# Changes of Intrinsic Membrane Potentials Induced by Flip-Flop of Long-Chain Fatty Acids†

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ABSTRACT: The passive transbilayer movement—flip-flop—was investigated on planar bilayer lipid membranes (BLMs), containing myristic, stearic, or linoleic long-chain fatty acids (FA). In response to a transbilayer pH gradient, a difference in the surface charges between inner and outer leaflets appeared. Because the BLM was formed from FA and neutral lipid, a surface potential difference was originated solely by a concentration difference of the initially equally distributed ionized FA. As revealed by ζ-potential measurements, the corresponding surface potential difference $\Delta\Phi_s$ was at least twice the value expected from a titration of the FA alone. The additional surface charge was attributed to FA flip-flop induced by the transbilayer pH gradient. $\Delta\Phi_s$ was derived from capacitive current measurements carried out with a direct current (dc) bias and was corrected for changes of membrane dipole potential $\Phi_d$. Dual-wavelength ratiometric fluorescence measurements have shown that $\Phi_d$ values of the pure DPhPC bilayers and BLMs containing 40 mol % FA differ by less than 6%. It is concluded that fast FA flip-flop is not restricted to membranes with high curvature. The role of pH gradient as an effective driving force for the regulation of FA uptake is discussed.

The question of how lipophilic acids move in cells is important for the understanding of long-chain fatty acid (FA)[1] metabolism and its intermediate role in signal transduction, ion channel activation (*1*−*3*), and oxidative phosphorylation (*4*). The central problem is whether the transport of FA proceeds spontaneously or the intervention of the membrane protein is required. The transmembrane step (flip-flop) is the most controversial one even in simple model systems (*5*). One of the essential points that makes research difficult is the impossibility of measuring the flip-flop directly. The movement of fluorescent-labeled FA frequently used as probes may not reflect that of native FA (*6*). It is also not clear whether the derivatized FA are representative of native FA (*7*−*9*). The poor solvation of long-chain FA creates additional difficulties. To overcome this problem, fatty acids bound to albumin are used in most uptake assays in vitro. Consequently, protein-related parameters have to be taken into account (*10*). Experiments with whole cells do not allow one to avoid the presence of membrane proteins. This circumstance complicates the separation of protein- and lipid-mediated processes.

On the model system level (vesicular membranes), it was shown that the transport rate for the flip-flop is fast enough to make transport mediation by proteins unnecessary (*5*). However, the relevancy of the liposome model for cellular processes is questioned because of differences in several parameters. Membrane curvature seems to be most important among them. Because membrane curvature appears to be carefully regulated by living organisms (*11*), this property is likely to have a significant effect on membrane function. Direct evidence for the influence of curvature on the activity of membrane proteins such as rhodopsin (*12*), the voltage-gated ion channel alamethicin (*13, 14*), and the ion channel gramicidin (*15, 16*) have been reported. Other processes such as aggregation of galactose vesicles (*17*) and budding and fission in mixtures of dimyristoylphosphatidylcholine with cholesterol (*18*), as well as the cholesterol transfer between vesicular membranes (*19*), were also shown to depend on spontaneous membrane curvature. Moreover, differences in the flip-flop rate of long-chain fatty acids were observed for small and large unilamellar vesicles (*7, 20*). It was shown that the transport rate increases with decreasing vesicle size although flip-flop of natural fatty acids was very fast in both types of vesicles ($t = 5-10$ ms and $t_{1/2} = 23 \pm 12$ ms for small and large liposomes respectively) (*21*). Measurements on whole cells, whose membranes are characterized by very low membrane curvature, have revealed a significant inverse correlation of the flip-flop rate with object diameter (*22, 23*). In the extreme case, i.e., at zero membrane curvature, passive FA exchange between the membrane leaflets may be supposed to be very slow, if not impossible. The goal of our investigations carried out on planar lipid membranes is to test this hypothesis.

In this work we have applied a method (IFC) that enabled us to measure the surface potential difference between two monolayers, caused by unequal amounts of fatty acids in

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[1] Abbreviations: BLM, bilayer lipid membrane; FA, fatty acid; $FA^-$, fatty acid anion; FFA, free fatty acid; PTFE, poly(tetrafluoroethylene); DPhPC, diphytanoylphosphatidylcholine; IFC, intramembrane field compensation; MES, 4-Morpholineethanesulfonic acid; CAPSO, 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid; TES, *N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid; di-8-ANEPPS, 1-(3-sulfonatopropyl)-4-[β-[2-(di-*n*-octylamino)-6-naphthyl]vinyl]pyridinio-betaine.

