Biochimica et Biophysica Acta, 1111 (1992) 263–269

BBAMEM 75803

The mechanism of the solute-induced chain interdigitation in phosphatidylcholine vesicles and characterization of the isothermal phase transitions by means of dynamic light scattering

Norbert E. Nagel, Gregor Cevc and Stephan Kirchner

Medizinische Biophysik, Urologische Klinik und Poliklinik der Technischen Universität München, Klinikum r.d.I., Munich (Germany)

(Received 13 August 1992)

Key words: Interdigitation; Phosphatidylcholine vesicle; Isothermal phase transition; Dynamic light scattering

A new method is introduced for the detection of chain interdigitation in phospholipid bilayers. The same method is used to measure the hydrocarbon tilt in the dipalmitoylphosphatidylcholine membranes as a function of the bulk concentration of the interdigitation-inducing solutes, such as ethanol. The hydrocarbon tilt in the phosphatidylcholine bilayers is demonstrated to be limited to angles below approx. 51°. The need for higher tilt values leads to bilayer interditigation. Solute-induced chain interdigitation is shown to be a cooperative process provoked by the excessively large lateral repulsion in the interfacial region and the concomittant excessive chain tilt. Ethanol-induced phosphatidylcholine interdigitation, for example, proceeds via interdigitated domains formation and finally gives rise to the bilayers with fully intercalated chains tilted by at least 30° (and sometimes as much as 50°) with respect to the membrane normal.

Introduction

Phosphatidylglycerol, more than a decade ago [1], was the first phospholipid proposed to be able to form membranes with interdigitated chains. Since then, a series of published reports has corroborated the existence of such 'anomalous' bilayers for many phospholipids under a variety of experimental conditions.

Interdigitated bilayers form spontaneously in aqueous lipid suspensions, for example, if the double-chain lipids have strongly asymmetric aliphatic tails (difference in the chainlength typically greater than 6 methylene groups) [2–7], if the phosphatidylcholine headgroup is in the *sn*-2-position [8] or if an alkyl-chain is in the *sn*-1-position [9–13].

The driving force of such hydrocarbon intercalation for the phospholipids with asymmetric chains appears to be the same in the dry [14–16] and hydrated [2–4] systems, its source being the packing incompatibility of the exceedingly different chain lengths (along the axis, 'transverse mismatch effect').

Chain interdigitation is also observed with symmetric chain phospholipids, such as phosphatidylcholine, after the addition of some amphiphilic substances, such as polymyxin B [17,18], choline and acetylcholine [19], drugs and anesthetics [20], including simple [21–23] as well as short polyvalent [24] alcohols, hepanoids [25], butylparabene [26,27], buffer molecules [28], and anions [29,30]. High pressure also has been reported to provoke chain interdigitation in phosphatidylcholine bilayers [31].

In spite of all these observations the precise mechanism and the reasons for isothermal, solute-induced chain interdigitation were obscure to date. In this report we resolve this question and also describe in detail the sequence of structural transformations which a lipid must undergo in order to end-up in an interdigitated state. We show, moreover, that the phospholipid chains interdigitation caused by solute molecules is a result of the 'lateral mismatch effect'. More specifically, we argue that in consequence to the solute binding at the lipid/water interface an excessive lateral intermolecular repulsion is created. This extra repulsion in the interfacial region first causes the lipid chains to increase their tilt perpendicular to the bilayer normal, in order to conform with the large interfacial molecular area, and once the tilt can not be increased any more, leads to the hydrocarbon interdigition.

We introduce a simple experimental method, based on the dynamic light scattering measurements of the

Correspondence to: G. Cevc, Medizinische Biophysik, Urologische Klinik und Poliklinik der Technischen Universität München, Klinikum r.d.I. Ismaningerstr. 22, W-8000 München 80, Germany.

liposome size, suitable for the rapid detection and characterization of the lipid chain interdigitation. We show that the latter phenomenon is cooperative and occurs via the formation of interdigitated chains domains. Last but not least, we provide estimates of the packing properties of the phosphatidylcholine chains in the interdigitated phases, which were not known to date.

Materials and Methods

Chemicals. 1,2-Dipalmitoyl-*sn*-3-phosphatidylcholine (DPPC, purity > 99% according to HPLC) was purchased from Boehringer (Mannheim, Germany). Ethanol (EtOH, p.a.) was obtained from Merck (Darmstadt, Germany). Water (18 MΩ) was doubly distilled in an all glass apparatus and reprocessed by a water purification unit (Elgastat HQ) including filtration just before the preparation of the samples.

