Hydration forces between bilayers in the presence of dissolved or surface-linked sugars

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1. Introduction

Non-electrostatic mechanisms for hydration forces were first proposed by Langmuir in 1938 [1] to explain the stability of bipolar coacervates, i.e. liquid–liquid phase separation, including lecithins in water. The stability of coacervates was described in detail by Bungenberg de Jong in the text book edited by Kruyt [2], but could not be predicted. The osmotic stress method [3,4,5] allowed the determination and modelled between bilayers of phospholipids. We distinguish between primary and secondary hydration forces. The first type is always present and linked to adsorption of water at the interface, while the second type requires the presence of an additional solute such as a salt or a carbohydrate and is linked to the competition of lipid and solute for the reservoir of water available at a given water activity. Low molecular solutes “immobilising” a large amount of water are present in large quantities in all living cells and are referred to as osmolytes [9].

Orders of magnitude of the surface activity of carbohydrates can be translated into depletion isotherms similar in magnitude to simple electrolytes. Using this method, it has been demonstrated that sucrose is similar to chaotropic salts, while glycerol is “neutral” like sodium chloride, and ethylene glycol is analogous to cosmotropic salts [10].

Ions near bilayers have been simulated at several levels of approximation. Simulation of carbohydrates near bilayers are more scarce, especially since, in principle, the chemical potential of sugar and water must be fixed. A remarkable example is shown in Fig. 1, with trehalose at biologically relevant concentration near model bilayers could be derived independent of any model or artefacts due to devices requiring adsorption of a bilayer on a substrate such as AFM or modified surface force apparatus (SFA) [6].

Two reviews of the field covered here identify the role and the relative magnitude of protrusion effects, the effect of the membrane bending modulus and enthalpy, and the entropy of water adsorption on the bilayer–water interface [7,8].

We focus in this review on hydration forces quantitatively determined and modelled between bilayers of phospholipids. We distinguish between primary and secondary hydration forces. The first type is always present and linked to adsorption of water at the interface, while the second type requires the presence of an additional solute such as a salt or a carbohydrate and is linked to the competition of lipid and solute for the reservoir of water available at a given water activity. Low molecular solutes “immobilising” a large amount of water are present in large quantities in all living cells and are referred to as osmolytes [9].

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zwitterionic bilayers [11]. This simulation suggests a larger concentration of trehalose near the interface, suggesting preferential binding at the lipid bilayer [12,13]. Adsorption of sugar should profoundly modify the intensity of the hydration force.

In a seminal paper, Lyle and Tiddy [14] demonstrated the equivalence of the hydration force as measured via osmotic stress and the speciation of free/bound water partition as measured by NMR. If one considers as “free” all water molecules that rotate fast, with a net free energy of interaction with the bilayer of less than 1 kT, and as “bound” all water molecules with slow motion, large NMR proton relaxation due to free energy higher than 1 kT, one can derive an exponential value of the hydration force. This force is seen as a derivative of the free energy versus spacing from NMR and vice-versa in the whole domain of existence of lamellar phases of neutral linear surfactants containing polyoxyethylene head-groups. These experiments have been a direct proof of the dehydration with constant decay length when varying temperature. In binary systems containing hydrated uncharged head-groups, the “molecular balance force” is the simplest known, since hydration forces compensate attractive van der Waals forces. One considers only the interplay between two major mechanisms when analysing experimental results obtained via direct thermodynamic methods, implying some control or measurement of the water activity, including via relative vapour pressure.

From a thermodynamical point of view, forces between water–oil interfaces in the presence of sugar can be quantified from surface tension data only, since partial exclusion or adsorption from a solute on a liquid–liquid interface must be considered. In this thermodynamical approach, sucrose and glucose are seen as repelled from the water–air interface, while glycerol is “neutral” towards the same interface, i.e. it is neither depleted nor adsorbed (a situation largely exploited in freeze fracture electron microscopy techniques). The situation at the air–water interface is linked to the water penetration “into” the phospholipid layer [15].

