Methods

An optimized filter trap assay for detecting recombinant authentic tau fibrils

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Abstract

The development and optimization of the Filter Trap Assay (FTA) for the detection of authentic tau fibrils *in vitro* mark a pivotal advancement in the realm of tauopathy research, particularly by addressing the limitations of using polyanion-induced tau fibrils, which structurally differ from those isolated from tauopathy patients. Recently it has been shown that truncated tau fragment (297-391), also termed dGAE, can form authentic tau fibrils in the absence of polyanions. This study introduces a refined protocol that reliably detects authentic tau fibrils in a physiologically relevant framework, utilizing nitrocellulose membranes to achieve heightened sensitivity. Our investigation highlights the superior efficacy of sarkosyl, an anionic surfactant traditionally used to prepare protein lysates from brains and cultured neurons, in preserving the aggregated state of tau dGAE fibrils *in vitro,* underscoring its potential for further exploratory studies. By offering a user-friendly and economically feasible approach, this technique enables a broad range of laboratories to measure the presence of authentic tau fibrils. This methodological enhancement propels our understanding of tauopathies forward and bridges the gap between basic research and advanced structural analyses, enriching the scientific community's methodologies for studying neurodegenerative disorders.

Keywords: Truncated tau fibrils, Filter trap assay, Sarkosyl, Tauopathies, Protein aggregation

Introduction

The microtubule-associated protein tau forms disease-specific filamentous aggregates in tauopathies, including Alzheimer's diseases (AD). Recent advances in cryo-electron microscopy (cryo-EM) have made it a powerful tool for determining the 3D structure of various proteins, including tau aggregates. The structure of aggregated tau from AD patients was determined: Tau filament cores are made of two identical protofilaments comprising residues 306-378 of Tau, which adopt a combined cross-β/β-helix structure and define the seed for tau aggregation[1]. To model tau aggregation *in vitro*, polyanions,

such as heparin, have been widely used to mimic tau aggregation[2,3]. However, the 3D structure of heparin-induced tau fibrils is quite different from those found in patients. Unlike the uniform structure observed in disease-related tau, heparin-induced tau fibrils are a mixture of three distinct structures: snake, twister, and jagged[4]. Thus, heparin-induced tau aggregates may not represent a physiological model for *in vitro* studies. This raises important considerations about the relevance and accuracy of using heparin-induced models in tau aggregation research. To overcome this issue, methods to generate tau aggregates without the need for polyanions were identified using the aggregation core region, such as truncated tau 297-391, termed dGAE tau[5,6]. This breakthrough offers new avenues for studying tau aggregation more physiologically.

The filter trap assay (FTA), also known as the filter retardation assay, is a widely recognized, user-friendly *in vitro* method for detecting protein aggregates including polyanion-induced tau aggregates. Given the structural differences between polyanion-induced tau aggregates and those extracted from tauopathy patients, work on the aggregation of the dGAE tau variant is expected to increase. A common approach to assess dGAE tau aggregation involves the use of Thioflavin T (ThT) fluorescence assays. However, these fluorescence-based assays sometimes yield inconsistent results, necessitating alternative methods to verify findings.

In this context, FTA, which utilizes a distinct mechanism for detecting tau aggregates, emerges as an essential complementary technique. While FTA detection methods for polyanion-induced tau aggregates have been previously established, the methods for detecting authentic tau fibrils remain largely unexplored[7]. The notable structural differences between these fibrils suggest distinct biochemical characteristics. Herein, we present an optimized FTA protocol for analyzing dGAE tau aggregates. This protocol is designed to provide more reliable and reproducible results than previous protocols, thereby enhancing the study of tau aggregation dynamics *in vitro* and contributing to a deeper understanding of tauopathies.

Results

Initially, we followed the FTA protocol published in 2008[7]. According to this protocol, tau protein samples are diluted in 2 % (w/v) sodium dodecyl sulfate (SDS) and the FTA is conducted using three types of membranes: nitrocellulose, cellulose acetate, and polyvinylidene fluoride (PVDF)[7]. Our findings indicated that the nitrocellulose membrane yielded the highest sensitivity. Notably, the sensitivity of dGAE tau fibrils in the FTA diminished when

