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Dehydroamino Acids in Alzheimer's Disease Protein Aggregates and a Proposed Mechanism for Their Formation

By

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I. Introduction

Scientists have a responsibility to make our work transparent and digestible to as many people as possible. Basic research is important, but it is rarely communicated outside of scientific circles. Our field is full of jargon, and it can feel like we are speaking a different language sometimes. My goal in this chapter is to distill my thesis down to the most important points by conveying the *story* of the research and what we learned along the way, rather than drowning the reader in the details. I am thankful to Professor Bassam Shakhashiri, Elizabeth Reynolds, and Cayce Osborne for their assistance in writing what follows.

II. Modifications are important

I joined Lloyd Smith's research group in 2021. Lloyd's group is interested in studying the molecular machines that are the primary actors in cells, called proteins. Proteins are polymers, meaning that they are made up of several, simple, constituent parts. There are twenty of these constituent parts called amino acids, and they can be shuffled into myriad orders to do radically different tasks around a cell, allowing for a lot of complexity from fairly simple molecules. There are around 20,000 different proteins in a human cell.

For comparison, the University of Wisconsin-Madison (UW) currently has ~27,000 employees. Think of proteins like employees on campus. Many scientists will flippantly make the claim that a single protein has a single function. This would be like reducing a UW employee like Lloyd, a college professor, to "someone who teaches". But as many of us would assume, Lloyd wears many hats. He *does* teach classes and write and grade exams. He is also a researcher, and as such serves as a mentor to me and my lab mates and writes grant applications and academic articles. He is also a part of the UW community and serves on committees and helps organize events. So distilling Lloyd to "someone who teaches" feels inaccurate to the whole picture, even if it is one of the hats he wears. Similarly, proteins can carry out multiple functions. Of course, a protein is not sentient like Lloyd or other UW employees and does not dictate the function it carries out intentionally. Instead, the function of a protein is heavily dictated by chemical modifications made to that protein. While some modifications have more significant effects on protein function than others, you can think of these modifications as the "hats" an employee wears.

Let's look at the complexity of the cell with these modifications in mind. Twenty amino acids make up approximately 20,000 proteins. Each protein, on the extremes, has between 80 and 27,000 amino acids in it, with the average sitting close to 400 amino acids per protein. In addition, we know of at least 650 modifications that can be made to these proteins, and each protein can be modified multiple times. Just one of these 650 known modifications has been documented at least *1.6 million* times across the 20,000 proteins.[1] It is difficult to comprehend

this level of complexity, and even harder to try to deconvolute what modifications are present at what times and what they mean. Lloyd's group specializes in identifying these modifications.

To study protein modifications, our group uses a technique called "mass spectrometry," which is basically like a scale that measures the weight of molecules. Within the last ~30 years, scientists have built very sophisticated scales and designed sophisticated techniques to identify molecules like proteins. Because we are just measuring the weight of these molecules, we can also measure the shift in weight that corresponds to protein modifications. We can make millions of these measurements in just a couple of hours, which we then can translate to thousands of proteins and sometimes tens of thousands of modifications.[2]

When I joined the lab, a senior student was using our molecular scale to identify a protein modification in HIV particles grown in human cells.[3] The modification, called a dehydroamino acid (DHAA), had never been discovered in HIV and was rarely reported in humans. Protein modifications are generally installed by other proteins, and neither HIV nor human cells had the machinery necessary to install a DHAA, which made her observation incredibly perplexing.

As a young graduate student, I began reading about the modification to see what was known. It turns out, "not much" would have been a good way to sum it up. When DHAAs were reported in humans, it was the result of a bacterial infection or the result of decades of protein aging (more on this later).[4,5] The viral particles were made in bacteria-free conditions and over the course of 48 hours, and thus we suspected there must have been some other way for the DHAAs to be produced. Since HIV is a relatively simple system that is known to hijack human cellular machinery, we proposed that the DHAAs were being generated by the human cell. This basic information frames two central questions: If neither HIV nor humans can generate DHAAs, then how did they come to be? And if they are found in this simple sample, is it possible they are more prevalent in humans than previously thought? These two questions would be the motivation for my research for the next five years.