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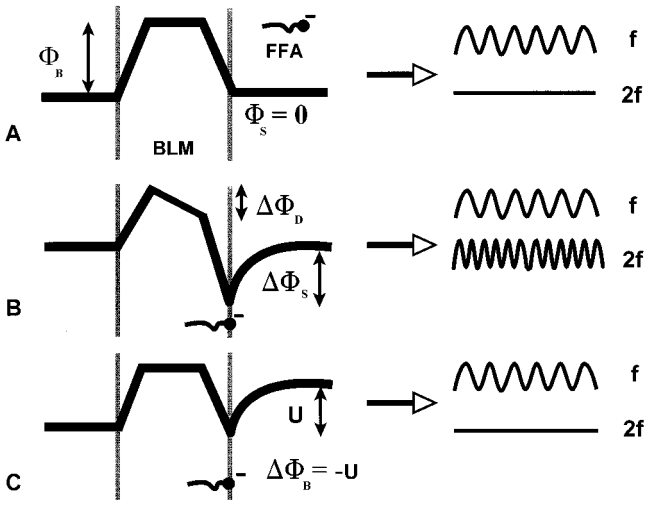


FIGURE 1: Principle of the intramembrane field compensation method (IFC). (A) The capacitive current of a symmetrical bilayer does not contain an overtone (2*f*) of the capacitive current after application of a sine wave with frequency *f*. (B) It appears due to the adsorption of fatty acid anions ($FA^-$) to one of the monolayers. (C) This signal disappears if the boundary potential difference $\Delta\Phi_b$ is compensated by a dc voltage equal to $\Delta\Phi_b$ in amplitude but opposite in sign.

each monolayer. The transmembrane step of the transport was measured on planar bilayer membranes directly, without any FA modifications (Figure 1). The combination of the IFC method with measurements of vesicle electrophoretic mobility and dual-wavelength ratiometric fluorescence measurements allowed us to separate the contribution of FA transport to membrane dipole and surface potential.

## MATERIALS AND METHODS

*Chemicals.* Diphytanoylphosphatidylcholine (DPhPC) was obtained from Avanti Polar Lipids (Alabaster, AL). The lipid was stored at −70 °C and used without further treatment. For the preparation of buffer solutions, MES (Fluka, Buchs, Switzerland), Tris (Fluka, Buchs, Switzerland), TES (Fluka, Buchs, Switzerland), CAPSO (Sigma Chemical Co., St. Louis, MO), and KCl (Fluka, Buchs, Switzerland) were used. Stearic, myristic, and linoleic acid were obtained from Fluka Chemie AG (Buchs, Switzerland). The potential-sensitive fluorescence dye 1-(3-sulfonatopropyl)-4-[β-[2-(di-*n*-octylamino)-6-naphthyl]vinyl]pyridiniobetaine (di-8-ANEPPS) was obtained from Molecular Probes (Eugene, Oregon).

*Formation of BLM.* The BLMs were formed by a conventional method (*24*) in a hole, 0.5−1 mm in diameter, of a diaphragm of a PTFE chamber. The membrane-forming solution contained DPhPC (maximum 20 mg/mL) and an appropriate amount of fatty acid (10−40 mol %), dissolved in *n*-decane (Merck, Darmstadt, Germany). The bilayer surrounding solution contained typically 10 mM Tris, 10 mM MES, and 50 mM KCl. It was agitated by magnetic bars. The experiments were carried out at room temperature (23−25 °C).

*Preparations of Liposomes.* DPhPC and FA (10−40 mol %) were dissolved in chloroform. Removal of solvent by evaporation resulted in a thin lipid film on the wall of a round-bottom flask. An appropriate volume of buffer (10 mM Tris, 10 mM MES, 10 mM CAPSO, and 50 mM KCl) was then added and the solution was vortexed for 3 min. Large unilamellar vesicles were prepared by an extrusion technique (*25*) using the small-volume apparatus LiposoFast (Avestin Inc., Ottawa, Canada) with filters of 100 nm pore diameter. The final lipid concentration was 0.2 mg/mL.

*Monitoring of the Surface Potential Drop between the Lipid Monolayers by the Inner Field Compensation Method.* The boundary potential of lipid membrane $\Phi_b$ (Figure 1A) is originated from outer-side (surface) and inner-side (dipole) potential (*26*). To measure differences between the boundary potentials at both sides of a bilayer we have used the inner field compensation method. It was introduced by Shoch et al. and Sokolov et al. (*27*−*30*).

The main idea of the method is shown in Figure 1. The application of a sine wave with frequency *f* (1 kHz) and amplitude *A* (10−30 mV) to the BLM (Figure 1A) leads to the formation of an overtone of capacitive current (2*f*) only if the boundary potential of the cis monolayer $\Phi_{b\ cis}$ is not equal to the boundary potential of the trans monolayer $\Phi_{b\ trans}$ (Figure 1B). This is the case if (i) charged molecules or dipole modifiers adsorb to one side of the membrane or (ii) if the BLM is asymmetrical, i.e., if $FA^-$ anions are incorporated in only one monolayer. If the dc signal, transferred to reference electrodes in addition to the ac signal, coincides with the boundary potential difference $-\Delta\Phi_b$, the signal harmonic to the fundamental frequency vanishes (Figure 1C). The magnitude of the dc signal *U* is given by

$$U = -(\Delta\Phi_s + \Delta\Phi_d) = -\Delta\Phi_b \qquad (1)$$

where $\Delta\Phi_s$ and $\Delta\Phi_d$ are, respectively, the surface potential and the dipole potential difference between two monolayers.