The situation is totally different for glycolipids, i.e. when the carbohydrates made from one up to seven sugar rings are bound to the bilayer via covalent binding. In this case the dominating repulsion originates from the water molecules bound to the sugar headgroups exposed to the solvent. Glycolipid binary phase diagrams indeed resemble phase diagrams in the presence of chaotrophic ions [16] or hydrotropes [17].

However, osmotic pressures of zwitterionic lipids below and beyond chain melting temperature have not been demonstrated to be qualitatively different. In the frozen-chain form, protruding head-groups are bound to a crystalline plane. To our knowledge, dynamical protrusion mechanism has not been detected experimentally as dominant for a short range primary hydration force [18,19].

In the case of grafted head-groups, i.e. the case of glycolipids, an exponential repulsive primary hydration is expected, albeit with larger contact pressure. This is the case for neutral glycolipids, while the presence of charged glycolipids, e.g. those containing sialic acid functions, are expected to be also affected by secondary hydration forces [7,20,21]. In the latter case, the surface layer can even be depleted from the surface. In this review, the hydration forces will be considered separately for the two cases.

Since the introduction of the SFA [22] and of the more reliable “colloidal probe method” based on the AFM combined to a small glass bead [23], a dominating “long range” attractive interaction has sometimes been reported. The sugar hydration layer has a lower dielectric constant than pure water since water dipoles are “immobilised” by the semi-rigid sugar ring. Therefore, the van der Waals attraction considered in the so-called triple film approximation is amplified [24]. In the force balance, this enhanced van der Waals interaction could dominate all repulsive hydration mechanisms. We do not consider this phenomenon in the present review, since it is an effect of the presence of sugar on the van der Waals attraction which is always present [25–28]. A typical example where short range hydration with 0.2 nm decay can be distinguished from electrostatic repulsion due to low ionic strength is shown in Fig. 2 [28]. In this case of a membrane made of GM1 and DPPC, the attraction mechanism is linked to the intermediary range located between the two exponential decays. Close to 40 nm, a damping of the force is measured. However, when osmotic stress at equilibrium is used, all molecular mechanisms including lateral fluctuations and in-plane miscibility effects are participating and combine together [8]. This is not the case in AFM or SFA indirect experiments since hysteresis effects are strong. Hysteresis effects due to lateral segregation have also been observed using a gemini glycolipid mixed with DPPC [29]. In this case, the hydration force could not be determined quantitatively since bilayers...
fragment into bicelles when the more hydrophilic component segregates to the edges.

In the case of the cryoprotective disaccharide trehalose, Crowe has reviewed arguments in favour of phospholipid head-group dehydration, i.e. reduction of area per head-group and hence chain melting temperature reduction. Therefore, the \( L_\alpha \) domain is larger in the presence of the cryoprotectant. This effect is only indirectly linked to the hydration force mechanism: since lipids are dehydrated, contact pressure of hydration should be reduced in the presence of trehalose [30].

Finally, it is crucial to pay attention to carbohydrate vitrification, which occurs at a temperature that may be below or above the chain melting temperature of the lipid system investigated. Indeed, the hydration force can dominate mechanical properties only above the chain melting and sugar vitrification temperatures in the mixed sample [31].

2. Direct evidence of depletion from the lipid–water interface

Using small-angle neutron scattering (SANS) and refined contrast variation method, a \( q \)-independent extinction of the average contrast is observed at low \( q \) in lamellar phase (\( L_\alpha \)) suspensions containing deuterated sugar [32]. As shown on Fig. 3, data collected in the situation where multilayer vesicles coexist with excess sugar solution show a \( q \)-independent contrast match point. The scattering intensity at fixed \( q \)-value, typically below \( 5 \times 10^{-2} \) nm\(^{-1} \), is a measure of scattering length fluctuations in H/D density per unit volume due to phase separation. Therefore, one has direct access to the concentration of labelled sugar inside the multilamellar vesicles and in the solution in excess. This value is directly linked to the amount of deuterated sugar present in the interbilayer region and therefore measures adsorption or depletion of the sugar from the interface, in the same way that surface tension does at the water–air interface.