I would not stay in the HIV-human cell system, as DHAAs are somewhat sporadic there. Surprisingly, I wound up finding them consistently in the human brain, particularly within Alzheimer's disease.[6] The path this research takes is somewhat winding, unexpected, and exciting. Like in a good mystery, we will go over the clues that we had available to us to begin with that led us to Alzheimer's disease, the facts we found once we got there, and what we know now.

III. Dehydroamino acids

DHAAs are inherently interesting from their unique chemistry. Outside of a cell, forming DHAAs requires extreme conditions, like a lot of acid, a lot of base, or boiling hot temperatures. This is one of the reasons our observation of the modification in human cells is so odd.

Further, DHAAs behave differently than other amino acids and modifications. Think about them like a puzzle piece, as shown in Figure 1. Proteins naturally contain a lot of puzzle piece donors (3 out of 20 amino acids contain donors), but it is *extremely* rare that a protein would contain a puzzle piece acceptor. DHAAs are this rare example of the puzzle piece acceptor.[7] These puzzle piece acceptors don't like to be alone, and luckily, they don't have to since cells contain a lot of puzzle piece donors. This pairing leaves two possible fates. 1) DHAAs may react with small, non-protein molecules, like an individual amino acid or 2) DHAAs can react with other proteins, tethering together two, extremely large molecules. This latter endpoint, what I will call DHAA-based protein tethers, has been implicated in a couple of diseases previously. To better understand why, it will be important to understand how these DHAA-based tethers might fit into the larger world of proteins sticking together.

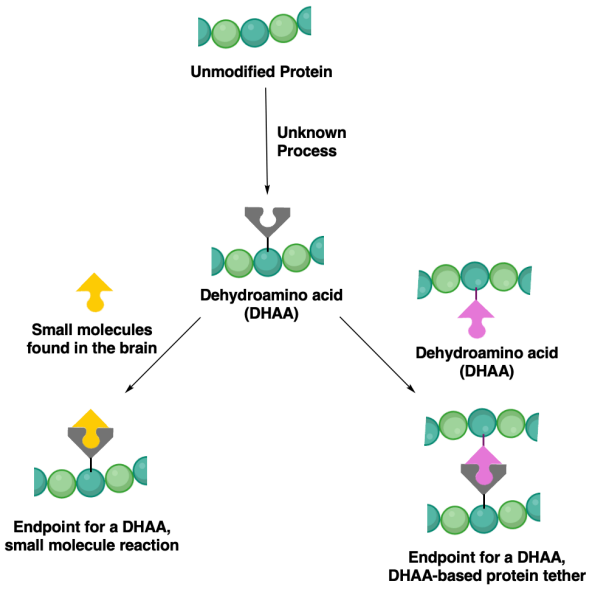

Figure 1: DHAAs are like puzzle pieces, joining together two molecules.

IV. Protein stickiness

Many diseases involve proteins sticking to each other in a process called protein aggregation. Though not all aggregation is the same, protein aggregation commonly happens in a snowball effect-like manner. Often due to some unknown cause, there is an initial sticking event, which in our analogy, is like forming the first, packed snowball. Then, like a snowball rolling down a hill, that initial protein clump picks up more and more protein molecules forming very large aggregates. Though the identity of the aggregating protein varies between diseases, protein aggregates are observed in type II diabetes, cancer, neurodegenerative disorders like Parkinson's disease (PD), Amyotrophic Lateral Sclerosis (ALS), and Alzheimer's disease (AD), and many more.[8,9]

Protein aggregates are unwieldy and interfere with the status quo in a cell. To return to the UW analogy used in describing proteins earlier, it would be like if someone started sprinkling objects the size of aircraft carriers around campus. It would still be possible to go to class (provided the aircraft carrier wasn't *in* your classroom), but it would make everything less convenient. Roads and sidewalks would be blocked, buses would stall, and other employees

would need to turn their focus to clearing the aircraft carriers from campus, which would take a lot of energy and effort. This is one obvious problem with protein aggregates.

