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Glycation alter the process of Tau phosphorylation to change Tau isoforms aggregation property

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ABSTRACT

The risk of tauopathies depends in part on the levels and modified composition of six Tau isoforms in the human brain. Abnormal phosphorylation of the Tau protein and the shift of the ratio of 3R Tau to 4R Tau are presumed to result in neurofibrillary pathology and neurodegeneration. Glycation has recently been linked to dementia and metabolic syndrome. To determine the contribution of Tau protein glycation and phosphorylation on Tau aggregation propensity, the assembled kinetics were examined in vitro using Thioflavin T fluorescence assays. We found that glycation and phosphorylation have different effects on aggregation propensity in different Tau isoforms. Different Tau proteins play important parts in each tauopathies, but 3R0N, fetal Tau protein, has no effect on tauopathies. Conversely, 4R2N has more modified sites and a higher tendency to aggregate, playing the most important role in 4R tauopathies. Finally, Glycation, which could modulate Tau phosphorylation, may occur before any other modification. It also regulates the 3R to 4R ratio and promotes 4R2N Tau protein aggregation. Decreasing the sites of glycation, as well as shifting other Tau proteins to 3R0N Tau proteins has potential therapeutic implications for tauopathies.

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1. Introduction

Tauopathies are neurodegenerative disorders resulting from massive accumulations of intracellular filamentous aggregates of microtubule-associated protein Tau. These disorders include Alzheimer's disease (AD), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), frontotemporal dementia with Parkinsonism linked to chromosome 17(FTDP-17), Pick's disease (PiD) and so forth [1]. The Tau protein is a kind of microtubule-associated protein mainly expressed in the neuron, which plays a role in microtubule assembly and disassembly. There are six predominant Tau protein isoforms in the adult human brain that are generated by alternative splicing of the MAPT gene [2,3] and show different expression during development [4,5]. For example, 3R isoforms are predominant in the fetal brain. These isoforms differ from each other by inclusion or exclusion of one or two N-terminal sequences (exon 2 encoding 29 residues or exons 2 and 3 encoding 58 amino acid residue), as well as by the absence or presence of a second 31 residue long microtubule binding repeat domain encoded by exon 10 in the C-terminal half (Fig. 1A) [3,6]. Some mutations in the Tau protein cause FTDP-17 by increasing the proportion of Tau transcripts that include exon 10, which encodes 4R Tau isoforms [7–10]. MAPT H1 haplotype, associated with CBD and PSP forms of FTLD, plays a role in the selective deposition of 4R Tau isoforms and promotes exon 10 inclusions in MAPT mRNA [11–13]. However, granule cells of the dentate gyrus mainly express 3R isoforms, which resist Tau aggregation in AD, but accumulate 3R aggregates in Pick's disease [14]. 3R Tau is a major Tau isoform in laser-microdissected Pick bodies [15]. The discussion above indicates that the alteration of the Tau isoforms composition makes people susceptible to several tauopathies.

Tau proteins have several types of post-translational modifications. The main function of the Tau protein is to stabilize the neuronal cytoskeleton by interacting with microtubules. The process depends on the Tau protein's dynamic changes through phosphorylation and dephosphorylation. In most tauopathies, hyperphosphorylation of Tau is found in fibrils. It is widely understood that an increase in Tau phosphorylation reduces its affinity for microtubules, which results in neuronal cytoskeleton destabilization [16]. Tau phosphorylation at S262, S293, S324 and S356, found in KXGS motifs of R1, R2, R3 and R4 domains respectively also called microtubules binding domains (MBDs), has been shown to decrease Tau binding to microtubules that result in the destabilization of the neuronal cytoskeleton [17–19]. Tau phosphorylation in proline-rich regions, such as S202, S235, T231 and

