Review

Functional role of water in membranes updated: A tribute to Träuble

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ABSTRACT

The classical view of a cell membrane is as a hydrophobic slab in which only nonpolar solutes can dissolve and permeate. However, water-soluble non-electrolytes such as glycerol, erythritol, urea and others can permeate lipid membranes in the liquid crystalline state. Moreover, recently polar amino acid's penetration has been explained by means of molecular dynamics in which appearance of water pockets is postulated. According to Träuble (1971), water diffuses across the lipid membranes by occupying holes formed in the lipid matrix due to fluctuations of the acyl chain trans–gauche isomers. These holes, named "kinks" have the molecular dimension of CH_2 vacancies. The condensation of kinks may form aqueous spaces into which molecular species of the size of low molecular weight can dissolve. This molecular view can explain permeability properties considering that water may be distributed along the hydrocarbon chains in the lipid matrix. The purpose of this review is to consolidate the mechanism anticipated by Träuble by discussing recent data in literature that directly correlates the molecular state of methylene groups of the lipids with the state of water in each of them. In addition, the structural properties of water near the lipid residues can be related with the water activity triggering kink formation by changes in the head group conformation that induces the propagation along the acyl chains and hence to the diffusion of water.

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Contents

1. Introduction

1.1. The selective barrier of the lipid membrane

The classical picture of a cell is a compartmentalized system in which the membrane is the selective barrier of contention of the cellular

material [1,2]. In this view, the core of the membrane is the lipid bilayer described as an autonomous rigid phase in which partition rules the thermodynamics of transport processes. In addition, from the electrical standpoint, the bilayer is considered as a slab of low dielectric permittivity that should be impermeable to ions and polar solutes.

Water transport through biological membranes has been thoroughly revised by different authors [3–5]. Briefly, water transport can occur in three ways: 1) by diffusion through the lipid matrix; 2) through transport proteins such as channels and some occlusion transport proteins such as glucose transporters, and 3) through water channel proteins specifically expressed by cells for such purpose. A common feature in all these ways is that water transport is passive, i.e. driven by a gradient of water chemical potential between the inner and the outer compartment.

Biological membranes appear to be composed of a complex mixture of lipids varying in head group size and chain length. The isolation and characterization of lipids from cell membranes allowed preparation of experimental model systems, which mimic several properties of biological membranes. Lipids swollen in an excess of water above the transition temperature form closed particles trapping an aqueous solution. These multilamellar liposomes are able to swell and shrink according to if they are dispersed in hypotonic or hypertonic solutions, respectively in comparison to the solution in its interior. This osmometer behavior is due to the selectivity of the bilayer to solutes according to size and charge and to the high rate of diffusion of water across the lipid matrix [6].

The lipid barrier is selective but not an ideal semipermeable one. This is to say that for some solutes it is completely impermeable while for others it displays a wide range of permeability properties. This partial permeation denotes that water can flow through the lipid bilayer provided that a gradient of water chemical potential is present and that aqueous soluble solutes can also go through the membrane. In terms of the thermodynamics of irreversible processes, these two fluxes are coupled, i.e. they interfere and connect to each other, contrary to the primitive view that solutes flow through some region and water across other. The assumption that solute and water permeations take place as independent processes is opposed by the findings of Hill and Cohen and van Zoelen [7,8], who demonstrated a strong solute–solvent interaction in the membrane phase. Kedem and Katchalsky [9] described by means of the formalism of thermodynamics of irreversible processes the simultaneous non-independent permeation of non-electrolytes and water. This approach clearly demonstrates that both fluxes are not independent i.e. water permeation affects the permeation of the nonelectrolytes and vice versa.

The formalism of the irreversible thermodynamics defines a reflection coefficient which is equal to one when the solute is completely reflected and only water can flow through the membrane, i.e. in this case, the membrane is ideally selective or semipermeable. Large solutes such as sucrose, dextran or ions fit into this classification. Other solutes such as glycerol, urea, ethylenglycol, erythritol permeate the bilayer at different rates, i.e. they are described by different values of reflection coefficients, all of them lower than one [10].

Two structural views of the membrane have been proposed to interpret these results. One of them considers a solubility model in which a ternary mixture of water, lipid and solute is formed in some degree during the transport process.

Another model suggests that water and solute diffuse through pores formed in a rigid matrix. This proposal disregards lipids as influencing the solvent properties of water i.e. solute is dissolved in water pools enclosed by the lipid matrix. However, these descriptions are quite extreme and membrane responses are a complex combination of the two proposals due to its unique physicochemical properties. One of these properties is that the lipid walls (hydrophilic and hydrophobic groups) organize water in a different structure than in bulk phase, which, in consequence, has different thermodynamic (surface tension) properties. Thus, the lipid membrane is able to admit different amounts

of water according to the presence of different solutes and to the different type of lipids. This makes that the membrane has a notable versatility to act as a non-polar solubility barrier or a polar sieve, which implicitly demands a dynamics of water in the lipid matrix. Therefore, a clarification of the organization of water molecules in different regions of the bilayer and its thermodynamic properties in comparison to bulk water is required.

From a molecular point of view, according to Träuble (1971), the movement of water molecules across membranes can be produced as a consequence of the thermal fluctuations of the conformational isomers in the hydrocarbon chains of the membrane lipids resulting in the formation of so-called "kinks" [11].

"Kinks" are described as mobile structural defects of free volumes in the hydrocarbon phase of the membrane (Fig. 1A). Kink diffusion is a fast process with a diffusion coefficient of kinks of c.a. 10^{-5} cm^2/s. Low molecular weight molecules such as urea, glycerol and some aminoacids can fit in the free volume of the kinks and migrate together with the kinks across the membrane. Thus, kinks are intrinsic polar carriers in the lipid membranes.

