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Effect of free fatty acids on the permeability of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine bilayer at the main phase transition

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Abstract

We measured the influence of saturated and unsaturated free fatty acids on the permeability and partition of ions into 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) bilayers. The bilayer permeability was measured using the depletion of *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphatidylethanolamine (*N*-NBD-PE) fluorescence as a result of its reduction by dithionite. We observed a distinct increase of dithionite permeability at the main gel–fluid phase transition of DMPC. When vesicles were formed from a mixture of DMPC and oleic acid, the membrane permeability at the phase transition was reduced drastically. Stearic acid and methyl ester of oleic acid have little effect. Similar results in the quenching of pyrene-PC in DMPC vesicles by iodide were obtained. Again, the increase of iodide partition into the lipid phase at the main phase transition of DMPC was abolished by the addition of unsaturated free fatty acids. Free fatty acids, in concentrations up to 5 mol%, do not abolish DMPC phase transition when measured by differential scanning calorimetry. It seems that unsaturated, but not saturated, free fatty acids reduce the lipid bilayer permeability to dithionite and iodide ions at the main phase transition of DMPC, without altering the thermodynamic properties of the bilayer.

Keywords: Free fatty acid; Lipid bilayer; Molecular packing; Phase transition; Ion transport

1. Introduction

Free fatty acids (FFA) are known to modify a variety of cellular functions, although their mechanisms of action and their location within a cell are still uncertain. A substantial amount of work has been devoted to determine the influence of FFA on a variety of biological systems. For example, the modification of immunological responses to an increasing level of FFA has been reported [1–4]. In the extreme case, FFA could modify plasma membrane properties to such a degree that hemolytic activity of the Sendai virus was abolished [5].

Several studies have shown that FFA affect properties of the plasma membrane [4,6–8]. FFA influence the activities of certain membrane proteins as well as the physical properties of the bulk membrane lipids [2,9–11]. The effective concentration of FFA in plasma membranes necessary to cause cell response is difficult to establish. This task is hindered by the easiness with which FFA exchange between membranes and by the presence of FFA binding proteins [12–14].

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To understand the effect of FFA on the plasma membrane, several studies using model membrane systems have been undertaken. The influence of FFA on the activity of reconstituted membrane proteins has been demonstrated. These include, for instance, acetylcholine receptor [15], protein kinase C [10,16], and brain phosphatidylinositol phosphodiesterase [17]. The ability of FFA to modify properties of membrane lipids has also been investigated. Major effort has been directed towards measurements of the effect of FFA on the bulk properties of membrane lipids (fluidity and thermotropic properties). However, most of the observed effects were produced by using an amount of FFA that is well above the physiological level (FFA are a minor fraction of membranes, below 1 mol% of total lipids [18]) [19–23]. Consequently, it has been argued that if FFA modifies biological activity, they do so mainly through interaction with plasma membrane proteins [18] rather than through the alteration of the physical properties of the lipid matrix.

A number of studies were undertaken to measure the membrane permeability. Only a few reports showed that FFA modifies membrane permeability in selected biological systems [15,33]. For example, Andreasen and McNamme [15] showed that unsaturated FFA inhibits the transport function of acetylcholine receptors. Studies of the influence of FFA on the lipid membrane permeability to ions in model lipid systems are even scarcer [14]. Theories which followed early permeability experiments on model systems correlated directly the membrane permeability for ions to the number of packing defects in lipid bilayers [24,25]. Those defects have been associated with boundaries of lipid domains [26,27] and with protein–lipid complexes [18].

It is known that at the gel-to-liquid phase transition, when the largest number of defects (domain boundaries) in the lipid matrix appear, membrane permeability reaches its maximum [27–30]. In this paper, we present data concerning the influence of FFA on the permeability of dithionite and iodide ions through lipid bilayer at the main phase transition using fluorescent assays recently developed in our laboratory to measure membrane permeability for dithionite and iodide [31,32]. The results show an unexpected differential effect of saturated and unsaturated free fatty acids on modifying the ion permeability of DMPC bilayers. This observation may lead to the understanding of an unknown biophysical mechanism of modifying bilayer permeability.

2. Materials and methods

2.1. Chemicals

1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) was obtained from Avanti Polar Lipids (Alabaster, AL). 3-Palmitoyl-2-(1-pyrenedecanoyl)-L-α-phosphatidylcholine (pyrene-PC) and *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-*sn*-glycero-3-phosphatidylethanolamine (*N*-NBD-PE) were purchased from Molecular Probes (Eugene, OR). Octadecanoic (stearic), *cis*-9-octadecenoic (oleic), *cis*-9,12-octadecadienoic (linoleic), hexadecanoic (palmitic), *cis*-9-hexadecanoic (palmitoleic), tetradecanoic (myristic), *cis*-9-tetradecanoic (myristoleic) acids, methyl ester of oleic acid and sodium hydrosulfite (dithionite) were purchased from Sigma (St. Louis, MO). All other chemicals were purchased from Fisher Scientific (Fairlawn, NJ). Phosphate buffer (0.02 M, pH 7.4) was used in all experiments.

