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# Hypothesis:
# Slowing the growth of β-amyloid fibrils with deuterated amino acids.

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**Abstract**

It may be possible to slow the growth of β–amyloid fibrils found in the brains of Alzheimer's disease patients by using side chain deuterated amino acids supplied either in the diet or intravenously. The deuterated amino acids would be incorporated into $A\beta_{40}$ peptide, producing a deuterated $A\beta_{40}$ (d-$A\beta_{40}$) peptide, which would then be incorporated into growing β–amyloid fibrils. The deuterated side chains in d-$A\beta_{40}$ would make the peptide more polar than its undeuterated counterpart, and, therefore, less prone to associate to form the nonpolar core structure of β–amyloid fibrils.

## Introduction and Hypothesis

The brain of an Alzheimer's disease patient has been shown to contain β–amyloid (or amyloid β; Aβ) fibrils, consisting of stacks of 40-residue Aβ ($A\beta_{40}$) peptide trimers arranged in a regular and repeating fashion (Figure 1) [1-3].

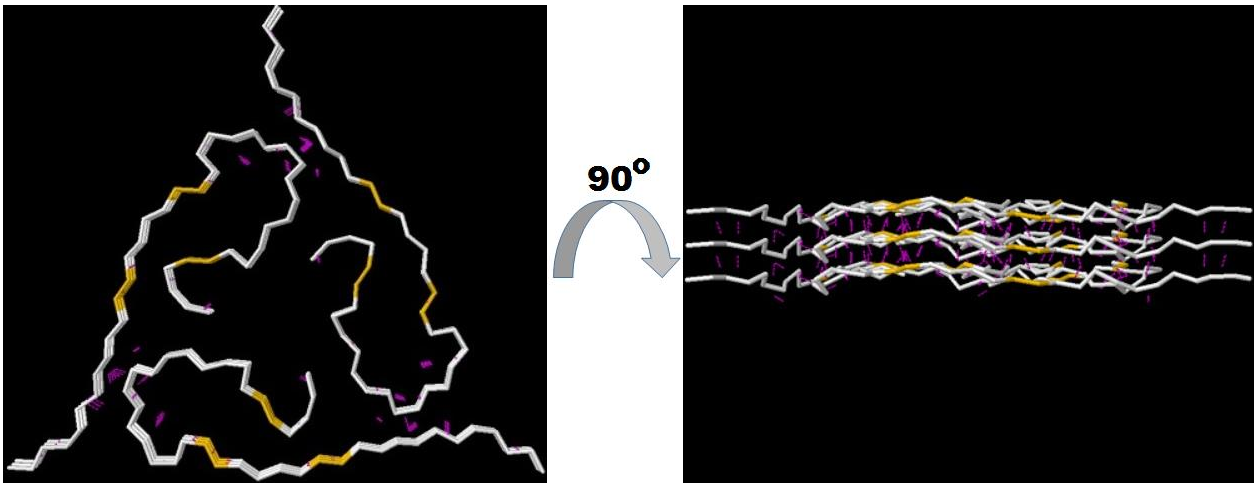


**Figure 1.** Protein Data Bank model 2M4J for the repeating subunit of a fibril found in the brain of an Alzheimer's disease patient [1-3]. The view on the left is a cross section through a fibril, showing the α-carbon backbones of three $A\beta_{40}$ peptides, and how they aggregate to form the subunit trimer. The view on the right illustrates how three subunit trimers stack, and the hydrogen bonds (purple) that stabilize the stack.

In the presence of a "seed" fibril and abundant $A\beta_{40}$ peptide, these fibrils grow at a relatively rapid rate [2]. An examination of the amino acid sequence, and relative positions of the $A\beta_{40}$ peptides, in the repeating subunit of a fibril reveals a clustering of nonpolar amino acid residues within the core of the trimeric subunit (Figure 2). Twenty-three (58%) of the 40 amino acid residues in the $A\beta_{40}$ peptide have nonpolar side chains [4]. If it were possible to modify the nonpolar residues in such a manner as to reduce their nonpolar character, without modifying the types of residues, that might affect the clustering of nonpolar side chains and slow fibril growth.