Precise determination of contrast match-points by this method requires availability of deuterated carbohydrates but gives access to the amount of excluded sugar from the interbilayer aqueous space and to the “hydration water”, i.e. the number of water molecules firmly bound to the polar heads and “inaccessible” to the sugar. In the case of glucose and DMPC [32], 28 water molecules per DMPC were found. Since the area per molecule is 0.6 nm\(^2 \) and the volume of water is 0.03 nm\(^3 \), typically seven layers of water are inaccessible to sugar using the Gibbs definition of adsorption. This in our opinion rationalises the surprisingly low increase of the decay observed. The depletion layer for sugar near bilayers contains water “bound” to bilayers as well as to the osmolyte. As long as this layer essentially contains water molecules, the decay length of the observed force is expected to remain 0.2 nm.

Two other studies describe sugar exclusion due to water layers inaccessible to sugars. Kent and coworkers have studied reverse hexagonal phases of DOPE which has a smaller head-group and a strong curvature: in this case, ten molecules are inaccessible to glucose [33]. Lenné and coworkers [34,35] have confirmed the molecular depletion mechanism of sugars from the interbilayer space in samples close to maximum swelling containing no excess sugar solution.

3. Experimental and theoretical view of contact pressure

We point out the distinction introduced by McIntosh and Simon between hydration and an indirect form of hydration via a thermally equilibrated protrusion mechanism which can be associated to a short range decay, appearing only in the molten chains state (\( L_\alpha \)) and not in gel and sub-gel states [8].

A simulation using molecular dynamics demonstrated that trehalose replaces water in osmotically stressed samples of phospholipids, some of them being “inserted” laterally as a wedge between polar headgroups. This mechanism would induce an increase of the contact pressure at the same area with sugar present in the sub-phase. Since molecules would be inserted laterally between lipids in the bilayer, local in-plane fluctuations would be enhanced due to the presence of adsorbed sugar. An ubiquitous consequence of solute insertion is the softening of the membranes; undulations are enhanced. This may be the origin of the peak broadening shown in Fig. 4. Since one observes a simultaneous peak shift to low-\( q \), there is no proof of the direct link between adsorption and softening [36].

Using optical analysis of fluctuations, Genova et al. have shown that apparent liposome fluctuations and hence intrinsic bilayer rigidity of SOPC vesicles does not decrease by more than 40%, decaying from 25 \( kT \) to 15 \( kT \) in the presence of mono and di-saccharides up to 20% in weight [37]. This is far from the order of magnitude that would be required to

Fig. 3. (Left) \( q \)-independent determination of the contrast match point in a lipid-deuterated sugar mixture (DMPC-2D-glucose) as determined by small-angle neutron scattering. (Right) Comparison of the contrast match point between multilamellar vesicles and excess sugar solution obtained with pure DMPC (■) and in the presence of 2D-glucose (□) (taken from [32]); copyright International Union of Crystallography 2000.
observe a fluctuation-enhanced apparent repulsive hydration force. This fluctuation-enhanced apparent force would translate experimentally into an enhanced contact pressure without change of the decay length.

4. What is the typical decay length in carbohydrate solutions?

If hydration is associated with a decay in the ordering of dipoles as suggested by Marcelja [38], it would be expected that the decay length in concentrated carbohydrate solutions is larger than the typical 0.19 to 0.2 nm found in pure water, with a monotonic increase towards 0.6 nm, the size of a monosaccharide ring. The experimental situation is completely different. As can be seen on Fig. 5, the decay length in concentrated solutions remains roughly the same as in pure water. How can this be understood?

