Functional Differences between Synthetic and Recombinant Amyloid Peptides

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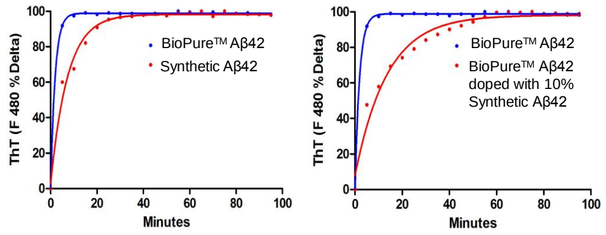
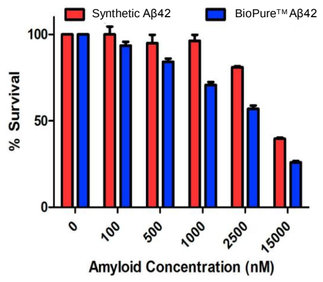
Thioflavin T Assay for Fibrillation



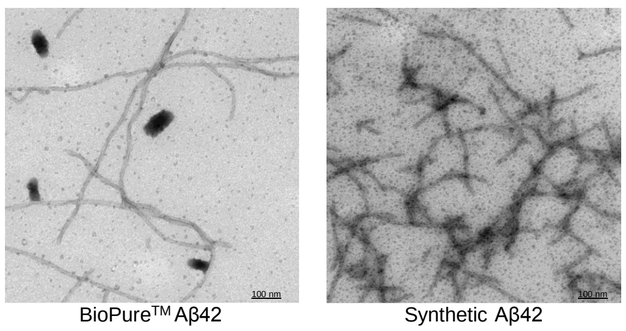
Thioflavin T (ThT) dye fluorescence is commonly used to monitor and quantify the formation of amyloid fibrils. Interaction between ThT and β-sheets of Aβ fibrils is monitored by the increase in 440 nm excitation – 490 nm emission and thus increases with amyloid fibrillation. BioPure™ Aβ42 reaches the maximum Δ480 significantly faster and this rate is consequently slowed when mixed with 10% Synthetic Aβ42.

MTT Assay for Cytotoxicity



Undifferentiated PC12 cells were treated with both BioPure™ Aβ42 and synthetic Aβ42. Cell death was monitored using the MTT assay and showed BioPure™ Aβ42 to be significantly more cytotoxic than its synthetic counterpart.

Electron Micrograph of Aβ42 Fibrillation



BioPure™ Aβ42 | Synthetic Aβ42

Aβ42 was oligomerized in 100mM NaCl, 10mM NaH_2PO_4, 1mM HCl, pH 7.4 and incubated at room temperature for 1 hour. Samples were visualized using a Philips CM100 TEM at 80 kV. BioPure™ Aβ42 (left panel) shows longer, more ordered fibrils than Synthetic Aβ42 (right panel).

Amyloid-β 1-42

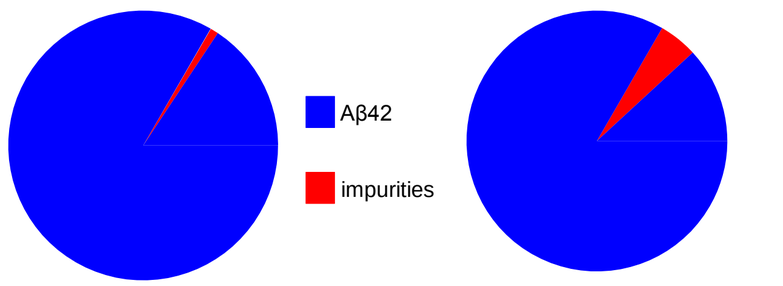
Amyloid-β 1-42 (Aβ42) plays a central role in the pathogenesis of Alzheimer's disease. As a reagent, the purity of Aβ42 is crucial for drug development.

Recombinant (BioPure™) Aβ42 has reproducibly been shown to have higher *in vitro* toxicity and aggregate significantly faster than synthetically prepared Aβ42 due to a higher level of purity. These traits allow for more accurate and consistent results.

