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Original Research Article

Studies of Molecular Energy Changes in AQP_5 in the Presence and Absence of Water Using Computational Method

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HIGHLIGHTS

- AQP_5's function and structure are dependent on the presence and absence of water.
- The presence of water molecules around AQP_5 protein causes changes in dynamic properties.
- The optimum functions of AQP_5 are arisen at low energy levels and the presence of water.
- kinetic energy for AQP_5 protein in mode of no water has the lowest fluctuations but in presence of water considerable fluctuations are seen.

ABSTRACT

Aquaporins (AQPs) are water channel proteins. Up to now, 13 AQPs have been known in mammals. AQPs play a key role in water osmotic flow in various cells. the members of aquaporin protein family have been identified as H_2O transposters across organelle and plasma membranes. AQPs of the aquaammoniaporin type are highly permeable for water and ammonia. In this study, we have evaluated the structure of AQP_5 using two computational methods. We investigated the potential and kinetic energy, as well as maximum and minimum difference of atomic charge for AQP_5. The atomic study of AQP_5 protein showed that the minimum and maximum value of atomic charge in the presence and absence of water were related to sections (1-15). The water effect is generally considered to be the major driving force in the folding of AQP_5. different sections of AQP_5 behaved different in the presence or absence of water, and have different functionalities. Also, the absolute value of atomic charge difference for AQP_5 sections was proven as an important feature in protein structural changes.

Keywords:
Aquaporins
Energy
Membrane channel
Computational simulation
Water

Introduction

In recent decades, using advanced computers and new methods to study lipids, membrane proteins and their atomic details has been decoded, as well, computer simulations provide accurate information about the structure and dynamics of membrane (Direito *et al*., 2016; Lee *et al*., 2016). Membrane proteins are of great importance and since their crystallization are difficult, little information is available to explain their structures. these limited information shows that these proteins seem to be similar to soluble proteins (Ishikawa *et al*., 2005; Borgnia *et al*., 1999). Many proteins like ion transmitter channels are able to act properly in ion transmission only when they are placed in the cell membrane. If proteins exit from the membrane, it is difficult to explain their process, exactly. Regarding that many of these carrier proteins are not easily crystalized, protein simulation in membrane is considered as one of the most applicable methods to investigate their molecular mechanism (Zeuthen and Klaerke, 1999; Huang et al., 2010; Pooladi et al., 2014).

Aquaporins (AQPs) are a member of water channel

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proteins family. Up to know, thirteen Aquaporins has been known in mammals (Mobasheri and Barrett-Jolley, 2014). These small proteins in membrane selectively transmit water across cell plasma membrane. Aquaporin function occurs in response to osmotic pressure. Although not fully known, it is said that Aquaporins are playing a key role in water flow osmosis in lumen cells (Keden and Katchalsky, 1989; Jackson, 2006; Wang and Tajkhorshid, 2007). AQPs compose a class of integral biomembrane proteins that exist in a wide range of living organisms, having protein channels located across the cell membrane for water transport through the cell membrane. AQP_5 is one of these trans-channel membranes that facilitate water passage.

In the study of complex systems and molecules, simulation method is an efficient one. In this method, a pattern or model of the system containing necessary information for analysis is used. Substantially, the more detail of biology system is incorporated in the model, to increase the similarity with real system and better evaluation of real behavior. In other words, more details in the model make it difficult to study. As a result a balance is required between two modes (Zeuthen, 2000; Ge-Groot and Grubmuller, 2005; Zhang and Chen, 2013). Computational chemistry is a mthod to describe and predict the stability of chemical systems, evaluation of energy difference (potential and kinetic) between different modes, and route explanation and finding the reaction mechanism at the atomic level (Schonfelder et al., 2016; Yu et al., 2016).

Due to the complexity of biological systems, the use of simulation and modeling to investigate the behavior of these systems is essential. Modelling protein in membrane has been performed for many purposes; for example, predicting the function of membrane protein, folding of proteins in the membrane, the possible assignment of protein in membrane and the study of environmental protein reaction with cells (Hub et al., 2009; Khaghani et al., 2015).

