WATER

Enzyme-Water Mutualism: Implicational Speculation

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Abstract

Water exists as a high density or low density liquid with different physical properties resulting from either of two forms of hydrogen bonds between the H_2O molecules. This is significant intracellularly because solutes may favor one form of water or the other setting up local gradients with differing water activities that may not be able to equilibrate by water flux because of volume constraint but by a transition of one water form into the other. It is very likely that this remarkable property of water was exploited by evolutionary forces resulting in a mutualism between vicinal H_2O molecule clusters and macromolecules. One outcome of this is the variety of substrate specific enzymes endowed with molecular structures that also select the most advantageous composition of the two water structures available, thereby enhancing the catalytic reactions. Any low density/high density water composition change, due to selective partitioning of enzyme reaction products, provides the mechanism for protein folding; a prerequisit for enzyme cycling.

Introduction

There is firm belief that earliest life originated, at some point, from an aqueous molecular mixture and macromolecules evolved from it as a result of endergonic reactions probably not yet completely understood. This communication is an attempt to reconcile earliest formation of solvated macromolecules destined to become enzymes with the unanswered question of the role of structured water in their catalytic reactions.

Evidence including that from -OH stretch spectra shows that water exists as a mixture of high density water (HDW) and low density water (LDW), (Maestro *et al.* 2016, Pollack, 2013, Vermouth *et al.* 1994, Co *et al.* 1997, Robinson, 1999, Wiggins, 2008, Chaplin, 2004). These are liquid polymorphs in rapid exchanging equilibrium with hydrogen-bonded H_2O clusters in two forms: H_2O clusters with long straight hydrogen bonds (LDW) or short bent weaker ones (HDW), with different physical and solvent properties conferred on each type such that partitioning of solutes depends upon size and charge. For example, it is known that Na+ ions partition differently from K+ ions, (Wiggins, 2008). Due to ionic size, the former prefers HDW while the latter prefers LDW. Furthermore, due to solvent preference an osmotic pressure gradient can arise across an interface between unlike contiguous water domains. Solutes not only partition into one type of water or another but also modify the structure and properties of a water type by displacing the equilibrium between domains when conversion of LDW into HDW takes place,

and vice versa, if a changed water activity in either domain creates a gradient that cannot equilibrate by osmotic flux (Wiggins, 2008). These displacements have a thermodynamic cost. When that cost is high enough, protein molecules may fold when endowed with configurational options.

A change in HDW/LDW composition may have not only significant effects on partitioning but also upon the free energies of solvation of dissolved solutes. In fact any change in the solvent properties of water will have profound changes on the hydration of ions and macromolecules alike such that the magnitude and sign of energies of solvation can change. Evidence of important changes in free energy of solvation of phosphate ester compounds has been provided which endorses the possibility that an enzymic reaction can reverse due to a change in solute solvation energy (George *et al.*, 1970).

In cellular microenvironments HDW and LDW can coexist and the HDW/LDW composition affected by solute preference for either polymorph because an ion or solute can bring about change in the water into which it favorably partitions. This phenomenon can thereby permit a cycling of macromolecular events; a concept that has been developed and supported experimentally with implications for cyclical enzyme action (Wiggins, 2008). A mechanism for the Na-K-ATPase based upon phosphorylation and cycling of HDW and LDW has been proposed and the Wiggins model (2008) also accounts for the role of two-phase water in a number of biochemical reactions including membrane transport, mechanical activity, dehydration, and even poly-L-lysine synthesis based upon the concept of two different H_2O solvents and changes in solvation energies. The Na-K-pump, ATP synthesis and other bioenergetic and enzymic reactions can be explained by a phase change in the proximal water of a liganded enzyme bringing about changes in partition and/or solvation free energies when H_2O-H_2O bonding changes. There is evidence of the synthesis of of ATP from ADP and inorganic phosphate spontaneously in a LDW environment without involvement of any enzyme (Wiggins, 2008). The fact remains, however, that enzymes carry out a multiplicity of biochemical reactions and although the energy pool provided by two-phase water may support endergonic reactions in specific environments, it does not preclude the probability that specificity and efficiency of reaction are inherent in the enzyme-water association.

