Optogenetic Analysis of the Effects of Nonhydrolyzable ATP Analogues and Cofilin Mimicking Peptides for Potential Alzheimer's Disease Prevention

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15 P 30.97 Phosphorus | 77 Ir 192.22 Iridium | 85 At 210 Astatine | 99 Es 252 Einsteinium

ABSTRACT

Alzheimer's disease (AD) is a major cause of age-related dementia affecting millions of people.[1] Within the next few decades, it is predicted that the US will encounter a drastic increase in the number of Alzheimer's cases due to the aging population. As a result, the need to investigate new approaches for treating the root causes of AD is paramount. However, generating new therapies is a major challenge, as the relationship between the biochemical hallmarks of AD (amyloid deposits, neurofibrillary tangles)[2] and the resulting cell death and cognitive impairment remains poorly understood.

In this research, we will use optogenetic techniques to simulate the protein clusters formed in AD neurons undergoing oxidative stress and investigate possible strategies for their inhibition. In one strategy, we will investigate the potential of nonhydrolyzable ATP analogues for inhibition the cytoskeletal abnormalities observed under oxidative stress. In a second strategy, we will investigate peptides derived from known human cofilin-1 actin G- and F-binding sites as potential inhibitors of cytoskeleton dysregulation in cells undergoing oxidative stress. The goal of this study is to identify small molecules and peptides that could be developed into therapies for reactive oxygen species-linked neurodegeneration.

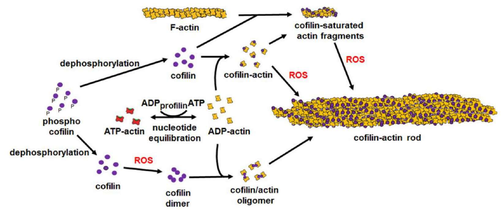


Figure 1: Likely molecular models of cofilin-actin rod formation under reactive oxygen species (ROS).

RESEARCH OVERVIEW

Overview of Actin dynamics:

One of the major conditions that are present in cells of AD patients in a degradation of synapses. During the normal process of aging, neuronal loss is at a minimum with even the hippocampal neurons remaining normal. However, with patients with AD, up to even 50% of widespread synaptic loss occurs with selective, localized neuron loss. Overexpression of normal proteins (i.e Cofilin; depicted in figure 2) or accumulation of abnormal proteins have been shown to bring about this neurodegeneration.[3] Recent studies suggest that amyloid formation can lead to synapses toxicity.[4] In the presence of ATP, actin will polymerize into a semi-immobilized state where the stability of this state is described as the critical concentration for polymerization. The Critical concentration of polymerization is described as the concentration of free monomers in equilibrium with the filaments.[5] This is known as actin "treadmilling" and is shown in figure 4.

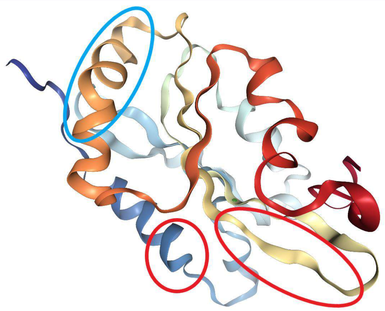


Figure 2: 3D structure of (WT) human cofilin-1, The blue and red circles indicate the G and F-binding sites. Where the α-helix and β-sheet are F-site 1 and F-site 2 illustrated below in the full amino acid sequence.

Amino acid sequence: **F-binding sites** **G-Binding site**

MASGVAVSDGVIKVFN**DMKVRKS**STPEEVKKRKKAVLF
CLSEDKKNIILEEGKEILVGDVGQTVDDPYATFVKMLP
DKDCRYALYDATYETKES**KKEDLVFIFW**APESAP**LKSK**
MIYASSKDAIKKKLTGIKHELQANCYEEVKDRCTLAEK
LGGSAVISLEGKPL

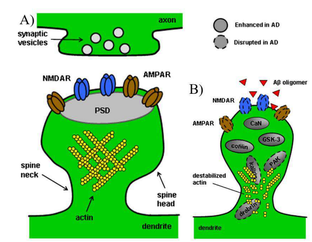
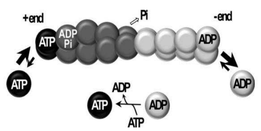


Figure 3 (Left): Synaptic actin cytoskeleton destabilization in AD through overexpression of actin intrusion proteins and disruption of actin stabilizing proteins. (A) healthy synapses, (B) Decay of synapses in AD.[2]

Figure 4 (Right): Actin treadmilling. Hydrolyzing ATP to ADP within the actin structure then releasing the ADP back into the system at the less stable (-) end of the filament.[5]



ACTIN-COFILIN BINDING COMPLEX

The theoretical approach is to introduce polymerization inhibitors of cofilin-1. In humans, cofilin-1 binds to the actin structure allowing for a structural rearrangement that allows cofilin-2 to bind which in turn creates the cofilin/actin amyloids.[6] Based on structural information given in previous studies[6], cofilin-1 has three binding domains as shown in figure 2; F-site 1, 2 and G-site.