Träuble noted that water molecules can fit neatly in g–t–g kinks between two chains calculated by Flory as low energy motion for chain polymers [12]. Water molecules filling the vacancies may jump from vacancy to vacancy, randomly according to its formation by thermal agitation. In this case, water would be molecularly dispersed in the hydrocarbon matrix. This picture suitably applies to the diffusion of water molecules in a liquid crystalline state of saturated phospholipids.
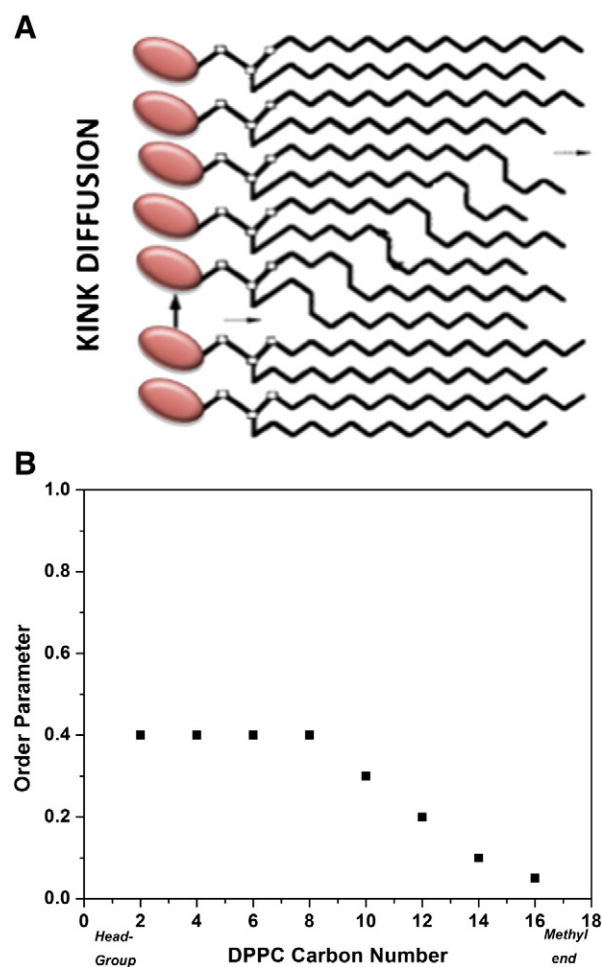
Fig. 1. A. Schematic representation of "kinks" in the lipid bilayer pictured as mobile structural defects. This picture is only a partial view and should be extended to the whole membrane in the liquid crystalline state. B. Order parameter in relation to kink formation.

In an extension of this mechanism, the copermeation of solutes and water has been interpreted. However, this process is not well understood and experimental results are forcedly interpreted within the frame of one of the two alternative models described above for permeation.

According to de Gier [13], water exchange across the membrane under equilibrium conditions is disturbed by the solute producing a net additional flux of water molecules coupled with it. This assumes that non-electrolyte is acting as a carrier or inducing an additional flow of water into the membrane. This can be explained considering a chemical equilibrium when lipids are dispersed in an excess of water. Membrane structure is stabilized with a given amount of water forming part of its structure. When a solute enters the lipid matrix, probably dissolving in water between the acyl chains occupying the kinks, a difference in the chemical potential of water is produced and a water influx occurs to reach again the equilibrium. This process is similar to osmosis and the swelling of the membrane matrix may produce water paths expanding the membrane. This is congruent with the leak of ions contained in the liposome interior during hypotonic swelling.

The possibility that water may be found in the interior of the lipid matrix has been suggested in the light of the behavior of polar aminoacids, which should not be able to permeate the lipid membrane if this is a pure non-polar phase.

This picture has emerged nowadays to explain unexpected results in the penetration of charged and polar aminoacids, that deviates from the predictions of the permeability mechanisms based on the partition of solute in the lipid considered as a nonpolar matrix (see below). This idea was coined first by Träuble and extended for solute behavior by de Gier [14].

Kink theory considers that the process of copermeation is due to the diffusion of the solute across the membrane, from the surface to the interior of the bilayer, of a large cavity filled with a solution of the permeant molecule, that disturbs the lipid packing.

The present review considers that Träuble theory can be updated taking into account that lipid matrix can influence the thermodynamic properties of water enclosed between the acyl chains and this gives place to a new ability of water to dissolve polar solutes in it. In other words, in addition to the probability of finding water in the lipid matrix (the kinks) the possibility that this water may have different solvent properties will be analyzed.

1.2. Permeability and partition. Failure of the classical picture

Permeability (P) is a phenomenological coefficient defined as

$$P = KD/d$$

where D is the diffusion coefficient of the permeant in the lipid membrane and d the membrane thickness. This coefficient is obtained from the integration of the first Fick's law of diffusion, introducing the partition coefficient of solute (K) between the membrane and the aqueous phase. If the diffusion of molecules into a cell is governed by the solubility of the permeating molecules in the lipids, small hydrophobic molecules generally cross the membrane more easily than water-soluble molecules or ions (Overton's rule) [15].

Generally, permeation of solutes in lipid membranes is understood as a consequence of the solute partition between the bulk aqueous phase and the nonpolar core of the membrane. Partition is a measure of the relative solute solubility between the membrane and water, both considered as isotropic pure phases. The partition coefficient is given by the difference between the standard chemical potential (free energy) of the solute in water and that of the solute in membrane. Membrane interior is a low dielectric phase, ascribed to the hydrocarbon phase excluding water. Therefore, highly hydrophobic solutes are expected to dissolve in the lipid matrix while polar and charged

molecules should be excluded from it. However, the extension of what can be considered a homogenous nonpolar phase is restricted to the center of the bilayer. In fact, regions imbibed in water compose the bilayer, and therefore partitioning is not the proper description for the insertion of several types of solutes.

For example, the penetration of some charged and polar aminoacids such as Arg, does not obey a simple partitioning between water and a hydrophobic phase as predicted by the Henry's law [16]. Arg may be either charged or uncharged at the center of the membrane as shown by molecular dynamics studies. Thus, the primary picture of the bilayer as a pure dielectric media is not valid.

In order to explain the thermodynamics of Arg insertion and to justify the high demand of energy to insert a charge in a nonpolar phase, water clustered in defects or pockets in the membrane phase has been postulated [17].

Presence of water pockets may acceptably explain the insertion of highly hydrophilic aminoacid moieties in pools between acyl chains [16]. Penetration of amphipathic helices into the hydrophobic interior of membranes was interpreted by a "snorkeling" mechanism. The "snorkel effect" of positively charged amino acids, such as Lys or Arg may be due to insertion into the acyl chain region favored by the long hydrocarbon side chains.

Thus, water domains would allow the penetration of high positively charged guanidinium group into the membrane [18].

On one hand, Phe has a broad distribution in the membrane but Trp and Tyr localize strongly at the interfacial region, specifically at the carbonyl group level. Thus, different solutes may stabilize in different regions of the bilayer according to their size and polarity. This evidence suggests that the bilayer has different regions bearing different degrees of affinity by the different aminoacids and thus it cannot be considered as a homogeneous solvent on which foundation of the simple partitioning approach was sustained. In this regard, molecular dynamics calculation has suggested that partitioning of charged and polar side chains is accompanied by water defects connecting the side chains to bulk water [20,23,24].

The number of water molecules that follows the permeating molecule, increases with the probability of formation of additional cavities at the membrane surface, a kind of cooperative process. If this is the case, water clusters in lipid membranes should give place to water distribution along the membrane thickness and topology according to the type and phase state of the lipids. In terms of membrane structure, this implies the location of water in the lipid matrix in correlation with the lipid chemical residues and the thermodynamic properties of water in these restricted domains as a result of the interaction of compounds with the membrane.