2.2. Preparation of vesicles and fluorescence measurements

DMPC and fluorescence labeled phospholipids were dissolved in chloroform for complete lipid and probe mixing. The chloroform was then evaporated under nitrogen, and the lipid was dispersed in the phosphate buffer at a temperature well above the main phase transition (24°C; [34]). Unilamellar vesicles (mean diameter of a Gaussian size distribution = 190 nm, with χ^2 of 3.5) were formed by forcing vesicle suspensions through a 0.2-μm polycarbonate filter (Costar, Cambridge, MA) [35,36]. The size of DMPC vesicles was determined by using the dynamic light scattering method (Submicron Particle Sizer Model 370, NICOMP, Santa Barbara, CA). The lipid concentration in all samples was 0.05 μmol/ml. The amount of a fluorescence probe in a membrane never exceeded 0.5 mol% of the total lipid.

All fluorescence measurements were performed on an SLM 8000 fluorometer. Fluorescence of pyrene-

PC and *N*-NBD-PE was measured when probes were excited at 347 and 475 nm, respectively.

Potassium iodide (KI) was used to quench pyrene-PC as described previously [31,37]. In short, KI was added from the stock solution into lipid suspensions in consecutive steps. After each addition of the quencher, the content of the cuvette was mixed, and the fluorescence emission measured. The quenching efficiency was estimated using at least five quencher concentrations, applying the Stern–Volmer equation and the least squares fitting of the data. The fluorescence intensities used in the estimation of the quenching constant were corrected for the inner filter effect as described elsewhere [37,38]. The absorptance of any sample did not exceed 0.02 for both excitation and emission wavelengths.

The permeability experiments were performed according to the method developed in our laboratory [32]. The dithionite interaction with *N*-NBD-PE results in a permanent fluorescence depletion of the dye [39]. The dithionite penetration through the lipid membrane is reflected in the time-dependent depletion of fluorescence. The dependence of the fluorescence intensity on time was monitored continuously at the emission wavelength of 530 nm. The permeability of dithionite through the membrane was estimated using a single exponential approximation for data points collected for 5 min after the addition of dithionite. Slopes shown in Figs. 4 and 5 represent the least squares approximation obtained from plots of a natural logarithm of *N*-NBD-PE fluorescence, normalized to the initial value (prior to the treatment with dithionite), as a function of time. Concentrations of lipids and lipid fluorescence probes were estimated by phosphate analysis [40].

2.3. Calorimetric and gas chromatography studies

The thermograms of lipid suspensions in the buffer were obtained with a Microcal MC-2C (Northampton, MA) differential scanning calorimeter at a heating rate of 45°C/h. Samples for gas chromatography were prepared as described elsewhere [41,42]. A Perkin Elmer 900 Gas Chromatograph was used to measure FFA compositions, and data were collected and analyzed using a Chromatochart-PC (Interactive Microwave, PA).

3. Results

3.1. Effect of FFA on quenching of pyrene-PC by iodides

The quenching efficiency of pyrene-PC by iodide is a measurement of the lipid molecular packing against iodide ion penetration into the hydrophobic core of the bilayer. The quenching of pyrene-PC by iodide is based on collision and is described by the Stern–Volmer formula [38]. This quenching is possible only when the fluorophor and iodide are in close proximity. The pyrene residue is attached to the end of the 10 carbons long hydrocarbon chain of phosphatidylcholine. We have shown previously that pyrene is located predominantly inside the lipid bilayer and its position does not change throughout the applied range of temperatures [31]. Therefore, a change in quenching efficiency reflects alteration of