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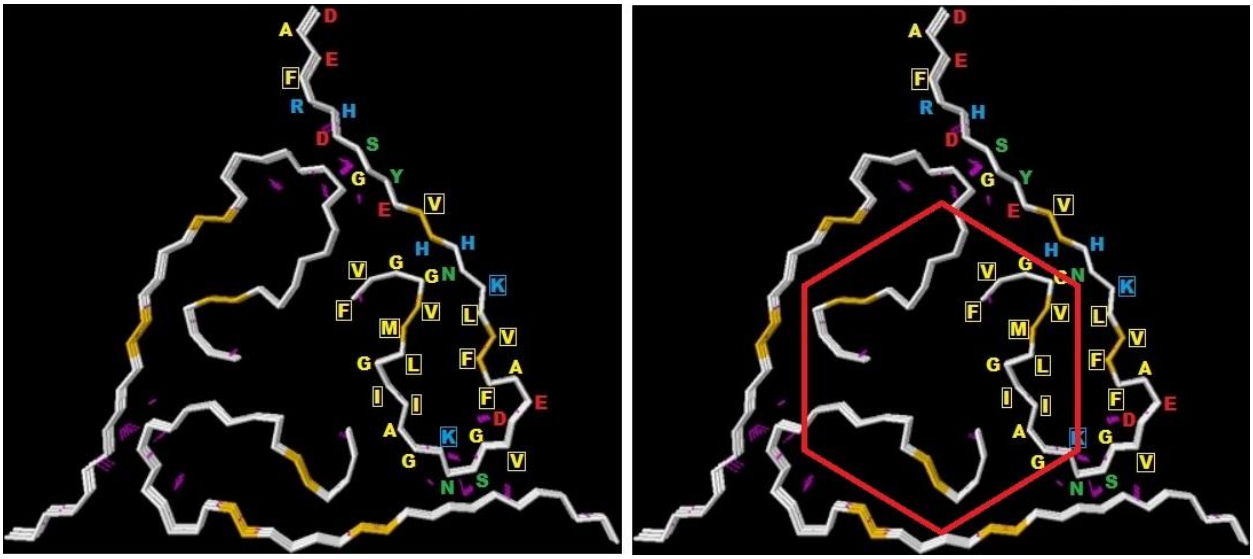

**Figure 2.** (Left) Amino acid sequence of the Aβ$_{40}$ peptide that comprises the monomeric subunit, of a trimeric repeating subunit, of the fibrils obtained from the brain of an Alzheimer's disease patient [1, 2]. Amino acid types are indicated by their IUPAC single letter symbols [4]. The color scheme is: nonpolar amino acid residues are yellow, fully or partially positively charged (at pH 7.4) residues are blue, negatively charged (at pH 7.4) residues are red, and uncharged polar residues are green. Amino acids that are essential in the diet of adult humans [4] are indicated by a box around the one letter symbol for the amino acid. (Right) The red hexagon encloses the core amino acid residues of three Aβ$_{40}$ peptides that are associated in the trimeric subunit. All of these amino acids [Phe (F), Val (V), Gly (G), Met (M), Leu (L), Ile (I), Ala (A)] have nonpolar side chains.

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The only way to modify the nonpolar character of amino acid residues without modifying the type of amino acid is by replacing their constituent atoms with stable isotopes; for example, replacing amino acid side chain hydrogen atoms with deuterium. The deuterated analog would be expected to have a more polar character than its undeuterated counterpart [5], and to be less prone to form the nonpolar core structure of fibrils.

All of the nonpolar amino acids that comprise the Aβ$_{40}$ peptide are available in their side chain deuterated forms from Cambridge Isotope Laboratories (Table 1) [6]. This means that it would be possible to either supply these amino acids in growth media when making recombinant deuterated Aβ$_{40}$ (d-Aβ$_{40}$), or they could be used in the chemical synthesis of d-Aβ$_{40}$ (Table 2). An *in vitro* test of the effect of deuterated amino acids on fibril growth could use the same methodology described in reference 2, where amyloid fibril growth was measured in the presence of an amyloid fibril "seed", and abundant Aβ40 peptide. Deuterated Aβ$_{40}$ peptide, obtained by recombinant techniques or by chemical synthesis, would simply be substituted for unlabeled Aβ$_{40}$ peptide in the assay.

There is evidence that Aβ peptides are prions, and it has been proposed "that targeting the formation of Aβ prions in the brain may constitute an ideal therapeutic strategy for treating AD during its earliest stages" [7]. However, one of the major problems of developing drugs that target the central nervous system is the presence of the blood brain barrier, which is highly selective in

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Wade Research Foundation Reports (2015) 7 (2)