The quantitative expressions in these studies allow scientists to calculate the total potential energy of a protein in a particular configuration. In fact, a major effort has been made to find good quantitative representations of the potential energy of a protein as a function of the positions of all atoms. These protein conditions have generally considered as the sum of various factors, including the presence of water, changes in protein expression, and variety of diseases associated with protein molecules (Wang and Tajkhorshid, 2007; Yubao and Bastien, 2011; Martinez-Ballesta-Mdel and Carvajal, 2016).

Hartree-Fock (HF) theory is a principal fact of electronic structure theory, as well as, the basis of molecular orbital (MO) theory, which posits that each electron's motion can be described by a single-particle function (orbital), which does not depend explicitly on the instantaneous motions of the other electrons (Sherrill., 2000).

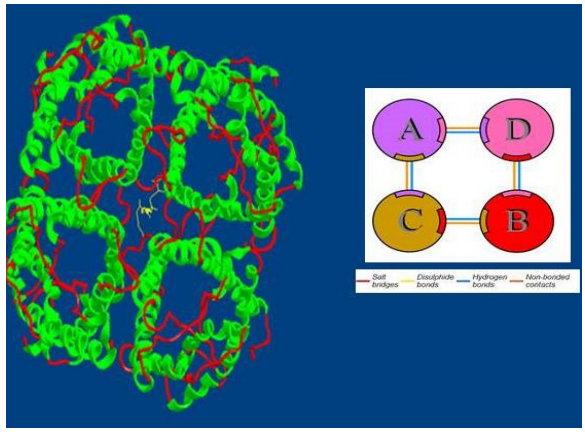

Figure 1. The shape and structure of AQP_5 in HyperChem software, have been extracted from protein database bank (www.pdb.org).

The aim of the study was to evaluate the structure of AQP_5 from the view point of maximum and minimum differences of atomic charge, as well as the absolute difference of atomic charge and energy (potential & kinetic) in the presence and absence of water.

Materials and Methods

The potential and kinetic energy in molecular mechanics mode were used to evaluate membrane proteins of AQP_5 using two computational methods: Monte Carlo simulations, by Gaussian 98 (version 5.1), and Hyperchem (HyperChem professional release 7.01 package of program for molecular modeling).

Crystallographic structure of AQP_5 has been extracted from protein database bank (www.pdb.org). This structure with the code of 3D9S, has 15198 atoms in 4 chains (A.B.C.D). In Fig. 1, the crystallographic tertiary structure of one $HsAQP_5$ monomer was presented. In this research, due to the bigness of molecule and maintenance of protein and peptide structure, the atoms in protein chain have been divided into 15 parts. To start the application, using Hyper Chem software, for each piece, two command lines along with 6 codes (2 2, 1, 1, 0 1, 0 2, 1 2, and 2 1) were applied (Shahmansoorian et al., 2014; Tohidi et al., 2015).

Calculations of total atomic charge and HF

For each obtained output, the values of total atomic charges were registered. Also, for each segment, maximum and

Table 1. The values of atomic charge difference for 15 sections in AQP_5.

Section	Min atomic charge	MaxAtomic charge	Absolute Value Of Atomic Charge Difference	HF
1	-1.974898	2.043664	4.018562	-5048.772359
2	-0.668049	0.674314	1.342363	-4380.256279
3	-0.938227	0.765317	1.703544	-4392.128862
4	-0.631761	0.66713	1.298891	-4617.760395
5	-0.922347	0.687667	1.610014	-4720.545659
6	-0.716844	0.665094	1.381938	-4445.675808
7	-0.740507	0.637814	1.378321	-4447.515233
8	-0.926981	0.736302	1.663283	-4558.552318
9	-0.705533	0.663319	1.368852	-4657.517569
10	-0.770505	0.740815	1.51132	-4899.795791
11	-0.640173	0.670239	1.310412	-5034.743285
12	-0.938531	0.807862	1.746393	-4828.593265
13	-0.728089	0.684761	1.41285	-4654.724558
14	-0.974346	0.774048	1.748394	-5364.585996
15	-0.942853	0.778545	1.721398	-4856.856378

minimum values were specified and absolute value of atomic charge difference were calculated. Finally, the diagram was also drawn. Furthermore, HF values were calculated for each segment and the diagram was drawn.

