurately, and in situ. Alternatively, the relative quenching efficiency of iodide could be used as a relative measure for the presence of a $\Delta\psi$. A more quantitative interpretation of these data is complicated, because of secondary effects of iodide on R_{18} self-quenching and the possibility of cotransport of iodide with the valinomycin/K^+-complex at smaller values of $\Delta\psi$. It might also be possible to apply R_{18} in biomembranes by introduction via exogenous addition. In combination with iodide quenching, R_{18} could be used as a qualitative $\Delta\psi$-probe in such systems.

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References

[1] Struck, D.K., Hoekstra, D. and Pagano, R.E. (1981) Biochemistry 20, 4093–4099.
[2] Hoekstra, D., De Boer, T., Klappe, K. and Wilschut, J. (1984) Biochemistry 23, 5675–5681.
[3] MacDonald, R.I. (1990) J. Biol. Chem. 265, 13533–13539.
[4] Wilschut, J., Bron, R., Dijkstra, J., Ortiz, A., Van Ginkel, L.C., De Grado, W.F., Rafalski, M. and Lear, J.D. (1991) in Progress in Membrane Biotechnology (Gomez-Fernandez, J.C., Chapman, D. and Parker, L., eds.), pp. 317–334, Birkhäuser Verlag, Basel.
[5] Zellmer, S., Cevc, G. and Risse, P. (1994) Biochim. Biophys. Acta 1196, 101–113.
[6] Leenhouts, J.M., Chupin, V., De Gier, J. and De Kruijff, B. (1993) Biochim. Biophys. Acta 1153, 257–261.
[7] Rouser, G., Fleischer, S. and Yamamoto, A. (1970) Lipids 5, 494–496.
[8] Hope, M.J., Bally, M.B., Webb, G. and Cullis, P.R. (1985) Biochim. Biophys. Acta 812, 55–65.
[9] Killian, J.A., Timmermans, J.W., Keur, S. and De Kruijff, B. (1985) Biochim. Biophys. Acta 820, 154–156.
[10] Lehrer, S.S. (1971) Biochemistry 10, 3254–3263.
[11] Sixl, F. and Watts, A. (1982) Biochemistry 21, 6446–6452.
[12] Seelig, J., MacDonald, P.M. and Scherer, P.G. (1987) Biochemistry 26, 7535–7541.
[13] Aiuchi, T., Daimatsu, T., Nakaya, K. and Nakamura, Y. (1982) Biochim. Biophys. Acta 685, 289–296.
[14] Emaus, R.K., Grunwald, R. and Lemasters, J.J. (1986) Biochim. Biophys. Acta 850, 463–448.
[15] Op den Kamp, J.A.F. (1979) Annu. Rev. Biochem. 48, 47–71.
[16] De Kruijff, B., Van den Besselaar, A.M.H.P. and Van Deenen, L.L.M. (1977) Biochim. Biophys. Acta 465, 443–453.
[17] Van Zoelen, E.J.J., De Kruijff, B. and Van Deenen, L.L.M. (1978) Biochim. Biophys. Acta 508, 97–108.
[18] Ganong, B.R. and Bell, R.M. (1984) Biochemistry 23, 4977–4983.
[19] Hope, M.J. and Cullis, P.R. (1987) J. Biol. Chem. 262, 4360–4366.
[20] Hope, M.J., Redelmeier, T.E., Wong, K.F., Rodrigueza, W. and Cullis, P.R. (1989) Biochemistry 28, 4181–4187.
[21] Eastman, S.J., Hope, M.J. and Cullis, P.R. (1991) Biochemistry 30, 1740–1745.