The electrical circuit was described earlier (*31*, *32*). In brief, it contained three computer-controlled devices: a function generator (Model 33120A, Hewlett-Packard, Loveland, CO), a Lock-In amplifier (HMS Elektronik, Leverkusen, Germany), and a current to voltage converter (Model 428, Keihley Instruments Inc., Cleveland, OH) and various self-made filters.

*Dual-Wavelength Ratiometric Fluorescence Measurements of the Membrane Dipole Potential.* Lipid bilayers possess a large membrane potential, positive inside, the so-called dipole (inner side) potential (*26*, *33*, *34*). By use of a fluorescence dye, di-8-ANEPPS, incorporated in unilamellar vesicle membranes, the absolute values of the dipole potential, $\Phi_d$, for pure and FA-containing liposomes were obtained (*35*, *36*). The intensity of dye fluorescence was measured by a fluorescence spectrophotometer (Oriel Instruments, Stratford, CT) at emission wavelength of 670 nm (Figure 3). The dipole potential, $\Phi_d$, was calculated as (*36*)

$$\Phi_d = \frac{R - 0.4}{5.4 \times 10^{-3}} \qquad (2)$$

where *R* is the ratio of the dye fluorescence excited at 420 nm to that excited at 520 nm in a lipid vesicle suspension.

*ζ-Potential Measurements.* Measurements of the electrophoretical mobility of FA-containing liposomes were carried out with the Coulter DELSA 440 (Langley Ford Instruments, Coulter Electronics of New England, Inc.). The velocity of liposome movement in an electrical field was deduced from the Doppler shift of a scattered laser beam. To determine the surface potential, $\Phi_s$, from the electrophoretical mobility

of liposomes, we have used the Gouy−Chapman theory (*37*; compare also ref *32*). $\Phi_s$ was calculated according to the (37)

$$\Phi = \frac{2kT}{ze} \ln \frac{1 + \alpha \exp[-\kappa\delta]}{1 - \alpha \exp[-\kappa\delta]} \quad (3)$$

with

$$\alpha = \frac{\exp[ze\Phi_s/2kT] - 1}{\exp[ze\Phi_s/2kT] + 1} \quad (4)$$

and

$$\kappa = \left(\frac{2e^2z^2CN_A}{\epsilon\epsilon_0 kT}\right)^{1/2} \quad (5)$$

where e, $N_A$, $z$, $T$, $\epsilon$, and $\epsilon_0$ have their usual meanings and $C$, $1/\kappa$, $k$, and $\delta$ are, respectively, the concentration of the electrolyte, the Debye length, the Boltzmann constant, and the distance of the shear plane from the surface. The shear plane is an imaginary sphere around the liposome, which limits the solvent moving with the liposome as it moves through the solution. Assuming $\delta = 0.2$ nm (*38*, *39*), we obtain $\Phi = \zeta$.

The measurements of the electrophoretical mobility were carried out in the absence of a pH gradient, i.e., the pH values of buffer inside and outside liposomes were the same. Consequently, there was no difference in the FA content of inner and outer leaflets. It was also assumed that the membrane curvature does not influence the fatty acid distribution between both monolayers, because relatively large liposomes (100 nm) were used.

It must be taken into account that the standard deviation (SD) of $\zeta$-potential measurements is 2−3 mV. For a pH gradient from 7.5 to 6.8 an acceptable signal to SD ratio ($\Delta\Phi_s = \Phi_{s,7.5} - \Phi_{s,6.8} = 4$ mV; see Figure 2) was obtained only for high fatty acid concentration (40%). Because the comparison between surface potential and boundary potential changes at low FA concentration and small ΔpH was subject to a great experimental error, the measurements were carried out at maximal FA concentration, increasing the established ΔpH from 0.8 to 4.

## RESULTS

To estimate the contribution of surface charge changes due to FA dissociation at different pH values of the buffer, the electrophoretic mobility of liposomes was measured in the pH range from 3 to 10.5. Figure 2 shows the $\zeta$-potential of liposomes containing 40 mol % stearic acid. Because the true p*K* of the stearic acid in a lipid bilayer is not definitively known and the pH value in the vicinity of charged lipid surface generally differs from that in the bulk phase, the maximal value of the $\zeta$-potential can hardly be attributed to the ionization of all fatty acid molecules.

