THE UNDULATIONS OF HYDRATED PHOSPHOLIPID MULTILAYERS MAY BE DUE TO WATER-MEDIATED BILAYER–BILAYER INTERACTIONS

Gregor CEVC, Boštjan ŽEKŠ and Rudi PODGORNIK

Institute for Biophysics, Faculty of Medicine
and Institute J Stefan, E Kardelj University of Ljubljana, 61000 Ljubljana, Yugoslavia

To establish the stability criteria of flat and undulated (phospho)-lipid (multi) bilayers, the main free energy contributions are analysed Any flexible system of layers interacting via water-mediated forces will tend to undulate One possible explanation is thus obtained for the existence of rippled water–phospholipid mixtures which agrees with most of the data on the pretransition in lipid bilayers.

The pretransition in phospholipid bilayers ($L_{\beta'} \rightarrow P_{\beta'}$ or $smB_C \rightarrow smB_{CA}$ at T_p) [1] is generally thought of as involving a change in the membrane surface structure. This change is observed as a transformation from a planar (hexagonal lateral packing symmetry) to a rippled surface (monoclinic lateral packing symmetry) [2]. However, recent reports argue in favour of the existence of undulated bilayers even below the T_p [3–5], these ripples being distinct in form from those observed between the pretransition and the main, order–disorder phase transition. The pretransition in lipid bilayers thus remains far less understood and explored than the main transition of lipid bilayers [6]. It has now been observed in bilayers formed from most common diacyl glycerophospholipids, although often only under restricted experimental conditions: in fully hydrated zwitterionic phosphatidylcholine [7–9], anionic phosphatidylethanolamine [10] and phosphatidylglycerol [11], and in doubly ionized phosphatidylserine [12] and phosphatidic acid [13]. No pretransition was seen in large unilamellar vesicles [14,15] and in bilayers with tilted chains but with relatively small degrees o hydration [16,17]. The pretransition was further found to disappear when ions at high concentration or water structure affecting molecules were added to the water subphase, both of which affected the main transition width and temperature only slightly [18,19].

None of the four models for the occurrence of the rippled bilayer phase introduced so far [3,20–22]

could fully account for the experimental data, which are even contradicted by some of them. It is therefore our aim to show that the surface of hydrated phospholipid bilayers may be rippled because of the water-mediated interlamellar coupling. We propose that the pretransition should be considered as a transformation which is at least partly due to the change of the intrinsically generated tension to which the bilayers are exposed (phenomenologically resembling the undulation of the smectic liquid crystals under external tension, i.e. the Helfrich–Hurault effect [23]).

Let us estimate the interaction energy of two flat, infinite bilayers immersed in excess water and separated by a water layer of thickness d_w. The repulsive part of the free energy/layer/ unit area of bilayer, $g_{BB}^{rep}(d_w)$, can be determined by a Landau expansion in power series using the orientational polarization of water dipoles as a parameter [24] ‡. If the orientational polarization changes sign at each subsequent water–lipid interface, this repulsive free energy density can be written as

$$g_{BB}^{rep}(d_w) = P_0^2 \xi \epsilon / 4\epsilon_0 (\epsilon - 1)[\mathrm{cth}(d_w/2\xi) - 1]$$

$$= 2g_{LO}^{hyd} \exp(-d_w/\xi),$$

with P_0 denoting the absolute value of the orientational polarization at the bilayer surfaces, ξ the correlation

‡ We should note that regardless of its simplicity this approach gives excellent agreement with experiment.

length of this orientational order within the water, and
ϵ, ϵ_0 the dielectric constants of the water and free
space, respectively We should note here, that one
(major) part of the free energy of bilayer hydration,
g^{hyd}, cannot be determined in the above procedure
It corresponds to the free energy of the phospholipid
polyhydrate formation This energy is highly negative
[25] and nearly independent of the water layer thick-
ness once the full occupancy of the inner hydration
shell(s) has been achieved [26] (which must anyhow
be the case if the Landau expansion is to be justified)

