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In vitro tau fibrillization: Mapping protein regions

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Abstract

We have investigated the propensity to form fibrillar aggregates of a variety of fragments and variants of the tau protein under the influence of a tau fibrillization inducer: coenzyme Q_0. To better identify fibrillization hotspots, we compare the polymerization propensity of tau fragments containing the sequence of putative hotspots with that of tau variants with that same sequence deleted. We also investigate the effects of biologically occurring modifications such as phosphorylation and deamidation. We found that residues 305 to 335 are essential for in vitro tau fibrillization. Residues 306 to 311 facilitate in vitro assembly, but are not sufficient to mimic the in vivo fibrillization of tau. Furthermore, the propensity of the 306–311 sequence to form fibrils is highly decreased by chemical modifications of tyrosine 310 that are commonly found in vivo.

Keywords: Tau; Polymerization; Coenzyme Q_0; Tau variant

1. Introduction

Alzheimer's disease is characterized by the appearance of two aberrant structures in the brain of patients: senile plaques (SP) and neurofibrillary tangles (NFTs) [1]. NFTs are intracellular inclusions made of clumps of protein fibers termed paired helical filaments (PHFs) [2]. About 20 years ago it was found that the microtubule associated protein tau is a major component of PHFs [3–9] and that purified tau was able to assemble in vitro into fibrillar polymers that resemble the morphology of ex vivo PHFs (from here on we use the term PHF exclusively to denote in vivo produced material) [10]. Subsequent studies revealed that PHFs were rich in a phosphorylated protein [11], which turned out to be a hyperphosphorylated form of tau [12,13]. Aberrant phosphotau polymers also appear associated with other neurological diseases like Pick's disease, frontotemporal dementia linked to chromosome 17, corticobasal degeneration, and progressive supranuclear palsy [14]. Interestingly, all these disorders are characterized by the presence of dementia symptoms [15].

Intriguingly, and in contrast to the β-amyloid peptides [16], tau protein has very low propensity to aggregate. This property has made difficult the development of in vitro procedures that faithfully mimic PHF formation. For instance, the hanging drop assay, as used for protein crystallization, renders tau fibrils with PHF-like ultra-structure, but requires extremely high protein concentrations [17]. Such limitation is often overcome by enzymatically modifying tau, or with the addition of compounds that facilitate tau assembly (i.e., inducers). Protein phosphorylation [18], glycation [19,20], deamination [10,21] and truncation by protein cleavage [22], have all been reported to increase the propensity of tau to fibrillize in vitro. Some examples of molecules that act as inducers are sulfated glycosaminoglycans (e.g., heparin [23,24]), other polyanions [25], fatty acids such as arachidonic acid [26,27], or products resulting from the oxidation of arachidonic acid like hydroxinonenal (HNE) [28]. More recently, Coenzyme Q_0 and other

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quinones have been reported to act as inducers of tau fibrillization [29]. In all these cases the concentration of tau required for its assembly is dramatically decreased (for a review see [30]).

Tau is also a large protein (i.e., 441 residues for the human full-length tau) with 6 different isoforms produced by alternative splicing [31,32], thus suggesting that only a fraction of the molecule should be involved in its fibrillization. Indeed, analysis of the PHF core showed that only a fragment of ~100 residues from the tubulin binding domain of tau is involved in forming the PHF scaffold [33]. The tubulin binding domain of tau comprises three (tau 3R) or four (tau 4R) repeated regions with similar aminoacid sequences [34,35]. It has later been proposed that it is the third repeat region of the tubulin binding domain the one primarily involved in tau fibrillization, with the second repeat playing a surrogate role in the assembly reaction [24]. Following these ideas, in this work we investigate the in vitro fibrillization of tau using Coenzyme Q_0, as inducer for polymerization of different tau fragments and/or variants with the goal of identifying the regions of tau that participate in PHF formation.

2. Materials and methods

2.1. Materials

2,3-dimethoxy-5-methyl-1,4-benzoquinone (coenzyme Q_0, ref no. D-9150), p-Benzoquinone (Ref. B-1266) was obtained from Aldrich (Steinheim, Germany). Acrylamide/bisacrylamide solution was supplied by Bio-Rad. Synthetic oligonucleotides were purchased from Isogen (Belgium) and peptides were obtained from NeoMPS (Strasbourg, France).

2.2. Construction of expression plasmids of three different tau deletion mutants

The plasmid pRKT42 [24], which encodes a human 4-repeat tau isoform with two N-terminal exons, was used a template. To obtain other constructs, mutagenesis was carried out in the parent plasmid pRKT42 using the polymerase chain reaction (PCR). Oligonucleotides R2D1 (AAACACGTCCCGGGAGGCGGCCAGGTGGAAGTAAAATCTGAGAAGCTTGACGTCAAGCTTCTCAGATTTTACTTCCACCTGGCCGCCTCCCGGGACGTGTTT),R2D2 (AAACACGTCCCGGGAGGCCCAGTTGACCTGAGCAAGGTGACCTCCGGAGGTCACCTTGCTCAGGTCAACTGGGCCGCCTCCCGGGZCGTGTTT) and R2D3 (TACAAACCAGTTGACCTGAGCCAGGTGGAAGTAAAATCTGAGAAGCTTGACGTCAAGCTTCTCAGATTTTACTTCCACCTGGCTCAGGTCAACTGGTTTGTA) were used as 3′ primers.