Total Atomic Charge = [Maximum – Minimum]

Calculations of E_{Kin}, E_{Pot}, E_{Tot}

In order to compute potential energy, kinetic and total, the files with extensions of mol were evaluated by Hyperchem software, applying Molecular Mechanic mode, then, in Compute section and in part Average, three modes of E_{Kin}, E_{Pot}, E_{Tot} were chosen in two forms of Average Only and Avg & graph, respectively (Tohidi et al., 2015).

$$E_{Total} = \Sigma E_{Potential} + \Sigma E_{Kinetic}$$

$$K = 1/2\ (\Sigma mv^2) = 3/2\ KT$$

The study of AQP_5 energy changes in presence of flowing water

The values of E_{Kin}, E_{Pot}, and E_{Tot} for studying water passage from Aquaporin has been used and to this goal, three modes (Z, Y, X),from section periodic box, for two forms of Average only and Avg & graph were selected, respectively.

Statistical analysis

Diagram scatter and trandline drawings were considered for error analysis of modeling and calculations.

Results

The atomic study of AQP_5 protein demonstrated that the minimum and maximum value of atomic charge was seen in section 1. Regarding this, the highest absolute value of atomic charge difference, also, belongs to the same part (Table 1). Fig. 2 shows the difference of absolute value of atomic charge between section 1 and sections 2-15. The sections of 2-15 had the atomic charge of approximately zero. These results stand for that the sections 2-15 have little impact in comparison with section 1, because of the higher absolute value of atomic charge difference (More than three times).

Regarding to the data of HF values (Fig. 3 and Table 1), it was seen that the minimum value is reported in section 13-14 and the maximum value, in 2-3 section. According to these results, the instability of section 2 is the highest and section 14 is the lowest. The reduction of energy level, which was seen in the diagram could be an

Table 2. The amount of kinetic, potential and total energy in the presence or absence of water in 15 sections.

Sections	E_{Kin}	E_{Pot}	E_{Tot}	$E_{Kin} + H_2O$	$E_{Pot} + H_2O$	$E_{Tot} + H_2O$
1	89.42363	232.7947	322.2184	175.2703	423.7771	599.0474
2	85.84668	225.9716	311.8183	185.1069	436.6323	621.7392
3	84.95244	308.2257	393.1782	235.1841	410.253	645.4371
4	89.42363	202.2319	291.6555	290.6268	352.2532	642.88
5	88.52939	226.205	314.7344	293.5432	346.3541	638.4321
6	84.95244	214.1178	299.0702	213.7225	420.7618	634.4842
7	86.74092	189.4004	276.1413	194.0493	384.7335	578.7828
8	86.74092	231.9201	318.6611	255.7516	428.2264	683.978
9	85.84668	190.6555	276.5022	324.6078	336.6225	661.2303
10	84.95244	197.3034	282.2558	194.9435	309.8334	504.7769
11	88.52939	238.3647	326.8941	169.0107	395.2161	564.2267
12	89.42363	211.0599	300.4835	287.9441	343.8592	631.8033
13	89.42363	202.6414	292.065	194.0493	343.0478	537.097
14	86.74092	216.8742	303.6151	191.3666	382.0298	573.3964
15	86.74092	249.9517	336.6927	274.5305	516.3726	790.9031

index for stability; as observed, section 14 showed lower energy level than sections 13 and 15, and also, section 11 showed lower energy level than sections 10 and 12, moreover, section 2 had higher energy level than sections 1 and 3. Generally, section arrangement regarding the lower energy level was: Section14>section1>section11.

In addition, section 1, which having the highest charge difference, was stable regarding to HF values and showed the lowest HF value after section 14. Furthermore, section 13 like section 14 showed little HF and stable mode. However, this section is less stable than sections 1 and 14.

Fig. 4 and Table 2 show the values of potential, kinetic, and total energy of all 15 AQP_5 sections in two modes, the lack of water and in the presence of water.

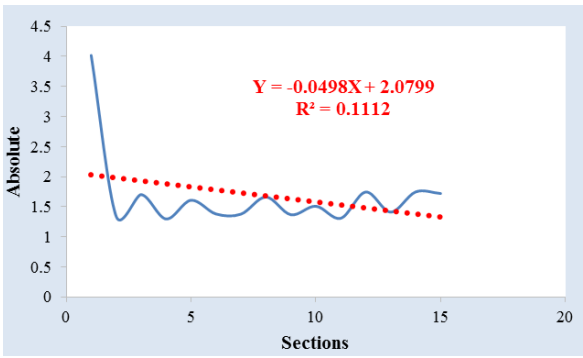

Figure 2. The differences and changes of the atomic chage in 15 section AQP_5.