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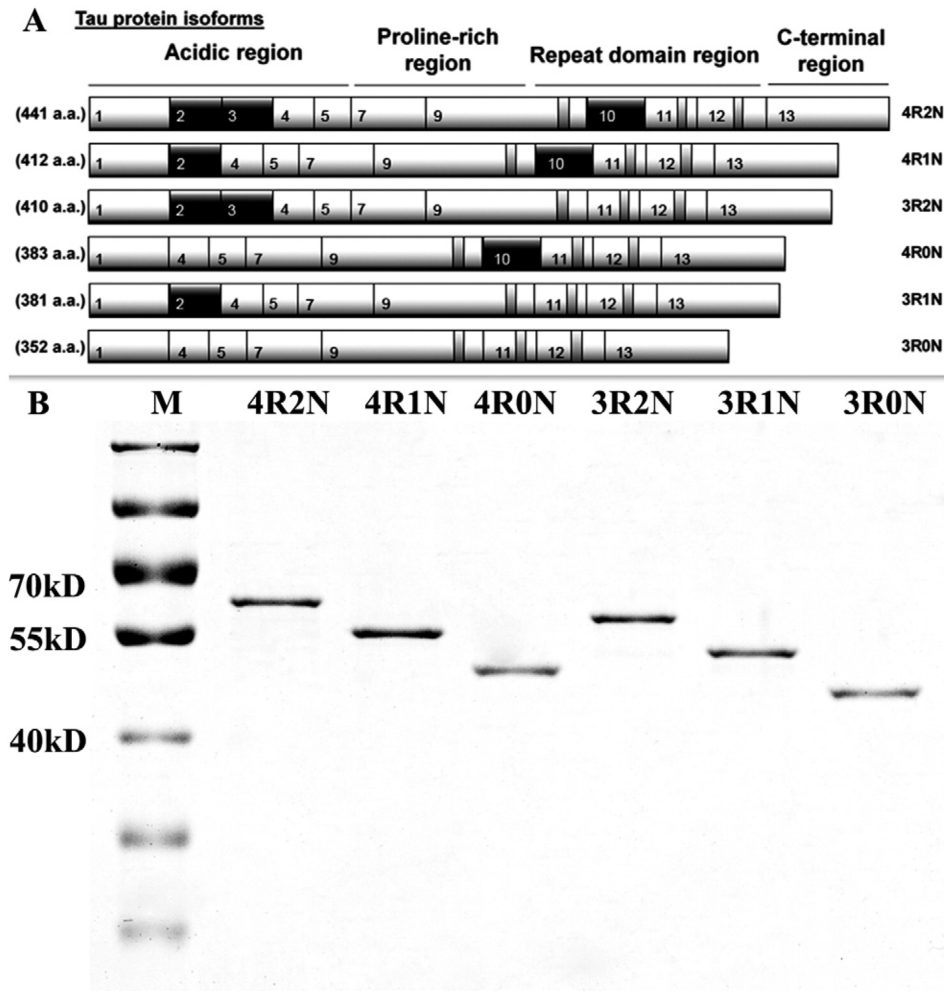

Fig. 1. Schematic representation of the human Tau and Coomassie blue-stained gel of recombinant Tau. (A) The 6 different Tau isoforms are generated by alternative splicing of exons 2, 3, and 10 as shown in the dark cube. (B) Purified recombinant Tau proteins used in the study were loaded heavy at 4 μg per well and separated on10% SDS-PAGE gels before being stained with Coomassie brilliant blue R-250to demonstrate purity.

S235, also contributes to the dissociation of Tau from microtubules. However, only phosphorylation in proline-rich regions is not sufficient for its dissociation [20]. GSK3β,which is a key protein in insulin signaling, is able to phosphorylate Tau at several sites that are then hyperphosphorylated in the PHF [21–23]. The most favorable phosphorylation sites of GSK-3β are S396, S400 and S404 in the C-region of tau [24,25]. In these areas, the Tau protein phosphorylated by another kinase can enhance GSK-3β's activity [24].

A correlation was found between the reduction of brain insulin signaling and the hyperphosphorylation of Tau [26]. Insulin resistance occurs both in the brain in AD and T2D [26–29]. Epidemiological studies have demonstrated that diabetic patients have a significantly higher risk of getting AD [30,31]. Some researchers refer to AD as type 3 diabetes [27]. The level of glycated proteins is relatively high in diabetes and normal aging.

Glycation is a reaction between decreasing sugars and amino residues, known as the Maillard reaction. It is a kind of non-enzymatic catalysis. The increased glucose increases the ratio of glycation. This situation is followed by rearrangements and cross-linking to generate AGEs. The level of glycated hemoglobin in the serum is one of diagnostic tests for diabetes mellitus.

Glycated proteins may not be degraded or released from the cell where they were accumulated. They are also more sensitive to oxidation and promote the generation of free radicals [32]. The AGEs (advanced glycation end products) are preferentially found in PHFs [32–34] and Tau. Additionally, AGE antigens have been reported to co-localize in NFTs [32,35]. The effect of glycation in formation of NFTs is still unknown.

In this study, we explored the aggregation property of different Tau isoforms under phosphorylation and glycation, as well as the relationship between the two in in vitro. We purified six Tau isoforms and modified them in in vitro, measured their assemble kinetics, and identified their post-translational modification sites. The results show that glycation of Tau protein can advance Tau phosphorylation in most of the Tau proteins. Furthermore, different modified Tau isoforms have different aggregation propensity that can accelerate the imbalance of Tau isoforms and formation of NFTs.

2. Results

2.1. Human phosphorylated Tau isoforms have different modified sites and differ in effect of aggregation tendency with unmodified Tau

Recombined Tau proteins were expressed by *E. coli* BL21 (DE3) and purified by Ni-agarose. The purity was shown by SDS-PAGE in Fig. 1B. Tau proteins were phosphorylated by GSK-3β. In vitro, recombinant Tau protein spontaneously, but slowly polymerizes into aggregate. Adding heparin drastically accelerates Tau aggregation [36]. All Tau isoforms, both with phosphorylation and un-modification, showed aggregation in the presence of the heparin inducer. Their assemble kinetics were measured by a Thioflavin T (Th T) fluorescence assay (Fig. 2A–F). The extent of aggregation was observed by fluorescence microscopy

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