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Development of a Protofibril-Selective Sandwich ELISA to Analyze Alzheimer's Amyloid-β Levels

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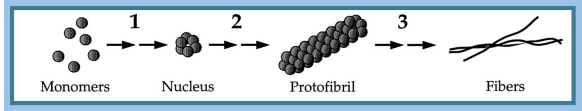
Introduction

- Protein aggregation is recognized as an important contributing factor to Alzheimer's disease (AD).
- One of the proteins involved in AD is amyloid-β (Aβ), a 40-42-residue peptide and the primary component of the senile plaques found in AD brains.
- Aβ monomer aggregation can occur through soluble protofibril intermediates on the pathway to forming insoluble fibrils.[1]
- Previous data from the Nichols laboratory demonstrate that soluble Aβ protofibrils are highly active and inflammatory.



DM Walsh et al., 1997, J Biol Chem

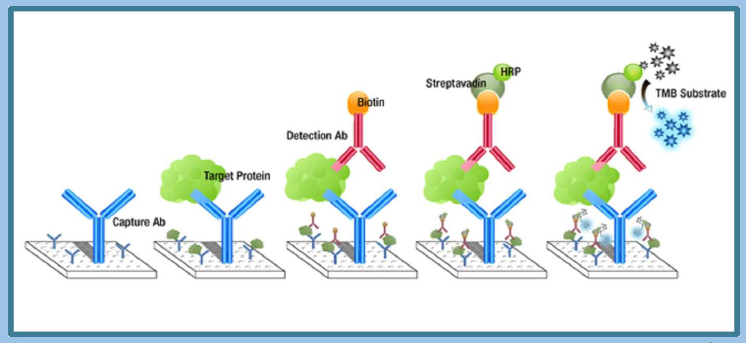
Purpose

- To develop a novel AbSL-based sandwich enzyme-linked immunosorbent assay (ELISA) that is able to selectivity and accurately measure Aβ protofibrils compared to monomers or fibrils.

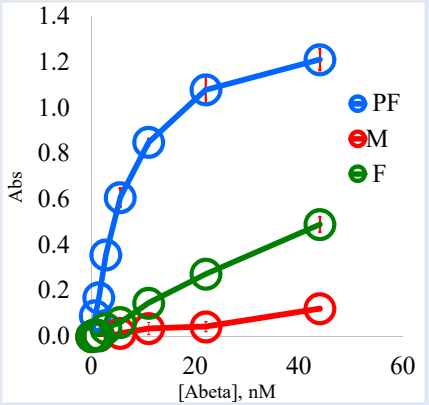
Methods

Sandwich ELISA. Enzyme-linked immunosorbent sandwich assay protocol was followed by using a 96-well plate coated with monoclonal antibody St. Louis 113 (mAbSL113) diluted in PBS and incubated overnight. With washing between each step, blocking buffer was added, followed by various amounts of isolated Aβ42 protofibrils, monomers, and fibrils diluted in aCSF, biotinylated-affinity-purify antibody St. Louis 40-4 (Biot-apAbSL 40-4), and Streptavidin conjugated with HRP. Finally, TMB substrate was added followed by sulfuric acid to stop the reaction. The signal was read at 450 nm using an ELISA plate reader.

Indirect ELISA. Different amounts of isolated Aβ42 protofibrils, monomers, and fibrils in sodium bicarbonate buffer pH 9.6 were adsorbed to a 96-well plate overnight. With washing between each step, Bloking buffer containing 10% milk was added, followed by the primary antibody mAbSL113, and secondary antibody anti-rabbit IgG-horseradish peroxidase. Finally, TMB substrate was added followed by sulfuric acid to stop the reaction.

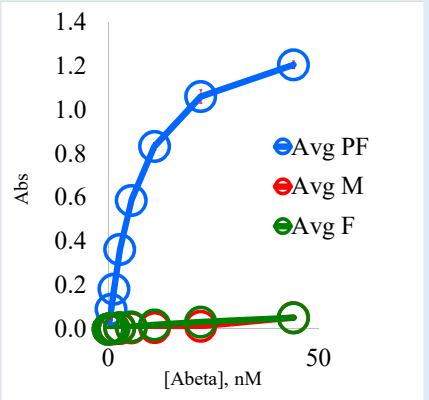


Design of a sandwich enzyme-linked immunosorbent assays (ELISAs).[2]
The plate is coated with a capture antibody. The sample is added, and any antigen present binds to capture antibody. The detecting antibody is added, and binds to antigen. Streptavidin protein that is covalently conjugated to horseradish peroxidase (HRP). Streptavidin binds to biotin in apAbSL 40-4 and the conjugated HRP provides enzyme activity for detection using an appropriate substrate system. This allows us to quantify the specificity of such antibody for the different Aβ intermediates.



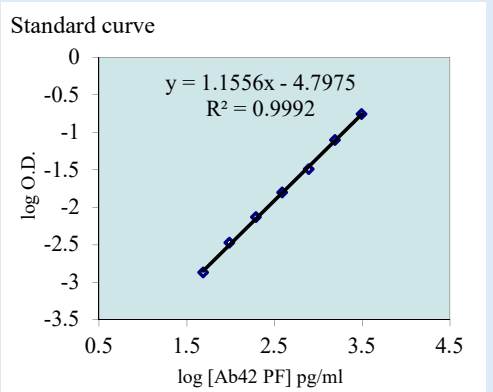
Selectivity Sandwich ELISA shows selectivity for protofibrils over monomers and fibrils.

- Aβ42 protofibrils, monomers, and fibrils concentration range was 44nM – 0.68nM.
- The greater absorbance (Abs) is reflective of a greater affinity of the antibody for a particular Aβ42 species.

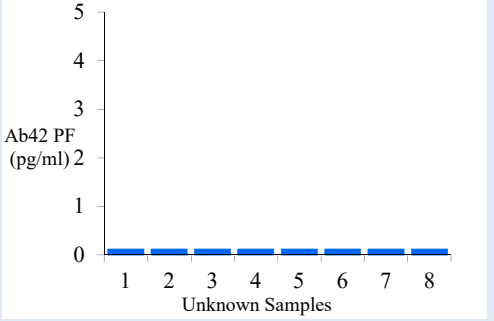


Indirect ELISA shows even greater selectivity but may not be as accurate as a sandwich ELISA.

- mAbSL 113 is selective for protofibrils over monomers and fibrils.
- Aβ42 protofibrils, monomers, and fibrils concentration range was 44 nM-0.68 nM.



A. Sandwich ELISA to create a standard curve for assessing the concentration of Aβ42 protofibrils in CSF samples.



B. Histogram showing the analysis of unknown CSF samples from AD patients.

Results & Conclusion

- The assay clearly demonstrated the selectivity of the AbSL antibodies for Aβ protofibrils over monomers and fibrils.
- The assay was very sensitive in measuring Aβ protofibrils levels in the low nanomolar range.
- The sandwich ELISA format detected the fibrils better than the indirect ELISA. We are currently trying to determine the reason for this.
- The applications of this antibody and assay may allow determination of Aβ protofibril levels in AD specimens along with other uses as a biomarker, detection agent, research tool, or potential therapy for Alzheimer's disease.
- Future research: additional testing of antibodies and their affinity for the different Aβ intermediates using the developed sandwich ELISA.

References

1. DM Walsh et al., 1997, J Biol Chem
2. ELISA. (2011) Epitomics. Web. *Google Image Search*. February 15th 2014.