Table 1: Cofilin-1 sections selected for experimental investigations. All primers were ordered with a GFP tag off the C-terminus of the fragment.

Name Length-First 3-Last 3	Amino acid sequence α-helix β-sheet Bold- Binding site
Short 19-MAA-DMK	MAAGVAVSDGVIKVFN**DMK**
Long 31-MAA-VKK	MAAGVAVSDGVIKVFN**DMK**VRKSSTPEEVKK
F-α-helix 59-MAA-VGD	MAAGVAVSDGVIKVFN**DMK**VRKSSTPEEVKKRKKAVLFCLSEDKKNIILEEGKEILVGD
F-β-sheet 48-VGQ-APE	VGQTVDDPYATFVKMLPDKDCRYALYDATYETKES**KKEDLVFIFW**APE
G-α-helix 59-SAP-KPL	SAP**LKSKMIYASSKDAIKKK**LTGIKHELQANCYEEVKDRCTLAEKLGGSAVISLEGKPL
F-1-2 104-MAA-IFW	MAAGVAVSDGVIKVFN**DMK**VRKSSTPEEVKKRKKAVLFCLSEDKKNIILEEGKEILVGD VGQTVDDPYATFVKMLPDKDCRYALYDATYETKES**KKEDLVFIFW**

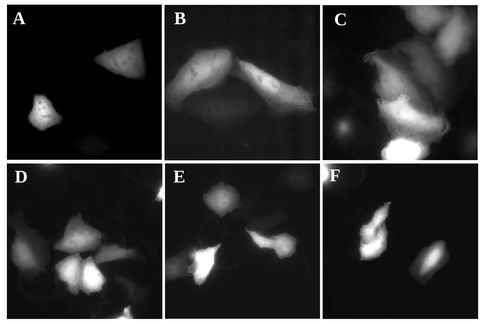


Figure 5 (A-F): HeLa cell transfected with the Cofilin fragments; (A) GFP control, (B) Short (19AA) fragment, (C) Long (31AA) fragment, (D) F-α-helix (59AA) fragment, (E) F-β-sheet (48AA), and (F) G-α-helix (59AA) fragment. The F-1-2 fragment was not pictured because of an error in the bacteria transformation process that is being addressed.

NONHYDROLYZABLE ATP ANALOGUES

Observing the changes from the control to the oxidant control, the amyloid formation is clearly present. Going forward, we were looking for a change in the cell's behavior in the presence of the oxidants. Based on the data gathered, we assume that the reactions for ATP-αS, AppNHp, and ATP-γS are potential candidates for moving forward with the optogenetic proteins as they exhibit none or slight evidence of actin amyloid formation which agrees with our hypothesis.

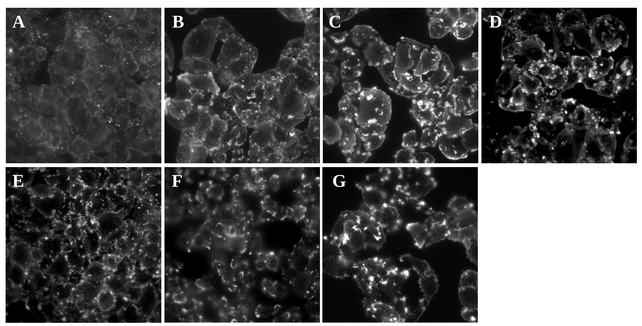


Figure 6 (A-G) (Above): Leica microscope images of HeLa cells treated with nonhydrolyzable ATP analogues under oxidative stress conditions; (A) Control cells, (B) Oxidant control cells, (C) ATP-α Sulfur treated cells, (D) ApCpp treated cells, (E) AppCp treated cells, (F) AppNHp treated cells, (G) ATP-γ Sulfur treated cells.