Sample preparation. For most of the measurements, a suspension of multilamellar lipid vesicles in doubly destilled water (50 mg DPPC/ml) was sonicated (Heat Systems W 380) at 42°C until the average vesicle diameter was approximately 50 to 60 nm. The resulting suspensions of unilamellar liposomes were filtered through a 0.2 μm filter and kept at room temperature. Alternatively, small lipid vesicles were prepared by multiple manual extrusion through a 100 nm pore filter, using LiposoFast (Avestin, Ottawa, Canada). Vesicle size in the resulting (sterile) preparations was controlled prior to each experiment and proved to be stable for several weeks.

To get intermediate size vesicles an aliquot of the original suspension of small lipid vesicles was kept at 42°C (i.e., at the chain-melting phase-transition temperature of DPPC) for a couple of days. This has catalyzed substantial intervesicle fusion. When the average vesicle diameter has increased to approx. 100 nm (or 150 nm) the high-temperature incubation was terminated and the resulting liposomes were used for experiments.

Scanning calorimetry (DSC, MicroCal, Northampton, MA) was used to confirm that all investigated vesicles were in the L_β phase at 20°C.

Samples for the dynamic light scattering measurements were prepared by diluting 10 μl of the original suspension in 1 ml of corresponding ethanol/water mixture to yield final lipid concentrations between 0.5 and 1 mM. After filtering of the resulting suspension through a 0.2 μm Millex GV13 filter each sample was filled in an acryl, 1 cm light-path cuvette (prewashed twice with freshly filtered water) and sealed with parafilm. Preparation and manipulation of the samples in the dust-free environment and free of contaminations was found to be essential for the high quality and reproducibility of all measurements!

Dynamic light scattering measurements were done in triplicate with a Zetasizer 2C instrument (Malvern Instruments, Malvern, UK). They were anylized by the modified Contin programme from the Autosizer software package. The required solvent viscosity data were taken from Ref. 32.

For the osmotic stress experiments vesicles prepared in 10 mM NaCl were used. After the addition of appropriate amounts of ethanol and equilibration in the resulting ethanolic solution the vesicle diameter was measured for the first time. Then, each sample was diluted with a corresponding ethanol/water mixture to decrease the final extravesicular salt concentration to 8 mM. This resulted in the starting transbilayer osmotic pressure gradient of 3 milliosmoles.

Results and Discussion

Vesicle size

When mixed with an alcoholic solution the dipalmitoylphosphatidylcholine (and other PC) suspensions tend to change their properties. This is owing to the redestribution of the amphiphilic alcohol molecules between the aqueous and organic subphases which leads to the accumulation of some of these molecules in the interfacial region, encompassing polar phospholipid headgroups, by and large.

One of the most striking consequences of this alcohol redestribution is the change in bilayer packing properties; particularly prominent is the lateral bilayer expansion and the concomittant vesicle size variation as a function of the bulk alcohol concentration. (In the present report we confine our interest to ethanol but this is only for the sake of brevity and similar conclusions also hold for other short chain alcohols.) Similar, although far less strong, vesicle size variations are induced by temperature changes (to be published). In this contribution we therefore report only the data obtained under isothermal conditions in the lamellar lipid gel ($L_{\beta'}$) phase.

Relaxed dipalmitoylphosphatidylcholine liposomes with a diameter of approx. 100 nm at 20°C are in such an ordered (gel) lamellar ($L_{\beta'}$) phase. This means that all lipid chains are orientationally ordered (in an all-*trans* configuration), tilted by approximately $\phi_{tilt} = 30°$ relative to the bilayer normal [33].

