Alzheimer’s Disease is Terrible and Costly

Alzheimer’s disease (AD) affects 5.4 million Americans today and may affect some 16 million by 2050. Its worst symptom is dementia, which includes loss of memory, intellectual capacity, and personality. It has risen to the 6th leading cause of death in the US. Deaths related to AD have increased 66% from 2000 to 2008 while deaths from the 5 leading causes of death have decreased in that same time period (Figure 1). AD’s effects far outweigh just those affected with this mind altering ailment. For each AD patient there are nearly 3 caregivers providing 17 billion unpaid hours of care with an estimated value of nearly $203 billion. The total cost to the health care system could reach $1.1 trillion by 2050. This will cost the US taxpayers, through Medicare and Medicaid, $130 billion in 2011; increasing to $706 billion by 2050.

Alzheimer’s is a Molecular Disease

The mechanism by which AD results in cognitive deterioration is currently unknown, but there is strong evidence that the amyloid-β protein (Aβ) is a key player. An understanding of the molecular structure of Aβ molecules associated with AD would lead to cures and/or preventative agents. For the past 30 or so years much of the study of AD has been directed by the Amyloid Cascade Hypothesis (Figure 2). This hypothesis states that the main cause of AD is the accumulation of Aβ proteins to form cell destroying plaques. Dr. S. Hardy, Selkoe and others suggest that there are numerous observations that support this hypothesis, including that the genetic code for Aβ precursor protein (APP) is located on chromosome 21. This same chromosome is involved with Down’s Syndrome and many Down’s patients develop amyloid plaques with accompanying AD dementia. Conversely, the number of amyloid plaques does not correlate with the presence of AD. A subsequent idea, the Oligomer Hypothesis, states that oligomers (a formation of several Aβ-42 peptides) are the toxic agents in AD.

The present work is guided by the following observations:

- Two disease-related isoforms exist in the brain: Aβ40, Aβ42
- Aβ42 associated with oligomer (particle formed by 2-50 protein molecules) formation

We want to know Aβ oligomer structure and why Aβ42 is more likely to form oligomers.

The difference between Aβ40 and Aβ42 is the addition of two amino acids – isoleucine and alanine, seemingly a small difference. Yet, they act very differently. When they self-assemble and aggregate with other like peptides, 42 tends to form oligomers while 40 aggregates into sheets. AD patients have elevated plasma Aβ42 levels relative to Aβ40.

To understand the relationship between Aβ42 and AD, we propose to investigate the solid state NMR structure of Aβ oligomers.

Aβ40 Amino Acid Sequence

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVY

Aβ42 Amino Acid Sequence

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVY

Hypotheses: Proposed oligomer structures from the literature

NMR research led Smith, et al. to propose the following structure for self-assembly of Aβ42 (Figure 2). They suggest that F19 is very close to L34 and would create a molecule folded itself on itself (Figure 3). Since the C-terminus of each monomer is hydrophobic they would tend to accumulate toward the center of the oligomer (Figure 4).

Alternatively, Olejniczak, E. et al. show a close proximity of F19 to I31 suggesting the more hairpin shape seen here (Figure 5). The coupling of other amino acids in their results led to a model of two Aβ42 molecules assembling toward the C-terminus with the hairpin sections pointing away from one another. As with the previous model, the hydrophobic C-termini would aggregate toward the center of an oligomer.

DR. Paravastu’s Experimental Approach: Solid State NMR

Solid State NMR can show intermolecular distances thus predicting the structural design of a protein. By labeling certain amino acids with carbon-13 a 2D spectrum is created and the amino acid interactions can be identified (Figure 6). The colored line patterns each represent the carbon atom chain in a labeled amino acid, and indicate the assignments of off-diagonal NMR peaks amino acids.

In Figure 7 the 2D RAD spectrum (left graph) shows that F19 has more peaks associated with interactions among neighboring amino acids. In the center graph the orange, dotted, vertical lines represent proximity interactions between F19 and I31 and leads us to the hypothesis that the structure of Aβ42 is closer to Olejniczak’s model (Figure 5). However, the green, dotted line shows interaction between F19 and A35 suggesting a closer relation than in Olejniczak’s model. We believe that the intra-chain from D23 through V18 by flipped so that F19 is on the inside of the curve. It should be noted that the lab is using solid state NMR and more dense Aβ42 samples so the other hypotheses discussed in this paper could be accurate for their samples.

Future Research: Test predictions of Olejniczak et al.

- Use an isotopic dilution experiment to see if F19 to I31 is intramolecular.
- Label amino acids within the C-terminal region to identify intermolecular dipolar coupling.
- Label only A21 carbonyl carbon to try to prove the molecules in a beta sheet are lined up parallel, not anti-parallel.
- Label selectively at N-terminal region to show intermolecular intra-chain distance.

References


NMR research led Smith,

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Figure 2. The sequence of pathogenic events leading to AD proposed by the amyloid cascade hypothesis.

Figure 3. Distinctive NMR peaks suggest the presence of an oligomerization process in Alzheimer’s disease.

Figure 4. Two disease-related isoforms exist in the brain: Aβ40, Aβ42

Figure 5. Aβ42 associated with oligomer (particle formed by 2-50 protein molecules) formation

Figure 6. Aβ42 Full Spectrum

Figure 7. 2D RAD spectrum (left graph) shows that F19 has more peaks associated with interactions among neighboring amino acids. In the center graph the orange, dotted, vertical lines represent proximity interactions between F19 and I31 and leads us to the hypothesis that the structure of Aβ42 is closer to Olejniczak’s model (Figure 5). However, the green, dotted line shows interaction between F19 and A35 suggesting a closer relation than in Olejniczak’s model. We believe that the intra-chain from D23 through V18 by flipped so that F19 is on the inside of the curve. It should be noted that this lab is using solid state NMR and more dense Aβ42 samples so the other hypotheses discussed in this paper could be accurate for their samples.

Figure 8. Test predictions of Olejniczak et al.

- Use an isotopic dilution experiment to see if F19 to I31 is intramolecular.
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