The boundary potential $\Phi_b$ is known to consist at least of two parts (outer and inner potentials, eq 1). The contribution of FA to the inner-side (dipole) potential $\Phi_d$ was investigated by the fluorescence method. As it was shown previously (*35*, *40*), the dual-wavelength ratiometric measurements of the fluorescence of a potential-sensitive dye, di-8-ANEPPS, do not depend on membrane charge and can be used to measure

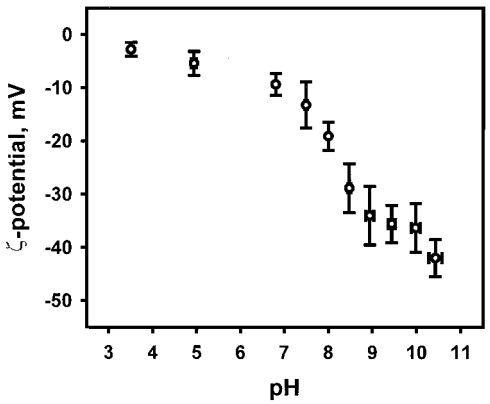


FIGURE 2: Dependence of $\zeta$-potential on buffer pH. Liposomes were made from DPhPC and stearic acid (60:40 mol %). The buffer solution contained 50 mM KCl, 10 mM Tris, 10 mM MES, and 10 mM CAPSO.

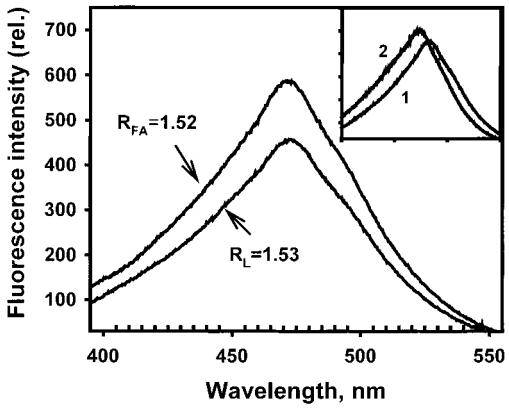


FIGURE 3: Examples of fluorescence spectra of di-8-ANEPPS bound to stearic-acid-containing (40 mol %) and to pure DPhPC liposomes. The buffer solution contained 50 mM KCl, 10 mM Tris, 10 mM MES, and 10 mM CAPSO (pH = 7.5), $T = 25$ °C. $R_L = 1.53$ and $R_{FA} = 1.52$ were calculated as the ratio of the dye fluorescence exited at 420 nm to that excited at 520 nm. $\lambda_{em} = 670$ nm. (Inset) Effect of phloretin on the fluorescence spectrum of di-8-ANEPPS-labeled liposomes. Excitation spectra in the presence (1, $R_1 = 0.89$) and in the absence (2, $R_2 = 1.44$) of 50 $\mu$M phloretin. The buffer solution contained 100 mM KCl and 30 mM TES, pH = 7.5.

changes of the local electric field associated with the variation of membrane dipole potential. In the inset of Figure 3, the spectrum shift (1) in the presence of the well-known dipole modifier phloretin is shown. $R_1 = 0.89$ and $R_2 = 1.44$ are the fluorescence ratios in the presence (1) and the absence (2) of phloretin. The dipole potential change $\Delta\Phi_d = 101$ mV, induced by 50 $\mu$M phloretin (pH = 7.5) and calculated according to eq 2, is in good agreement with that obtained with electrical methods previously (*31*). Figure 3 (main figure) shows typical fluorescence spectra obtained in the presence and in the absence of FA. $R_L$ and $R_{FA}$ were calculated as the ratio of the dye fluorescence excited at 420 nm to that excited at 520 nm in a lipid vesicle suspension. The difference between $\Phi_d$ of pure DPhPC membranes (195 ± 10 mV; eq 2) and $\Phi_d$ of DPhPC membranes with stearic acid was small (less than 6% at pH = 7.5). The alteration of $\Phi_b$ (see eq 1) mediated by fatty acids was supposed to be mainly determined by $\Phi_s$ changes.

To study the transmembrane step the following assumptions were made based on the knowledge obtained from