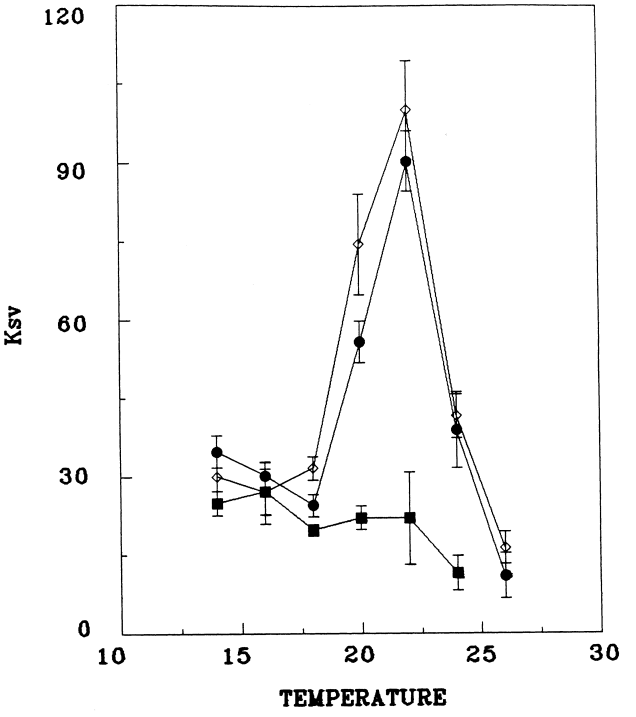

Fig. 1. The quenching efficiency of pyrene-PC in DMPC vesicles by iodide as a function of temperature. The quenching efficiency is expressed in terms of Stern–Volmer quenching constant. Diamonds represent DMPC vesicles alone, whereas circles and squares show results for DMPC vesicles with stearic and oleic acids, respectively. FFA concentrations are 10 mol%. Temperature is in centigrade.

iodide concentration in the lipid matrix. We have previously measured the iodide partition into DMPC lipid bilayer, and found that it depends strongly on lipid packing (it reaches its maximum near the main lipid phase transition [31]).

Fig. 1 shows the quenching efficiency of pyrene-PC by iodide in DMPC unilamellar vesicles at different temperatures. The quenching efficiency reached its maximum at temperatures when DMPC vesicles were at, or around, the main phase transition [34]. When the same vesicles were prepared with oleic acid, this maximum disappeared. Such an effect was not observed when stearic acid was added. This result does not depend on the procedure with which FFA was added into lipid vesicles. The potency of oleic acid when added to the vesicle suspension from the ethanol solution did not differ from that when mixed with lipids prior to the vesicles formation. This is expected since the majority of FFA partition into a lipid phase as shown by Anel et al. [43]. Consequently, in all experiments that followed, FFA was added to the vesicles suspension from a concentrated ethanol solution.

Fig. 2 shows the dependence of quenching efficiency on the amount of FFA in the membrane measured at 22°C (maximum of the quenching efficiency). When the fraction of stearic acid in lipid membrane increased, the quenching efficiency decreased slightly, whereas 2.5 mol% of oleic acid was sufficient to prohibit quenching almost entirely. A similar effect was observed for linoleic acid (data not shown).

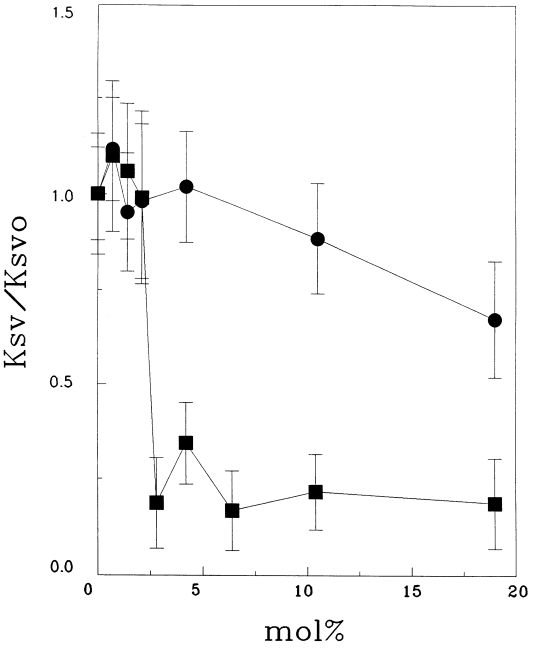

Fig. 2. The quenching of pyrene-PC by KI as a function of the amount of FFA in DMPC vesicles at 22°C. Circles and squares represent data for DMPC vesicles with stearic and oleic acid, respectively.