Typical consequences of the ethanol addition to a suspension of lipid vesicles with a diameter of 106 nm in the $L_{\beta'}$ phase, as studied by the photon correlation spectroscopy, are illustrated in Fig. 1. From this picture, vesicle size upon increasing ethanol concentration is seen first to increase gradually to approx. 119 nm; this is the case until the bulk concentration exceeds approx. 0.4 mol EtOH per litre. An increase in the bulk alcohol concentration above this value diminishes the measurable vesicle size significantly. An absolute

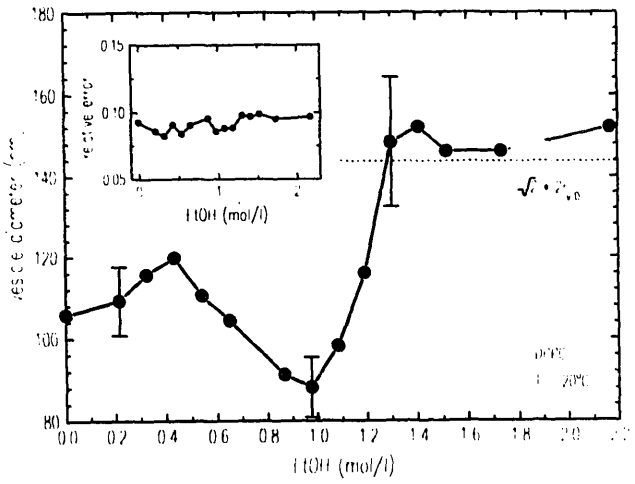

Fig. 1. Effect of the changing bulk ethanol concentration on the diameter of dipalmitoylphosphatidylcholine unilamellar vesicles, as measured by the dynamic light scattering at 20°C in the gel phase. Error bars give typical standard deviations, which are nearly concentration independent (inset).

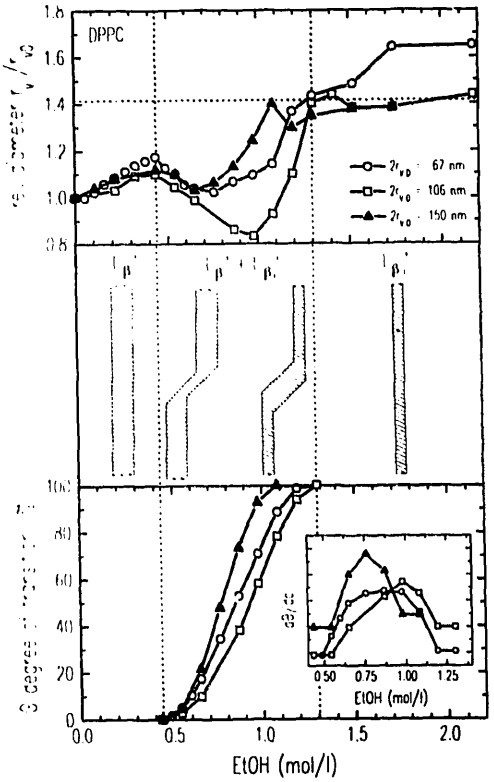

Fig. 2. Relative size increase (top) and the degree of chain interdigitation (bottom) for the DPPC vesicles as a function of the bulk ethanol concentration. Middle panel shows schematically the corresponding sequence of phases. Inset: estimated lipid amount involved in the phase transition ($= d\theta/d[EtOH]$). Vertical dashed-lines indicate the position of phase boundaries; horizontal dashed line in the top panel corresponds to $2r_v \equiv 2\sqrt{2}r_{v0}$. To estimate the degree of phase transition, θ, the points at 0.4 M EtOH and the first point in the pure interdigitated, L_{β_i} phase were connected and the resulting difference curve of the measured and expected size was integrated.

size minimum is reached for ethanol concentrations between 0.65 and 1.0 mol EtOH per litre, where the vesicle size is approx. 88 nm. For the bulk ethanol concentrations between 1.0 and 1.3 mol/l the vesicle diameter increases rapidly to approx. 142 nm. This is by a factor of $\sqrt{2}$ greater than in pure water and a sign of lipid chain interdigitation (if the initial and final chain tilts are the same; see further discussion). Finally, at concentrations higher than 1.3 mol EtOH per litre, vesicle size becomes rather insensitive to further increase in the bulk ethanol concentration.

The strongest ethanol-induced vesicle size variation is thus observed in the concentration range between 1.1 and 1.3 mol EtOH per litre (cf. Fig. 1). This is in good agreement with the results of the lipid phase transition [34] and X-ray diffraction measurements [21], which suggest that the chains in DPPC bilayers interdigitate in the same concentration range. The non-monotonous vesicle size variation with the changing bulk solute concentration is not a consequence of the vesicle non-sphericity: the fact that sample-polidispersity and standard deviation of the vesicle diameter are nearly concentration independent suggest this (Fig. 1, inset) [35].