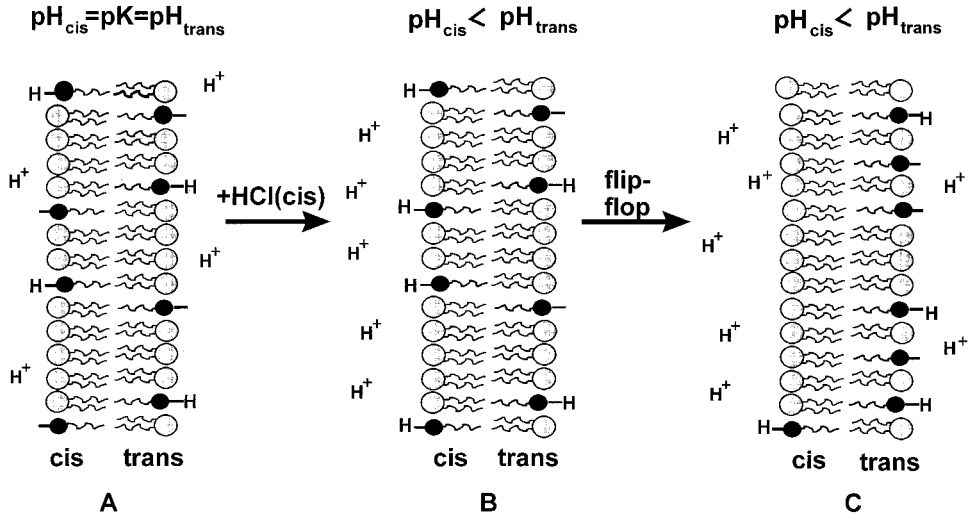


FIGURE 4: Modified diagram [after Kamp and Hamilton (*41*)] 1992) of FA movement across planar bilayer. (A) FA molecules are equally distributed in both leaflets of the BLM at the beginning of the experiment. (B) Lowering of the pH value on the cis side of the membrane leads to proton binding by FA. (C) FA diffuses to the trans side of the membrane and dissociates.

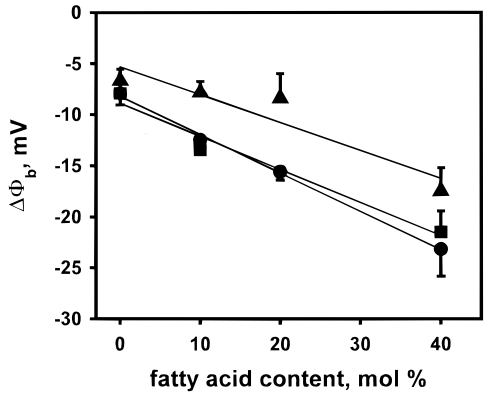


FIGURE 5: Dependence of boundary potential difference $\Delta\Phi_b$ on the fatty acid content in the DPhPC bilayer (●, linoleic acid; ▲, myristic acid; ■, stearic acid), measured by the IFC method. The buffer solution contained 50 mM KCl, 10 mM MES, and 10 mM Tris. pH$_{cis}$ = 6.8, pH$_{trans}$ = 7.5.

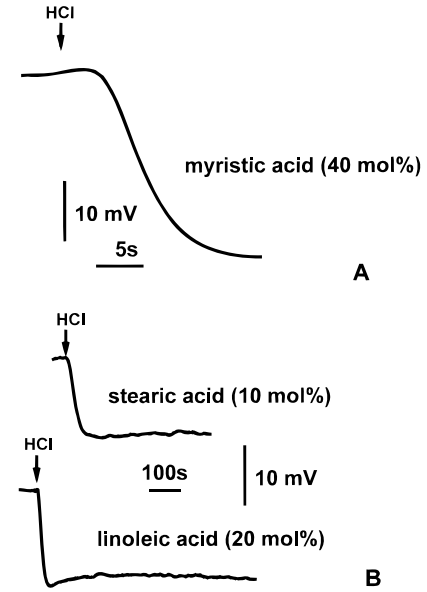


FIGURE 6: Kinetics (A) and time dependence (B) of boundary potential changes. The conditions are similar to those in Figure 5. Examples for different FA content are given.

experiments on liposomes (*41*). Because the apparent p*K* of the FA incorporated in the PC bilayer is about 7.5 (*42, 43*) the bound fatty acids were assumed to be to 50% ionized and to be equally distributed between cis and trans leaflets of the bilayer at the beginning of experiments (Figure 4A). After the pH is lowered on the cis side (Figure 4B), the ionized FA molecules become protonated and flip to the trans side where they release their protons (Figure 4C). The concentration difference of fatty acid anions between the two monolayers causes a boundary potential difference, which can be detected by the IFC method (IFC). If our model is correct, the FA movement from one BLM leaflet to the other will produce surface potential changes that should be about twice the surface potential $\Phi_s$ obtained from $\zeta$-potential measurements. It is known from previous experiments that the flip-flop rate of the acid anion is lower by at least some orders of magnitude (*41, 44*), so it was assumed not to contribute to the formation of membrane asymmetry.

Figure 5 shows that the acidification of the cis buffer from 7.5 to 6.8 was followed by rapid formation of a boundary potential difference up to 23 mV similar for all studied long FA (myristic, stearic, and linoleic acids). The changes in $\Delta\Phi_b$ were proportional to the mole fraction of FA in the membrane-forming solution but did not depend on the type of FA. The pH-related changes of potential were completed within seconds (Figure 6). The period of time, $\tau_{1/2}$, that elapsed from the beginning of potential changes to the time where half the steady-state potential was achieved was similar for stearic, linoleic, and myristic acids and was equal to 33.2 ± 19, 21.1 ± 13, and 24.1 ± 15 s, respectively. Three representative examples indicate that the kinetics and time constant of potential changes do not depend on the amount of FA dissolved in lipid. An example of the detailed kinetics for myristic acid is shown in Figure 6A. An upper limit to the true flip-flop rate was given by the time resolution of our experimental setup, i.e., the mixing time of the buffer solution and the time needed for the buffer diffusion through the unstirred layer adjacent to the membrane (*45*). It was shown previously that the half-time required for diffusion, $t_{1/2}$, can be calculated as $t_{1/2} = 0.38\alpha^2/D$ if the buffer diffusion