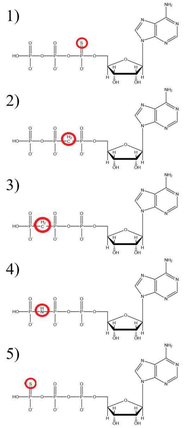
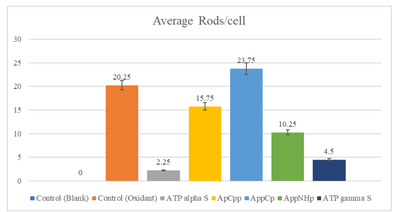


Figure 7 (Right): Structure of Nonhydrolyzable ATP analogues with the changes to the original structure labels in red; (1) ATP-α Sulfur, (2) ApCpp, (3) AppCp (4) AppNHp (5) ATP-γ Sulfur



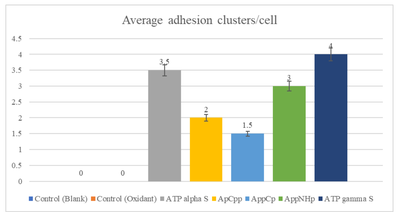


Figure 8 (Left) and 9 (Right): Bar graphs corresponding to the average actin rods and cell adhesions per cell in the sample where n=100 cells for each condition.

OPTOGENETIC ANALYSIS OF SMALL MOLECULES

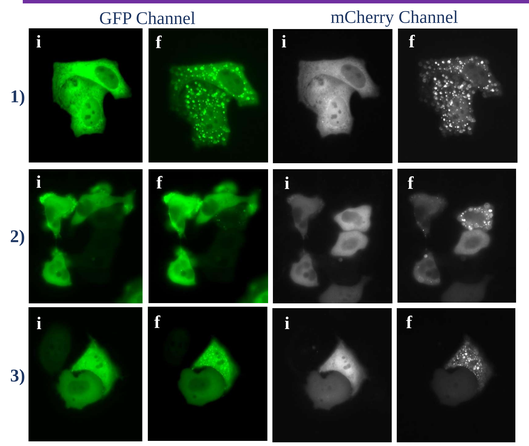


Figure 10 (Above): Optogenetic experimentation with nonhydrolyzable ATP analogues under oxidative conditions on both the GFP and mCherry channels. ATP analogues were double transfected with Actin-CIB-GFP and CofilinS3A-Cry2-mCherry. (i) is before light activation, (f) is after light activation. (1) Oxidant control, (2) ATP α Sulfur, and (3) ATP γ Sulfur.

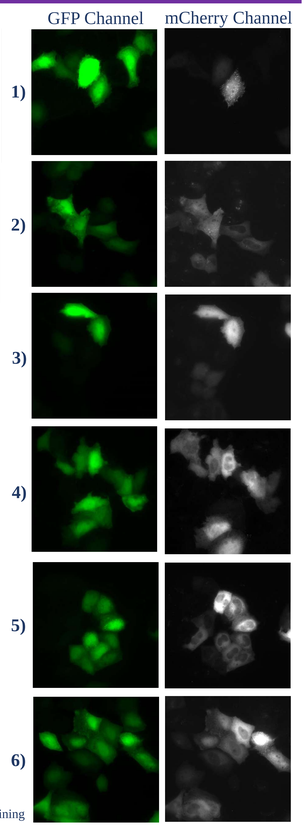


Figure 11 (Right): Optogenetic experimentation with cofilin fragments under oxidative conditions on both the GFP and mCherry channels. The cofilin fragment experiments were triple transfected with Actin-CIB-STOP to remove the GFP, CofilinS3A-Cry2-mCherry, and the cofilin fragment. (1) GFP only control, (2) Short (19AA) fragment, (3) Long (31AA) fragment, (4) F-α-helix (59AA) fragment, (5) F-β-sheet (48AA), and (6) G-α-helix (59AA) fragment.

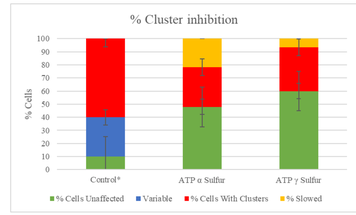


Figure 12: Bar graph comparing the optogenetic cluster inhibition capabilities of the sulfur containing nonhydrolyzable ATP analogues where n=20 cells per samples. *Control data from source[7]

CONCLUSIONS & FUTURE WORK

Regarding the preliminary data collected from the optogenetic experiments, the sulfur containing ATP analogues gave some surprising results. We would like to go forward in attempting a concentration study based on these two compounds to find the most efficient concentration for inhibition of actin clustering. The cofilin fragments have shown interestingly different dynamics in the HeLa cell model. We would like to investigate these differences as well as compare the cofilinS3A and WT strands and their corresponding fragments as WT cofilin has slightly different clustering dynamics.

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