The ER-Resident Acetyltransferases: Novel Targets for Alzheimer's Disease

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Abstract

Age-associated diseases are diseases that have a predisposition toward persons of advanced age. Common examples of these types of diseases are cataracts, osteoporosis, arthritis and certain types of dementia. One of the most common types of age-associated diseases is Alzheimer's disease (AD), the leading form of dementia (1). The disease occurs through improper cellular protein processing, resulting in malformed bundles of non-functional proteins forming in the brain that reduce neuronal signal transduction and contribute to brain deterioration. Currently there are no known preventative or curative treatments for AD, so investigation into new targets for AD treatment is of high importance. One novel therapeutic approach is to use cellular autophagy, or the cells ability to stop protein production and to degrade and recycle malformed proteins, to prevent or slow AD progression. It has been determined that autophagy can be turned on by inhibiting the enzymes ATase1 and ATase2, components of the endoplasmic reticulum (ER) acetylation machinery. These enzymes transfer acetyl from acetyl coenzyme A, a common metabolite in cells, onto properly folded proteins (2). Excitingly, when this inhibition occurs in an AD-mouse model there is no progression of AD as determined by reduced neurodegeneration and cognitive decline. Current efforts are focused on identifying novel ATase1 and ATase2 inhibitors; however, these efforts are hindered by a lack of accurate structural information on the enzymes. Here, we refined a previously published structural model of truncated forms of the enzymes (shortened at F58) using new computational approaches, resulting in structures more closely matching their templates. Additionally, we analyzed how well and in what orientation a small class of known ATase1 and ATase2 inhibitors bind to these models. This strategy resulted in a cluster of high-affinity binding events near the hypothetical acetyl-CoA binding pocket, likely signifying where substrate docks on the protein. Further experiments using hydrogen/deuterium exchange mass spectrometry, a method for determining the surface of an enzyme structure, provided additional structural information for ATase1. The results of this early study have implications on our knowledge of chemical families that may induce autophagy by affecting the ER acetylation machinery at the ATase step. Future studies will use this information to understand the mechanism of these enzymes, design inhibitors that are more effective and, thus, produce alternative autophagy inducers and potential AD therapeutics.

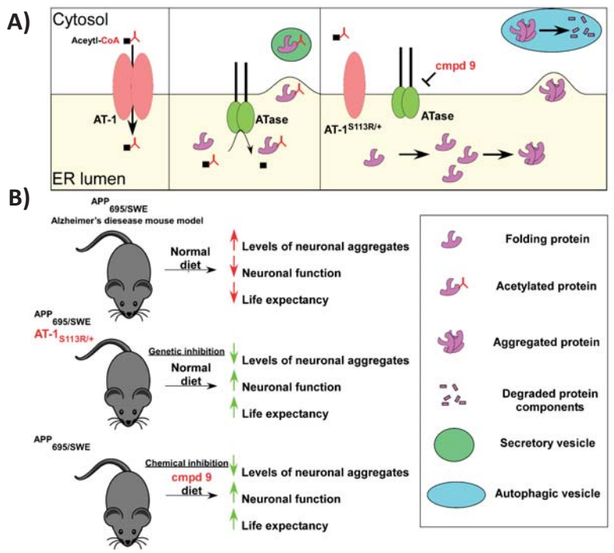

Figure 1: The ER-acetylation machinery and the effect of its inhibition on proteins transiting the secretory pathway and the Alzheimer's disease mouse model $APP_{695/SWE}$. A, left panel: The acetyl-CoA cytosol:ER-lumen transporter acetyltransferase-1 (AT-1) allows for acetyl-CoA entry into the ER. **A, middle panel:** The ER-resident lysine acetyltransferases, ATase1 and ATase2 (ATase), label stably folded protein intermediates with an acetyl group, marking them for export through the secretory pathway for further processing before they are utilized elsewhere in the cell or body. **A, right panel:** Genetic inhibition of AT-1 or chemical inhibition of ATase results in an accumulation of misfolded proteins, eventually forming large aggregates and triggers the cellular process of autophagy, which exports and degrades large aggregates within the ER. **B, top:** The mouse model of Alzheimer's disease, $APP_{695/SWE}$, presents with increased protein aggregates of amyloid beta (the key pathologic aggregate seen in the human disease) internal and external to the neurons, decreased neuronal function and a greatly reduced lifespan relative to wild-type mice. **B, middle and bottom:** Genetic or chemical inhibition of the ER-acetylation machinery results in a decreased observation of amyloid beta aggregates, greatly improved neuronal function, and a near complete restoration of life expectancy.