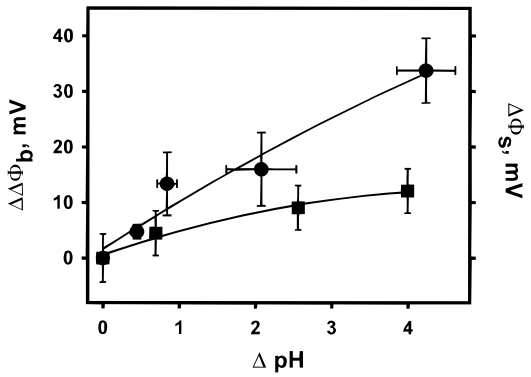


FIGURE 7: Alterations in the boundary potential difference, measured by IFC ($\Delta\Delta\Phi_b$, ●), and surface potential differences, calculated from ζ-potential values ($\Delta\Phi_s$, ■) induced by $\Delta pH = pH_0 - pH_x$. $pH_0$ (7.5) and $pH_x$ denote the pH values at different sides of the planar membrane or the initial and actual pH values for the experiments carried out on liposomes. Liposomes and BLM were made from DPhPC and stearic acid (60:40 mol %). The buffer solution contained 50 mM KCl, 10 mM Tris, 10 mM MES, and 10 mM CAPSO.

coefficient ($D$) and the unstirred layer thickness (α) are known (*46*). For our experiments $t_{1/2}$ was found to be similar to $\tau_{1/2}$. Further alterations of $\Phi_b$, which would correspond to the flip-flop of the ionized form of FA, were not detected over the whole registration time (up to 2 h; Figure 6B).

To visualize the contribution of each process (flip-flop and titration) to the measured alteration of membrane potentials, the surface potential difference $\Delta\Phi_s$, calculated from ζ-potential values, and the difference of $\Delta\Phi_b$ ($\Delta\Delta\Phi_b$), estimated by IFC on BLM, are compared in Figure 7. With the IFC method it is not the absolute boundary potential ($\Phi_b$) but the boundary potential difference between both membrane leaflets ($\Delta\Phi_b$) that is measured. From the measurements of the electrophoretical mobility, absolute values of $\Phi_s$ are calculated. To compare these data the potential changes ($\Delta\Delta\Phi_b$ and $\Delta\Phi_s$) in the same pH interval (ΔpH) were used. The $\Delta\Phi_s$ values were calculated as the difference of the potentials $\Phi_{s,x}$ and $\Phi_{s,0}$ at acidic $pH_x$ and $pH_0 = 7.5$, respectively ($\Delta pH = pH_0 - pH_x$). To estimate the $\Delta\Delta\Phi_b$, pH was decreased only on the cis side of the BLM. ΔpH represents the pH difference between cis ($pH_x$) and trans ($pH_0 = 7.5$) sides of the membrane. The $\Delta\Delta\Phi_b$ values were corrected for values received in the absence of FA. As shown in Figure 5, the acidification of pure DPhPC membranes gives an offset of about −7 mV.

The addition of FFA to pure DPhPC membranes on the cis-side buffer solution did not lead to changes of the boundary potential (data not shown). The lack of the effect can be interpreted in terms of two processes: (i) a very fast redistribution of FA between the two monolayers (flip-flop) or (ii) the failure to detect any adsorption because the resulting membrane concentration of the long-chain acids is below the detection threshold. Using the adsorption coefficient calculated for some fatty acids (*44*), we estimated that the added amount of FA should have caused a surface potential drop of several millivolts. Because the IFC method has a sensitivity of better than 1 mV, the absence of measurable $\Phi_b$ changes represent strong evidence in favor of the first hypothesis.

## DISCUSSION

Many research groups have shown that transport of fatty acids is very sensitive to the nature of the FA as well as that of the membrane (*7, 10, 47*−*50*). In our experiments we have used planar bilayer lipid membranes with zero radius of curvature, which reflects the low curvature of biological membranes more accurately. Into protein-free membranes were incorporated unmodified and unbound long-chain fatty acids. The transmembrane transport step has been studied separately from adsorption and desorption processes. As predicted (*7*), our results indicate that also in planar membranes with zero surface curvature the flip-flop rate is comparable with the diffusion rate through the unstirred layer, existing also in cells (*46*). Our protein-free, two-component system demonstrates that proteins are not absolutely necessary for the transmembrane diffusion of the unionized form. However, the diffusion of the acid anion was not detected in our experiments. On the basis of known rate constants [$k = 10^{-4}$ s$^{-1}$ (*44*); $t_{1/2}$ = minutes (*41*)], we supposed that anion diffusion is slower than lateral lipid diffusion and, consequently, is not detectable in our experiments. To occur on a physiological time scale the $FA^-$ transport probably requires the participation of FA transport proteins (*5, 51, 52*).