Now imagine that rather than randomly adding in new objects, these aircraft carriers housed important employees. Say that all the custodial staff, fed up with dealing with aircraft-

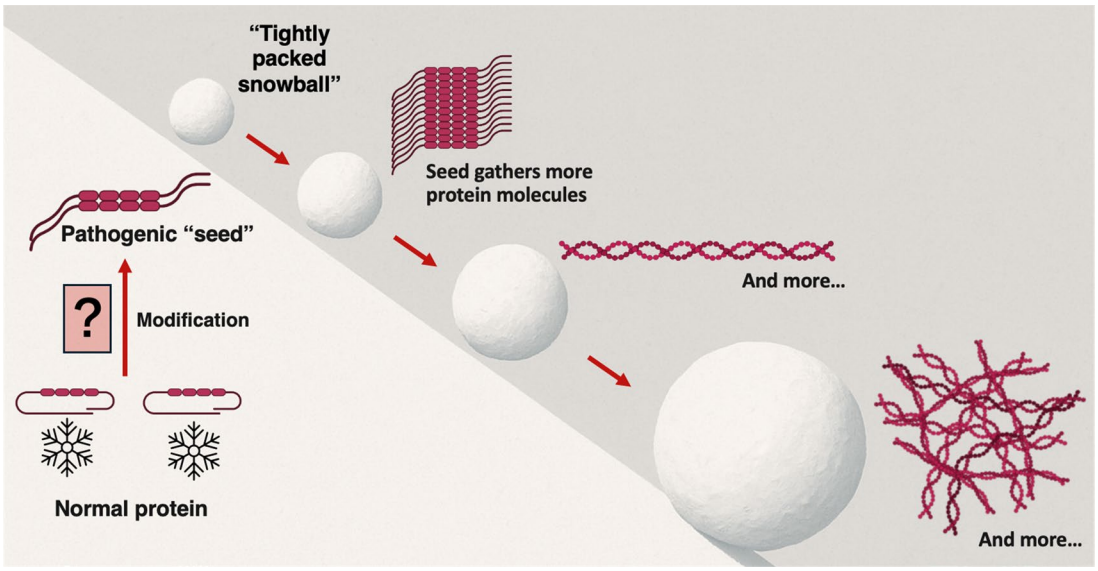

Figure 2: Like a snowball rolling down a hill, protein aggregates are formed by first forming a tightly packed protein snowball called a "seed" and picking up individual protein molecules to create a large aggregate.

carrier related wreckage, went on strike, and sat in the aircraft carriers and refused to leave. In this analogy, even if the university tried to replace the custodial workers, they would also immediately strike and board the aircraft carriers as well. While the university might carry on for a couple days without the staff, it wouldn't take long for their absence to be noticed. The reason that the university employs the custodial staff is because they are vital to the way the university is run. Analogously, the reason that a cell makes a protein is because it is vital to the way the cell is run. With the protein being effectively sequestered by protein aggregates, there are consequences. The consequences, in the case of neurodegeneration, are widespread cell death. This directly causes the symptoms we observe in response to neurodegeneration, such as the memory loss accompanying AD.[10]

V. Dehydroamino acids have been linked to protein aggregation

This section is meant to discuss the clues that were available to me when I began working on this project, and the clues that led me towards looking into DHAAs in Alzheimer's disease. I mentioned previously that DHAA-induced protein tethers, like those illustrated in Figure 1, have

been linked to disease. DHAAs have a reputation for being linked to protein stickiness, but crucially, they are not known to play a causal role.