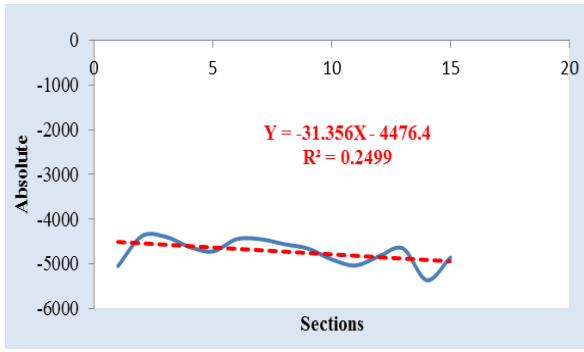

Figure 3. The differences and changes of the HF in 15 section AQP_5.

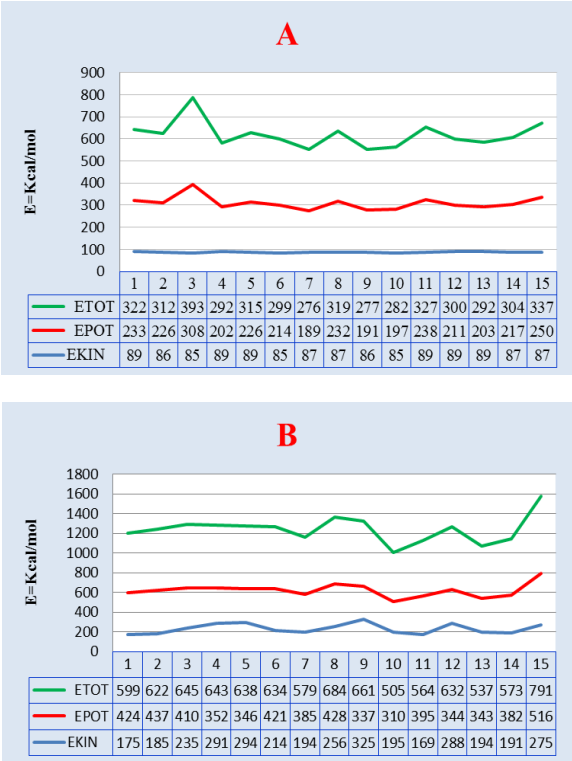

Figure 4. AQP_5 general comparison in space, **A)** in the absence of water **B)** in the presence of water.

According to calculations, section three in the mode of no water showed the highest value of potential and total energy; as a result, it had the lowest stability. Section 15 also had the same value as section 3. It seems that there is a significant relationship between this situation and the way of putting these two sections after sections 1 and 2 (instable sections with acute quality) and sections 13 and 14 (with lowest HF and highest stability).

We have compared the changes of E_{Kin}, E_{Pot}, and E_{Tot} in two modes. The lowest kinetic energy in the mode that protein enclosed in water molecules, associated with section 1, showed the high movement of this section in presence of water. In contrast, section 15 prefered no water mode, that showed its instability, according to high potential energy, and high total energy.

According to obtained results of Fig. 4 (A and B), kinetic energy in mode of no water had the lowest fluctuations, however, in the presence of water, considerable fluctuations were seen. The other interesting point is that in Fig. 4A, in the mode of no water, more fluctuations were seen in preliminary sections (1, 2 and 3). Our approach to the end was that the extent of fluctuation reduced. This fact was opposite in the presence of water. In reality, we can come to the conclusion that water contributes to structure stability and creation of appropriate functional condition for Aquaporins and especially, preliminary amino acids.