On the structural standpoint, several assumptions are usually made on the water location in the bilayer structure that greatly influences the final values of area per lipid and thickness of lipid bilayers [19].

Water buried in the lipid membrane would be facing the lipid head groups and apolar alkyl chains [20]. In this regard, it has been recognized that water may penetrate the lipid bilayer reaching the region of the carbonyl groups [21,22].

In this context, Israelachvili and Wennestrom (1996), Israelachvili (1977), and Pashley et al. (1981), [25–27] showed that water structure near hydrophobic surfaces has modified solvent properties over distances up to 2 to 3 nm.

Formalisms of classical thermodynamics usually employed to understand biological transport processes consider water as a continuous solvent where macroscopic properties of bulk water are thought to be still valid. This is against the idea that water may be occluded in small regions conforming water clusters whose properties are different than bulk water.

In conclusion, sieving properties of membranes are not just a matter of simple diffusion, but also rely on complex mechanisms of relaxation and cooperativity between different regions of the bilayer.

1.3. Confirmation of kinks hypothesis: experimental evidences

1.3.1. Phase transition and water states

Small Angle X Ray Scattering SAXS indicated that the phase transition of lipid membranes is accomplished with a membrane thickness decrease and an area per lipid increase [19]. Concomitantly, in this condition, the increase in the trans–gauche isomers in the lipid acyl chain lipid membranes produces higher disorder state [28]. Parallel to the increase in chain mobility, the water amount in the bilayer increases from 7 to 20 water molecules per lipid in the case of phosphatidylcholines (PC) and from 4 to 7 in phosphatidyl ethanolamines (PE) with a slight variation with the chain length [29].

Träuble suggested that the creation of kinks is easier above the phase transition temperature i.e. kink formation is favored by the membrane expansion at the phase transition [11]. Let us analyze next the molecular properties of kinks and water enclosed by them.

The global cooperativity of the transition measured by the enthalpic change obtained by Differential Scanning Calorimetry (DSC) [30,31] can be interpreted in terms of the cooperative units of the molecules, which main contributors are the acyl chain residues [32]. On the other hand, fluorescent anisotropy using probes inserted in the hydrocarbon region indicates that order in the membrane phase decreases along the hydrocarbon chain into the center of the bilayer [33,34].

The decrease in order at the phase transition is measured by an increase in polarity using Laurdan as a fluorescent probe [35,36]. Change in polarity is ascribed to an increase in water in the membrane phase. The generalized polarization in the gel state of DMPC and DMPE is 0.6–0.7 and above T_t, − 0.1 and 0.2 respectively. Generalized polarization (GP) values are related with the number and motional freedom of the water molecules around the fluorescent probe, assumed to reflect indirectly the state of the lipid environment. Thus, independent methodologies indicate a correlation between membrane structure and water properties at different phase states of the lipid membrane.

1.3.2. The molecular picture

The formation of kinks is based on the ability of CH_2 residues to rotate along the C–C bonds. Both, fluorescent anisotropy and Electron Paramagnetic Resonance (EPR) [37] indicate that the rotational isomers of the CH_2 residues along the C–C bonds result in an elevated number of configurations. Thus, order parameter decreases noticeably beyond the first 4–5 carbon atoms of the acyl chains [38,39]. In Fig. 1B, the order parameter is described schematically along the kink formation according to Deuterium quadrupole coupling-NMR. It must be considered that this disorder propagates in the plane of the membrane.

Fourier Transform Infrared Spectroscopy (FTIR) provides evidence of the membrane state at molecular level. This methodology measures the shift to higher values of the frequency corresponding to the CH_2 groups at the phase transition temperature [40,41].

The results of Fig. 2A show that the frequency of the CH_2 asymmetric stretching of different lipids increase at the reduced temperature. This shift is due to a reinforcement of the CH bond constant force by the decrease in the lateral CH_2 interactions in the gel phase when the membrane goes to the liquid crystalline state. An interpretation of the FTIR results is that the increase in isolated CH_2 populations corresponds to the appearance of kinks [42]. These results consolidate the idea that above the phase transition places due to the decrease in contacts between the CH_2 groups i.e. kinks are opened. These kinks are formed by thermal agitation of the acyl chain moieties. However, it must be noticed that values obtained above the transition temperature are not the same for the different lipids. This is an important observation denoting that the formation of isolated populations depends on the lipid nature. See for example the increase of the frequency value in the fluid state with the acyl chain composition above the phase transition temperature in Fig. 2A. This indicates that although all the lipids are in the so-called "fluid state", at molecular level there are important differences in the CH_2 populations. The longer the acyl chain, more
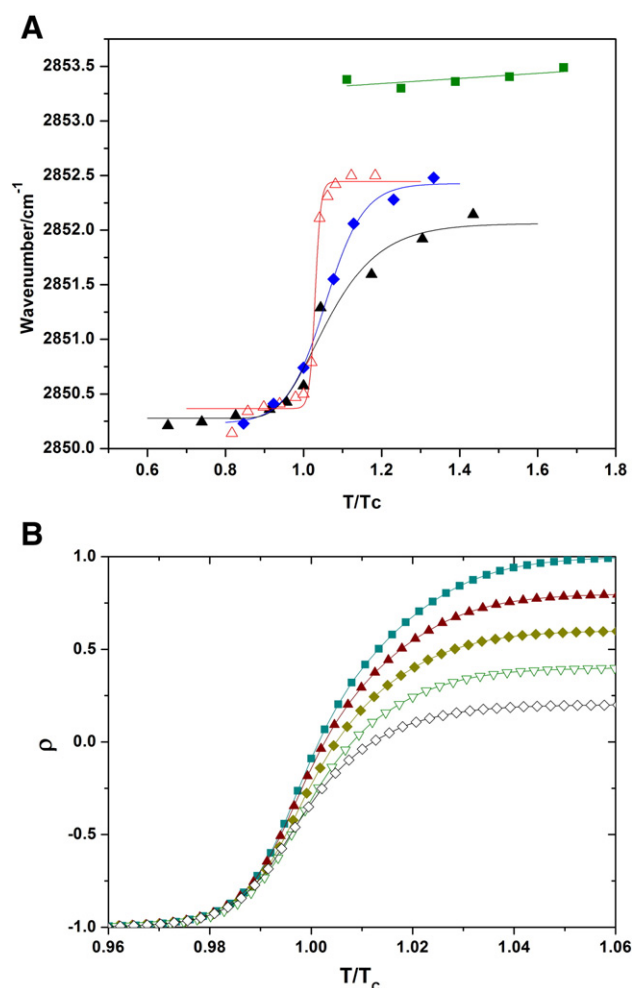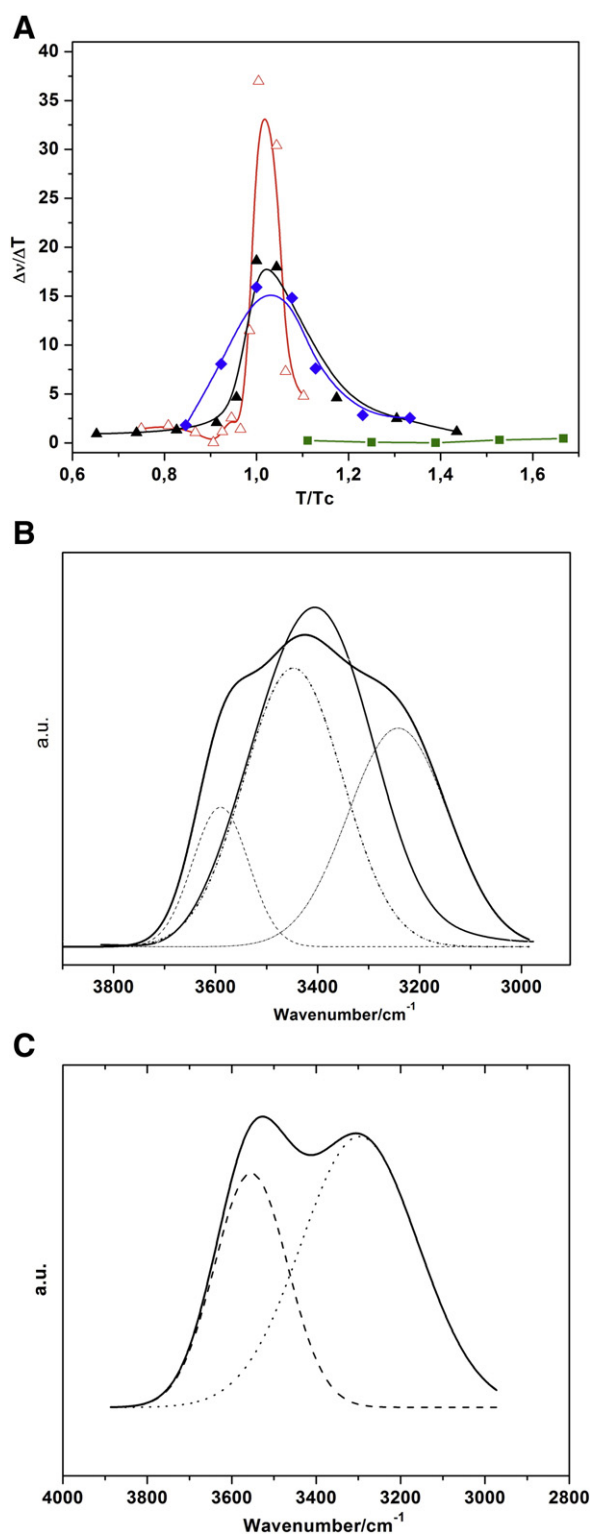