Oligonucleotide A6 (CGGGATCCATAATGGCTGAGCCC), which includes the initiation codon as well as *Bam*HI and *Nde*I sites for a proper cloning onto the vector, was used as 5′ primer. The obtained fragment was used as template for a second PCR. Oligonucleotide A8 (GCGAATTCTCACAAACCCTGCTTGG), which includes a stop codon as well an *Eco*RI site downstream, was used as the 3′ primer. The fragments generated by PCR amplification were digested with *Nde*I and *Eco*RI and the ligated into *Nde*I–*Eco*RI-digested pRK172. A similar approach was done to isolate tau deletion mutants using the plasmid pRKT30, which encodes a human three-repeat tau isoform.

2.3. Protein and peptide preparation

Recombinant human tau (whole molecule), tau fragment 1 (containing the amino-terminal half of tau protein), tau fragment 2 (containing the four tubulin binding motifs and the carboxyl-terminal region), tau fragment 3 (containing the four tubulin binding motifs), tau fragment 4 (containing the second and third tubulin binding motifs), and tau fragment 5 (containing the carboxy-terminal region), were isolated as previously described, [29] (see Fig. 7 for a scheme of the tau fragments used here). Among, tau variants we have used tau protein with the four tubulin binding repeats (tau 4R) but lacking residues 306–311 (tau $4R\Delta_{306-311}$), or tau protein with three tubulin binding repeats (tau 3R), lacking the equivalent residues (first six residues located at the 3rd repeat) of tau 4R (tau$3R\Delta_{275-281}$). Also, we have used those tau proteins lacking the whole 3rd repeat (tau $4R\Delta_{305-335}$) or residues 321–335 (tau $4R\Delta_{321-335}$). In this work, also we use the following peptides, which have been synthesized with solid-phase methods and purified as previously reported [36]. Peptide I (first repeat): QTAPVPMPDLKNVKSKIGSTENLKHQPGGGK; peptide II (second repeat): VQIINKKLDLSNVQSKCGSKDNIKHVPGGGS; peptide IIa: VQIINK; peptide IIb: NVQSKCGSKDNIKHVPGGGS; peptide III (third repeat): VQIVYKPVDLSKVTSKCGSLGNIHHKPGGGQ; peptide IIIa: VQIVYK (also tested with tyrosine in phosphorylated form, or replaced by phenylalanine); peptide IIIi: PVDLSKVTS; peptide IIIb: KCGSLGNIHHKPGGGQ and peptide IV (four repeat): VEVKSEKLDFKDRVQSKIGSLDNITHVPGGGN.

2.4. Assembly of Tau peptides into filaments

Filaments were grown by vapor diffusion in hanging drops, as previously described [24]. For assembly of peptides in the presence of quinones, tau protein (0.5–2 mg/ml) in final volume between 20 μl and 1 ml, was incubated at 4 °C, in a buffer containing 0.1 M MES pH 6.4, 0.5 mM $MgCl_2$, 2 mM EGTA (buffer A) plus 50 mM NaCl, in the absence, or the presence, of 0.25 mM to 4 mM of

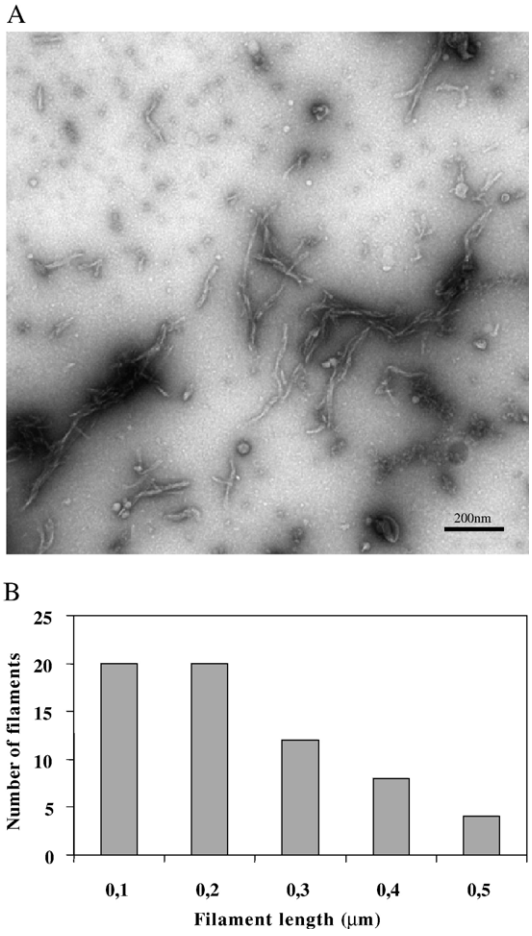

Fig. 1. Assembly of tau in the presence of quinones. Tau protein (1 mg/ml) was mixed with Coenzyme Q_0 (1 mM) and the assembled polymers from tau were visualized by electron microscopy (A). The length distribution of the polymers is shown in panel B.