Sodium acetate effects on the helical stability of a poly-alanine peptide

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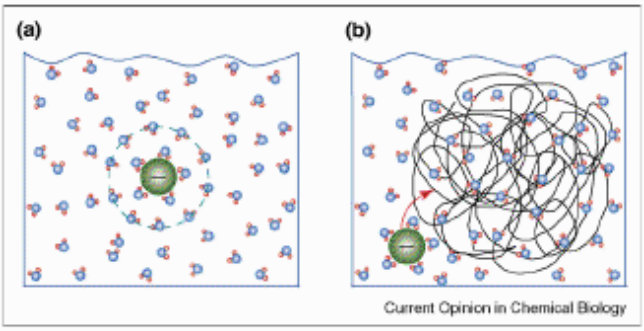
I. Introduction

The conformations and activities of proteins can be altered by the influence of ions in water. Though we know ions affect proteins in solution, the exact mechanisms are not well understood. The categorizing of ions into chaotropes (traditionally known as water-structure breakers) and kosmotropes (traditionally known as water-structure makers) were attempts to explain how different ions changed the water structure of a solution. Chaotropes are defined as low-charge density ions and are able to disrupt "water-structure". On the other hand, kosmotropes are small and/or high-charge density ions that are able to induce order by creating hydrogen bonding throughout water, as demonstrated in Figure 1. [1]

Figure 1.



Anions and water structure. **(a)** Organized water beyond an anion' first hydration shell would be needed for structure-making and breaking effects to occur. **(b)** The direct interaction of an anion with a macromolecule in aqueous solution. The relative sizes of water molecules, macromolecules, and the anion are not generally to scale. However, the relative size of the anion with respect to the water molecules is approximately correct for SO_4^{2-}.

The ability of ions to make hydrogen bonds with water, thereby "making" or "breaking" water structure lead to the observation of salting-out and salting-in of proteins, first noted by Franz Hofmeister in 1888. [1] The salting-out of a protein refers to the ion's ability to increase the protein's stability thereby inducing precipitation of the protein. This phenomenon is traditionally observed when kosmotrope ions are introduced into an aqueous solution. Conversely, salting-in refers to the ion's ability to decrease the protein's stability in solution and causing it to remain hydrated by the water molecules. Therefore, the protein experiences greater solubility from increased interaction with the water via hydrogen bonding and remains in solution. The salting-in phenomenon is traditionally observed when chaotrope ions are present in an aqueous environment. [3]

Hofmeister also observed that the ions varied in how they interacted with the protein and to what degree the protein unfolded when in solution with the ion present. Also, the ions varied in their ability to affect the surface tension of the solution. Therefore, Hofmeister created a ranking, known as the Hofmeister series that ordered these different ions based on their ability/tendency to salt-out proteins by increasing protein stability:

For Anions [1,2]:

$$H_2PO_4^- > SO_4^{2-} > CH_3CHOO^- > F^- > Cl^- > Br^- > NO_3^- > I^- > ClO_4^- > SCN^-$$

For Cations [1]:

$$Mg^{2+} > Li^+ > Na^+ \sim K^+ > NH_4^+$$

Though these general observations have been accepted for simple ions, the effects of more complex ions that contain both charged and hydrophobic or hydrophilic groups is presently unknown. [1] In order to further investigate this unknown field, the effects of the acetate anion are going to be studied. In attempts to accurately observe these unknown interactions between the acetate anion with the water and the poly-alanine peptide, molecular dynamic simulations will be conducted. The resulting trajectories throughout the simulations should provide meaningful insight to possibly how this large and negatively charged ion interacts with water molecules and the peptide.

II. Methodology[1]

The effects of the acetate anion will be studied using the poly-alanine peptide (AP): AAAAA(AAARA)$_3$A , where A represents alanine and R represents the three arginine side chains. The simulations will be conducted using the AMBER 9 package with a modified version of the AMBER-99 force field, FFSB99 which was developed for mainly-alanine peptides. The atomic charges of the acetate anions were fitted to the electrostatic potentials calculated at the Hartree-Fock level using the 6-31* basis set.

The peptide will be simulated in a water box containing 0.2 M NaCH$_3$-CHOO solution, which will be achieved by adding approximately 400 acetate anions to the system along with the appropriate quantity of sodium cations. The coordinates of these ions will be added to the coordinates of the peptide. Then the system will be solvated upon the addition of approximately 100,000 TIP3P water molecules to the water box as explicit solvent.

Once the construction of the water box is complete, the system will be minimized, followed by an equilibration at 300K. After equilibration, the total volume and density of the system will be adjusted at a constant

pressure of 1atm. The time step of 2 fs will most likely be used, while saving the trajectory data every 1 ps. The data collection will start after the first 3 ns of the molecular dynamics simulations to avoid initial biasing of the system.

Once the simulations are complete, the trajectory files will be compiled into a master file in order to watch the movements and interactions between the different species throughout the simulations. The change in the peptides angles (ϕ and ψ) will be measured and graphed in Ramachandran plots. This will provide analytical analysis for each angle between juxtaposed residues. For example as the peptide transforms from a folded into an unfolded-like state, that can be defined by characteristic ϕ and ψ angles. Also, the distribution of ions around the peptide will be measured in Angstroms (Å) through a radial distribution plot. This will show how the acetate anions moved and distributed themselves around the peptide throughout the simulation.

III. Research Objectives

The objectives for the research this summer is to create two different water boxes that solvate the AP-acetate system:

1. To create a large water box, as per described in the previous Methodologies section, and observe how the acetate anions interact with the poly-alanine peptide
2. To create a smaller water box with the additional REMD parameters (Replica-exchange molecular dynamics) and observe how this additional parameter affects the interactions between acetate, water, and the peptide

The data from each of the above simulations will be analyzed by creating the master trajectory files, Ramachandran plots, and radial distribution plots as described in the Methodologies section. The data should show if indeed the acetate anion interacts more preferably with water or the peptide directly, as predicted by the Hofmeister series.

IV. Conclusions

Hopefully as a result of conducting independent molecular dynamic simulations on acetate, the effects of more complex ions on ion-protein, ion-water and water-protein interactions will become better understood. By examining these interactions, whether or not the acetate anion interacts intimately with the peptide or not should shed light on the stabilization or

destabilizations affects of this bulkier, negatively charged ion on the native helical secondary structure of AP.

V. References

1. Asciutto, Eliana K and Madura, Jeffry D. "Sodium perchlorate effects on the helical stability of a mainly alanine peptide." *Manuscript in progress.*

2. Chaplin, Martin. "Hofmeister Series." Water Structure and Science. 31 May 2009. London South Bank University. 19 July 2009 < http://www.lsbu.ac.uk/water/hofmeist.html>.

3. Zhang, Yanjie and Cremer, Paul S. 2006. "Interactions between macromolecules and ions: the Hofmeister series." *Current Opinion in Chemical Biology*. Vol 10:658-663.