PREPARATION AND CHARACTERIZATION OF TOXIC A\textbeta{} AGGREGATES FOR STRUCTURAL AND FUNCTIONAL STUDIES IN ALZHEIMER’S DISEASE (AD) RESEARCH

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Introduction: The amyloid cascade hypothesis, supported by strong evidence from genetics, pathology and studies using animal models implicates Amyloid-\textbeta{} (A\textbeta{}) oligomerization and fibrillogenesis as central causative events in the pathogenesis of Alzheimer's disease. Today, significant efforts in academia, biotech and the pharmaceutical industry are devoted to identification of the mechanisms by which the process of A\textbeta{} aggregation contributes to neurodegeneration in AD and the identity of the toxic A\textbeta{} species.

Aims: Herein, we describe methods and detailed protocols for reproducible preparation of A\textbeta{} aggregates of defined size distribution and morphology, including monomers, protofibrils and fibrils, using size exclusion chromatography (SEC). In addition, we describe detailed biophysical procedures for elucidating the structural features, aggregation kinetics and toxic properties of the different A\textbeta{} aggregation states, with special emphasis on protofibrillar intermediates.

Methods: Size exclusion chromatography, electron microscopy, thioflavin dye binding, primary neuronal cultures, cytotoxicity assays.

Results: The protocols reproducibly allowed the preparation of different A\textbeta{} species including monomer, protofibrils, and fibrils of wild type and fAD associated mutant A\textbeta{} peptides. The protocols were equally valid for synthetic A\textbeta{} peptides from various sources and also to the recombinant A\textbeta{} peptides.

Conclusions: The information provided by this approach allows for consistent correlation between the properties of the aggregates and their toxicity towards primary neurons and/or cell lines. A better understanding of the molecular and structural basis of A\textbeta{} aggregation and toxicity is crucial for the development of effective strategies aimed at prevention and/or treatment of AD.