

CHAO/KOSMOTROPIC PROPERTIES OF BRINE SOLUTIONS IN THE PRESENCE OF ANCIENT PROTEINS AND THEIR ASSISTANCE IN THE BIOAVAILABILITY AND PRECIPITATION OF LIFE-NECESSARY ORGANIC MOLECULES. Shelby D Osborne¹, Mortaza Derakhshani Molayousefi², Mahmoud Moradi², and Vincent F Chevrier³; ¹University of Arkansas, Environmental Dynamics, Fayetteville, Arkansas, ²University of Arkansas, Department of Chemistry and Biochemistry, Fayetteville, Arkansas, ³University of Arkansas, AR Center for Space and Planetary Sciences, Fayetteville, Arkansas

Introduction: The formation, stability, and precipitation of proteins that emerge in naturally occurring brine solutions (water and salt) across the solar system may have significantly aided in providing the essential environmental conditions for organic interactions necessary in subsequent life formation [1,2,3]. The relationship between brine solutions and the behavior of life-necessary organic proteins in these solutions may provide us with insight into the components and interactions that were required during life's earliest developmental stages.

Solutes in water are defined as kosmotropic (order-making) if they contribute to the stability and structure of water-water interactions. Kosmotropes cause water molecules to favorably interact, which in effect stabilizes intramolecular interactions in macromolecules such as proteins. On the other side, chaotropicity (order-destroying interactions) describes the consequent disordering of protein structures when dissolved in water. Various cations and anions have been ordered in terms of their degree of chao- or kosmotropicity on a scale called the Hoffmeister Series, and some work has been attempted to quantify this property [5].

The relationship between water and salts in brine solutions (kosmo- or chaotropic behavior), has a substantial influence on the precipitation and bioavailability of these proteins [4]. Although universally significant thermodynamic parameters, such as temperature and water activity, exist within specific boundaries for life, the relationship between primordial biochemistry and water-salt composition remains unclear. How exactly these early proteins react in the presence of these brines across the solar system is largely unknown, but some observations of these conditions have accumulated due to the large variety of brines probably present on icy moons or terrestrial bodies like Mars and early Earth.

The scope of this research pertains to the most ancestral amino acid sequences required for the beginning stages of life and their interactions with brines that exhibit various kosmo- or chaotropic properties, including: Mg^{2+} , Ca^{2+} , Na^+ , NH_4^+ and chloride Cl^- , perchlorate ClO_4^- , carbonate CO_3^{2-} , and sulfate SO_4^{2-} , which accounts for the majority of putative brines across the solar system [6]. The subsequent precipitation, renaturation, and chemistry that occurs between the brine environment and the ancient protein gives us insight into the initiation of these primordial life-

forming structures in brine environments. This abstract presents preliminary results of the AncC ribonuclease sequence in the presence of NaCl and $MgCl_2$ at two different concentrations.

Methods: The ancestral sequence of the Anc1 bacterial ribonuclease, the most recent common bacterial ribonuclease ancestor of *E. Coli* and *T. Thermophilus*, is estimated to have existed approximately 3 billion years ago [6]. AncC is a slightly more recent, more *E. Coli* specific ribonuclease sequence. The Anc1 sequence is largely disputed, while the sequence of AncC is known [7]. We have used all-atom molecular dynamics (MD) simulations to investigate the differential structural dynamics of the AncC protein in different salts and at different salt concentrations. The initial structure is based on an X-ray crystal structure which was obtained from RCSB protein databank with the PDB entry 4LY7 [6]. All simulations were performed using the NAMD 3.0 [8] simulation package and the CHARMM36m all-atom additive force field [9].

The protein was solvated in a box of TIP3P waters, with 0.15 M or 1.5 M of NaCl or $MgCl_2$, resulting in four independent simulation systems. Systems were composed of approximately 60,000 atoms with a simulation box dimension of $\sim(84 \times 84 \times 84) \text{ \AA}^3$. The simulations for 0.15M concentrations ran for 100 nanoseconds, while the 1.5M concentrations ran for 90 nanoseconds. Simulations were carried out using a 2-fs time step at 273 K using a Langevin integrator with a damping coefficient of $\gamma = 0.5 \text{ ps}^{-1}$ at 1 atm of pressure.