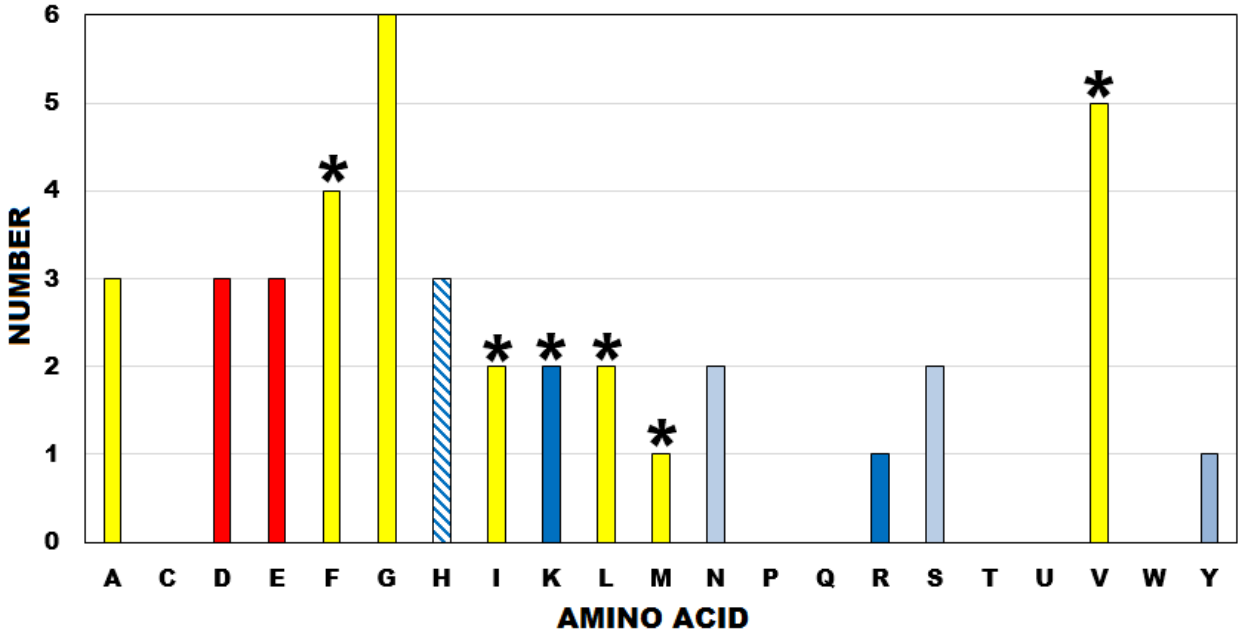


**Figure 3.** Amino acid composition of the $A\beta_{40}$ peptide present in amyloid fibrils from an Alzheimer's disease patient. In regard to the type of amino acid side chain [4], the color and pattern scheme is: solid yellow for nonpolar; solid red for polar acidic [negatively charged (-) at pH 7.4)]; solid dark blue for polar basic [positively charged (+) at pH 7.4]; diagonal blue lines for only partially charged, polar basic {ratio of uncharged to charged ($[H]/[H^+]$) = 25/1 at pH 7.4} solid light blue for polar uncharged. If the three partially positively charged Histidine residues are excluded from consideration, the peptide has a net charge of -4 at pH 7.4. Essential amino acids are indicated by asterisks.

**Table 1**. Amino acids in the $A\beta_{40}$ peptide that are available in their deuterated forms from Cambridge Isotope Laboratories, Tewksbury, MA [6]. Several are also available in α-amino group protected forms that would be suitable for chemical synthesis of the peptide.

| Amino acid: | Deuterated version available: |
|---|---|
| Alanine (A) | L-Alanine (2,3,3,3-D4, 98%) |
| Aspartic acid (D) | L-Aspartic acid (2,3,3-D3, 98%) |
| Glutamic acid (E) | L-Glutamic acid (2,3,3,4,4-D5, 97-98%) |
| Phenylalanine (F) | L-Phenylalanine (D8, 98%) |
| Glycine (G) | Glycine (2,2-D2, 98%) |
| Histidine (H) | L-Histidine:HCl:$H_2O$ (Ring-2,4-D2; Alpha, Beta, Beta-D3, 98%) |
| Isoleucine (I) | L-Isoleucine (D10, 98%) |
| Lysine (K) | L-Lysine:2HCl (3,3,4,4,5,5,6,6-D8, 98%) |
| Leucine (L) | L-Leucine (D10, 98%) |
| Methionine (M) | L-Methionine (2,3,3,4,4-D5; Methyl-D3, 98%) |
| Asparagine (N) | L-Asparagine:$H_2O$ (2,3,3-D3, 94% |
| Arginine (R) | L-Arginine:HCl (<5% D) (4,4,5,5-D4, 94%) |
| Serine (S) | L-Serine (2,3,3-D3, 98%) |
| Valine (V) | L-Valine (D8, 98%) |
| Tyrosine (Y) | L-Tyrosine (D7, 98%) |