For decades structured (fourth phase) water has been invoked to provide mechanisms for various bioenergetic reactions (Szent-Gyorgyi, 1957). It has been shown that ATP can change the structure of vicinal water indicating that solvation energies in bioenergetic reactions can change (Wiggins and Knight, 1979, Wiggins, 1982, Wiggins, 2002). Changes in the microenvironment of active enzyme sites on phosphorylation following ATP binding may be of a very general nature and it is of interest in this regard that the same phosphoenzyme moiety of the Na-K-ATPase is required for the Na-K-pump to operate as well as synthesis of ATP when the pump is reversed (Garrahan and Glynn, 1967). This is the phosphorylated aspartic acid residue. Wherever ATPases are involved it is invariably the aspartic acid residue of the enzyme that becomes phosphorylated. However, classical thermodynamic principle is not adhered to in the energy coupling mechanisms put forward.

Consequently, in conjunction with this, the concept of biochemical energy transduction has remained a problem for bioenergetics as there is no molecular mechanism detailed in texts whereby (for example) a product of any of the exergonic redox events in the electron transport chain becomes a reactant in the uphill endergonic reaction:

ADP + inorganic phosphate (Pi) = ATP. $\Delta G = +30kJ/mole$

which would conform to classical thermodynamics.

ATP is an unstable molecule which hydrolyses in the presence of an ATPase enzyme to ADP and inorganic phosphate (Pi) and comes to equilibrium with H_2O in an exergonic reaction:

ATP + H_2O = ADP + inorganic phosphate (Pi). $\Delta G = -30kj/mole$

The free energy in this case is regarded as the equivalent of that produced by the Na-K-ATPase and somehow linked to the mechanism of the Na-K-pump. Here also there has been no acceptable molecular mechanism for a truly coupled reaction in the classical sense, although the link between both events has never been disputed.

The energy coupling according to Wiggins (1982) must come about, not by a so-called energy transduction but by the environmental HDW/LDW change induced by the phosphorylation of the aspartic acid residue. Without ignoring classical thermodynamic principles, Wiggins, (1982) has elegantly shown how active transport of ions can be explained by a change in vicinal water at a cell membrane when ion transport takes place. Furthermore, when ATP is added to a suspension of actomyosin, a change in light scatter and other parameters provide evidence that a change in hydrogen-bonded water molecules had taken place which persists until all ATP has been hydrolysed, in all probability by the Ca-ATPase of the contractile protein (Knight and Wiggins, 1979). Over half a century ago it was proposed that the contraction and relaxation of muscle was dependent upon collapse and reforming of water structure by actomyosin, and that water and macromolecules generally form one single functional unit not to be regarded separable into molecular components (Szent-Gyorgyi, 1957).

In the past mitochondria swelling studies have been extensive and linked to oxidative phosphorylation. Experiments have shown that what would have been interpreted in earlier studies as swelling could not be reconciled with changes in actual size of mitochondria when determined using laser technology (Knight *et al.*, 1981). In these experiments the light scatter changes could only be due to a change in the LDW/HDW composition.

Valinomycin induces ATP synthesis in non respiring mitochondria suspended in a K^+-free medium (Cockrelle *et al.*, 1966). The preferred interpretation here is that the ValK^+ species unaccompanied by an internal anion diffuses outward and this diffusion, limited by the build-up of external positive charge, results in hyperpolarisation of the coupling membrane. This increase in external positivity is believed to cause a configurational change in the coupling membrane and an increase in LDW resulting in ATP formation. This suggests that a critical increase in LDW/HDW closely associated with the F1-ATPase could well bring about ATP synthesis. In contrast, the decrease in light scatter when coupled mitochondria are suspended in hypertonic KCl, supports the view that depolarisation of the coupling membrane by inward diffusion of K^+ converts matrix LDW to HDW by a critical amount, uncouples and prevents ATP synthesis (Knight V. unpublished results).

From these highlighted studies, the view held here, which supports the Wiggins model (1990), is that for muscle as well as for active transport and oxidative phosphorylation, oscillating changes in the properties of a small zone of strategically placed water contributes a molecular mechanism for doing work and gives a role to coexisting, interconvertible high density water (HDW) and low density water (LDW) clusters. As long ago as 1957 it was proposed that biological functions consisted of the building

and destruction of water structures, all part and parcel of the living machinery, including electronic excitation (Szent-Gyorgyi, 1957).

The two-phase water model has been fully described in the monograph "Life requires two forms of water" (Wiggins, 2008) and of particular importance to the discussion here is the fact that HDW and LDW are two microdomains that probably directed chemical reactions in micropores of inorganic clays in pre-biotic times. ATP, or a polyphosphate precursor could have formed under primitive earth conditions, participated in energy driven reactions predictably synthesising molecules that played their role in macromolecular evolution. Coincident with the macromolecular evolution of solvated enzymes must have been perfection of specific substrate binding sites together with optimal vicinal water clusters, benefiting from molecular mutualism between enzyme and one form of water or the other. The complexities of surface water-enzyme associations have been highlighted (Wiggins, 2009).

Presumably the earliest solvated enzymes would have folded and provided a cleft, or something similar, with a complex molecular structure that determined not only substrate specificity but also the nature of its vicinal water. The latter would contribute to substrate specificity (Wiggins, 2009), while influencing positively the efficiency of the catalytic reaction. The interconversion of the two forms of water provides the mechanism for the release of product and cycling of the enzyme molecule. Szent-Gyorgyi (1957) stated that water molecules around dissolved macromolecules may have different crystalline structure dependent upon the polar or non polar nature of the atomic groups on that molecule. If this is so, as H_2O-H_2O bondings change, different polar groups on the enzyme would become exposed and hydrated as the complex, with configurational options, folds or unfolds until the preferred energy state with stability is arrived at.

Therefore in cellular microdomains there are effectively two water solvents (HDW & LDW) where one can convert into the other when subjected to an osmotic gradient. It is inconceivable that this remarkable property of H_2O based upon the hydrogen bonding of contiguous H_2O molecules and inherent energy pool was not exploited by evolutionary forces. The endless variety of solvated enzymic macromolecules with the capacity for reacting specifically with high efficiency is the outcome. This coexistence of enzymic reactions in an aqueous phase with one structure of water (HDW) or the other (LDW) supporting and enhancing the specific catalytic reaction can be appreciated and interpreted best when considered conceptually as molecular mutualism; a phenomenon which is evident and ubiquitous throughout the living world (Lanier *et al.*, 2017). The interaction between H_2O and ATPase in order for the Na-K-pump to function is a profound example of molecular mutualism, evolved as a sophisticated mechanism necessary for survival of the living cell (Wiggins, 1990).

In conclusion, selected hydrated macromolecules had bestowed upon them specific enzymic functions. Solvated enzymes would then manifest enhanced performance due to an association with the preferred water type determined by the molecular structure of the enzyme. In the event that the enzyme folds with water filled clefts, the $(H_2O)_x$-enzyme association enhances the catalytic reaction and products of that reaction would bring about a change in the HDW/LDW composition. This shift in water equilibrium provides the energy pool change (Δg_w) required for the folding or unfolding of macromolecules, a pre-requisite of enzyme cycling. This catalytic mechanism, based on enzyme-water mutualism, is a very plausible alternative to the substrate-template model believed to function due to precise

geometric fitting of hydrogen bond donors of substrates and/or dipoles of opposite charge; an arrangement of weak bonds with hitherto no molecular explanation supposedly transforming into a potent chemical reactor as the enzyme changes shape.

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