Fig. 2. A. Frequency of the CH_2 asymmetric stretching of different lipids as a function of the reduced temperature (■) DOPC, (△) DMPE, (◆) DPPC, (▲) DMPC. B. Simulation of the phase transition for different lipid species for different values of θ. See text for details. θ: (■) = 0.00, (▲) = 0.10, (◆) = 0.20, (▽) = 0.30, (◇) = 0.40.

isolated populations can be formed (see DMPC and DPPC) and the increase is more pronounced when double bonds are introduced. Cooperativity units derived from DSC can be correlated with the rotational isomers of the CH_2. In this regard, the highest CH_2 connected population corresponds to the highest order parameter. Similarly, low order is concomitant with an increase of isolated CH_2. In this condition, the distribution of connected and isolated populations of methylene groups is not similar for different lipids although they are in the same phase state (Fig. 3A). Thus, at the phase transition, the microscopic configurations may be quite different depending on the lipid species.

In the condensed state, the CH_2 are connected in a solid-like lattice, but in the liquid crystalline state, the number and kind of isolated CH_2 are not the same for all lipids. The sequence that follows the dependence with the acyl chain length is DMPC, DPPC and DOPC. However, the change at the transition temperature is steeper for DMPE. This could be a consequence of strong head group–head group interaction accomplished by hydrogen bonding between the phosphate and the amino groups. It must be noticed that the higher values of frequency are comparable to DPPC, which has a longer acyl chain. The transition temperature of DMPE is around 52 °C, which is needed to disrupt the intermolecular H-bonds. Thus, when this is achieved more degrees are apparently gained by the CH_2 in comparison to PC. Thus the rupture of the H bonds produces a higher population of isolated CH_2.

The "kink" theory analyzed by modeling of small molecules permeating the lipid bilayer showed that the probability values (P) decrease

A tetha-parameter (θ) denoting the probability of a CH$_2$ group in each lipid chain unaltered during the transition was defined. When $\theta = 0$, all the groups in the lipid unit are able to reach a high frequency state. If it is considered that some CH$_2$ groups that cannot undergo the transition the final state in the liquid crystalline state (the so called fluid phase) is dependent on the lipid species and on the cholesterol ratio [44]. The density of states at the membrane along the phase transition for different values of θ is plotted in Fig. 2B. These results are congruent with the experimental finding that the methylene groups contributing to the phase transition are those corresponding to carbon atoms beyond 5 or 6 with respect to the polar head group as described in Fig. 1B. The θ parameter denotes the ratio between the internal modes of the lipid molecules (identified as the CH$_2$ groups) that cannot undergo a transition with respect to the total can be defined [39,45].
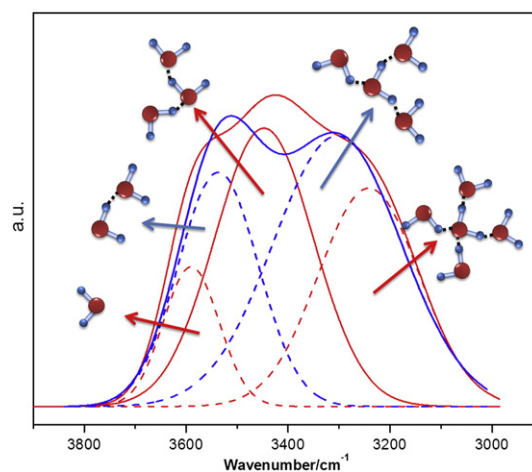
1.3.3. Water states in kinks

In addition, the shift to higher frequencies of the νCH$_2$ symmetric stretching as a function of the water content has been also reported for DMPC [46]. That is, a lyotropic transition also leads to isolated CH$_2$ populations. Taken together, at the phase transition, CH$_2$ isolated populations give place to kinks in which water can be located.

The water state, defined in terms of hydrogen bonding populations as observed by the variation the frequencies of the OH bands, correlates with the phase state of the lipids (Fig. 3B and C). In fully hydrated lipids above the transition temperature, two clear bands are observed one at 3620 and another at 3250 cm^{-1} bands. This distribution is quite different than that observed in pure water.