On Fig. 5, we see that the presence of sugar (up to 30% w/w in the water phase) induces an increase of the contact pressure term by only less than one decade. This is consistent with the unchanged value of the number of hydrogen bonds to water per lipid, remaining constant independently of the possible presence of adsorbed carbohydrate [39]. The order of magnitude at contact pressure lies between $10^9$ and $10^{10}$ Pa. With a one-layer decay of 0.2 nm and a molecular volume of 0.03 nm$^3$, the order of magnitude of water adsorption enthalpy is estimated to 180 kJ/mol, stronger than hydrogen bonding alone.

The presence of inaccessible water, the invariance of contact pressure, and the competition between zwitterionic headgroups and osmolyte explain the unexpected invariance of the decay length of the hydration force. On Fig. 5 this has been tested up to 30% w/w of sugar in water. The crossover regime from 0.2 to 0.6 nm is expected only when sugar molecules replace water as first coordination neighbour, well beyond 30% w/w.

Dehydration associated with the presence of an osmolyte induces a decrease of the area per lipid but also changes the surface dipole of the lipid, but not its sign. Therefore, the part of contact pressure due to water–lipid dipole couplings should also decrease. In the end, the contact pressure should vary whenever sugar molecule exchange with water in the hydration layer is involved [41,42]. The two competing effects – dehydration of head-groups and replacement volume by volume when trehalose is adsorbed – as shown in Fig. 1, have been distinguished by molecular simulations [43].

The hydration force here is an interaction perpendicular to the water–lipid interfacial plane. It should be noticed that the lateral repulsion term can be accessed directly by the P–A isotherm of a monolayer. The case of sucrose and fructose present in the sub-phase has been studied [44].

Strong binding as inferred from buckling transitions of surface monolayers would correspond to a large increase of contact pressure in the case of trehalose, and to our knowledge those have not been measured [45].

The hydration force is obtained from scattering curves as shown in Fig. 4. (left) Small-angle X-ray scattering curves showing the swelling and the broadening of a lamellar phase upon addition of fructose to DMPC suspensions (from 0 to 40% sugar/water (w/w) as indicated); (right) Increase of the lamellar periodicity showing the swelling of the lamellar phase in equilibrium with excess sugar solution as determined for a monosaccharide (glucose) and a disaccharide (fructose). (taken from [36]).

Fig. 5. Osmotic pressure versus water layer thickness directly determined by small-angle neutron scattering of DMPC suspensions hydrated with a large excess of glucose solutions. The ternary samples are under osmotic stress of dextran 110 solutions of known osmotic pressure. The glucose concentration in water ranges from 0 to 30% w/w glucose/water. The two lines are fits to the exponential regime showing the invariance (same slope) of the decay length between pure lipid (dots) and the highest sugar concentration investigated (30% w/w glucose, dashes) (taken from [40]).
The oil–water interfacial tension at the bilayer–carbohydrate interface has been calculated via molecular dynamics and would be consistent with a strong increase in contact pressure [46]. In all cases, the first-order transition between liquid condensed (LC) and liquid expanded (LE) phases vanishes and total surface pressure increases in the presence of sugar.

5. How does hydration combine with other colloidal interactions?

The additivity of pressures corresponding to supposed uncoupled interactions is the basis of molecular force balance used to predict phase diagrams including maximum swelling limits. This simple addition of different derivatives of the free energy is evidently a rough approximation. For example, competition for water implies that free energies of adsorption of water on the sugar and on the lipid are completely independent and. This is not true because the same ensemble of water molecules is considered. Moreover, electrostatic interactions involve ions near interfaces, leading to secondary hydration force only present when surface charges and background salt coexist. The only general way to explore this additivity is to determine osmotic pressures in a full ternary phase diagram.