The matching attractive part of the interbilayer
interaction energy has been shown to be $g_{BB}^{att}(d_w) =$
$-M(d_w)/12\pi d_w^2$ ($\approx -M/d_w^2$ since $M(d_w)$ is nearly a
constant provided that $d_w < 3$ nm) [27] If we denote
the free energy of chains/layer/unit area by g^{ch}
$\neq g^{ch}(d_w)$, we can write the total surface density of
the free energy of interacting flat bilayers as

$$g^{tot}(d_w) = 2g_{LO}^{hyd} \exp(-d_w/\xi) - M/d_w^2$$
$$+ g^{ch} + g^{hyd}$$

Alternatively, this energy can also be expressed in
terms of the displacements of the neighbouring lipid
surfaces perpendicular to their average plane, i e of
$u(z)$ At given g_{LO}^{hyd}, d_{wo}, and ξ, the total free energy
of flat bilayers is then minimum when the layers are
at $u_1(z) = d_L$ and $u_2(z) = 2d_L + d_{wo}$ (d_L being the
bilayer thickness) whereupon the parameter M can be
determined from

$$M = (d_{wo}^3/\xi)g_{LO}^{hyd} \exp(-d_{wo}/\xi). \qquad (1)$$

because in the stable state

$$(\partial g^{tot}(d_w)/\partial d_w)_{d_w = d_{wo}} \equiv 0$$

We proceed to calculate the free energy of the
rippled system by assuming that the bilayers become
unidirectionally undulated, $u(z) \to u(x, z)$. In this case,
g^{hyd} is not affected and $g_{BB}^{att}(d_w)$ is only slightly af-
fected by the surface corrugations This is because the
former depends solely on the local head-group–water
interactions of quantum origin and the latter is due to
the rather long range van der Waals forces which are
nearly insensitive to the shape of the interacting sur-
faces at small distances [28,29]. But $g_{BB}^{rep}(d_w)$ becomes
smaller. This is so because of the high directional aniso-
tropy of the bilayer–bilayer repulsion (this can be
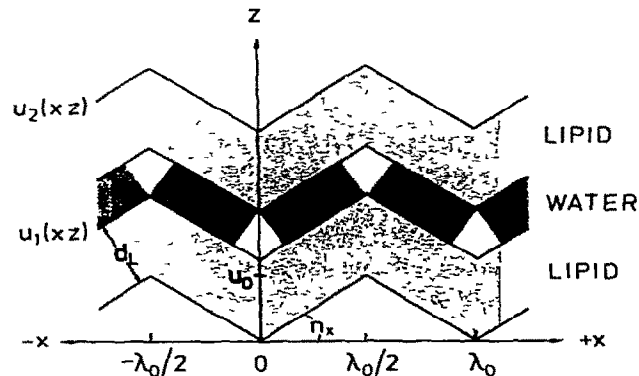readily seen from the generalization of eq. (2) of ref.

Fig 1 *Schematic* representation of the (lipid)bilayer–water–
(lipid)bilayer system with undulated surface The non-shaded
area symbolizes the interbilayer space in which the bilayers
lost their repulsive, water-mediated contact, $u_1(x, z), u_2(x, z)$
denote the lipid surface displacements

[24] to three dimensions) but also because of the very
short range of this force The repulsive contact between
the adjacent surfaces is thus partially lost (schematically
represented by the unshaded interbilayer space in fig 1)
The decrease of $g_{BB}^{rep}(d_w)$ is in the first approximation
proportional to the tilt angle of the local bilayer surface
with respect to the average bilayer plane, $n_x = |\partial u(x, z)/
\partial x|$, and to the reciprocal (wave) length of the periodi-
cal undulation, $k = 2\pi/\lambda$, formally corresponding to a
second-order term in n_x (fig. 1) The resulting gain in
energy is partly cancelled by the compression of the bi-
layer interior brought about by the surface undulations
The elastic free energy required for this compression,
$g^{el}(n_x)$, can be assumed to account for most of the
change in g^{ch} [30] The interlamellar interaction modi-
fications due to the curvature effects and to the inter-
bilayer distance changes are of higher orders and can
be shown to yield essentially the results that were obtain-
ed for flat bilayers. In fact, no other terms below the
fourth order need be included and the total free energy/
layer/unit area of the interacting bilayers is

$$g^{tot}(d_w, n_x, k) \approx 2g_{LO}^{hyd} \exp(-d_w/\xi)(1 - 2d_w n_x |k|/\pi)$$
$$- M/d_w^2 + g^{el}(n_x) + g^{ch} + g^{hyd}.$$

Here, $g^{el}(n_x)$ is proportional to the product of the bi-
layer elastic constant (which is by our choice $u(z)$
$\to u(x, z)$; $K \triangleq K_x \ll K_y$) and the surface curvature,
$(\partial n_x/\partial x)^2$ [30]. Given that $uk \ll 1$ [2], the other ener-

gy terms are of the same form for the triangular and the sinusoidal undulation of amplitude u and after averaging with respect to x the total free energy of the corrugated interacting bilayers system/layer/unit area can be written as

$$g^{tot}(d_w, u, k) \approx 2g_{LO}^{hyd} \exp(-d_w/\xi)(1 - 2d_w u k^2/\pi)$$

$$- M/d_w^2 + K u^2 k^4/2 + const. \qquad (2)$$

In deriving the above equations we have omitted the higher-order terms. Therefore, from the minimization condition,