BioPure™ Aβ42 produced using AmideBio's proprietary technology produces material that offers the following advantages:

- >99% purity by RP-HPLC and SDS-PAGE, with mass confirmation by ESI-MS.
- Devoid of *E. coli* protein contaminants.
- Absence of (n-1) deletion products and racemized amino acids that are characteristic of chemical synthesis and highly difficult to remove.
- Batch-to-batch consistency for reproducible results.

BioPure™ Aβ42 Has Higher Purity Than Synthetic Aβ42



>99% BioPure™ Aβ42 >95% Pure Synthetic Aβ42

Most Aβ42 preparations are >95% pure. AmideBio's BioPure™ Aβ42 boasts a purity of >99%, which does not contain the same synthetic artifacts that have been shown to inhibit bioactivity.

Conclusions

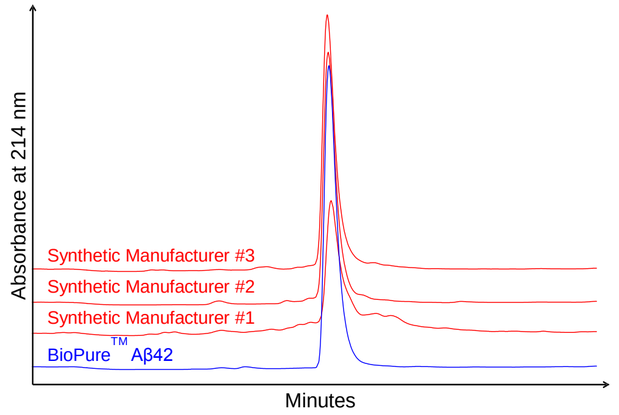
BioPure™ Aβ42 is More Biologically Active

- ✓ Faster rate of fibrillation as shown by the ThT Assay
- ✓ Higher *in vitro* cytotoxicity as shown by the MTT Assay
- ✓ Longer fibrils due to higher homogeneity

Minor Impurities Can Have Major Impacts

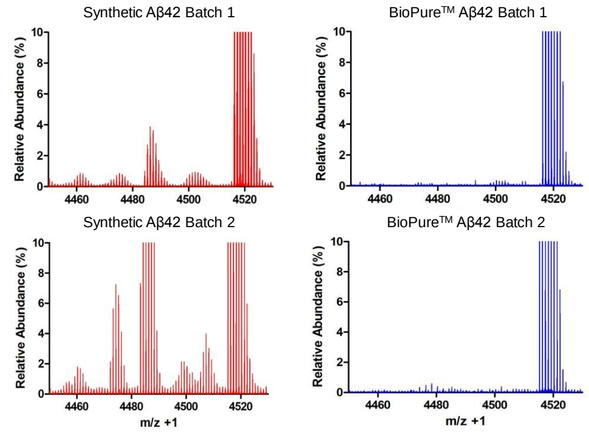
- ✓ Can alter activity
- ✓ Can alter formulation stability
- ✓ Cannot be easily removed from synthetic preparations

BioPure™ Aβ42 Lacks (n-1) and Racemic Impurities



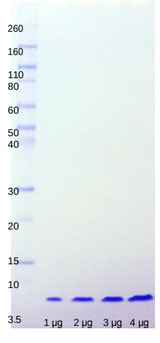
Comparative C18 RP-HPLC analysis of BioPure™ Aβ42 to various commercially available synthetic Aβ42 preparations. **A** H_2O + 0.05% TFA; **B** CH_3CN + 0.05% TFA; 60°C; 214 nm, 30-50% B over 20 minutes.

BioPure™ Aβ42 has consistent batch-to-batch purity



Two separate batches of BioPure™ Aβ42 produced by AmideBio and a synthetic competitor compared by ESI-MS to show both the improved purity of BioPure Aβ42 and the batch-to-batch variation of impurities in synthetic preparations that can lead to inconsistent results.

SDS-PAGE Characterization of BioPure™ Aβ42



BioPure™ Aβ42 analyzed on 10-20% Tris-Glycine gel to confirm the removal of all *E. coli* protein contaminants.

Poster presented at the 7th Annual Peptide Therapeutics Symposium, October 24th, 2012 at the Salk Institute for Biological Studies in La Jolla, California

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