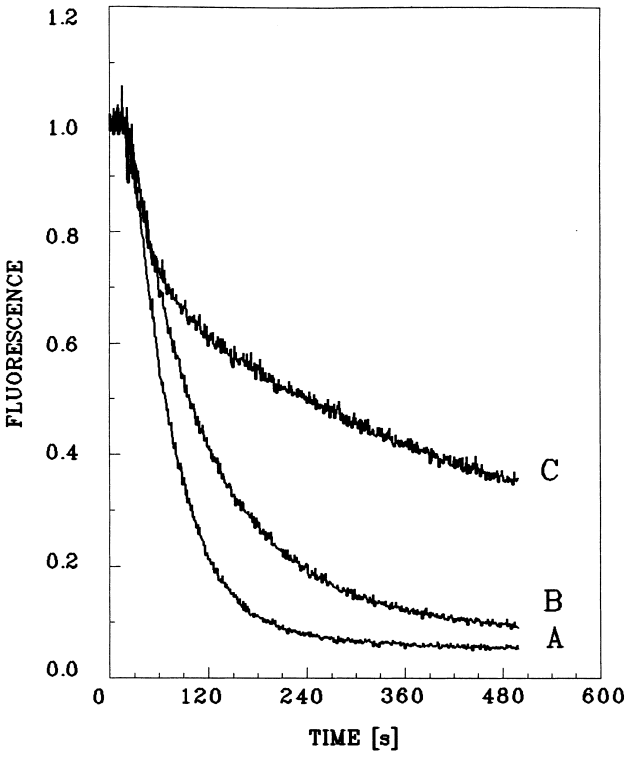

Fig. 3. The fluorescence of *N*-NBD-PE in DMPC vesicles after the addition of dithionite at 24°C. Curve A represents a plot for DMPC vesicles alone, whereas curves B and C show plots for vesicles with 10 mol% stearic and oleic acid, respectively. The fluorescence intensities were normalized to that before dithionite treatment.

To exclude any possibility of extensive FFA-iodide reaction, we incubated stearic and oleic acids with iodide at 24°C for 30 min (this time is longer then that applied during experiments). The incubation was followed by an analysis of the FFA using standard gas chromatography. The oxidation of oleic acid never exceeded 10%. There was no measurable oxidation of stearic acid. This experiment excludes the possibility that the decrease of quenching efficiency in the presence of unsaturated FFA is due to their oxidation.

3.2. Effect of FFA on the dithionite permeability through membrane

The partition of iodide into lipid bilayers has been modified by unsaturated FFA; therefore, the membrane permeability is expected to be affected as well.

We measured the permeability of dithionite through the DMPC membrane with and without FFA using a technique developed in our laboratory [32]. Water-soluble dithionite reduces *N*-NBD-PE fluorophor and permanently abolishes its fluorescence. The *N*-NBD-PE moiety is attached to the phosphatidylethanolamine headgroup, and is at the aqueous phase near the membrane surface [44]. When dithionite is added to the vesicle suspension, *N*-NBD-PE exposed to the outer aqueous phase is immediately quenched. Further quenching of *N*-NBD-PE requires the permeation of dithionite through the lipid bilayer. We used this slow decrease of fluorescence intensity as a measure of membrane permeability.

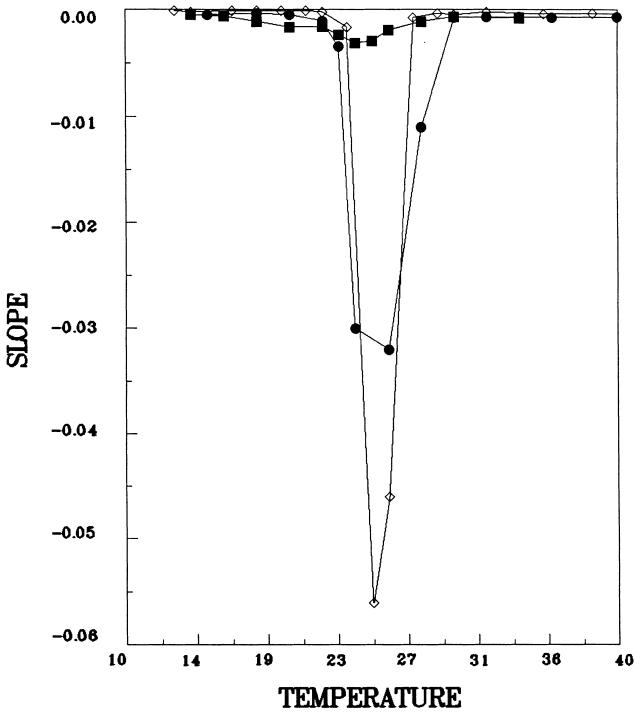

Fig. 4. The dependence of the permeability of DMPC vesicles to dithionite on temperature. The permeability is measured by quenching rates, which are derived from slopes of traces of *N*-NBD-PE fluorescence intensities as a function of time. Diamonds represent DMPC membranes alone, whereas circles and squares represent membranes with 10 mol% stearic and oleic acids, respectively.