One of the more high-profile connections of DHAAs to proteins sticking together is the case of cataracts. Cataracts are protein aggregates that occur inside the lens of the eye comprised of a fascinating protein called crystallin. Most proteins in human cells are constantly being destroyed, with new protein molecules being generated in their place. Crystallin, however, is never destroyed. The crystallin protein molecules in your eye right now are the same exact molecules that were synthesized by your cells before birth, which makes them amongst the oldest proteins in the body. Crystallin is arguably the most common place to find DHAAs in humans.[5]

There is rock solid evidence of DHAAs and their puzzle-piece like tethers in the crystallin molecules that comprise cataracts. The scientists that made this discovery suggest that DHAA-based protein tethers form the initial "snowball" in protein aggregation.[5] This is a great example of how protein aggregates themselves disrupt standard function – the aircraft carrier-like aggregates cloud your vision, making it more difficult to see.

Another common place DHAAs are observed in human cells is as part of particular bacterial infections, including salmonella and pseudomonas.[11] These bacteria intentionally introduce DHAAs into *very* important human proteins. The protein target of this DHAA-based attack is like the cell's alarm system that would enable the rest of the cell to act in response to the bacterial invader. This attack is almost like if an arsonist removed all the smoke detectors from a building and left them in a pile by the front door, setting the building on fire on the way out. The DHAA-modified protein rapidly forms DHAA-based protein tethers and is effectively sequestered from the rest of the cell, unable to set off the alarm. In the case of the arsonist, the building burns to the ground without anyone noticing until it is too late. In the case of the bacterial infection, the cell dies because it didn't react like it was being infected.[4,11] This is like the second reason why these protein aggregates are bad – like the custodial staff filing into the aircraft carriers, the proteins that are modified with DHAAs are unable to do their normal job.

This tie between protein aggregation and DHAA-induced protein tethers is one of the reasons I chose to look for the modification in Alzheimer's disease, where protein aggregation is known to be a driving force in the disease.[10] The proposed solution to preventing the DHAA-mediated devastation seen in both cataracts and the cell-based attack is stopping the protein-tether from forming, particularly by forcing the small molecule tether to occur.[5,12] Conveniently, the human cell has a way of doing this.

We all make a protein called LanCL, that had an unknown function for a long time. Several scientists spent years on the case, trying a variety of methods, including looking at how models for neurodegeneration responded to the protein. Some of these models involve mimicking the disease in mice, making small changes to the mouse's genetic code that leads to them developing hallmark characteristics and symptoms of the disease they are mimicking.

Fascinatingly, mice model systems for both AD and ALS showed that large quantities of LanCL slow the progression of neurodegeneration, indicating that this protein is likely important to protect against the diseases.[13,14] In 2021, for the first time, it was reported that one of the "hats" this protein employee wears is to force DHAAs to create small molecule tethers, therefore preventing DHAA-based protein tethers from forming.[12]

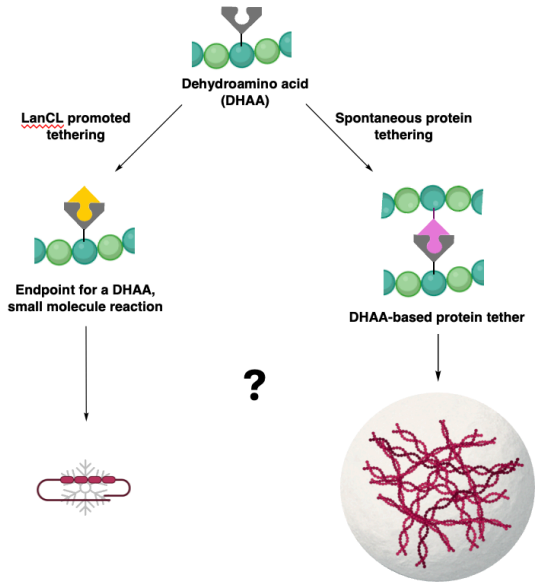

Figure 3: We hypothesize that LanCL prevents protein aggregation by creating a DHAA-small molecule tether, thus preventing the DHAA-mediated protein tethers.

Combining the neurodegeneration studies with the relatively new knowledge that LanCL prevents DHAA-based protein tethering strongly indicates that DHAAs are likely present in neurodegenerative disease. This theory is presented in Figure 3. Further, because DHAAs have been intimately tied to protein aggregation, we would suspect that they would be most abundant in human protein aggregates. Combining these clues, I decided it would be best to look for DHAAs in a human system where LanCL is relevant and contains large amounts of proteins stuck together. I chose to investigate Alzheimer's disease.