When fully hydrated lipids are displaced to 18 °C, at which the lipids are in the gel-ripple state, a net band corresponding to the center of the pure water is evident but those observed above the transition temperature are still noticeable. This denotes that in the gel ripple state some liquid crystalline regions still coexist with the gel state due to the spontaneous curvature of the ripples. The pattern of solid partially disappears and shifts to higher frequencies, when acyl chains melt indicating a decrease in water bonding.

The inspection of the water bands denotes that water in DMPC at 18 °C, where the lipids are in the ripple-gel state is distributed in monomers and 4H bonded water molecule species coexisting with trimers of pure water [47] (Fig. 4). Instead when, isolated populations are present (i.e. kinks are formed) the water bands show an increase in the dimer and pentamer populations with a disappearance of the species corresponding to pure water. Apparently, an increase in the hydrogen bonded water populations appears when CH$_2$ groups surround water domains or water domains are created around CH$_2$ groups.

Fig. 3. Water states in lipid membranes below and above the phase transition temperature. (A) Derivative of the frequency values with respect to T as a function of the reduced temperature for lipids in Fig. 2A. Symbols are the same as in Fig. 2A. (B) Water bands in the presence of fully hydrated DMPC at 18 °C. Central band corresponds to pure water. (C) Water bands in the presence of fully hydrated DMPC at 30 °C. Dotted lines represent deconvolution of the bands denoting different water species.

Fig. 4. Distribution of water species in the gel ripple (red line) and liquid crystalline (blue line) states in comparison to pure water.
From Disalvo and Frias [22].

with temperature and (or) with area decrease. P calculated as probabilities of "kinks" or gauche-isomers for the chains in liquid-crystal bilayer, agree with the experimental data. In addition, cholesterol, introduced in the bilayer decreases its permeability as found experimentally [43].

The distribution was obtained from the deconvolution of the water bands for pure water, and overlapped with those obtained for water in the presence of lipids at 18 °C and in the liquid crystalline state. It is observed that the bands for pure water contain all the bands in the presence of lipids.

Since the frequency is a consequence of the OH strength in one molecule, the formation of H bonds between adjacent molecules makes weaker the covalent bond and then the frequency is displaced to lower values. It is inferred that the addition of subsequent discrete H bond to a central molecule will make weaker the H bonds and then the frequency will displace to lower values. It may be considered also that the shifts to lower frequencies is not an all of none phenomenon since its population of water in the deconvoluted bands is broad, meaning that even at each water populations several configurations of H bonds are possible, probably due to bending and scissoring modes.

More interesting and important, when kinks are formed in lipids with double bonds, such as in dioleoylphosphatidylcholine (DOPC), the water bands are comparable to that found in pure water. This strongly suggests that when water molecules condense in a large space, the water type resembles that of bulk water.

1.4. The extended model of Traüble–Haines–Liebowitz

Water permeability of phospholipid membranes is decreased by cholesterol in nearly one order of magnitude at 1:4 cholesterol:phospholipid ratio. This is concomitant with a reduction in the lateral mobility of the lipids.

According to the Haines–Liebowitz–Traüble model, permeability is connected to lateral diffusion [48]. In FTIR spectroscopy, there is a reduction of the ρ value indicating that the connected populations of CH_2 increase with the cholesterol ratio, which is totally compatible with the water permeability decrease via the reduction of kinks [44].

1.4.1. Contribution of the interphase

Traüble's proposal was developed further to understand the mechanism of water permeation in lipid bilayers by Haines and Liebovitz [48].

This model proposes that the generation of kinks in the lipid matrix is due to changes in the conformational state of the lipid head groups. In other words, the membrane is considered as a phase that is sensitive to the presence of solutes in the adjacent aqueous phase.

Headgroup lateral movement generates kinks or vacancies at the surface. The lateral interaction of the headgroups is qualitatively and quantitatively different than that between the acyl chains. Thus, the head group movement should be the rate-limiting step for water transport. In this regard, it has been demonstrated that energy input required to expand a monolayer beyond the hydration shell grows with the area per lipid for DMPC and DMPE being much higher for DMPE (Fig. 5A). As reported, polar head groups of PC's are more hydrated than those of PE's. In the liquid condensed state, the average number of water molecules per lipid is higher for DMPC than for DMPE [29,49]. In monolayers of PE's, polar heads of adjacent molecules interact laterally through internal hydrogen bonds between $(NH_3)^+$ and (PO_4) groups. In contrast, in PC's polar head groups of neighboring molecules interact laterally through indirect H-bonds mediated by a water molecule (water bridge), which are more labile than direct H-bonds [41]. As a result, DMPE membranes have a much lower hydration than DMPC's (4 mol per PEs in comparison to 20 in PCs in the fluid state), thus reducing the area per lipid [49].

A 30–40% expansion of the bilayer means a creation of 20–30% vacancies in the lattice of the head groups giving place to an exposure of the tops of the chains to form hydrocarbon cavities in contact to water. Fig. 5B shows the different orientations of the polar head groups according to their composition.
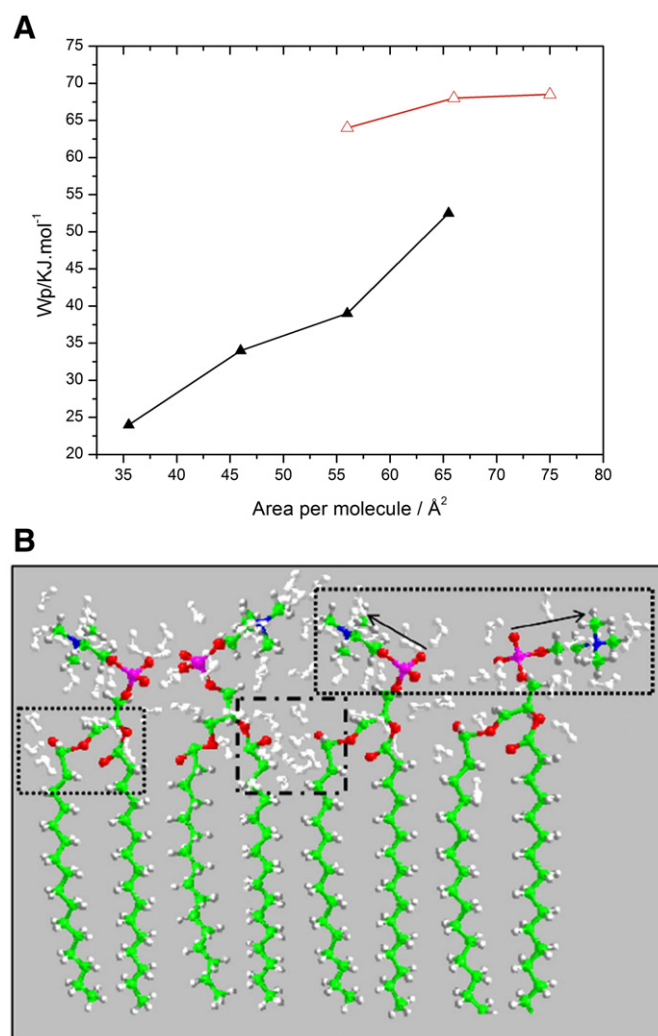

Fig. 5. A. Energy input required to expand DMPC and DMPE monolayers beyond the hydration shell with the area per lipid. (▲) DMPC, (△) DMPE. B. Interaction and conformational changes at the lipid interphase. (From Disalvo et al. [49].)