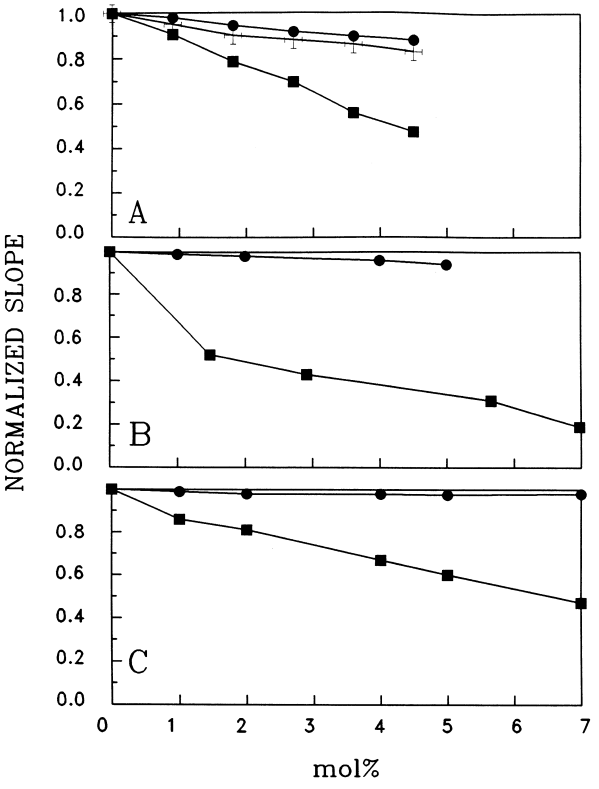

Fig. 5. The relative decrease of the *N*-NBD-PE fluorescence intensity upon reaction with dithionite is shown as a function of the amount of FFA present in DMPC vesicles. Squares and circles represent unsaturated and saturated FFA, respectively. The effect of a variety of FFAs is shown: (A) stearic and oleic acids; (B) palmitic and palmitoleic acids; and (C) myristic and myristoleic acids. Crosses in A represent the decrease of the fluorescence in the presence of methyl ester of oleic acid. All data were collected at the main phase transition of DMPC (24°C). The decrease of fluorescence is expressed as a ratio of slopes at a given FFA concentration to that obtained for DMPC vesicles alone. Slopes are calculated, as described in Section 2, from data points collected for 5 min after the addition of dithionite to the vesicle suspension.

Traces of the *N*-NBD-PE fluorescence intensity upon treatment with dithionite are presented in Fig. 3. These traces, as shown in Fig. 3, were obtained when DMPC vesicles were incubated at the temperature of main phase transition (24°C). The fluorescence intensity of *N*-NBD-PE incorporated into pure DMPC vesicles declined rapidly to the background level (curve A) after the addition of dithionite. The results showed that, in a short period of time, all *N*-NBD-PE moieties were reduced by di-

thionite, which easily penetrated the DMPC bilayer at this temperature. When saturated FFA (stearic, palmitic, or myristic) were present in the DMPC membrane, the fluorescence decay was only slightly reduced (curve B). Whereas unsaturated FFA (oleic, palmitoleic or myristoleic) drastically reduced the membrane permeability (curve C). Therefore, the addition of unsaturated FFA impeded the access of dithionite to the inner surface of vesicles.

While the effect of FFA on the permeability at the main phase transition was dramatic, the permeability of the DMPC membrane was not altered by FFA when measured in fluid or gel phases, at temperatures sufficiently different from the main phase transition temperature. The dependence of permeability on temperature in the presence and absence of FFA is shown in Fig. 4. Similar to the iodide quenching experiments, oleic acid drastically reduces the permeability peak at the transition temperature, whereas the effect of stearic acid is much less pronounced.

The dependence of the dithionite permeability through the DMPC membrane at the main phase transition was measured at various amounts of FFA present in the system, while all other conditions were kept constant. Fig. 5 shows that the decay of fluorescence intensity is reduced when the amount of unsaturated FFA in the membrane increases. The effect of saturated FFA, similarly to the partition experiment, is much smaller.

When methyl ester of oleic acid was added to the suspension of lipid vesicles the permeability of DMPC bilayer to dithionite at the main phase transition was not affected. This result shows that charged carboxyl-group and unsaturated hydrocarbon chain of FFA are essential to affect the permeability of DMPC vesicles.