VI. The molecules behind Alzheimer's disease

Alzheimer's disease (AD) is characterized by the snowball-like aggregation of two proteins: "amyloid-β" and "Tau". These protein clumps are widespread throughout portions of brains afflicted with AD, where amyloid-β aggregates outside of the cell and Tau aggregates inside of the cell (Figure 4). A common way to describe the relationship between these two protein clumps is that amyloid-β is the trigger, but Tau is the bullet. While amyloid-β aggregation happens prior to Tau aggregation, Tau aggregates seem to drive cognitive defects, making the Tau "snowballs" an intensely interesting subject. Further, several drugs have targeted the amyloid-β aggregates, but have been ineffective in treating the disease, so much of the focus in the drug development community has shifted towards clearing Tau aggregates as well.[10]

One of the central mysteries in AD is what mechanistically connects the amyloid-β trigger to the Tau bullet. Though we know much about Tau protein aggregates, including some factors and modifications that make Tau stickier, there is no widely agreed-upon first action for Tau to begin aggregating, though we know of several ways to induce Tau aggregation outside of

the brain. What is clear is that Tau aggregates lead to cell death by distracting other cellular employees with the task of clearing the clumps while also pulling Tau away from its normal duties.[10]

There are many hypotheses as to how AD develops invoking all kinds of complex biochemistry. One of these hypotheses involves metals, both intact and dissolved in solution (the same way that table salt can be dissolved in your pasta water).[15] Dissolved, salt-like metals are vital to a functioning cell, but as we all know, you can have too much of a good thing. Remember that protein modifications are important to dictating that protein's function, and that those modifications are commonly made by other proteins. Metals, both intact and dissolved, have access to odd modifications that often lead to cell death. Some believe that these metals play a role in generating aggregates.

Intact metals are rarely observed in and around human cells, but there is a growing body of evidence that they accumulate in Alzheimer's disease. When you make a snowman, you roll a ball of snow around just like you would in the snowball effect. But for those of us that have made a snowman, we know our snowball is contaminated with other things around that it can nonspecifically pick up like grass, mud, sticks, etc. This is exactly what happens for intact metal accumulation in AD.[15,16] The amyloid-β aggregates, as they snowball into the large protein aggregates observed in Alzheimer's disease, behave like a net, absorbing these metals and harboring them near brain regions, shown in Figure 4. The accumulated metal's role in AD progression has not been thoroughly investigated, but it has been suggested to contribute to AD pathology.

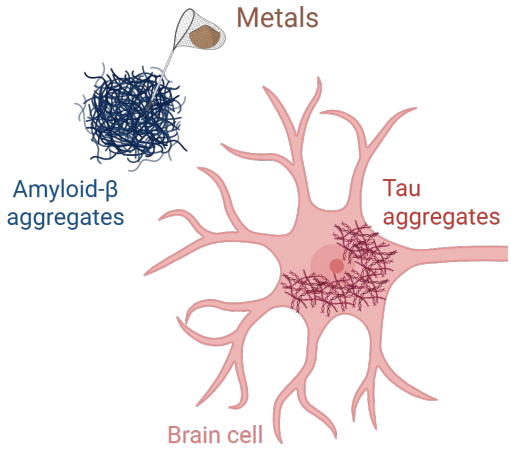

Figure 4: Amyloid-β aggregates build up outside of the cell, leading to metal accumulation. Tau aggregates build up inside the cell, leading to cell death.

VII. Dehydroamino acids in Alzheimer's disease

So far, we know that DHAA-based protein tethers are thought to make proteins sticky. We know that preventing DHAA-based protein tethers helps prevent neurodegeneration. We know that in the neurodegenerative Alzheimer's disease, proteins get sticky. So, I thought it was reasonable to hypothesize that DHAAs might be installed on sticky proteins like Tau as a modification. There, it could form a tether with other protein molecules, thus forming the "packed snowball" necessary for protein aggregation. The issue with this theory is that neither DHAAs, nor their tethers, were previously known to occur in the human brain. So, despite the circumstantial evidence we had accrued, it was a somewhat obscure and absurd hypothesis.