Dipalmitoylphosphatidylcholine vesicles thus react in a complex way to the addition of ethanol. In a series of experiments with liposomes of different size, ranging from 60 to 150 nm to date, we have been repeatedly and reproducibly observing that within one day after the ethanol addition to the DPPC suspension vesicle size varies with the bulk alcohol concentration in a highly non-monotonous manner, as shown in Fig. 1 *. Initial vesicle size affects this variation quantitatively but not qualitatively (Fig. 2).

Large lipid vesicles ($2r_{v0} = 150$ nm) at 20°C show a similar size-sensitivity to the bulk ethanol concentration as vesicles with an initial diameter of approx. 70 nm (cf. Fig. 2). In contrast to this, the size of very big lipid vesicles ($2r_{v0} = 230$ nm) at 20°C is nearly independend of the bulk ethanol concentration. This is true immediately after the addition of an ethanolic solution to the liposome suspension as well 24 h later. Even after two days of equilibration the final-to-initial vesicle size ratio (r_v/r_{v0}) is not greater than 1.10; this

* In order to reach equilibrium, lipid bilayers must be exposed to identical solutions on both sides. It therefore takes time after the addition of ethanol to the vesicle exterior before perfectly equilibrated bilayers are observed. Consequently, we have never seen any evidence of chain interdigitation immediately after the addition of alcohol. Depending on the bulk ethanol concentration and initial vesicle size it took several minutes, hours or even days for the intravesicle concentration to reach the critical level at which massive bilayer reorganization sets in. For the vesicles with a diameter of approximately 100 nm it is sufficient to wait for some 24 h, however.

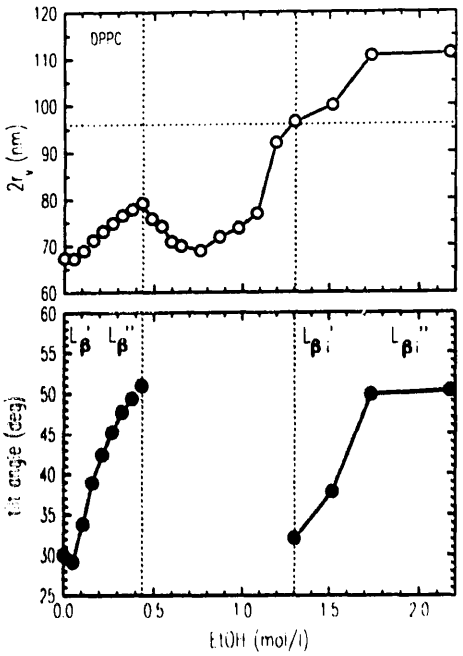

Fig. 3. Variation of the vesicle diameter (top) and hydrocarbon tilt-angle (bottom) with the bulk ethanol concentration for DPPC at 20°C. Tilt values in the $L_{\beta'}$ and $L_{\beta_1'}$ phases were calculated from Eqns. 1 and 2, respectively.

corresponds to an area increase of only 20% (data not shown). A detailed discussion of this phenomenon will be given in a separate paper.

Molecular mechanism of the hydrocarbon interdigitation

The observed ethanol-induced variations of the DPPC vesicle size in the gel lamellar phase can be explained by the following simple model of the $L_{\beta'} \rightarrow L_{\beta_1'}$ phase transition (Fig. 2, middle panel).

The first ethanol molecules to interact directly with the phosphatidylcholine headgroups lead to an expansion of the lipid/water interface. Owing to the strong inter-chain attraction this can only be achieved by simultaneously increasing the hydrocarbon tilt from a starting angle of approx. 30° [33] to 50–51°. This latter value is similar to the estimated upper limit for the tilt-angle of the aliphatic-chains, owing to sterical reasons (Fig. 3, bottom).

Vesicle size and the hydrocarbon tilt for standard dipalmitoylphosphatidylcholine bilayers are maximal when the bulk ethanol concentration is approximately 0.4 M (Fig. 3, lower panel).

The actual value of the hydrocarbon tilt angle in the ordered lipid lamellae (this is, in the $L_{\beta'}$ phase) can be calculated from the following phenomenological expression:

$$\phi_{tilt} = \arccos[(r_{v0}^3 / r_v^3)\cos 30°] \quad (1)$$

which is based on the assumption that the bulk lipid compressibility is negligibly low and that the total number of the lipid molecules in each individual vesicle is conserved. The above equation, moreover, implies that: $\phi_{tilt}(\text{water}) = 30°$.

