Osmolytes and Hofmeister Salts Dynamically Regulate and Promote Mutant Huntingtin Aggregation

Hannaan Choudhry, Principal Investigator: Dr. Alice Y. Liu
Department of Cell Biology and Neuroscience, Rutgers University, Piscataway, NJ 08854

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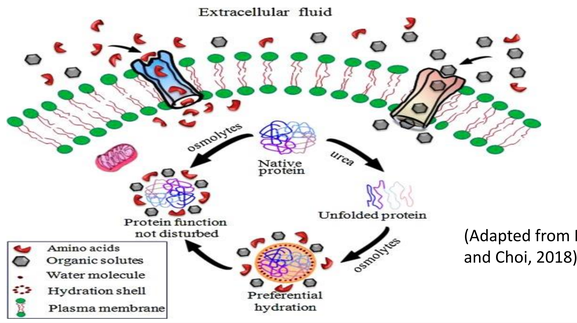
Abstract

A common pathological feature of age-dependent neurodegenerative diseases (ND), such as Parkinson's, Alzheimer's, and Huntington's, is the presentation of protein aggregation such as β amyloid in Alzheimer's disease (AD), Lewy bodies in Parkinson's disease (PD), and Inclusion Bodies (IB) in Huntington's disease (HD). The mechanism as well as the pathogenic role of protein aggregation in ND are not entirely clear. Heat Shock Factor 1 (HSF1) is a master transcription regulator for induction of Heat Shock Protein (HSP) chaperones. This quality control mechanism, known as the heat shock response, promotes the proper folding/refolding and disposition of cellular proteins, which is necessary to treat neurodegenerative diseases. The purpose of this research project is to assess the possibility that conditions that modulate protein structuring change the dynamics of mHtt protein to form IB for beneficial functional outcome. In this study, the effects of osmolytes, heat shock, and Hofmeister salts on the structure and expression of the mutant Htt protein found in Huntington's disease were analyzed in a cell model of HD. Osmolytes and heat shock promoted the structuring and compaction of mHtt to form IB whereas urea showed the opposite effect. Functionally, osmolytes and heat shock negated the mHtt-Induced sequestration of transcription factors. Hofmeister salts displayed a dose response, with higher concentrations driving diffuse into IB. Toxicity, however, was a side effect for many salts at higher concentrations. The results of this study indicate a potential approach to modulate the folding and aggregation of mHtt as a framework for therapeutics development in neurodegenerative diseases. Collectively, my results suggest that conditions which support protein structuring promote the aggregation of mHtt to form IB to alleviate TF repression.

Background

Age-related neurodegenerative diseases appear to share a similar pathogenic mechanism cause either by mutation or by epigenetic mechanism to result in the emergence of "pathogenic conformer" of the disease proteins and their aggregation to form fibrils and other higher order aggregate such as IB. Most of these accumulated misfolded proteins are found in amyloid, which is a β-sheet-rich fibrillar protein conformation. These amyloid deposits may be a beneficial coping or protective mechanism for the cell, while the cause of the degenerative processes may be the pre-amyloid species (diffuse) that appears before formation of the aggregated amyloid deposits. Mitigating protein misfolding or increasing the IB count slows the progression of Huntington's disease, and maintaining proper protein homeostasis may provide a key to treatment. Previous literature has indicated that osmolytes and salts have varying effects on the structure of these misfolded proteins via different mechanisms. The study hopes to discover one or more molecules or treatments that are capable of increasing the formation of IB while maintaining proper cell growth and viability without any significant side effects. By gaining a better understanding of the effect of osmolytes and salts in this preliminary study, a potential avenue for treatment of Huntington's disease and other neurodegenerative diseases opens for future research. Understanding the pathology and pathogenic conformation of mHtt (mutant huntingtin protein) can help narrow potential strategies to target the disease.

Figure 1: Structuring Pathway used by Osmolytes



(Adapted from Rabani and Choi, 2018)

Results

Figure 3: Stabilizing Osmolytes Significantly Diminish Diffuse and Total mHtt similar to Heat Shock

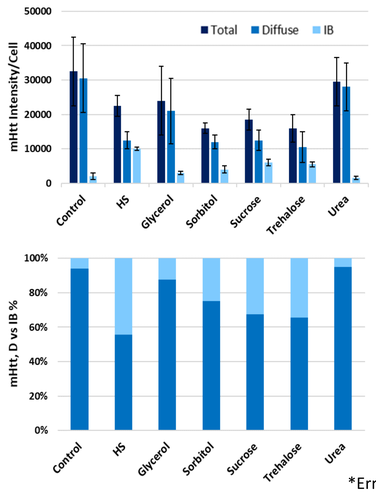
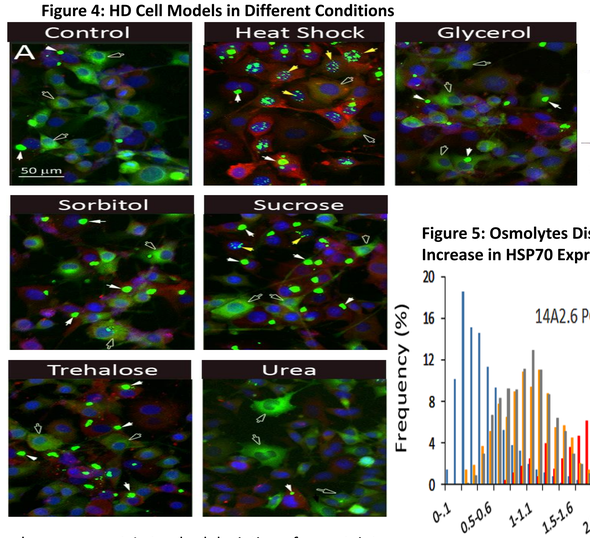


Figure 4: HD Cell Models in Different Conditions



*Error bars represent 1 standard deviation of uncertainty.