In 2023, I used our molecular scale to find the first evidence of DHAAs in human brains, a discovery I was *extremely* excited about. In total, I identified 404 DHAAs in 171 proteins from the protein aggregates from both healthy and AD brain specimens, all of which were identified by looking for DHAAs tethered to small molecules.[6] Of the many proteins found to be modified, one of the most commonly observed DHAA-modified proteins was Tau. Tau is one of the two primary aggregating proteins in Alzheimer's disease, and the one that tracks better with disease progression. This was a major breakthrough for our research, and it was exactly what we were looking for.

As part of this investigation, I searched (weighed molecules from) both the protein aggregates themselves (snowballs) and the non-aggregated healthy protein (snowflakes) to compare the prevalence of DHAAs. I found that DHAAs were identified at six times more sites in the aggregates than the soluble protein, ultimately resulting in them being almost *forty-fold* more abundant in the protein aggregates than the healthy protein. While this does not prove *causality*, the correlation is still fascinating.

Though at first glance, the finding that they are present in the healthy brain samples might indicate they are *not* involved in AD, it is well established that protein aggregation occurs well before the onset of the disease, and many healthy individuals are found with small quantities of Tau aggregates, so I was not surprised to see this trend. Of the 171 proteins identified, several of them are known to aggregate, and many of them are connected to cellular systems that become disoriented in AD.

While identifying the DHAAs themselves was exciting, it was not the end of the discoveries. Remember our hypothesis was that the DHAA-based protein tethers may contribute to protein aggregation, so direct evidence of the tethers was important to our theory. A known problem is that identifying these tethers, particularly by using the molecular scale, can be challenging as they do not have the defined shift in weight like most modifications do. Despite this challenge, I was able to find eleven of these DHAA-induced protein tethers, three of which were formed in the Tau protein. Once again, these eleven proteins have a track record of either aggregating or otherwise misbehaving in AD. It still doesn't prove causality, but it felt like another huge step forward.

Finally, although there was not the convincing binary of DHAAs present in AD but not in controls, I was able to measure how much of each individual DHAA was found in each condition. If some were more prevalent in the AD sample, that may warrant a further investigation into those specific DHAAs. Unsurprisingly, due to there being more of the protein Tau in AD aggregates, there were several DHAAs in Tau that were found to be significantly more present in the AD samples compared to the healthy samples. Comparing a different metric, how many DHAAs are there per Tau molecule, reveals some sites to be more important than others, including one of the three DHAA-induced protein tethers in Tau.[6] This site is part of a

cluster of sites known to be important to Tau aggregation, but the particular site and the DHAA installed there is now being further investigated for its role in Tau protein aggregation.

VIII. How did DHAAs get into the human brain?

The short answer here is that we don't know how the DHAAs formed in AD, but we have entertained many theories. I spent several years looking for a protein capable of generating the modification, but my searches never identified the modifier. While proteins are much more common modifiers, there had been reports that another biological polymer, DNA, could generate DHAAs under very specific conditions.[17] While investigating that possibility, I was shocked to find that a cocktail of three metal salts, in close-to-brain-like conditions, could form DHAAs *extremely* efficiently from molecules mimicking Tau. This observation was always accompanied by an unknown brown gunk that formed in our tubes. I did not know the nature of the reaction, and I did not know what the brown gunk was, but I was nevertheless excited to have a new reaction that formed DHAAs.

This observation, like many observations in science, created more questions than it answered. How is this modification being installed? Are all the metal salts necessary or is one more important than the others? What is the brown gunk made out of? Is it responsible for the reaction? Can this modification happen to any protein or is it specific for Tau? Is there any biological precedent for this reaction/could this be a source of DHAAs in the brain?