Increasing the bulk – and consequently the interfacial – ethanol concentration beyond 0.4 M keeps on expanding the interfacial region. Owing to the maximum-tilt limitation this can be accomplished only if some of the lipid molecules give way and form interdigitated structures. Consequently, lipid lamellae are formed in which $L_{\beta'}$ and $L_{\beta_1'}$ phases co-exist.

The thickness of the lipid bilayers in the L_{β} and L_{β_1} phases differs appreciably, however. This causes incompatibility and separation of such phases. In ethanolic solutions containing more than 0.4 M EtOH one therefore must expect lipid domains to form which contain (nearly) pure $L_{\beta'}$ and $L_{\beta_1'}$ phase and give rise to vesicle surface undulations (see the middle panel in Fig. 2). It is such surface undulations which change the optically measurable vesicle surface by a sort of accordeon phenomenon (see further discussion).

The co-existance of $L_{\beta'}$ and $L_{\beta_1'}$ phases in DPPC bilayers first leads to a vesicle-area decrease and then to a vesicle-area increase, as the $L_{\beta_1'}$ domains grow at the expense of the $L_{\beta'}$ domains (see upper panel in Fig. 3). To monitor the progress of the lipid chain interdigitation it therefore suffices to determine the relative change in the lipid vesicle area between the pure $L_{\beta'}$ and $L_{\beta_1'}$ phases. The bottom panel in Fig. 2, which shows the progression of lipid interdigitation, demonstrates this. The size minimum, which for the DPPC small unilamellar vesicles is observed in 0.65–1 M ethanol solutions, consequently is indicative of the existence of lipid bilayers in which half of the area is in the $L_{\beta'}$ and half of the area is in the $L_{\beta_1'}$ phase.

Homogeneous $L_{\beta_1'}$ structures for the dipalmitoylphosphatidylcholine bilayers are unlikely to exist before the vesicle radii ratio has reached a characteristic value of $r(L_{\beta_1'})/r(L_{\beta'}) = \sqrt{2}$. This happens for the DPPC vesicles between 1.1 and 1.3 mol EtOH per litre and is illustrated in the lowest panel of Fig. 2 and in the inset to this figure.

Intermediate size vesicles in the fully interdigitated phase apparently have a constant tilt angle. For the dipalmitoylphosphatidylcholine molecules, this tilt is nearly identical to the value measured in the lamellar gel phase (not shown). This can be seen from expression

$$\phi_{tilt} = \arccos[(2r_{v0}^3 / r_v^3)\cos 30°] \quad (2)$$

which is an appropriate modification of Eqn. 1 for the $L_{\beta_1'}$-phase systems.

It is worth to note that it is not necessary, albeit very helpful, for the detection and identification of lipid chain interdigitation if the hydrocarbon tilt in the ordinary and interdigitated lamellae is identical. By all

means, the deviations from this rule are expected for the lipids with other chain lengths. Some variability in the hydrocarbon tilt-angle in the interdigitated phase is observed even with small lipid vesicles. Lower panel in Fig. 3 documents this by suggesting that the tilt-angle value, as calculated from the measured vesicle radii by means of Eqn. 2, in fact, may reach 51 degrees in the highly concentrated ethanol solutions, for some vesicles at least.

Tension effects

To confirm such a scenario of the lipid interdigitation we have attempted to prove directly that the surface of each lipid vesicle in the mixed phase region ($L_{\beta'} + L_{\beta_i'}$) is, indeed, corrugated. For this purpose we have exerted an inside-out transmembrane pressure on the vesicles suspended in different ethanolic solutions (0.4–1.1 M) and determined the vesicle size at different times after the induction of such tension.

The required transbilayer pressure difference was created by diluting the original suspension in a NaCl solution (10 mM) with an appropriate salt-free ethanol/water mixture. Such a dilution gave rise to a small transbilayer salt concentration gradient (2 mM) and to a transbilayer osmotic pressure difference of the order of a few milliosmoles. This led to a solvent inflow into the lipid vesicle interior. If vesicles were spherical, this inflow would result in only a small vesicle surface (and thus radius) expansion, due to the rather low lipid lateral compressibility modulus [36]. For corrugated vesicles, conversely, the corresponding area increase should be much greater, as only little effort is required to make an undulated vesicle surface flatter and larger [37].