$$\partial g^{tot}/\partial d_w \equiv \partial g^{tot}/\partial u \equiv \partial g^{tot}/\partial k \equiv 0,$$

only the optimal d_{wo} and the product $u_o k_o$ can be determined. The optimal d_{wo} of eq. (2) is nearly equal to the one used in eq. (1). But the solution

$$k_o^2 = 4 d_{wo} g_{LO}^{hyd} \exp(-d_{wo}/\xi)/\pi u_o K$$

shows that the stable state of interacting bilayers is one which is undulated with a period

$$\lambda_o = \pi [\pi u_o K \exp(d_{wo}/\xi)/d_{wo} g_{LO}^{hyd}]^{1/2} \qquad (3)$$

The experiments suggest the following values of the parameters of eq. (3) for the case of phosphatidylcholines at $T < T_t$: $d_{wo} \approx 2u_o \approx 1.6 \text{ nm}^2$, $g_{LO}^{hyd} \approx 0.28$ J m^{-2}, $\xi \approx 0.2$ nm [*]. Unfortunately no experimental data are available on K at $T < T_t$. An estimation based on the approximate relation between the interfacial energy/unit area, γ, and the constant $K \cdot K \approx d_L^2 \gamma$ [34], as well as on the data for the changes at T_t of γ [#] and of d_L [2] suggests that $K(T < T_t)/K(T > T_t) \approx 2-3$. Using the measured value for $K(T > T_t)$ [37] we then get 4×10^{-21} J $< K < 4 \times 10^{-19}$ J at $T_p < T < T_t$. The calculated ripple periods, 22 nm $\lesssim \lambda_o \lesssim 220$ nm, compare well with those determined experimentally,

13.5 nm [2] $\leqslant \lambda_{exp} \leqslant 100$ nm [38]. In light of the fact that eq. (3) contains no free parameters this qualitative agreement thus yields strong support to our starting assumption that it is the water-mediated bilayer —bilayer interactions [24,34] which is mainly responsible for the lipid bilayer undulations. It should be borne in mind, however, that other strains such as those due to tilted chains (which were in part considered already with our choice $u(z) \to u(x, z)$) may assist in the creation of surface ripples but *cannot be the sole factor.*

Besides yielding λ_o alike to λ_{exp}, the model introduced in this work explains why the surface undulations are seen in multi- (at least bi-) lamellar systems only [14,15] but not a fortiori in all systems with tilted chains [16,17] and a first-order main transition. It elucidates the disappearance of the pretransition in systems in which the water-mediated interbilayer forces are largely changed because of the water structure affecting molecules [12,18,19]. Eq. (3) further suggests, also in accord with the experimental findings, that only those lipid systems which are sufficiently elastic (no intermolecular bonds and full hydration [2–5, 7–13]) and consist of rotationally asymmetric molecules to possess a small value of $K = K_x \ll K_y$ will have observable ripples at $T < T_t$. (The pretransition and the change of the ripples size at T_p could then be associated with an abrupt alteration of K at this temperature.) At $T > T_t$, on the other hand, $K_x \approx K_y < K$ and the bilayer surface will be jumbled rather than rippled [3–5]. Our formalism cannot be strictly applied to this case; still using eq. (3) with the parameter values of phase L_α we conclude that the lateral size of the L_α phase humps should be $\lesssim \lambda_o$. This has been also established experimentally [9–11].

In summary, we are able to explain most of the firmly established facts about the rippling (and the existence of a pretransition) in phospholipid bilayers. However, the actual contribution to g^{tot} from chain tilt, and the correlation between the parameters used in our model and the thermodynamic data on the pretransition of lipid multibilayers, remain to be established.

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[*] Theoretical considerations suggest that g_{LO}^{hyd} $(T < T_t)/g_{LO}^{hyd}$ $(T > T_t) \approx 1.3$ [31] but experimentally no phase dependence of g_{LO}^{hyd} for phosphatidylcholines could be established [32]. Our value for g_{LO}^{hyd} is the best fit to the data from ref. [32] after the suppression of the data points at $d_w < 1$ nm, for which the Landau expansion cannot apply $\xi(T > T_t)/$ $\xi(T < T_t)$, on the other hand, was found to be ≈ 1.25 [33].

[#] The change of γ at T_t is probably $(3-4) \times 10^{-2}$ J m^{-2} since the lateral pressure of chains then increases from ≈ 20 mN m^{-1} [35] to 50–60 mN m^{-1} (the latter value corresponds the phase L_β; see ref [36]).

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