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Historical perspective

Beyond the hydrophobic effect: Critical function of water at biological phase boundaries — A hypothesis

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ABSTRACT

Many life-sustaining processes in living cells occur at the membrane–water interface. The pertinent questions that need to be asked are what is the evolutionary reason for biology to choose the membrane–water interface as the site for performing and/or controlling crucial biological reactions and what is the key physical principle that is singular to the membrane–water interface that biology exploits for regulating metabolic processes in cells? In this review, a hypothesis is developed, which espouses that cells control activities of membrane-bound enzymes and receptor activated processes via manipulating the thermodynamic activity of water at the membrane–water interfacial region. In support of this hypothesis, first we establish that the surface pressure of a lipid monolayer is a direct measure of a reduction in the thermodynamic activity of interfacial water. Second, we show that the surface pressure-dependent activation/inactivation of interfacial enzymes is fundamentally related to their dependence on interfacial water activity. We extend this argument to infer that cells might manipulate activities of membrane-associated biological processes via manipulating the activity of interfacial water via localized compression or expansion of the interface. In this paper, we critically analyze literature data on mechano-activation of large pore ion channels in *Escherichia coli* spheroplasts and G-proteins in reconstituted lipid vesicles, and show that these pressure-induced activation processes are fundamentally and quantitatively related to changes in the thermodynamic state of interfacial water, caused by mechanical stretching of the bilayer.

Contents

1. Introduction . . . 22
2. Interfacial activation of enzymes . . . 24
3. Physical nature of the surface pressure . . . 25
4. Alternate definition of surface pressure . . . 26
5. Interfacial water activity concept . . . 26
    5.1. Potential role of interfacial water in biology . . . 28
    5.2. Critical function of interfacial water in receptor-mediated cellular processes . . . 29
6. Mechanosensitive ion channels . . . 30
7. Mechanosensitive G protein and G protein-coupled receptors (GPCRs) . . . 31
8. Conclusions . . . 32
References . . . 33

1. Introduction

It is well recognized that liquid water is quintessential for the genesis of terrestrial life. The fundamental driving force responsible for this process is the ‘hydrophobic effect’, a phenomenon by which nonpolar and amphiphilic molecules tend to spontaneously aggregate when dissolved in an aqueous medium [1,2]. In Tanford's words, “The hydrophobic effect is a unique organizing force, based on repulsion by the solvent instead of attractive forces at the site of organization” [3]. However, there has been considerable debate in the literature about the use of the term ‘hydrophobic’, as there is no absolute experimental evidence for ‘phobia’ or ‘repulsion’ between water and nonpolar substances.

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Two schools of thought exist to explain the insolubility or immiscibility of nonpolar substance in water. The first, which is based on interfacial energy between two immiscible liquids (such as water and octane), argues that since the ‘work of adhesion’ between two immiscible liquids is positive (for example 43.76 erg/cm^2 between water and octane), meaning that it is attractive, there is no phobia between water and hydrocarbons [4,5]. However, the energy of this attractive interaction (the origin of which is the dipole–induced-dipole interaction between water and hydrocarbon) is not strong enough to break apart the hydrogen bonds of water in order for the hydrocarbon to go into solution [4]. For instance, assuming that the concentration of water at the air–water interfacial region is about 5.7×10^{-10} mol/cm^2 [6], the work of adhesion of 43.76 erg/cm^2 between water and octane corresponds to attractive interaction energy of only about -1.85 kcal/mol. On the other hand, the average hydrogen bond energy of bulk water is about -6 kcal/mol. Thus, the attractive interaction energy between water and octane is not large enough to break hydrogen bonds in bulk water, and this energy inequality limits the solubility of octane (and similar nonpolar substances) in water.