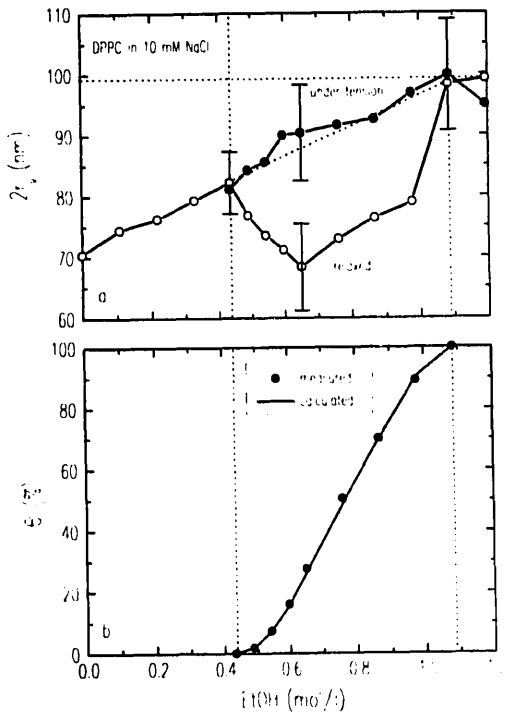

Fig. 4. Diameter (top) and degree of chain interdigitation (bottom) for the DPPC vesicles at 20°C as a function of the bulk ethanol concentration before and after the generation of a transbilayer osmotic tension. Open symbols represent the data obtained with fully equilibrated samples prepared in 10 mM NaCl. Full symbols were measured in an independent series of experiments right after the sample dilution to a final salt concentration of 8 mM. Dashed line represents the tentative, extrapolated functional dependence, as has been used in Fig. 2 to calculated the value of θ. Bullets in the lower picture give the corresponding directly measured results. Error bars denote typical standard deviation of the individual data points.

Data shown in Fig. 4 clearly indicate that only the latter supposition is correct: for a period of 6 to 9 min, vesicles under tension increase their size. Such a transient diameter increase makes vesicles nearly exactly as large as they would have to be for each given ethanol concentration if the maximum tolerable chain tilt-angle was not limited by the sterical intermolecular constraints (compare full symbols in panel a with dashed line).

Fig. 4 also vindicates the extrapolation procedure used in Fig. 2 to obtain the results presented in the panel 4b: the sum of the actually measured vesicle size deficits (upper curve minus lower curve from Fig. 4a) and the difference between the size of tensionless vesicles (lower curve in Fig. 4a) and the extrapolated vesicle sizes (dashed line in Fig. 4a) are nearly the same (bullets and line in Fig. 4b). The calculated progression of the isothermal lipid phase transition, shown in the lowest panel of Fig. 2 for three different systems as a function of the bulk ethanol concentration, consequently, is quite reliable.

We thus trust that the general reason for the solute (or solvent) induced hydrocarbon interdigitation of symmetric-chain phospholipids is the excessive lateral expansion in the interfacial region. In a previous paper [38] we have argued that at higher temperatures the excessive intrinsic lateral repulsion and expansion in the interfacial region, caused by the strongly hydrated and rapidly rotating lipid heads, will lead to the bilayer undulations and lipid pretransition. At relatively low temperatures ($T \ll T_m$) investigated in this work a sufficiently strong repulsion can only be induced by the addition of extrinsic (e.g., ethanol) molecules. These tend to first expand the lipid area by increasing the hydrocarbon tilt; when the tilt-value has reached its highest possible value an additional area increase is realized by the progressive chain interdigitation; once all lipid molecules are in the interdigitated state a secondary tilt-increase may occur. From this, we deduce that the necessary condition for the DPPC chains interdigitation is that the tilt angle exceeds 50°, approximately. Any external system modification that will fulfill this requirement is thus prone to induce chain interdigitation.