3.3. *The effect of FFA on lipid phase transition, as measured with differential scanning calorimetry*

The influence of FFA on the phase transition of DMPC was measured with differential scanning calorimetry (Fig. 6). The data collected were similar to that presented elsewhere [19,20]. DMPC phase transition was somewhat modified by the presence of 5 mol% of FFA. Saturated FFA slightly elevated

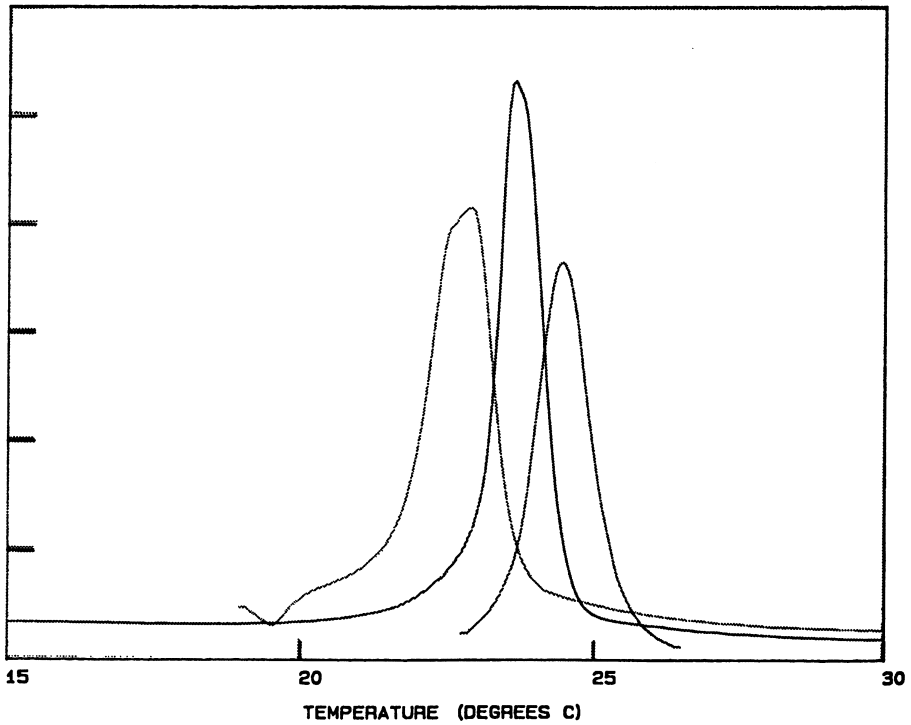

Fig. 6. Thermograms obtained from vesicle suspensions. The central peak represents the trace for vesicles formed from DMPC alone. The peaks at the right and left of the central peak represent traces obtain when 5 mol% of stearic acid and oleic acid was added, respectively. Ticks on the *y*-axis give the excess heat capacity in kcal/mol/deg.

(about 1°C) the temperature of the main phase transition, whereas unsaturated FFA depressed it slightly. Transition enthalpy decreased from 5.1 to 2.4 and 4.9 kcal/mol, respectively, from added stearic and oleic acids. FFA (2 mol%) caused even smaller effect, whereas 10 mol% broadened the phase transition considerably (data not shown).

4. Discussion

We applied a series of fluorescence techniques to determine the effect of FFA on the various aspects of molecular packing of DMPC bilayers. We concentrated our studies on lipid vesicles at the gel-to-fluid phase transition, where the number of defects reached its maximum [24–27]. The quenching of pyrene-PC by iodide measures both the tightness of lipid packing against the penetration of iodide into its hydrophobic interior, and the transient exposure of pyrene at the phase transition. These are relative measurements and do not show 100% quenching of pyrene fluorescence even at the peak of phase transition. The extinguishing process of *N*-NBD-PE fluorescence by dithionite indicates the permeability of dithionite (a larger ion than iodide) through the bilayer. It measures the partial loss of barrier functions of the bilayer. Both of these properties have the maximum value at the gel-to-fluid phase transition, when structural fluctuations are most extensive. The increased permeability of a lipid bilayer at the main phase transition is associated with the formation of defects due to the coexistence of the gel and fluid phases [45,46].

Transbilayer relocation of lipids (flip/flop) is also at a maximum at the main phase transition. One may argue that the quenching of fluorescence could represent the flip/flop rate of quenched fluorescent lipid molecules, rather than the penetration rate of quencher molecules through the bilayer. While the flip/flop rate of FFA is in the millisecond range [47], that for DMPC has a half time of 4 ± 2 h at the phase transition temperature [48], and even longer at other temperatures [49]. Recent measurements of the flip/flop half time of PC in LUV at liquid-crystalline phase temperatures gave values of ~ 7.5 h [49] and < 9 h [53]. Given that the flip/flop time for our labeled PE is shorter than PC by a factor of ten [50], the half time is still more than 20 min. The penetration rates we measured here are in the order of 1–3 min. In addition, in the original paper where the dithionite method was first described [39], the permeability of MLV was measured. The results were interpreted as dithionite penetrating the multilayers, and not the effect due to a non-permeable extinguishing probe (dithionite) acting on the flip/flop of lipids in the outer bilayer of MLV alone. Furthermore, the iodide quenching effect is independent of lipid flip/flop, unless the labeled lipids are preferentially positioned in the outer monolayer initially. Since we do not have asymmetric bilayers, and the iodide quenching results agree with the dithionite extinguishing results, it is unlikely that the flip/flop of labeled lipids has any strong influence on our measurement. The fluorescence decline is always a single exponential in our experiments, indicating that only a single mechanism is responsible for the observation. Therefore, we are confident that the fluorescence measurements represent what they are designed to do, i.e. the accessibility of the fluorophors to the quenching and extinguishing ions via a partial loss of the barrier function of the bilayer at the phase transition.