1.4.2. Interphase water activity

The process of expansion during the permeation process is not well understood. In order to interpret it in terms of physical chemical process, the mechanism of interaction and the kind of adsorption process should be analyzed. To proceed further an update of the membrane regions is required in which the properties of lipid membranes depends on water organization as part of the structure. In consequence, its thermodynamic properties must be redefined. The updated model is described in Fig. 6A. The regions of 1 nm thick at each side of the bilayer, denoted as an excluded volume for solutes in the previous session, contain around 18–20 water molecules per lipid in phosphatidylcholines [50,51]. The presence of those interphases describes the membrane as a composite element consisting of the lipid leaflets and the regions of hydrated polar head groups at each side.

The consideration of these membrane interphases in the structure of the membrane makes necessary to revise the thermodynamic properties of water and hydrated membrane components in energetic and entropic terms, that, as a result make the membrane to act as a non-autonomous phase in which interphases are active in relation to its response to changes in the adjacent media.

The approach consists in considering the interphase as a bidimensional aqueous solution in which the polar head groups are imbibed in water maintaining its hydration shell, similar to ions dissolved
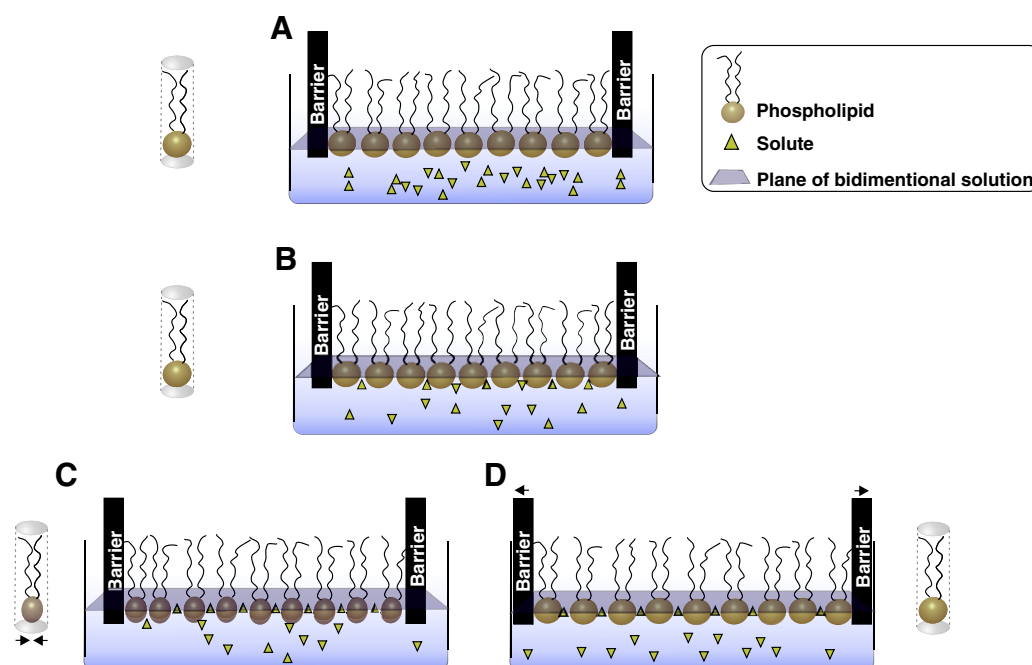
Fig. 6. Model of the membrane regions considering water (gold triangle) as part of the structure in a bidimensional solution. B) Solute insertion in the surface lattice of phospholipids. C) Water entrance at constant area. Surface pressure increases at expense of the packing between solute and phospholipids, in which hydration shell may be reorganized. D) Water entrance and membrane expansion against a constant pressure.

in water. The approach is based on the definition introduced by Defay–Prigogine [52,53] in which the surface pressure of a lipid monolayer can be described in function of the water activity at the interphase solution by Eq. (1)

$$\pi = \left(\gamma^{\circ} - \gamma \right) A = RT \ln a_w^i / a_w^L \tag{1}$$

This equation clearly denotes that the surface pressure (π) – which is the difference between the surface tension of pure water (γ°) and the surface tension in the presence of lipids (γ) – increases when α_w^L decreases from below 1 and becomes zero when $\alpha_w^i = \alpha_w^L$, i.e. the activity of pure water when lipids coverage is zero [54].

Thus, when water activity varies the surface pressure is varied. Thus surface pressure (surface tension) is a consequence of the changes in the properties of the solution at the interphase. Several reasons can concur to affect interphase water activity. One of them is relevant to this analysis considering Traüble's model. A permeant solute may interact at the interphase by dissolution in the interphase, which decreases the water activity. In consequence, the difference of water chemical potential between aqueous solution and the membrane interphase drags water increasing the surface pressure, as an osmotic process. (Fig. 6B). At constant pressure, barrier displaces to reach a new equilibrium position (Fig. 6C). At constant area, the surface pressure increases at expense of solute–lipid interaction and reorganization of the water interphase (Fig. 6D).

The perturbation of the monolayer pressure in terms of water activity by addition of a solute to the subphase is plotted vs. the initial surface pressure (Π_o) (Fig. 7). The slope of the curve is a function of the acyl chain composition and the cut-off is the limit pressure that depends on the head group hydration water. This is somehow the covolume in a real gas. It is clearly observed that the perturbation is only possible when an excess of area above the critical one corresponding to the limit surface pressure. This area increase corresponds to a relaxation of the surface pressure below the critical one ($\Pi_c - \Pi$) and can be identified as the excess of interfacial water beyond the hydration shell of the phospholipids. Bottom picture in Fig. 7 describes the increase of water

due to surface pressure reduction below the cut off necessary to peptide or solute interaction.

