Polymerization of tau peptides into fibrillar structures. The effect of FTDP-17 mutations

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Abstract The peptides corresponding to the four repeats found in the microtubule binding region of tau protein were synthesized and their ability for self-aggregation in presence of heparin or chondroitin sulfate was measured. Mainly, only the peptide containing the third tau repeat is able to form polymers in a high proportion. Additionally, the peptide containing the second repeat aggregates with a very low efficiency. However, when this peptide contains the mutation (P301L), described in a fronto temporal dementia, it is able to form polymers at a higher extent. Finally, it is suggested to have a role for the first and fourth tau repeats. It could be to decrease the ability of the third tau repeat for self-aggregation in the presence of heparin.

Key words: Tau; