Heme-based water aquareceptors

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Abstract

How cells sense water is a fundamental question in biology and for the evolution of life. The mechanisms underlying hygrosensation in numerous organisms and animals have been experimentally demonstrated. However, hygrosensation has mainly been investigated only in specialized sensory cells (hygroreceptors) that sense extracellular humidity. Even in single-celled microorganisms osmosensors are not sensing water molecule *per se*. In my opinion, cells must have the capability to sense water molecule *per se* via protein-based sensors or receptors (or aquareceptors) that would have enabled them to migrate and survive in water-scarce regions or conditions (via osmoadaptation or etc.,) and evolve into multicellular organisms. And, just as gas (solute)-sensing gasoreceptor functions in almost every cell, water-sensing aquareceptors must also function in almost every cell. Due to the potential capability of water molecules to regulate/antagonize the gas-binding sites in the heme moiety-containing sensor domains of gasoreceptors, I propose that some heme-based gas-sensing gasoreceptors may have a parallel role as water-sensing protein aquareceptors. And I wonder if hemoglobin can also be considered a putative aquareceptor.

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The evolution of animals to switch between aquatic to terrestrial habitat and vice versa must have also influenced the specialization of water sensation mechanisms. Invertebrates can sense water or humidity (hygrosensation) via specialized sensory cells (hygroreceptors), and several models have been proposed for hygrosensation.[1,2] However, water sensation must have been a fundamental process not just in invertebrates but also in single-celled microorganisms. For instance, some yeast can survive complete dehydration but still retain some water molecules (such as the approx.10% residual water content in dry yeast).[3] Hence, such organisms must also have other simpler water-sensing mechanisms especially for acute changes in water stress. In such microorganisms, whether water sensing majorly occurs via osmoreceptors/osmosensors or mechanoreceptors or direct water-sensing aquareceptors is unclear.

Acute sensing of water must be a fundamental and essential process. Some microorganisms can respond rapidly and exhibit water loss or uptake within the first minute or two, altering their bioenergetic properties as well. Therefore, even acute osmotic stress (<1-2 minute) can induce significant changes in numerous cellular pathways in microorganisms.[4] An organism's physiology and metabolism can be affected not just due to the presence or absence of water molecules, but also due to the presence of other metabolites that affect hydration of other proteins or biomolecules. Several proteins have been shown to act as osmosensors, but the majority of such proteins do not detect water molecules *per se*. For instance, one of the bacterial osmosensor, the betaine uptake carrier BetP activity is based on the concentration of ions such as K+ or Cs+ or Rb+.[5] It has been proposed that osmosensor proteins may also act by direct sensing, mediated by conformational changes resulting from solubility and/or solvent changes.[4] But the identity of such protein-based aquareceptors are largely unknown. In my opinion, if cells have aquaporins that allows them transport water molecules and due to the fragile nature of cell membrane that can be easily disrupted (via physical injury), a cell must have specialized aquareceptors that will allow it to sense water directly and almost instantaneously.[6]

Bacterial FixL is one of the O_2 gasoreceptors that can sense O_2 and exhibit increased kinase activity[7]. Now, if FixL can bind to O_2 and it can be called as an gas-sensing protein or gasoreceptor or receptor for O_2.[8] But then, what about similar proteins that can also bind with water molecule instead of O_2? Aren't such proteins attractive candidate for water-sensing aquareceptors? However, nearly every enzyme can bind to varying numbers of water molecules, and interactions with water, solute or even post-translational modifications can affect its conformation and activity, possibly by altering the accessibility of water

molecules. Does this imply that all these enzymes should be classified as aquareceptors or potential aquareceptor candidates? But then not all the bound water molecules can equally affect the enzymatic activity at the same level. Only particular water molecule bound at essential site will have the capability to affect the enzymatic activity that can trigger a cellular signal. And this is especially important if the water molecule can directly bind at ligand-binding site. Will it then make the water molecule an antagonist for that ligand? Or maybe it is the water molecule which is the agonist and the previously identified ligand is the antagonist.

If we consider hemoglobin for instance, 60-75 water molecules are associated with it.[9–11] At high O_2 concentration, O_2 is bound to the Fe(II) iron heme and also forms a hydrogen bond with the distal histidine (αHis-58 ; E7 helix). Upon exit of the O_2 as an neutral superoxide radical, water molecule binds to oxidized Fe(III) iron heme and also forms a hydrogen bond with the distal histidine residue. Furthermore at lower O_2 concentrations, water molecule can also bind at the O_2-binding site Fe(II) iron heme[9–12]. Water-binding will create a stabilization and must be displaced for O_2-rebinding, thereby acting as a gate for ligand rebinding.[13–17] Similar barriers to O_2 or CO binding has been also experimentally demonstrated based on sperm whale myoglobin distal histidine mutants (His64 at E7 helical position).[18,19] However, in this context, the water molecule is not referred to as a ligand but rather as an allosteric regulator or modulator. It is also not appropriate to consider all the other water molecules bound to hemoglobin as a ligand. Nevertheless, the fact that upon the release of the O_2 molecule, water molecule can bind near the previously O_2-bound heme protein active sites and create a barrier for O_2-rebinding warrants reconsideration of the role of the water molecule as merely an allosteric modulator or regulator. This reconsideration is important because, apart from its well-known O_2-transporting role, hemoglobin from different organisms has been demonstrated to exhibit diverse enzymatic activities such as nitrite reductase, anhydrase, peroxidase and deoxygenase.[20–25] We could infer that the hemoglobin enzymatic activity is not just under the control of O_2-bound water-less state but also water-bound O_2-less state. Biochemical evidence supporting the role of the distal heme pocket structure as an important requirement for the nitrite reductase activity has been demonstrated in Arabidopsis hemoglobin.[26] Specifically Arabidopsis hemoglobin AHb2 distal histidine (H66L) mutant can reduce nitrites and synthesize higher levels of NO (nitric oxide).[26] Similar reports has been also reported in hemoglobin of other organisms.[27,28] Likewise the role of distal histidine has also been shown to be a key regulator in enzymatic activity of cytoglobin and

neuroglobin.[29-32] To the best of my knowledge, it must be still demonstrated whether if hemoglobin's enzymatic activity is affected by the water binding states at heme or via the distal histidine.[16] But experiments to tackle such question must take into consideration of the possibility of potential noncanonical heme binding sites as well.[33]

Based on the literature data and arguments provide above, I wonder if mammalian hemoglobin is merely one of the aquareceptors, with its nitrite reductase activity likely modulated by the state of water-binding directly at its O_2-binding sites with or without interaction with the distal histidine.[22,23] In my opinion, we should reconsider the classic definition of allosteric modulator to include some of the heme-based gasoreceptors as mere water-sensing aquareceptors as well. In such proteins, we could debate whether O_2 is merely an allosteric modulator and water is the actual agonist, and this may help us to consider hemoglobin merely as an aquareceptor. Hence, I believe that some of the candidate aquareceptors may include other heme-based signaling proteins such as circadian regulator CLOCK (Clock Circadian Regulator), *Caenorhabditis elegans* O_2-sensing gasoreceptor GCY-35, *Escherichia coli* O_2-sensing gasoreceptor DosP and other yet to be demonstrated gasoreceptors such as spermatogenesis-inducing O_2-binding protein androglobin.[8,34]

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Savani Anbalagan: conceptualization, writing of the original draft, and review and editing.

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CONFLICT OF INTEREST

None.

DISCLOSURE

The author employed ChatGPT for correcting the scientific English. The author takes full responsibility for the content of this manuscript.

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