This water excess beyond the critical cutoff (Π_c) can be described by the difference ($\Pi_c - \Pi$). A plot of the perturbation vs. the excess of surface pressure is plotted in Fig. 8A. The value of slope k depends on the differences of the chemical potential of water at the interphase in the absence and in the presence of the perturbation [55]. In Fig. 8B, the increase of isolated CH_2 population (i.e. kink formation) with the increase in chain length and saturation is shown. The relative increase in isolated populations of CH_2 residues follows the same trend than the increase in the slope in the plots of $\Delta\Pi$ vs. excess of water (see inserted values of the slopes above columns corresponding to each lipid) [55]. Thus, the increase in the slope is related to the open spaces produced by water regions present in the membrane phase according to the acyl chain composition.

In conclusion, the presence of water in the membrane structure is evidenced in two states: hydration water at the polar head groups and confined water beyond the hydration shell. The first one is inert for peptides and solutes but the second one gives thermodynamic support to membrane response. It is reasonable then to recognize that water activity is an intensive parameter of the thermodynamic state of the lipid system. Water molecules present within the hydrocarbon region of the phospholipid membranes interact with phospholipid molecules through their chemical potentials (Gibbs–Duhem relation) implying the conformational state of the acyl chains [56–58]. This might be the reason for which similar structural compounds interact differently with fluid membranes of different lipid components.

1.5. Kinks may preserve water in hydrophobic environments in the membrane

As discussed above, water structure near hydrophobic surfaces has modified solvent properties over distances up to 2 to 3 nm [25–27]. Specifically, the dynamics of hydrophobic apolar surfaces is slowed down [60]. As reported elsewhere by Giovambattista et al. 2006 and Arsov et al. 2009 [54,59], the boundary structure greatly influences the water structure and dynamics. The dynamics is slowed significantly in
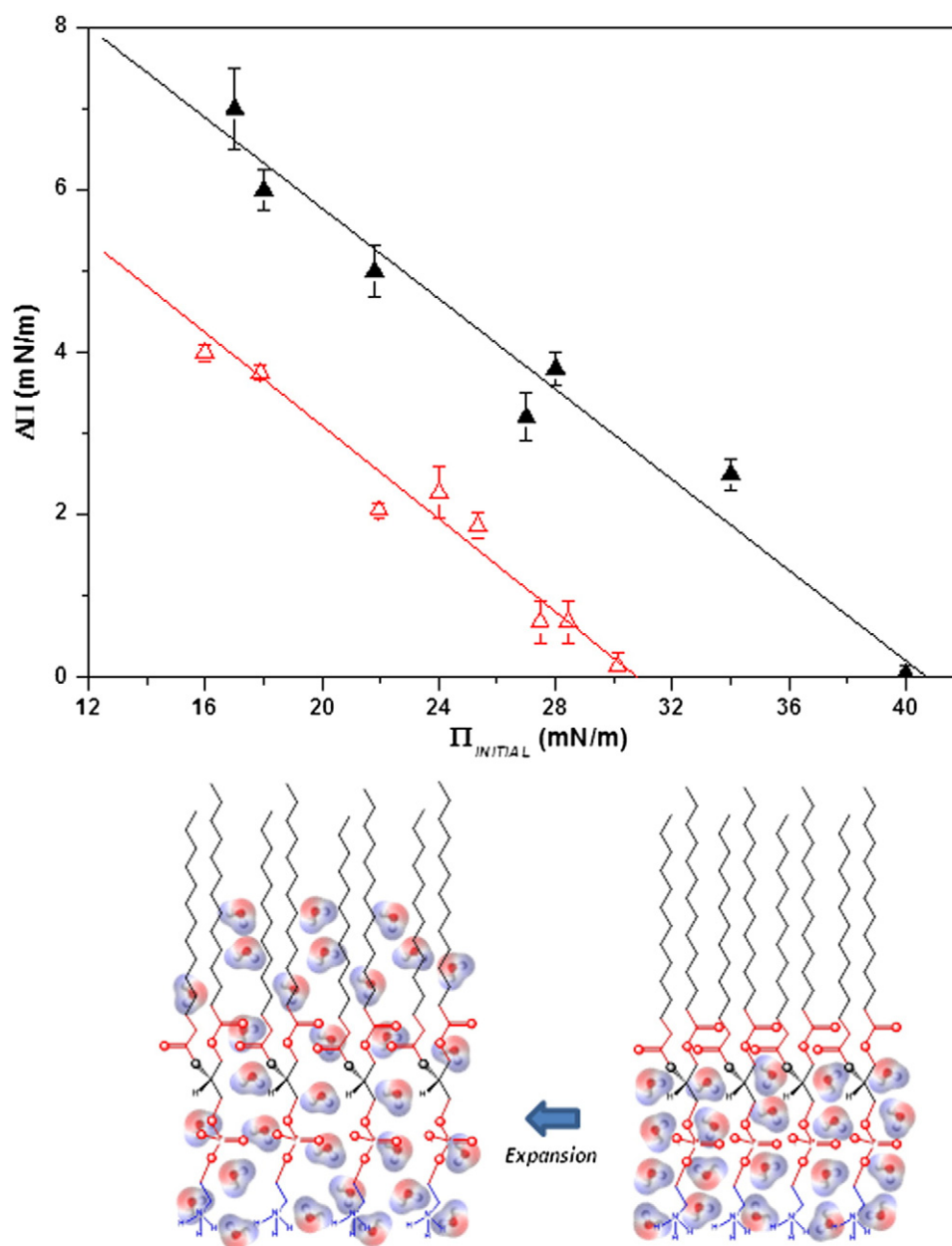
Fig. 7. Perturbation of DMPC (▲) and DMPE (△) monolayer surface pressures by addition of a solute to the subphase vs. at different initial surface pressure (Π_o). From right to left the surface pressure decrease means penetration of water in between the lipids, promoting a larger effect of the solute in the subphase.

systems with small dimensions, c.a. 3–8 Å so that the velocity autocorrelation function is similar to solid ice, i.e. low frequency populations [61,62]. However, recently a new set of information suggests that ice like formation is unlikely near the surfaces [63]. Histograms of the spatial dependence of the hydrogen-bond lifetimes show confinement or local template environmental ordering. It is inferred that the dynamics are significantly slower near the structured hydrophilic boundary. Thus, local environments affect the structure and dynamics of water. The magnitude of the effects of confinement greatly depends on the number of hydrogen bonds available per water molecule as well as lifetime of nearby hydrogen bonds.

The comparison of the shifts in the CH_2 region with the water bands suggests that in the ripple-gel state, CH_2 contacts (low frequency populations) are concomitant with low H-bonded water populations, i.e. small water clusters. Water band shifts to lower frequencies indicates a strengthening of the "local tetrahedral" structure because confined template environments are formed due to thermal fluctuations of the

acyl chains. Local order is prevalently dominant in smaller systems. Hence, frequency band of the water populations shifts to higher values while the larger systems tend toward bulk-like dynamics.