First, some background. Remember that proteins are made up of sequential amino acids. Tau has 441 of these amino acids, and I didn't need all of those to begin with. So instead, I used 8-11 amino acid chunks that are the same sequence of amino acids found in Tau, which comprised our Tau mimics. I used other small amino acid sequences for diversity as well, so that we could better understand how the reaction operated, and monitored if the modification was present using the molecular scale.

I mentioned way back at the beginning that DHAAs can be generated under extreme conditions, like a lot of acid, base, or heat, and these salts don't exactly rise to the level of "extreme". To understand why it makes sense for these metal salts to perform the way they do, I will need to briefly explain why acids and bases are so good at this reaction. For a DHAA to form, two things need to be kicked off the molecule. The acid is good at extracting one of them, so the other one is happier to leave without its partner. The base is good at extracting the other one, so the one the acid extract's is happier to leave, even in the absence of the acid. Some metals can serve as the acid, *and* generate the base for the reaction in the process.

Think about this reaction like a toddler holding and losing both a balloon and a lollipop, as shown in Figure 5. The DHAA is made when both the balloon and lollipop have been lost. In one scenario, call it the acid-mediated reaction, someone reaches in and snips the ribbon holding the balloon. The toddler, desperate to save the balloon, drops the lollipop and reaches for it with his other hand. In the opposite scenario, the base-mediated reaction, a bully smacks the lollipop from the child's hand. The child, fumbling around trying to catch the lollipop, drops the balloon and it floats away.

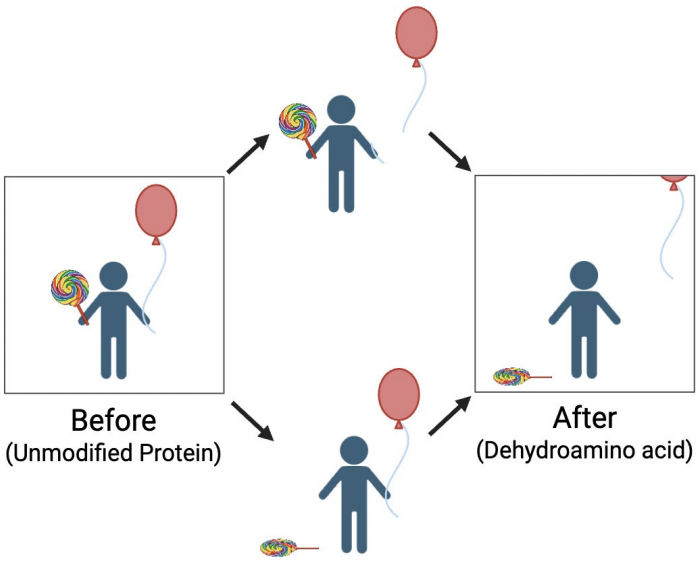

Figure 5: Dehydroamino acid formation is like a child losing a balloon and a lollipop.

Due to the abundance of metal salts in cells, this would be very bad if it happened every time a metal encountered a protein. Instead, metals can make DHAA formation more efficient, but they require an external influence (base), like the bully slapping away the lollipop. By treating the Tau mimics with particular metal salts (zinc and copper) I showed for the first time these two metal salts make for pretty good scissors by themselves, effectively acting as the acid that helps generate the DHAA. They're still not as strong as the extreme conditions normally required, but it is a proof-of-principle that these metal salts can accelerate this reaction.

Interestingly, my original observation that the metal salt cocktail (consisting of zinc, manganese, and magnesium) led to the efficient formation of DHAAs in the Tau mimics was *incorrect*. The soluble metal salts, while giving modest yields, were not enough on their own to recreate the extremely efficient reaction I had observed. Instead, it was a product of this salt cocktail, the brown gunk, that led to the reaction. I would later find that the brown gunk was a manganese and oxygen complex, like those found in dirt. This represents an insoluble metal complex (IMC). Remember that these IMCs accumulate in AD and possess the ability to modify proteins in ways that are not good for the brain. Shockingly, a small handful of these IMCs lead to DHAAs in our Tau mimics.