The data presented in this paper show that FFA with an unsaturated hydrocarbon chain prevents iodide and dithionite ions from entering the lipid bilayer, whereas saturated FFA has little effect. We speculate that the unsaturated FFA affects defected areas (domain boundaries) within the membrane and prevents ions from the crossing the lipid bilayer.

Earlier studies on a variety of model lipid systems show that the addition of FFA changes the global properties of lipid bilayers, such as 'fluidity' [47] or thermotropic properties [20–22]. The main phase transition temperature of the host lipid bilayer is shifted and the peak is broadened with increasing concentrations of FFA [22,23]. FFA also alter the organization of hydrocarbon chains in the lipid bilayer. The effect is most apparent in lipid membranes in the gel phase [45,51,52]. In our experiments, the penetrated permeability of the DMPC bilayer is reduced significantly with only 2.5 mol% of oleic acid (Figs. 2 and 5). At this low concentration of fatty acids, the thermotropic properties were only slightly altered (Fig. 6). Furthermore, the unique effect of unsaturated FFA on permeability could not be ex-

plained by changes in global (thermotropic) properties of the membrane, because there is no fundamental difference between the influences of saturated and unsaturated FFA on the phase transition of the host DMPC lipid. Furthermore, the significant effect of 5 mol% of oleic acid on permeability (Figs. 2 and 5) cannot be explained by its weak influence on the phase transition of DMPC (Fig. 6). In addition, the same amount of the methyl ester of oleic acid did not cause any changes in the membrane permeability (Fig. 5A).

Neither can the differences between saturated and unsaturated FFA be attributed to the variation in their partitioning into the bilayer lipid phase. Recent data presented by Anel et al. [43] show that the majority of both saturated and unsaturated FFA, when added to the vesicle suspension, partitions preferentially into the lipid phase (excess of 95%). In fact, stearic acid partitions into membranes better than oleic acid.

The ability of low concentrations of unsaturated FFA to reduce the permeability of lipid bilayers at the transition temperature indicates that the FFA modify the defect regions through their structure and differential localization. Data given in this paper show that both unsaturated hydrocarbon chains and carboxyl groups are necessary for this function. The actual molecular mechanism awaits further studies.

