Stabilizing osmolytes drive mutant huntingtin aggregation and alleviate CREB dysfunction in HD cell models

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Abstract

PolyQ-expanded huntingtin (mHtt) is a class of intrinsically disordered proteins (IDP) that are shown in models of Huntington's disease (HD) to misfold and aggregate into insoluble protein deposits termed inclusion bodies (IB). Recent evidence demonstrates that the highly reactive, diffusible monomers and oligomers of mHtt are responsible for inducing neurotoxic changes in striatal and cortical neurons rather than IBs. This research aimed to better understand the cellular dynamics that regulate the aggregation of the polyQ-expanded mutant Huntingtin protein (mHtt) in regard to the pathogenesis of Huntington's disease. For this, we elected to test the effects of organic osmolytes on the expression of Q103-Htt-GFP as well as the relative density of diffuse versus IB Q103-Htt-GFP in our cell model of HD. Osmolytes are organic solutes that aid cells in maintaining proteostasis by chaperoning protein folding; by elevating the chemical potential of the denatured state of IDPs, osmolytes shift the equilibrium towards more aggregated structures. In previous studies, the Liu lab demonstrated that diffuse mHtt in a dose-dependent manner represses crucial transcription factors involved in stress resistance and memory formation. In this work, we show that stabilizing osmolytes promote mHtt aggregation to alleviate CREB transcription factor dysfunction and consequently, reduce neuronal death.

Background

Mutant Huntingtin (mHtt)

- The intrinsically-disordered proteins implicated in neurodegenerative diseases (ND) are all related by their propensity to aggregate into insoluble, protein deposits (Turoverov et al., 2010).
- Specifically, Huntington's disease is an autosomal-dominant disease characterized by an expanded polyglutamine stretch (> 35 polyQ) within the N-terminus of the huntingtin (Htt) protein (Arrasate et. al, 2004).
- Recent studies provide evidence that the formation of inclusion bodies (IB) — insoluble, intranuclear amyloid deposits— behave as a cellular coping mechanism to reduce the more neurotoxic and highly interactive, diffusible monomers and oligomers (Haass & Selkoe, 20007).
- Previous work from the Liu Lab demonstrated that the induction of HSP chaperones via heat shock promotes the aggregation of diffuse mHtt into IBs This is concurrent with the reduced repression of key transcription factors: cAMP-response element-binding protein (CREB), heat shock factor 1 (HSF1), and nuclear factor ϰ light chain enhancer of activated B cells (NFϰB) (Chen et al., 2018).

Osmolytes

- Osmolytes are naturally-occurring, organic solutes that aid cells in maintaining proteostasis by chaperoning protein folding; this stabilizing effect on protein structure is accomplished through elevating the chemical potential of the denatured state of intrinsically disordered proteins, thus shifting the folding equilibrium towards more aggregated structures with less reactive, surface areas.
- Urea, as opposed to glycerol, sucrose, sorbitol and trehalose, is considered a chaotrope, a destabilizing osmolyte.

Purpose

- Therefore, the purpose of this study is to use physiologically relevant concentrations of stabilizing osmolytes as well as urea to evaluate the effects of protein structuring on the compaction and aggregation of mHtt from a diffusible ensemble into IB. The expectation is that these chemical osmolytes can reduce toxic, diffusible forms of mHtt-GFP, while simultaneously alleviating CREB dysfunction and reducing cellular death.

Results

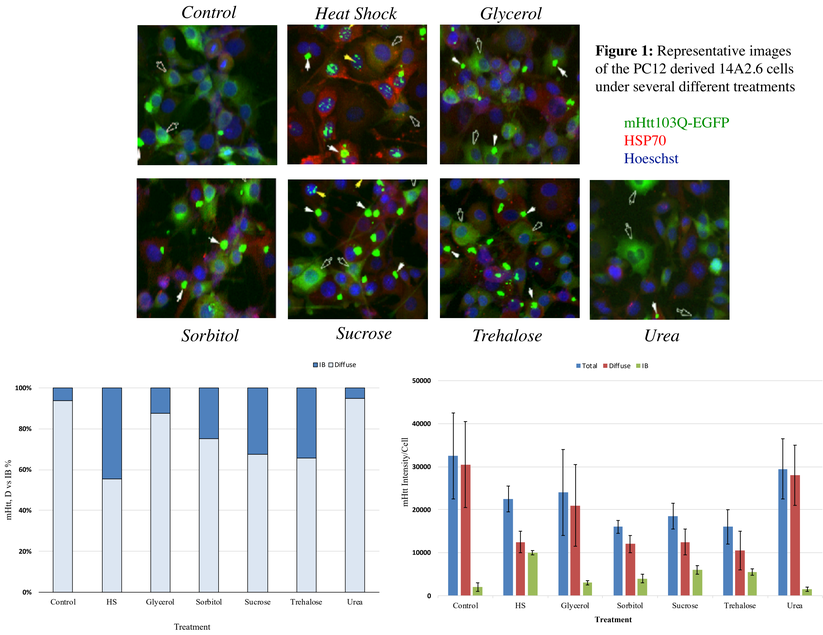

Figure 1: Representative images of the PC12 derived 14A2.6 cells under several different treatments