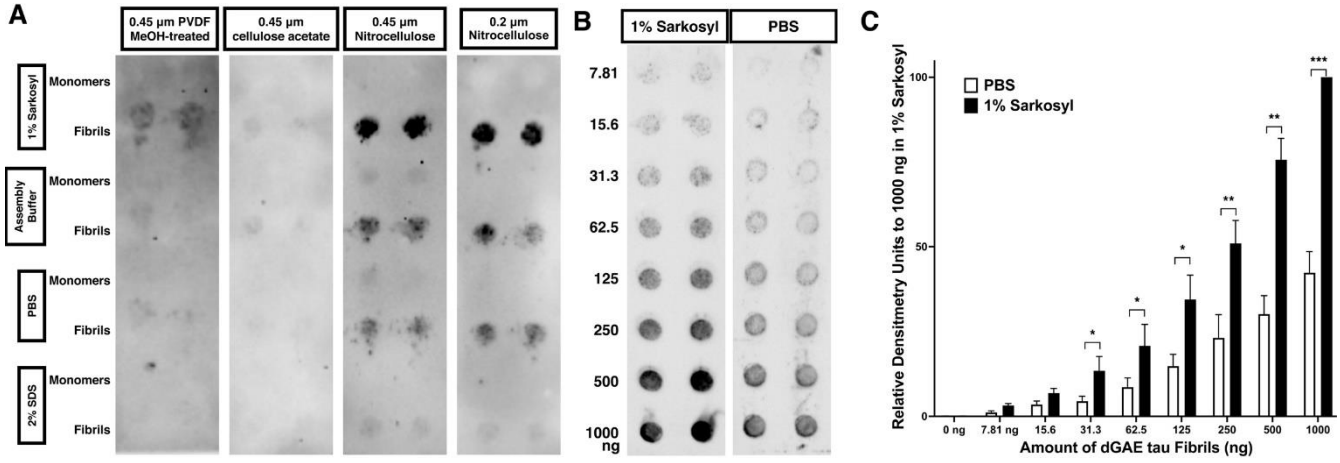

Figure 1. Optimal sensitivity in detecting dGAE tau fibrils is achieved using a 1 % sarkosyl solution in conjunction with a nitrocellulose membrane.

A. dGAE tau protein (100 ng) was applied in duplicate per well (50 µl/well) to nitrocellulose membranes and PVDF membranes pre-treated with methanol for 30 seconds using a 96-well Dot-Blot apparatus and visualized with an anti-dGAE antibody. Similar results were obtained from at least three independent experiments. **B.** Different amounts of dGAE tau protein were processed similarly, representative of three independent experiments. **C.** Densitometry analysis was performed using Bio-Rad ImageLab 6.1, with results normalized to the highest tau fibril concentration (1000 ng) in 1 % sarkosyl from three independent experiments.

diluted in 2 % SDS (Figure 1A). Subsequently, we explored FTA using various solutions, such as phosphate buffered saline (PBS), 1 % (v/v) sarkosyl, and assembly/ HEPES (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid) buffer, all of which have been previously documented for suspending whole tau fibrils[6,8,9]. Tau dGAE fibrils maintained their aggregated form more effectively when diluted in 1 % sarkosyl (Figure 1A). No significant difference in sensitivity was observed between nitrocellulose membranes with pore sizes of 0.2 µm and 0.45 µm (Figure 1A). As little as 7.8 ng of dGAE fibrils could be detected using a 0.45 µm pore size nitrocellulose membrane when diluted in 1 % sarkosyl (Figures 1B and 1C).

Using SDS-PAGE we found that the majority of dGAE tau aggregates were reduced to monomers when treated with Laemmli sample buffers containing SDS or lithium dodecyl phosphate (LDS), regardless of heat denaturation and reducing processes (Figure 2A). To corroborate our observations, we subjected protein samples, prepared in PBS, SDS, and sarkosyl, to native-gel PAGE followed by western blotting. Both the monomeric and fibrillar forms of tau dGAE protein, when in PBS, remained in the wells, presumably due to the high basic nature of tau dGAE, which has a theoretical isoelectric point of 9.59 and thus would be positively charged in the absence of an anionic surfactant[10]. When subjected to anionic detergents, the dGAE tau proteins displayed distinct behaviors: in SDS, the fibrils predominantly were broken down into monomers, whereas in sarkosyl, they retained their fibrillar integrity (Figure 2B).

Taken together, these data imply that for effective FTA detection, tau dGAE samples should be diluted in sarkosyl that preserves their aggregated state.

Discussion

The introduction of truncated tau variants such as dGAE tau offers a more representative *in vitro* model for studying tau aggregation as compared polyanion tau aggregation. We optimized a protocol for the FTA using dGAE tau aggregates, representing a notable stride towards more accurate and physiologically relevant *in vitro* models. The use of nitrocellulose membranes in the FTA emerged as a key factor in achieving high sensitivity in detecting tau aggregates, thereby providing a robust alternative to ThT fluorescence assays, which are prone to inconsistency.