This has been done once to our knowledge, by mixing a cationic lipid and a glycolipid [47]. Two critical points, i.e. compositions where fluctuations in relative concentration are large, are present in the phase diagram as shown on Fig. 6. These critical points can exist only if two different repulsive mechanisms coexist [48–51]. Full calculated phase diagrams with different hypothesis for combination of hydration and electrostatics are compared to the experimental one on Fig. 6. Contact pressures for pure glycolipid and pure cationic lipids are experimentally determined and are not adjustable parameters. The phase diagram the closest to reality (5) is constructed by simple addition of all interactions with two hypothesis: (a) that the electrostatic interaction is proportional to charge per unit surface, and (b) that the logarithm of the hydration contact pressure varies linearly with the mole fraction of glycolipid in the mixed bilayer. As for simple fluids, detailed shapes of phase limits in phase diagrams [52] are a direct translation of the complexity of hydration mechanisms [53] and this remains to be explored.

6. Observations linked to “hydrophobicity” and “Hofmeister effects”

For non-swelling glycolipids, the hydration force can be measured only in a limited range of less than a nanometre. Undeformable complex glycolipids cannot be swollen, and decay lengths of typically 0.2 nm of the hydration force have been found at high pressure >10^5 Pa [54]. However, this initial decay is followed by a second, surprisingly steep decay of 0.05 nm. To our knowledge, steep decays are expected only for 

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interactions. But in this case too, these interactions are supposed to be independent of primary and secondary hydration mechanisms; hence additivity of forces is a pre-requisite to measuring the forces linked to water interaction with the bilayer and direct interaction via sterically defined hydrogen bonds with carbohydrates. These specific forces should be very sensitive to the relative direction and density of hydrogen binding. This is demonstrated by the large variety of glycolipids involved in membrane recognition processes [63].

8. An open question: the influence of carbohydrates on the “lateral” equation of state

In this short review, we have mainly focussed on the hydration force perpendicular to bilayers and the link to the presence of carbohydrates as co-solvent or chemically bound to the bilayer. Interplay between sugar depletion and secondary hydration is subtle. We feel that the main open problem is the lateral equation of state, i.e. the relation

Fig. 6. Ternary phase diagrams of water, a synthetic cationic lipid and a synthetic glycolipid showing two critical points and equilibrium tie-lines. (1–5) as calculated with different hypothesis on the intensity of the repulsive hydration force and (6) as experimentally established by combining small-angle X-ray scattering and osmometry (taken from [47]); copyright The American Chemical Society 1998.
between area per molecule in the bilayer versus osmotic pressure [50]. Determining lateral equations of state needs delicate measurements not yet available in the presence of sugars. However, in the simple case of synthetic ionic lipids and in the absence of screening salt, the key role of the spontaneous curvature of each monolayer is evidenced in ternary phase diagrams by a strong shift in the thermodynamic equilibrium between vesicles and cylindrical micelles in the presence of carbohydrates [64].

Another effect is linked to the spontaneous curvature of the two monolayers forming the bilayer. If head-groups are too large to accommodate in a cylindrical shape, mesh-planes or planes punctuated by pores form. These structural transformations are not driven by a modification of the hydration force perpendicular to the bilayer plane, but by the variation of head-group area per lipid. This variation is also an effect of carbohydrate adsorption/desorption. This is another general mechanism that must be characterised in the frame of a “lateral” equation of state: the osmotic pressure is examined not as a function of area per molecule—lateral equations of state, could lead not only to predictive models for transport, but also to more general predictions of effects induced by molecules inserting laterally between lipid headgroups of the lipid bilayer.

References and recommended reading**


Fig. 7. Force–distance curves of two similar glycolipids with disaccharide headgroups: Gentibiose (left) and Lac (right), where two different regimes with characteristic decay lengths can be identified (taken from [54]); copyright The American Physical Society 2008.


A general model-free analytical method based on neutron contrast variation to determine carbohydrate depletion/adsorption in a two phase regime, i.e. when a lamellar phase is in equilibrium with excess sugar solution.

[33] Kent B, Garvey CJ, Lenne T, Porcar L, Garamus VM, Bryant G. Measurement of carbohydrate depletion/adsorption in a two phase regime, i.e. when a lamellar phase is in equilibrium with excess sugar solution.