Two of the best DHAA-forming IMCs I have discovered so far are goethite and hematite, which are both iron-based minerals that are comparable, though chemically distinct, from rust. Goethite and hematite are both naturally occurring and have been found to accumulate in the human brain over time, likely due to overwhelming iron content in cells or simply by breathing it in. Amyloid-β aggregates can exacerbate this problem by catching goethite and/or hematite in its

net, and accordingly, iron-based minerals like goethite and hematite have been shown to accumulate in AD. Goethite and hematite are the only IMCs we have found so far that both leads to efficient DHAA formation and accumulates in the brain of human Alzheimer's disease patients.

This was highly unexpected, but in retrospect, it is simple to understand why. When soluble metals are in water, they behave like water filling a pool and spread evenly across the available area. IMCs don't disperse evenly, like pennies thrown into a pool, and thus make pockets of active acid/base chemistry. The metal itself serves as the acid, but these IMCs actually have the ability to make water molecules near it more basic. The acidic, scissor-like, activity combined with the basic, bully localization make losing the balloon and lollipop much easier, and thus the modification forms quicker than it would with just soluble metals.

Once I had learned that IMCs could perform this unexpected modification, we suspected it would perform the modification on *all* proteins, due to the nature of this interaction. Other, comparable interactions, like the ones with soluble metals that require large amounts of base, affect practically all protein substrates. Shockingly, this modification mechanism is more specific than we originally imagined.

Proteins are *long* molecules, like an extension cord. Extension cords, while they may be stretched out, are often found folded back on themselves. Many proteins have specific ways they like to sit, like how most owners would like their extension cord wrapped up into a nice, neat bundle. Different proteins wrap themselves up in different ways, and most of them adopt some sort of bundle-like formation. Tau, the protein that aggregates in AD, is not one of these proteins. Tau is like if you put an extension cord in a tumble dryer. It never settles on one bundle but is constantly in flux. The IMCs seem to *exclusively* modify proteins like Tau, the ones in flux, while leaving the proteins packed neatly into their bundles unmodified. In simple terms, we suspect this is because a protein as wild and chaotic as Tau can't protect its candy from bullies in the way that more put-together, well-bundled proteins can.

IX. Summary of results and future work

I first wondered whether DHAAs could be found in the human brain due to observations by others that possibly implicated them in AD. I found, for the first time, that answer is *yes*. DHAAs are commonly found in the human brain, but that generated two, important and interesting questions. First, are they doing anything? And second, how did they get there?

We still do not know if DHAAs are contributing to protein aggregation, but there is reason to believe they might. DHAAs were found to be forty-fold more abundant in the snowball-like protein aggregates compared to the individual snowflake-like proteins. I found that DHAA-based protein tethers are only present in the aggregates, but not in the individuals. We also know that proteins who are responsible for preventing these tethers from forming are

extremely important to prevent neurodegeneration in mouse models. All of this adds up to a lot of right-time, right-place based accusations for DHAAs, but we are still working to prove a causal relationship between DHAAs and protein aggregation.

We also do not know how DHAAs form in the human brain. We did, however, find a new mechanism for this modification to arise from interactions between proteins and insoluble metal complexes. We do know that these metals, including the insoluble ones like goethite, accumulate unnaturally in Alzheimer's disease. It is thus even more fascinating that our newly discovered modification route is somewhat specific for the somewhat rare-protein type that describes Tau.

Our work here is not finished. It is the beginning of exciting new mystery. If DHAAs are proven to have functional significance, their unique puzzle-piece acceptor-like reactivity could be exploited for entirely new classes of drugs. My lab and I found DHAAs in Alzheimer's disease, but they are very likely present in other neurodegenerative diseases, like other dementias, ALS, and Parkinson's disease. As this modification has not been previously appreciated as one worth looking for, it is possible this is more widespread and could play functional roles in other protein aggregation diseases, like cancer and diabetes as well. Research never has a clear-cut conclusion; there will always be more to learn and new questions to ask. We are excited to have introduced the world to the prevalence of this underappreciated human modification. Our discovery reveals new questions to be asked about human neurodegeneration and protein aggregation.

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