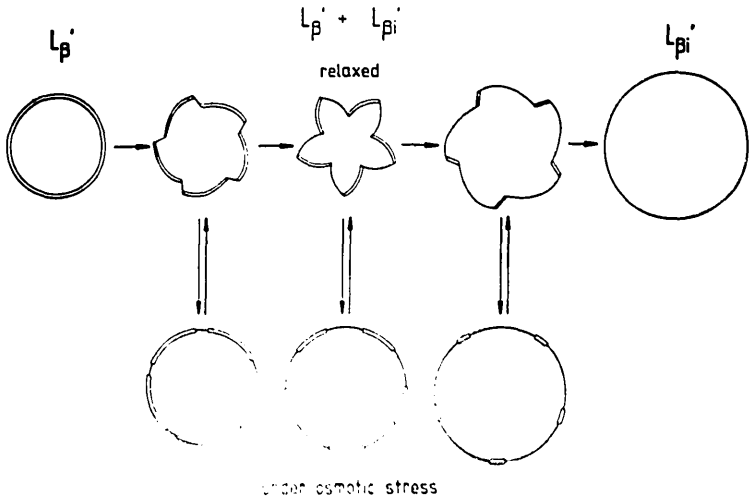

Fig. 5. Cartoon thought to represent the sequence of events associated with the transformation of standard unilamellar lipid vesicles into the interdigitated state.

We speculate that for the asymmetric chain phosphatidylcholines even lower amounts of inducer will suffice for the chain interdigitation owing to the larger spontaneous tilt of such lipids. On the other hand, for the lipids with a smaller starting tilt-angles higher critical concentration of the interdigitation-inducers are needed. In subsequent papers we will show how these conditions are affected by the lipid chain-length and headgroup type.

Our view of the events which are involved in the interdigitation of phospholipids with symmetric chains is summarized in Fig. 5. More specifically, this cartoon represents the sequence of bilayer surface modifications which we believe to occur upon the addition of ethanol to a suspension of the phosphatidylcholine vesicles. In Fig. 5 we show schematically why the vesicle size first decreases, then attains a minimum value, and subsequently increases due to this repulsion as the bulk ethanol concentration is increased to more than 0.4 M (for DPPC). The same figure also demonstrates how the vesicle surface corrugations, which are a consequence of the coexistence and formation-cooperativity of the $L_{\beta'} + L_{\beta i'}$ phases, can be eliminated by the transbilayer concentration (and thus pressure) gradients.

In summary, we have introduced a new method for the detection of lipid chain interdigitation. We have shown that doubling of the vesicle surface area (or an increase of the vesicle radius by a factor of $\sqrt{2}$), is indicative of hydrocarbon inderdigitation. (For different final chain-tilt angles (0–50°), r_v/r_{v0} values between 1.27 and 1.68 are possible.) We have determined for the first time the whole sequence of events which precede hydrocarbon interdigitation and have succeeded in measuring the precise values of the hydrocarbon tilts as a function of the bulk alcohol concentration and/or lipid phase state. Last but not least, we have shown that chain tilts greater than 50° are a prerequisite for the hydrocarbon interdigitation, for the symmetric-chain phosphatidylcholines at least.

Vesicle size determination method complements nicely – and often can replace – the previously known techniques for the identification of $L_{\beta i}$ phases. X-ray diffraction measurements [21] are straightforward, both in experiment and interpretation, but require expensive equipment and highly concentrated lipid samples. Electron spin resonance experiments using spin labels [5,39,40], on the other hand, can provide no direct means for quantitative determination of the lipid packing parameters.

We trust that our approach will prove to be valuable for further investigations on the lipid polymorphism, most notably for the studies on the interdigitated phases formed by lipids other than DPPC or provoked by inducers other than ethanol.

Acknowledgement

This study was supported by the Deutsche Forschungsgemenschaft through grant Ce 19/2-2.

References

1 Ranck, J.L., Keira, T. and Luzzati, V. (1977) Biochim. Biophys. Acta 488, 432–441.
2 Hui, S.W., Mason, J.T. and Huang, C.-H. (1984) Biochemistry 23, 5570–5577.
3 McIntosh, T.J., Simon, S.A., Ellington, J.C. and Porter, N.A. (1984) Biochemistry 23, 4038–4044.
4 Hui, S.W. and Huang, C.-H. (1986) Biochemistry 25, 1330–1339.
5 Boggs, J.M. and Mason, J.T. (1987) Biochim. Biophys. Acta 863, 231–242.
6 Mattai, J., Sripada, P.K. and Shipley, G.G. (1987) Biochemistry 26, 3287–3297.
7 Ali, S., Lin, H.-N., Bittman, R. and Huang, C.-H. (1989) Biochemistry 28, 522–528.