Above the phase transition, CH_2 frequency increases denoting a lower contact between the methylene groups, i.e. isolated population increases. In this condition, due to an increase in hydrophobic surfaces an increase in H-bonding between water molecules is likely. Thus, domains of water partially organized by H-bonding are formed in between acyl chains in the liquid crystalline state [64]. The formation of strong H-bonds between water molecules is consistent with the reinforcement of water structure in the presence of nonpolar residues such as the CH_2 groups. In conclusion, the increase in isolated CH_2 populations is congruent with the appearance of small nanoenvironments of water structure. This is compatible with the formation of water clusters in between the lipid acyl chains when the bilayer is in the liquid crystalline state [64,65] and also with the appearance of hydrophobic defects [66].
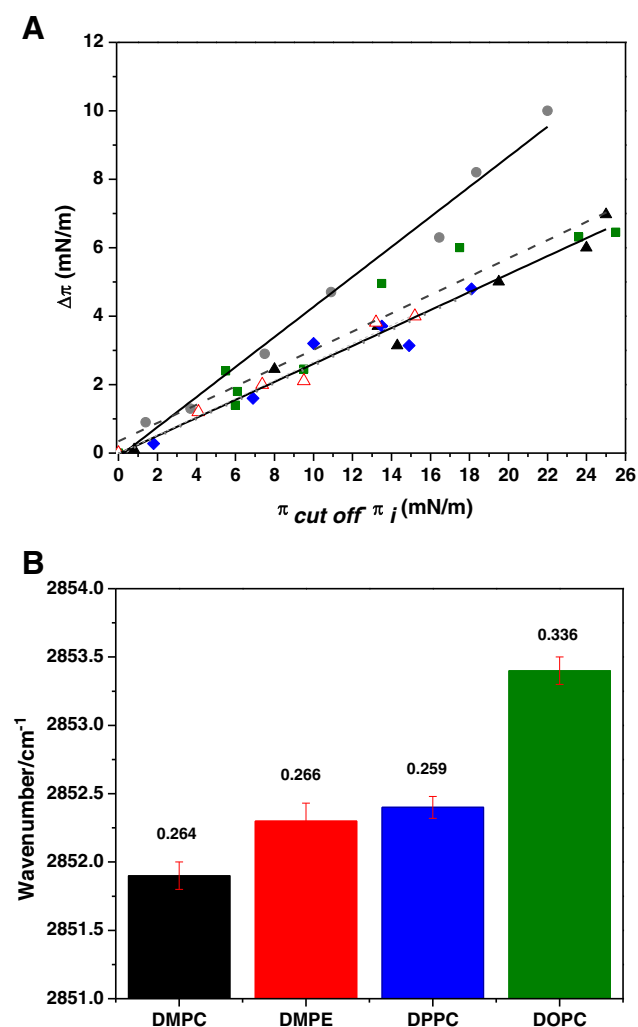
A

B

Fig. 8. A. Perturbation of lipid monolayers vs. the excess of surface pressure. (▲) DMPC, (△) DMPE, (◆) DPPC, (■) DOPC, (●) DPhPC. B. Increase of the frequency value for lipids above the phase transition temperature with the acyl chain composition.

An important point is to take into account that cooperativity may also include a contribution related to water reorganization, concomitant with the changes in the CH_2 populations. In this regard, FTIR data indicate that the liquid crystalline state is characterized by a low entropy phase due to water clustering in low-density networks due to an increase in H-bonds. H bonds are conventionally defined as the intermolecular interaction atoms of moderate (X) and strong electronegativity (Y) such as X–H–Y [67] in the carbonyl groups (–C = O–H). However, water may also form weak associations with the methylene groups (CH–OH). These interactions are classified as a non-conventional H-bond since the donor atom is not oxygen [68,69]. The spatial arrangement and the dynamical features displayed by these two kinds of association are very different [70]. The very small binding energies (similar to those of van der Waals interactions) imply that networks of weak hydrogen bonds often increase the stabilization of the dimer. Even in the presence of multiple bonds the partner molecules show a high degree of internal freedom within the complex [71–74].

1.6. Consequences of Träuble model on the conductance of lipid membranes

The consideration of water as a structural component of the lipid membrane has important consequences in the reinterpretation of several biologically relevant phenomena.

For example, electrical phenomenon in biomembranes is usually interpreted on the assumption that discrete ion conduction is due to ion channels inserted in the lipid membrane considered as an inert electrical insulator [75].

The admittance of water in the lipid matrix provides a reasonable way to explain with a single property permeation of polar solutes and conductivity of protons and ions. Water jumping in kinks and water interconnection would give place, according to Haines, to H conductive wires [76]. Lipid membranes in the liquid crystalline state are highly permeable to water and small molecules, and are therefore not always inert. However, in voltage-clamp experiments, Heimburg [75] found quantized conduction events through protein-free membranes in the liquid crystalline state similar to or even undistinguishable from those attributed to proteins. Thus, ions may migrate through pure lipid membranes. To give to this statement a rigorous treatment under thermodynamic grounds, water in membrane and its properties should be considered.

The lipid bilayer is an active element due to the fact that the lipid composition is responsible for the presence of domains of water with different interfacial energies.

Thus, permeability and conductivity of bilayers are consistent with a mechanism of the formation of microscopic holes due to fluctuations in the bilayers. The hole formation is considered as a nucleation process of water clusters in the lipid matrix [77].

2. Conclusions

The presently accepted fluid-mosaic model proposed by Singer & Nicolson is not applicable or may even be misleading in understanding the diffusion–solubility characteristics of solutes in a membrane. One reason, probably the most important, of this failure is that water is not considered as a structural thermodynamically active component of the lipid membrane.

The formation of pockets filled with water has been postulated in order to explain the insertion of polar peptides and aminoacids into lipid membranes. These defects can be formed as a consequence of membrane deformation, such as expansion–contraction processes, curvature and membrane phase transitions. The expansion via a thermotropic or a lyotropic transition increases water in the membrane, probably opening paths through which ions and small solutes may permeate.

It is likely that water molecules confined in those paths form pools of few nanometers in diameter in which hydrogen bond structural dynamics differ drastically from the dynamics they undergo in bulk water. Orientational motions of water require hydrogen bond network breakdown and rearrangement. The fine structure of the different water populations derived from FTIR (Fig. 5) makes to think that the bandwidth is due to water molecules with zero, one, two, three and four hydrogen bonds. As H-bonds are also cooperative, its energy depends on the coordination number of the water molecules giving the broad line spectra. The macroscopic state is composed by different microscopic components represented by CH_2 isolated populations, which corresponds to different intercrossed water species. Consequently, this water organization confers different free energy content that determines permeability and protein or peptide insertion.

The present review updates the Träuble's ideas in the light of new experimental evidences, which give a more realistic membrane concept.

3. Conflict of interest

The manuscript has been written in complete agreement of the coauthors and there is no conflict of interest with previous publications.

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