Objectives

- Identify potential areas of importance on ATase1 and ATase2 by:
  - Preparing an optimized protein homology model to identify potential areas of importance on the enzyme
  - Performing computational estimates on how inhibitors known to affect ATase1 and ATase2 function orient on the structure
  - Assessing the strength of the predictions using hydrogen-deuterium exchange mass spectrometry, a method of deterring protein structure by identifying the areas of the protein are exposed to liquid

Computational modeling of the ATases identified potential inhibitors of interest

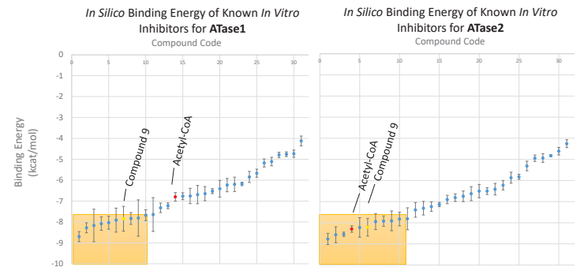

Figure 2: Computational predictions for binding energy of known ATase inhibitors identify tight binding inhibitors which all cluster in the same areas on each ATase. Yellow box: Compounds with low binding energy (high affinity) to the ATases were shown to cluster in two distinct regions of the protein models.

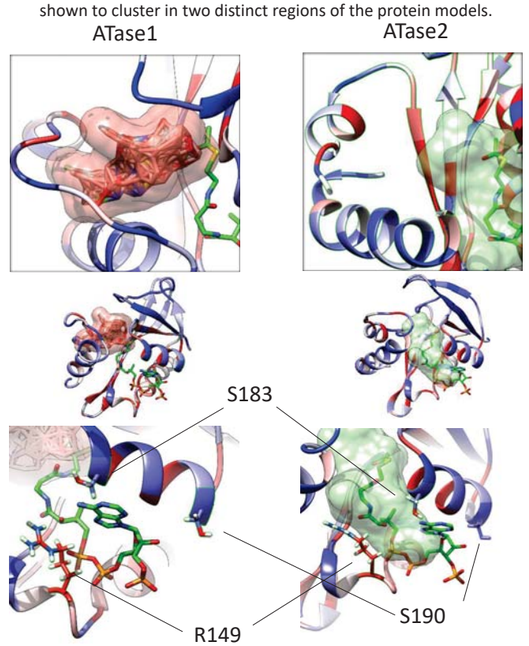

Figure 3: Refined homology models of ATase1 (left) and ATase2 (right). Above: surface fill of the nine highest-affinity binding orientations for potential ATase inhibitors, colored red and green, with distinct clustering proximal to the acetyl portion of acetyl-CoA on ATase1, likely corresponding to the peptidyl-lysine binding site. Proteins are colored by how well conserved their sequences are to other acetyltransferases. Blue – low conservation, Red – high conservation. **Below:** residues predicted to bind docked Acetyl-CoA. Acetyl-CoA from an aligned homologous structure from the *P. horikoshii* acetyltransferase is displayed for reference. Residues listed (R149, S190, S183) were observed to be important for enzymatic activity (not shown).

Preliminary hydrogen-deuterium exchange mass spectrometry studies give some evidence of overall protein structure

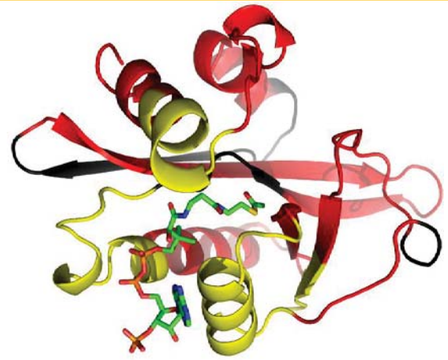

Figure 4: Preliminary data on solvent accessibility by hydrogen-deuterium exchange mass spectrometry on MBP-ATase1(F58) shows promise for defining the substrate binding pocket. The optimized homology model of ATase1(F58) colored to show observed segments of the protein in normal water (red) and heavy water (yellow). Regions where heavy water was shown to incorporate were less than expected, with a large amount of protein unlabeled. However, the areas that were labeled correspond to the predicted acetyl-CoA binding site shown as multicolored sticks, which could allow us to clarify the binding orientation of the substrate and further improve on our model.

Conclusions and future directions

1. Computational docking studies with the optimized homology models for ATase1 and ATase2 identified a subset of high-affinity binding compounds to be investigated further for ATase inhibition ability.
2. Structural studies with hydrogen-deuterium exchange mass spectrometry has the potential to characterize the hypothetical acetyl-CoA substrate binding pocket and further optimize our models.
3. Additional structural studies and inhibition studies on mouse animal models for protein aggregation diseases are underway. These could lead to novel therapeutics for age-associated protein aggregation diseases.

Acknowledgements and works cited

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