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References

[1] G.V. Richieri, A.M. Kleinfeld, J. Immunol. 143 (1989) 2302–2310.
[2] G.V. Richieri, A.M. Kleinfeld, J. Immunol. 145 (1990) 1074–1077.
[3] C.D. Stubbs, A.D. Smith, Biochim. Biophys. Acta 779 (1984) 89–137.
[4] A. Altman, K.M. Coggeshell, Adv. Immunol. 48 (1990) 227–360.
[5] R.C. MacDonald, V.D. Ore, R.I. MacDonald, Virology 134 (1984) 103–117.
[6] R. Bialick, R. Gill, G. Berke, W.R. Clark, J. Immunol. 132 (1984) 81–87.
[7] T.M. Buttke, Immunology 53 (1984) 235–242.
[8] C.J. Meade, J. Mertin, Adv. Lipid Res. 16 (1978) 127–165.
[9] H.G.P. Swarts, T.J.F. Van Uem, S. Hoving, J.A.M. Fransen, J.J.H.H.M. DePont, Biochim. Biophys. Acta 1070 (1991) 283–292.
[10] S.G. Chen, K. Murakami, Biochem. J. 282 (1992) 33–39.
[11] F.G. Herring, I. Tatischeff, G. Weeks, Biochim. Biophys. Acta 602 (1980) 1–9.
[12] A.M. Kleinfeld, J. Storch, Biochemistry 32 (1993) 2053–2061.
[13] M.C. Doody, H.J. Pownall, Y.J. Kao, L.C. Smith, Biochemistry 19 (1980) 108–116.
[14] F. Kamp, J.A. Hamilton, Biochemistry 32 (1993) 11074–11086.
[15] T.J. Andreasen, M.G. McNamee, Biochemistry 19 (1980) 4719–4726.
[16] S.G. Chen, D. Kulju, S. Halt, K. Murakami, Biochem. J. 284 (1992) 1–6.
[17] R.F. Irvin, A.J. Letcher, R.M.C. Dawson, Biochem. J. 178 (1979) 497–500.
[18] R.B. Gennis, Biomembranes, Springer-Verlag, New York, 1989.
[19] S.E. Schullery, T.A. Seder, D.A. Weinstein, D.A. Bryant, Biochemistry 20 (1981) 6818–6824.
[20] M.F. Omodeo-Sale, P. Palestini, M. Masserini, Chem. Phys. Lipids 61 (1992) 149–155.
[21] S.P. Verma, D.F.H. Wallach, J.D. Sakura, Biochemistry 19 (1980) 574–579.
[22] A.W. Eliasz, D. Chapman, D.F. Ewing, Biochim. Biophys. Acta 448 (1976) 220–233.
[23] S. Mabrey, J.M. Sturtevant, Biochim. Biophys. Acta 486 (1977) 444–450.
[24] S. Marcelja, J. Wolfe, Biochim. Biophys. Acta 557 (1979) 24–31.
[25] J.F. Nagle, H.L. Scott Jr., Biochim. Biophys. Acta 513 (1978) 236–243.
[26] P. van Hoogevest, J. de Gier, B. de Kruiff, FEBS Lett. 171 (1984) 160–164.
[27] D. Marsh, A. Watts, P.F. Knowles, Biochemistry 15 (1976) 3570–3578.
[28] C. Block, L.L.M. van Deenen, J. De Gier, Biochim. Biophys. Acta 433 (1976) 1–12.
[29] D. Papahadjopoulos, K. Jacobson, S. Nor, T. Isac, Biochim. Biophys. Acta 311 (1973) 330–348.
[30] T.Y. Tsong, Biochemistry 14 (1975) 5409–5414.
[31] M. Langner, S.W. Hui, Chem. Phys. Lipids 60 (1991) 127–132.
[32] M. Langner, S.W. Hui, Chem. Phys. Lipids 65 (1993) 23–30.
[33] M. Schramm, B. Eisenkraft, E. Barkai, Biochim. Biophys. Acta 135 (1967) 44–52.
[34] D. Marsh, Handbook of Lipid Bilayers, CRC Press, Boca Raton, FL, 1990.

[35] M.J. Hope, M.B. Bally, G. Webb, P.R. Cullis, Biochim. Biophys. Acta 812 (1985) 55–65.
[36] L.D. Mayer, M.J. Hope, P.R. Cullis, A.S. Janoff, Biochim. Biophys. Acta 817 (1985) 193–196.
[37] A.P. Winiski, M. Eisenberg, M. Langner, S. McLaughlin, Biochemistry 27 (1988) 386–392.
[38] J.R. Lakowicz, Principle of Fluorescence Spectroscopy, Plenum Press, New York, 1983.
[39] J.C. McIntyre, R.G. Sleight, Biochemistry 30 (1991) 11819–11827.
[40] G.R. Bartlett, J. Biol. Chem. 234 (1959) 466–468.
[41] B.L. Brian, R.W. Gracy, V.E. Scholes, J. Chromatogr. 66 (1972) 138–146.
[42] M. Kates, Techniques of Lipidology, Elsevier, Amsterdam, 1991.
[43] A. Anel, G.V. Richieri, A.M. Kleinfeld, Biochemistry 32 (1993) 530–536.
[44] D.E. Wolf, A.P. Winiski, A.E. Ting, K.M. Bocian, R.E. Pagano, Biochemistry 31 (1992) 2865–2873.
[45] R.D. Klausner, A.M. Kleinfeld, R.L. Hoover, M.J. Karnovsky, J. Biol. Chem. 255 (1980) 1286–1295.
[46] M.J. Karnovsky, A.M. Kleinfeld, R.L. Hoover, R.D. Klausner, J. Cell Biol. 94 (1982) 1–6.
[47] K.F. Zakim, D. Zhang, N. Noy, J.A. Hamilton, Biochemistry 34 (1995) 11928–11937.
[48] B. DeKruiff, E.J. van Zoelen, Biochim. Biophys. Acta 511 (1978) 105–115.
[49] J. Bai, R.E. Pagano, Biochemistry 36 (1997) 8840–8848.
[50] R. Homan, H.J. Pownall, Biochim. Biophys. Acta 938 (1988) 155–166.
[51] W. Ehringer, D. Belcher, S.R. Wassall, W. Stillwell, Chem. Phys. Lipids 54 (1990) 79–88.
[52] P. Meers, K. Hong, D. Papahadjopoulos, Biochemistry 27 (1988) 6784–6794.
[53] W.C. Wimley, P.E. Thompson, Biochemistry 29 (1990) 1296–1303.