8 Serrallach, E.N., Dijkman, R., De Haas, G.H. and Shipley, G.G. (1983) J. Mol. Biol. 170, 155–174.
9 Ruocco, M.J., Siminovitch, D.J. and Griffin, R.G. (1985) Biochemistry 24, 2406–2411.
10 Ruocco, M.J., Makriyannis, A., Siminovitch, D.J. and Griffin, R.G. (1985) Biochemistry 24, 4844–4851.
11 Laggner, P., Lohner, K., Degovics, G., Müller, K. and Schuster, A. (1987) Chem. Phys. Lipids 44, 31–60.
12 Lohner, K., Schuster, A., Degovics, G., Müller, K. and Laggner, P. (1987) Chem. Phys. Lipids 44, 61–77.
13 Haas, N.S., Sripada, P.K. and Shipley, G.G. (1990) Biohpys. J. 57, 117–124.
14 Hauser, H., Pascher, I. and Sundell, S. (1980) J. Mol. Biol. 137, 249–264.
15 Pascher, I. and Sundell, S. (1986) Biochim. Biophys. Acta 855, 68–78.
16 Pascher, I. and Sundell, S. (1985) Chem. Phys. Lipids 37, 241–250.
17 Ranck, J.L. and Tocanne, J.F. (1982) FEBS Lett. 143, 171–174.
18 Theretz, A., Ranck, J.-L. and Tocanne, J.F. (1983) Biochim. Biophys. Acta 732, 499–508.
19 Ranck, J.L. and Tocanne, J.F. (1982) FEBS Lett. 143, 175–179.
20 McIntosh, T.J., McDaniel, R.V. and Simon, S.A. (1983) Biochim. Biophys. Acta 731, 109–114.
21 Simon, A. and McIntosh, T.J. (1984) Biochim. Biophys. Acta 773, 169–172.
22 Kamaya, H., Matubayasi, N. and Ueda, I. (1984) J. Phys. Chem. 88, 797–800.
23 Veiro, J.A., Nambi, P. and Rowe, E.S. (1988) Biochim. Biophys. Acta 943, 108–111.
24 McDaniel, R.V., McIntosh, T.J. and Simon, S.A. (1983) Biochim. Biophys. Acta 731, 97–108.
25 Wolff, P., Bisseret, G., Albrecht, A.-M., Tanaka, T., Nakatani, Y. and Ourisson, G. (1983) Biochem. Biophys. Res. Commun. 110, 320–324.
26 Komatsu, H., Higaki, K., Okamoto, H., Miyagawa, K., Hashida, M. and Sezaki, H. (1986) Chem. Phys. Bull. 34, 3415–3422.
27 Komatsu, H., Okamoto, H., Miyagawa, K., Hashida, M. and Sezaki, H. (1986) Chem. Phys. Bull. 34, 3423–3430.
28 Wilkinson, D.A., Tirrell, D.A., Turek, A.B. and McIntosh, T.J. (1987) Biochim. Biophys. Acta 905, 447–53.
29 Cunningham, B.A. and Lis, L.J. (1986) Biochim. Biophys. Acta 861, 237–242.
30 Cunningham, B.A., Tamura-Lis, W., Lis, L.J. and Collins, J.M. (1989) Biochim. Biophys. Acta 984, 109–112.
31 Braganza, L.F. and Worcester, D.L. (1986) Biochemistry 25, 2591–2596.
32 Handbook of Chemistry and Physics (1986) 26th Edn. CRC Press, Boca Raton, FL.
33 Wiener, M.C., Suter, R.M. and Nagle, J.F. (1989) Biophys. J. 55, 315–325.
34 Rowe, E.S. (1987) Biochemistry 26, 46–51.
35 Canto, L., Corti, M., Lago, P. and Musolino, M. (1991) in SPIE, Proceedings of Meeting on Photon Correlation Spectroscopy: Multicomponent Systems, Vol. 1430, pp. 144–159.
36 Cevc, G. and Marsh, D. (1987) Phospholipid Bilayers, Chapter 14, Willey-Interscience, New York.
37 Needham, D., McIntosh, T.J. and Evans, E. (1988) Biochemistry 27, 4668–4673.
38 Cevc, G. (1991) Biochim. Biophys. Acta 1062, 59–69.
39 Boggs, J.M., Wang, H.-Y., Rangaraj, G. and Tümmler, B. (1989) Biochim. Biophys. Acta 985, 199–210.
40 Wang, H.-Y., Tümmler, B. and Boggs, J.M. (1989) Biochim. Biophys. Acta 985, 182–198.