The second school of thought stems from experimental data of thermodynamic changes that occur when a nonpolar solute is transferred from the gas phase or from the liquid hydrocarbon phase to an aqueous solution, as shown in Fig. 1. The enthalpy change (ΔH) for the transfer process is either negative or zero, depending on whether the transfer is from the gas phase or a liquid phase, but the transfer free energy change (ΔG_{tr}) for this process is always positive in both cases, meaning that the transfer of the hydrocarbon from the nonpolar phase to the aqueous phase is thermodynamically unfavorable. Since $\Delta G_{tr} = \Delta H - T\Delta S$ (where ΔS is the entropy change), it follows that when a hydrocarbon is transferred from a nonpolar medium to an aqueous medium, a large negative (unfavorable) change in entropy occurs in the aqueous phase, which more than offsets any negative (favorable) change in enthalpy, so that the net free energy change of the process is positive, i.e. $\Delta G_{tr} > 0$ [2]. The negative entropy change denotes that by its mere presence in an aqueous medium, a nonpolar solute imposes an increase in “order” or “structuring” of water. More importantly, theoretical analyses indicate that the geometry of this structured water is quite different from that of the normal hydrogen bonded water clusters. Stillinger [7] had reasoned that when a nonpolar solute is introduced into an aqueous solution, water is attracted to the nonpolar surface via dipole–induced-dipole interaction. However, in order to maintain its hydrogen bonding interactions with other water molecules in the vicinity of the nonpolar surface, water is forced to rearrange its orientation toward the nonpolar molecule so that all four of its hydrogen bonding orbitals (both donors and acceptors) are pointed away from the nonpolar surface in a straddled position. This reorganization, known as “hydrophobic hydration”, is distinctly different from ionic hydration or hydration of polar solutes where no such orientation requirement is imposed. The nonpolar solute with this type of hydration shell is known as ‘clathrate hydrate’ and water molecules associated with this hydration shell completely lose their rotational freedom. A major consequence of this structural reorganization of water around the nonpolar solute is that the hydrogen bonded water–water geometry in the clathrate structure is very different from that found in hydrogen bonded water clusters in bulk water and in ice: The water–water orientation in ice and in bulk water is in a staggered configuration [7], whereas in the clathrate hydrate it is in an eclipsed configuration, as shown in Fig. 2. In the eclipsed configuration, the lone pairs of electrons of oxygen atoms come closer to each other than in the staggered configuration, and the increased repulsive interaction between the lone pairs exerts a strain on the hydrogen bond. Together, the loss of rotational freedom of the hydrogen bond's dihedral angle and the strain on the hydrogen bond decrease the entropy of water, which renders the presence of the nonpolar solute thermodynamically unfavorable. To restore its entropy, it becomes imperative for water to minimize its association with the nonpolar solute. To accomplish this, water forces nonpolar solutes to aggregate/associate with each other so that the water released from the clathrate shells could return to their original higher entropy state. This process, which is the reversal of hydrophobic hydration with free energy change of $\Delta G < 0$, is known as the ‘hydrophobic effect’ or ‘hydrophobic bond’ [7]. It should be emphasized that the association of nonpolar solutes in aqueous solutions and/or expulsion of nonpolar groups from water to a non aqueous phase is driven not by the innate van der Waals attraction between them, but by the entropic force from water structure and therefore the energy of hydrophobic interaction is considerably stronger than van der Waals interaction. It has been reported that the transfer free energies of nonpolar amino acid side chains from water to ethanol, hexane, N-methyl acetamide, and the air/water interface were almost the same regardless of the polarity of the nonaqueous medium [8]. This further confirms that the free energy change for the transfer solely emanates from thermodynamic changes in water structure.

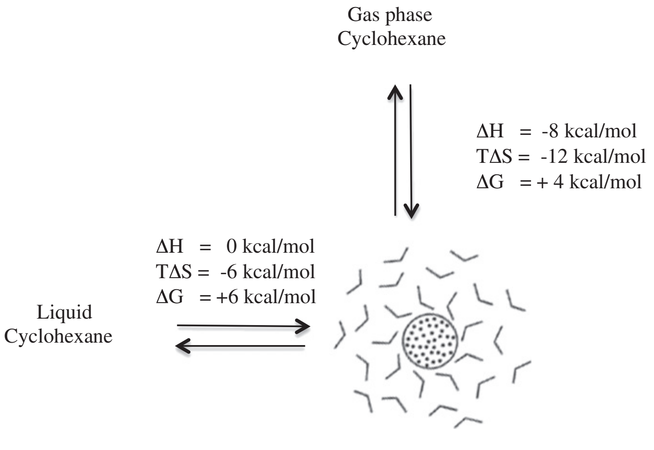

Fig. 1. Thermodynamic changes for transfer of cyclohexane (or similar size nonpolar substances) from the gas phase and liquid phase to an aqueous phase at 20 °C. (Adapted from Ref. 118).

There is a consensus among biologists/biochemists that the second school of thought is more appealing and probably the correct one for explaining thermodynamic incompatibility between water and nonpolar solutes. The imposition of nonpolar solutes on water to reorganize its structure, and water's proclivity to regain its higher entropy state is at the core of evolution of biological structures, such as protein structures, lipid bilayer membranes, and other cellular structures, and perhaps the evolution of carbon-based life itself.

Although the essentiality of water in biology is well established, the current perception of its role in biology is confined only to its ability to create organized structures via the hydrophobic effect. It is assumed that once the organized structures, i.e., uniquely folded protein structure [9], bilayer membranes, etc., are formed, cells use water mainly as a solvent medium and all the biological functions thereafter are performed by the macromolecular structures dissolved or suspended in this medium. What is being overlooked is the potential role of water at the phase boundaries of such organized structures in the very functioning of those structures. For instance, activator–receptor assemblies located in the plasma membrane carry out many communication and signaling reactions between exterior and interior of cells. The functioning of these membrane-associated signaling systems is explained mainly in terms of specific effects of activators on the conformation of receptors. However, the biochemical link, that is, how conformational changes in receptor proteins (which is a mechanical energy) in a membrane or dynamic shape fluctuations in membranes are translated into other forms of energy that subsequently drives a metabolic pathway is not well understood. The current theories propound that signaling activity at cell membranes is effected by alterations in membrane shape, i.e. expansion, deformation, bending, curvature, etc. [10–13]. It is believed that signaling occurs as a result of transient accumulation of