Results:

The original structure of the AncC ribonuclease can be seen in Figure 1 with two specific regions circled in red and blue. These circled regions in Figure 1 correspond to significant areas that indicated change (or no change) once in contact with the brine (Fig. 2). These red and blue circles in Figure 1 correspond with the blue and red arrows in Figure 2.

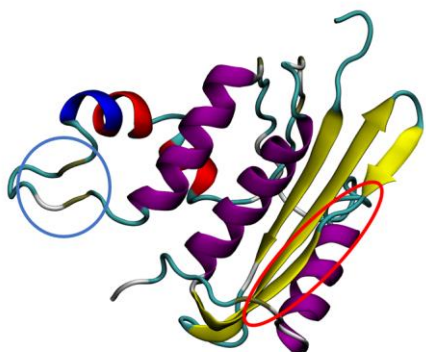


Figure 1: AncC ribonuclease structure with alpha helices (purple) and beta sheets (yellow); two regions are circled (in red and blue) which denote a location of interest after brine exposure.

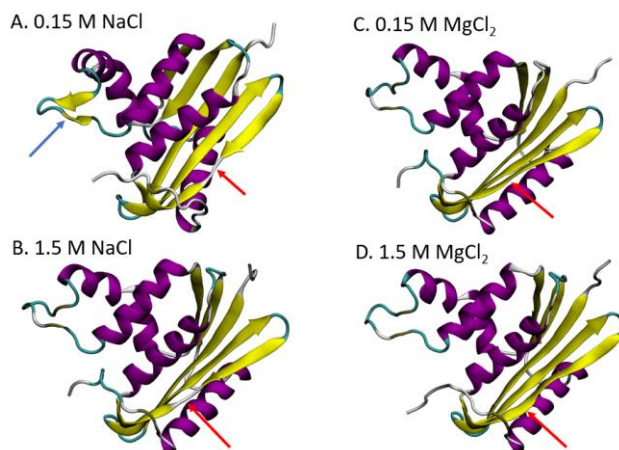


Figure 2: Resulting structures of AncC ribonuclease after exposure to designated salt concentration for 90-100 nano-seconds.

We observed 2 significant changes in the proteins exposed to the brine solutions, depending on concentration and nature of the cation. First, a secondary structure in the form of two beta sheets emerges in the 0.15M NaCl concentration (Figure 2A, blue arrow). These two small beta sheets only form at 0.15M NaCl, and not at the higher concentration or in MgCl₂ brines at all. These two beta sheets do not exist in the original structure (Fig. 1). The second major change concerns the beta sheet in the red circled region of Figure 1, which exhibits splitting. The original structure exhibits splitting in that region, and the beta sheet appears to retain its splitting feature upon exposure to NaCl (Fig. 2 A, B, red arrow), but not to MgCl₂ brines (Fig. 2 C, D, red arrow). In the MgCl₂ brines, the beta-sheet appears as one unit. Hence, both the nature of the cation and concentration of the salt promote different responses and effects in the secondary structures of the AncC protein.

According to the Hoffmeister Series scale, sodium is more kosmotropic and magnesium is more chaotropic. These two different salts with two different chao-kosmo properties create two different responses within the protein structure in that particular brine.

Beta Sheet Change	AncC	0.15 NaCl	1.5 NaCl	0.15 MgCl ₂	1.5 MgCl ₂
Emergence	NO	YES	NO	NO	NO
Splitting	YES	YES	YES	NO	NO

Table 1: Changes in Secondary Structures of AncC when exposed to NaCl and MgCl₂

This observation speaks highly to the significance of chao-kosmo influences on molecular level outcomes. Additional tests will utilize a wider range of salts at more varying concentrations in order to deduce whether the formation of additional secondary structures is more conducive to life-inducing characteristics. **Conclusion:** Both the type of cation and concentration of the salt promote different responses and effects in the overall structure of the AncC protein. The secondary structure of a protein is different in the presence of various salts at various concentrations. The ways ancient proteins react when exposed to brine solutions found on various planets in our solar system may represent an expansion of our current knowledge concerning habitability among worlds. The results of this study demonstrate the importance of understanding the complicated and specific outcomes of chao- and kosmotropic interactions between brines and proteins.

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