We have shown that the incorporation of fatty acids in lipid membranes does not alter considerably the dipole potential under the conditions used in our experiments. It leads to the conclusion that all FA molecules are located in one adsorption plane and, obviously, do not affect the transmembrane transport of ions or charged molecules sensitive to the intrinsic membrane potential. Cherny et al. (*53*) reported one experiment where the global change of boundary potential due to oleic acid adsorption was related to the variation of $\Phi_s$ and not $\Phi_d$. This result was obtained with the current relaxation method, which allows only indirect measurements of $\Phi_d$.

Usually, the discussion of the physiological importance of FA transport is focused on the production of intracellular acidification, which in turn plays a significant role in a variety of cellular processes, such as, e.g., fuel homeostasis, ion channels, and enzyme function (*6, 54, 55*). However, compared to classical protonophores fatty acids are inefficient $H^+$ carriers, mainly due to the slow translocation rates of the anionic species (*44*). On the contrary, the importance of a pH gradient as a driving force for FA uptake (*42, 56, 57*) is still underestimated. Our investigations on model membranes show that the distribution of fatty acids across the membrane is very sensitive to surrounding pH. The decrease of pH from 7.5 to 6.8 gives a considerable and fast alteration of FA content in each monolayer. Recent results obtained on cells (*56, 57*) support this point of view. So the acidification of the adipocyte cytoplasm was accompanied by rapid reduction of cellular oleate uptake, which recovered after normalization of cytoplasmic pH (*56*). Elsing et al. (*57*) have shown that FA transport was increased by 45% during cellular alkalosis and decreased by 30% during acidosis. The experiments on cells, however, do not allow to distinguish between flip-flop and the adsorption−desorption processes or between protein- and lipid-mediated steps. Our results show that at least the transmembrane step of fatty acid uptake

in a protein-free system can be regulated by intracellular/bulk pH.