As shown in Fig. 5, the structure is highly dynamic

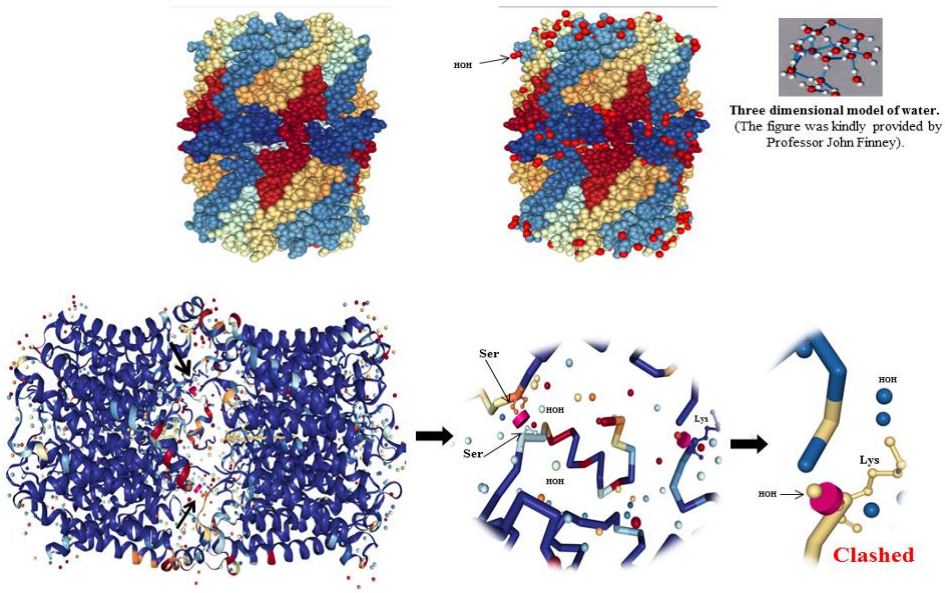

Figure 5. Normal configuration of AQP_5 in the presence and absence of water [(www.pdb.org), (www.rcsb.org) and (https://genome.ucsc.edu/)]. Three dimensional model of water. The 3D structure of Water was kindly provided by Professor John Finney.

with hydrogen bonds forming, breaking, and reforming in different patterns. In bulk water, each molecule has a configurational freedom for forming such bonds in all directions around itself. This is not the case in the presence of a solute. Forming a hydrogen bond leads to a decrease in enthalpy (negative: ΔH) while the configurational freedom of different partners, with which it form bonds, leads to an increase in entropy (positive: ΔS). Both features contribute to a decrease in free energy (ΔG= ΔH - TΔS) and are, therefore, favoured thermodynamically. Also, in Fig. 5, crystal structures of human AQP_5 in the presence and absence of water were presented.

Discussion

One might think that steric forces would make a biological macromolecule behavior, almost like a hard ceramic object. But, the atoms in a macromolecule are not fixed in place. They can move, as the bonds that hold them together strain and twist (Stansfeld and Sansom, 2011). Computational methods can simulate macromolecules such as proteins. The molecular forces between the different amino acid side chains and backbone groups are different and fold the protein into its compact state for proper function, named its native state. The native state is a global state, comprising a minute subset of all possible conformations available for a protein (Horsefied et al., 2008; Hub et al., 2009).

Water has unusual properties such as negative change in volume with melting, high dielectric constant, high melting point, high freezing point, and high heat capacity (Hashido et al., 2007). To account for the role of water, one must think of hydrogen bonding as an reversible reaction rather than a simple bond formation. The hydrogen bonding with water is an exchange process, in which the contacts with water are replaced by intermolecular contacts; the free energy change associated with hydrogen bond exchange is small. When a hydrogen bond forms between two groups confined within a protein interior, water has already been excluded. Hydrogen bonding is then, no longer an exchange process, so that the full bond energy is available to drive secondary structure formation (Parisi and Ibarra, 1996; Janosl and Ceccarelli, 2013). This decreases the energy that hydrogen bonds can contribute to structural stability (Hashido et al., 2007).

Conclusion

According to the results, the water effect is generally considered to be the major driving force for the folding of AQP_5. It results in the burial of the hydrophobic amino acid side-chains in the core of the protein. In fact, the optimum functions of AQP_5 are arisen at low energy levels and the presence of water. The aim of the present study in the presence of water, is to find the low level of energy for AQP_5, which results in better performance. It was also found that, the different sections of AQP_5 behaved differently in the presence or absence of water.

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Competing Interests

The authors declare that they have no conflict of interests.

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