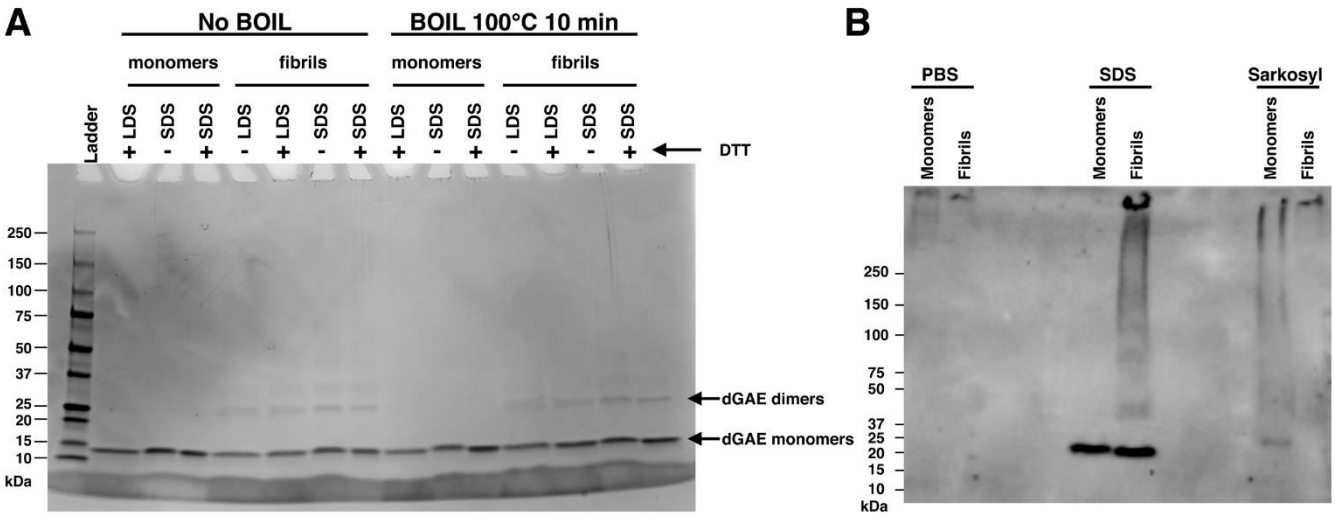

Figure 2. The majority of dGAE tau aggregates are reduced to monomers when treated with SDS.
A. dGAE tau protein (100 ng/lane) was treated with Laemmli buffer containing SDS or lithium dodecyl sulfate (LDS), with and without 100 mM dithiothreitol (DTT), and processed both unboiled and boiled for 10 minutes. Proteins were then subjected to SDS-PAGE and visualized using Coomassie Brilliant Blue staining. **B.** Samples in PBS, 2 % SDS or 1 % sarkosyl (100 ng/lane) were analyzed using native PAGE and Western blotting, detecting tau protein with an anti-dGAE tau antibody. Data shown are representative of two independent experiments.

Moreover, our exploration of different solvents such as PBS, 1 % sarkosyl, and assembly/HEPES buffer for FTA further refines the approach to study tau aggregation. Although sarkosyl has traditionally been used to prepare lysates from brain tissues and from cultured neurons to detect tau aggregates, the effectiveness of sarkosyl in maintaining the aggregated state of tau dGAE fibrils *in vitro* presents a promising avenue for future studies. The underlying mechanism for the enhanced efficiency of sarkosyl in detecting tau dGAE aggregates through FTA-compared to PBS and assembly/HEPES bufferremains elusive. We speculate that the unique physicochemical properties of sarkosyl may interact with tau aggregates in a manner that is less denaturing than SDS, or promotes the stabilization of tau aggregates, thus facilitating their capture on the membrane. Further investigations are required to delineate the specific molecular interactions and biophysical changes induced by sarkosyl, which may offer insights into its preferential efficacy and may potentially uncover novel aspects of tau aggregation dynamics. Thus, the assay we developed in this study is especially relevant given the need for more reliable and reproducible methods in tauopathy research, particularly in view of its simple, efficient, user-friendly and cost-effective approach. This ease of use and affordability make FTA an accessible tool for laboratories with varying resources, democratizing research in neurodegenerative diseases.

This method offers a practical alternative to more complex and expensive techniques, such as EM. While cryo-EM provides unparalleled detail in structural analysis, its high cost and technical requirements can be prohibitive. In contrast, the FTA protocol, especially with the use of nitrocellulose membranes, presents a straightforward and economical solution for initial screening and analysis of authentic tau aggregates. This approach not only facilitates routine laboratory investigations but may also serve as a critical preliminary screening step before employing more sophisticated methods. The capacity to detect minimal quantities of dGAE fibrils (as low as 7.8 ng) using affordable materials underscores its practicality for widespread application. The flexibility of the method, demonstrated by the effective use of different solvents like sarkosyl, adds to its appeal in diverse research settings.

This study therefore positions the optimized FTA protocol as an excellent tool in the arsenal of in vitro cell-free tauopathy research, enabling more laboratories to engage in meaningful investigation of tau aggregation. It paves the way for the use of more intricate techniques such as EM to further elucidate the complex mechanisms underlying tauopathies. By providing a cost-effective and user-friendly method, we bridge the gap between basic research and advanced structural analysis, fostering a more comprehensive understanding of tauopathies.

Author Contributions

Conceptualization, H.K.; software, H.K.; investigation, A.R.B. and H.K.; resources, H.K.; data curation, A.R.B. and H.K.; writing - original draft preparation, H.K.; writing - review and editing, A.R.B., T.L.R. and H.K.; visualization, H.K.; supervision, H.K.; project administration, H.K.; funding acquisition, T.L.R. and H.K.

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Data Availability Statement

The datasets generated and/or analyzed during the current study are available on demand.

Conflicts of Interest Statement

The authors declare no conflicts of interest.

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