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## REFERENCES

1. Huang, J.-M. C., Xian, H., and Bacaner, M. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 6452−6456.
2. Ordway, R. W., Walsh, J. V. J., and Singer, J. J. (1989) *Science 244*, 1176−1179.
3. Ordway, R. W., Petrou, S., Kirber, M. T., Walsh, J. V. J., and Singer, J. J. (1995) *J. Physiol. 484*, 331−337.
4. Samartsev, V. N., Smirnov, A. V., Zeldi, I. P., Markova, O. V., Mokhova, E. N., and Skulachev, V. P. (1997) *Biochim. Biophys. Acta 1319*, 251−257.
5. Hamilton, J. A. (1998) *J. Lipid Res. 39*, 467−481.
6. Hamilton, J. A., Civelek, V. N., Kamp, F., Tornheim, K., and Corkey, B. E. (1994) *J. Biol. Chem. 269*, 20852−20856.
7. Kamp, F., Zakim, D., Zhang, F., Noy, N., and Hamilton, J. A. (1995) *Biochemistry 34*, 11928−11937.
8. Storch, J., and Kleinfeld, A. M. (1986) *Biochemistry 25*, 1717−1726.
9. Kleinfeld, A. M., and Storch, J. (1993) *Biochemistry 32*, 2053−2061.
10. Abumrad, N., Harmon, C., and Ibrahimi, A. (1998) *J. Lipid Res. 39*, 2309−2318.
11. Osterberg, F., Rilfors, L., Wieslander, A., Lindblom, G., and Gruner, S. M. (1995) *Biochim. Biophys. Acta 1257*, 18−24.
12. Baldwin, P. A., and Hubbell, W. L. (1985) *Biochemistry 24*, 2624−2632.
13. Keller, S. L., Bezrukov, S. M., Gruner, S. M., Tate, M. W., Vodyanoy, I., and Parseian, V. A. (1993) *Biophys. J. 65*, 23−27.
14. Lewis, J. R., and Cafiso, D. S. (1999) *Biochemistry 38*, 5932−5938.
15. Scarlata, S., and Gruner, S. M. (1997) *Biophys. Chem. 67*, 269−279.
16. Lundbaek, J. A., Maer, A. M., and Andersen, O. S. (1997) *Biochemistry 36*, 5695−5701.
17. Chakrabarti, A., and Podder, S. K. (1990) *Biochim. Biophys. Acta 1024*, 103−110.
18. Dobereiner, H. G., Kas, J., Noppl, D., Sprenger, I., and Sackmann, E. (1993) *Biophys. J. 65*, 1396−1403.
19. Panakkezhum, D. T., and Poznansky, M. J. (1988) *Biochem. J. 254*, 155−160.
20. Kleinfeld, A. M., and Storch, J. (1993) *Biochemistry 32*, 2053−2061.
21. Kamp, F., Zakim, D., Zhang, F., Noy, N., and Hamilton, J. A. (1995) *Biochemistry 34*, 11928−11937.
22. Kleinfeld, A. M., Storms, S., and Watts, M. (1998) *Biochemistry 37*, 8011−8019.
23. Broring, K., Haest, C. W., and Deuticke, B. (1989) *Biochim. Biophys. Acta 986*, 321−331.
24. Mueller, P., Rudin, D. O., Tien, H. T., and Wescott, W. C. (1963) *J. Phys. Chem. 67*, 534−535.
25. MacDonald, R. C., MacDonald, R. I., Menco, B. P., Takeshita, K., Subbarao, N. K., and Hu, L. R. (1991) *Biochim. Biophys. Acta 1061*, 297−303.
26. Brockman, H. (1994) *Chem. Phys. Lipids 73*, 57−79.
27. Schoch, P., and Sargent, D. F. (1976) *Experientia 32*, 811.
28. Schoch, P., Sargent, D. F., and Schwyzer, R. (1979) *J. Membr. Biol. 46*, 71−89.
29. Sokolov, V. S., and Kuzmin, V. G. (1980) *Biofizika 25*, 170−172.
30. Sokolov, V. S., Cherny, V. V., Simonova, M. V., and Markin, V. S. (1990) *Bioelectrochem. Bioenerg. 23*, 27−44.
31. Pohl, P., Rokitskaya, T. I., Pohl, E. E., and Saparov, S. M. (1997) *Biochim. Biophys. Acta 1323*, 163−172.
32. Pohl, E. E., Krylov, A. V., Block, M., and Pohl, P. (1998) *Biochim. Biophys. Acta 1373*, 170−178.
33. Andersen, O. S., Finkelstein, A., Katz, I., and Cass, A. (1976) *J. Gen. Physiol. 67*, 749−771.
34. Franklin, J. C., and Cafiso, D. S. (1993) *Biophys. J. 65*, 289−299.
35. Gross, E., Bedlack, R. S. J., and Loew, L. M. (1994) *Biophys. J. 67*, 208−216.
36. Frank, J., Zouni, A., Van, H. A., Visser, A.-J. W., and Clarke, R. J. (1996) *Biochim. Biophys. Acta 1280*, 51−64.
37. Ohki, S., and Ohshima, H. (1995) in *Bioelectrochemistry: General introduction* (Caplan, S. R., Ed.) pp 212−287, Birkhäuser Verlag, Basel, Switzerland.
38. Hunter, R. J. (1981) *Zeta potential in colloid science. Principles and Applications*, Academic Press, London.
39. Ermakov, Y. A. (1990) *Biochim. Biophys. Acta 1023*, 91−97.
40. Clarke, R. J., and Kane, D. J. (1997) *Biochim. Biophys. Acta 1323*, 223−239.
41. Kamp, F., and Hamilton, J. A. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 11367−11370.
42. Hamilton, J. A., and Cistola, D. P. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 82−86.
43. Ptak, M., Egret-Charlier, M., Sanson, A., and Bouloussa, O. (980) *Biochim. Biophys. Acta 600*, 387−397.
44. Gutknecht, J. (1988) *J. Membr. Biol. 106*, 83−93.
45. Pohl, P., Saparov, S. M., and Antonenko, Y. N. (1998) *Biophys. J 75*, 1403−1409.
46. Barry, P. H., and Diamond, J. M. (1984) *Physiol Rev. 64*, 763−872.
47. Kleinfeld, A. M., Chu, P., and Romero, C. (1997) *Biochemistry 36*, 14146−14158.
48. Kleinfeld, A. M., Chu, P., and Storch, J. (1997) *Biochemistry 36*, 5702−5711.
49. Evtodienko, V. Y. U., Kovbasnjuk, O. N., Antonenko, Y. N., and Yaguzhinsky, L. S. (1996) *Biochim. Biophys. Acta 1281*, 245−251.
50. Evtodienko, V. Y., Bondarenko, D. I., and Antonenko, Y. N. (1999) *Biochim. Biophys. Acta 1420*, 95−103.
51. Garlid, K. D., Orosz, D. E., Modriansky, M., Vassanelli, S., and Jezek, P. (1996) *J. Biol. Chem. 271*, 2615−2620.
52. Richieri, G. V., Ogata, R. T., and Kleinfeld, A. M. (1999) *Mol. Cell Biochem. 192*, 77−85.
53. Cherny, V. V., Sikharulidze, M. G., Mirskii, V. M., and Sokolov, V. S. (1992) *Biol. Membr. 9*, 733−740.
54. Madshus, I. H. (1988) *Biochem. J. 250*, 1−8.
55. Garlid, K. D., Jaburek, M., and Jezek, P. (1998) *FEBS Lett. 438*, 10−14.
56. Trigatti, B. L., and Gerber, G. E. (1996) *Biochem. J. 313*, 487−494.
57. Elsing, C., Kassner, A., and Stremmel, W. (1996) *Am. J. Physiol. 271*, G1067−G1073.

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