Figure 5: Osmolytes Display only Modest Increase in HSP70 Expression

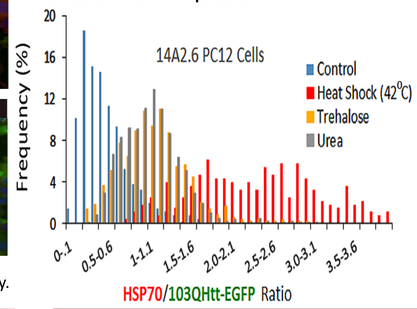
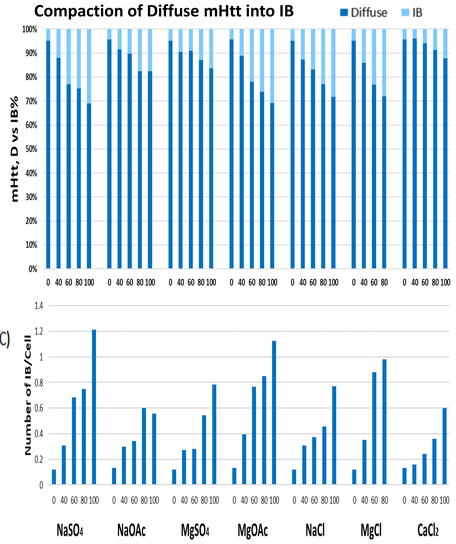


Figure 6: Salts Display Dose Response and Drive Compaction of Diffuse mHtt into IB



Methods and Materials

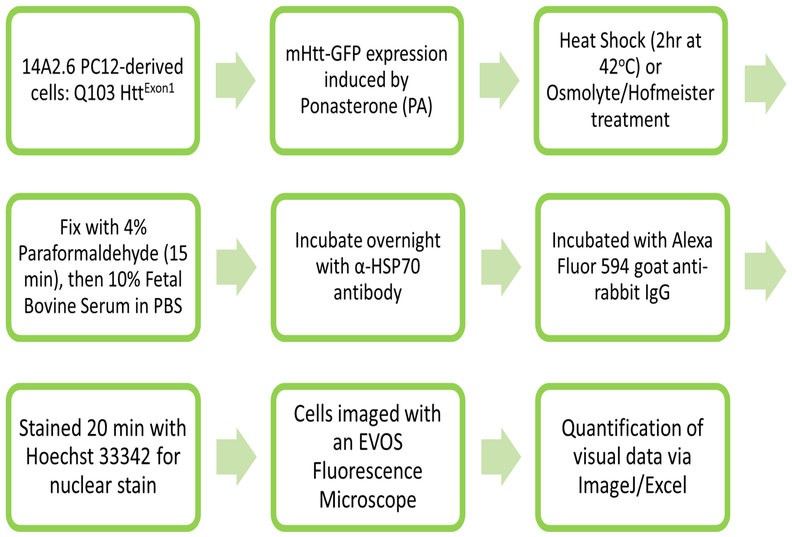


Figure 2: Flowchart depicting steps and timeframe abided by in this study

References

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Discussion and Future Direction

Overall, the experiments conducted have provided a strong base for future experiments related to the question. The experiment involved testing HD cell models in the presence of osmolytes and Hofmeister salts by analyzing their mHtt structure composition. Sucrose and trehalose clearly showed the greatest capability of driving diffuse into IB; osmolytes in general were able to decrease the diffuse:IB ratio. Additionally, heat shock showed a similar trend of reducing diffuse significantly. On the other hand, urea did the opposite and increased the diffuse:IB ratio. However based on the HSP70/103QHtt-EGFP ratio, osmolytes likely have a different mechanism than heat shock, since heat shock has a much higher ratio and therefore higher activation levels of HSP70. Finally, I tested increasing concentrations of Hofmeister salts, which demonstrated that increasing concentrations did promote the sequestration of diffuse Htt to IB form. However, the cells did not tolerate these higher concentrations of some salts, causing a very low count in some conditions.

It is clearly supported by the data that osmolytes and Hofmeister ions help drive diffuse into IB, indicating that structuring could play a major role in understanding the pathology of Huntington's disease. Future experiments could be done using in vitro studies, which would provide a set of results for different treatments outside the bacterial cell model. Another possible avenue would be to explore a potential osmotic effect that may be the primary cause for this sequestration, as opposed to any unique properties of the osmolytes themselves. After conducting trials in vitro, a future step could be to use animal models to better simulate treatment options for human use.

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