**Table 2.** N-protected, side chain deuterated, versions of amino acids that comprise the nonpolar core structure of the $A\beta_{40}$ peptide trimer in the amyloid fibril. All are available from Cambridge Isotope laboratories (CIL), Tewksbury, MA [6]. Two N-protected, side chain deuterated, amino acids, Ile (I) and Met (M), are not available. The N-protected, side chain deuterated, amino acids could be used for chemical synthesis of the core segment of $A\beta_{40}$ peptide.

| N-Protected, deuterated amino acid: | CIL product number: |
|---|---|
| L-Alanine-N-Fmoc (2,3,3,3-D4, 98%) | DLM-8168-0.5 |
| Glycine-N-Fmoc (2,2-D2, 98%) | DLM-7339-PK |
| L-Leucine-N-Fmoc (D10, 98%) | DLM-7575-PK |
| L-Phenylalanine-N-Fmoc (D8, 98%) | DLM-8752-PK |
| L-Valine-N-Fmoc (D8, 98%) | DLM-7784-PK |

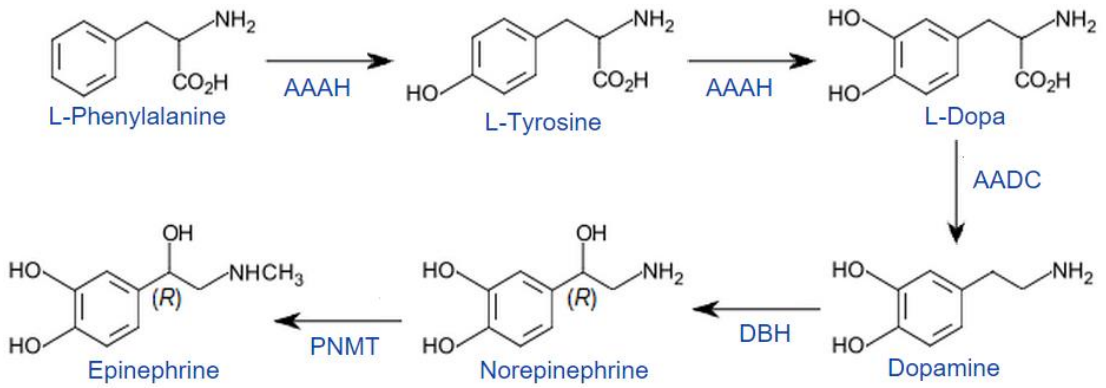


**Figure 4.** Metabolic reaction sequence for the conversion of phenylalanine to the neurotransmitters, dopamine, norepinephrine, and epinephrine [11]. Deuteration of the phenyl ring hydrogens of phenylalanine would slow the rates of hydroxylation of Phe ➔ Tyr and Tyr ➔ DOPA, and, in turn, the rates of synthesis of the three neurotransmitters.

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the types of compounds (e.g., peptides) that are permitted to pass through it [8, 9]. Amino acids are transported through the blood brain barrier [8, 10], and, interestingly, 16 (40%) of the 40 amino acids in the $A\beta_{40}$ peptide are essential amino acids for the adult human diet (Figure 3) [4]. Also, 14 (61%) of the 23 nonpolar amino acids in the $A\beta_{40}$ peptide are essential amino acids. Consequently, any slowing of fibril growth that can be produced using deuterated amino acids, *in vitro*, may also be possible *in vivo* by supplying the appropriate deuterated, essential amino acids in the diet or, perhaps more efficiently, via direct infusion into the brain via intravenous methods. One potential problem could arise with the use of deuterated phenylalanine, in that deuteration of its phenyl ring will slow the enzymatic conversion of phenylalanine to tyrosine, tyrosine to DOPA, and DOPA to the three neurotransmitters, dopamine, norepinephrine, and epinephrine (Figure 4). Studies with deuterated amino acids have been done extensively with rats and humans [9, 12], and this author is unaware of any reports of undesirable side effects or deaths. However, in order to prevent possible interference with normal brain function, it might be prudent to avoid the use of deuterated phenylalanine *in vivo*. The $A\beta_{40}$ peptide core amino acid sequence [Figure 2 (right)] contains only one phenylalanine ($1/12^{th}$ of the core peptide sequence, FVGGVMLGIIAG), so it may still be possible to slow the growth of amyloid fibrils without the use of deuterated phenylalanine.

## Concluding Remarks

Alzheimer's Disease International estimates that there are currently almost 47 million people worldwide living with dementia, that this number will double every 20 years, and that the global costs of dementia are currently US$ 818 billion [13]. According to the website of the Institute of Neurodegenerative Diseases, at the University of California, San Francisco, "The dementias are the only causes of death among the top ten in the U.S. that do not have a cure." [14]. Obviously, the need to find a cure, or treatments, for Alzheimer's disease should be a high priority.

## Aknowledgements

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