Figure 2: Osmolytes drive the aggregation of diffuse 103Q mHtt-EGFP into inclusion bodies (IBs) in PC12 derived 14A2.6 neuronal cells

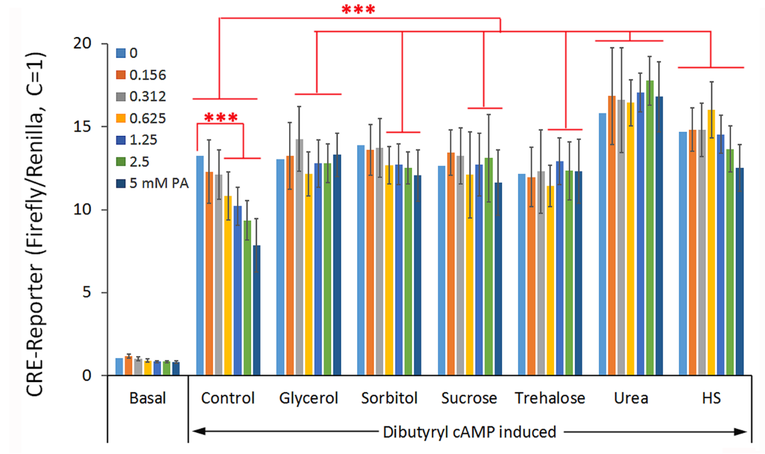

Figure 3: Effects of stabilizing and destabilizing (i.e. urea) osmolytes versus that of heat shock in alleviating the diffuse mHtt-EGFP dose-dependent repression of CRE-firefly luciferase reporter gene

Methods and Materials

Cell Culturing

14A2.6 PC12 derived cells: Q103-mHtt$^{Exon\ 1}$-EGFP
↓
mHtt-GFP expression induced by Ponasterone
↓
t=24hr: Heat shock (2h at 42°C) or Osmolyte treatment (at 37°C)
↓
t=48hr: Cells fixed with 4% paraformaldehyde, followed by 10% Fetal Bovine Serum in PBS
↓
Incubated overnight with α-HSP70 antibody
↓
Incubated with Alexa Fluor 594 goat anti-rabbit IgG
↓
Stain nuclei for 20 min with Hoechst 33342
↓
Cells imaged with an EVOS® Fl microscope
↓
Quantification of visual data via ImageJ/Excel

CRE-firefly luciferase Reporter Gene Assay

-24hr: Plated 14A2.6 line of PC12-cells
-12hr: Transfected cells with CRE-firefly luciferase reporter DNA along with the internal control CMV-driven humanized Renilla luciferase reporter DNA
0hr: mHtt-GFP expression induced by the addition of 0 – 5 μM Ponasterone
24hr: Treated cells with osmolytes (120 mM) at 37°C. Incubated designated HS cells for 2 hr at 42°C, followed by recovery at 37°C
48hr: CRE-reporter gene was induced by the addition of 1 mM dibutyryl cAMP at t=48 hr, then incubated for 6 hr at 37°C
52hr: Reporter gene activity was assayed using the Dual-Glo reporter gene assay kit from Promega Inc

Discussion and Future Direction

- Our results show that stabilizing osmolytes (i.e. glycerol, sorbitol, sucrose and trehalose) promote the aggregation of diffusible mHtt into IB, concurrent with reduced mHtt-dependent repression of CREB and thus increased CRE-firefly luciferase reporter gene activity.
- Optimal results were seen when treatments involved osmolyte concentrations of approximately ~100-120mM, with a minimum of ~50mM needed to a yield a statistically significant effect on protein structuring.
- As expected, urea did not drive the aggregation of diffuse mHtt into IB but nonetheless reduced the diffuse mHtt-dependent repression of CREB function. Unlike the stabilizing osmolytes that promote key transcription factor function by sequestering mHtt and reducing the highly reactive, diffusible form of the protein, urea disrupts protein-protein interactions between diffuse-mHtt and key regulatory factors
- In order to better understand HD pathogenesis for the future development of therapeutics, the Liu Lab is currently investigating the effects of Hofmeister salts on mHtt aggregation. The next steps include finding optimal concentrations for said salts, testing potential synergistic treatments, and understanding the mechanism that underlines salt-IDP interactions.

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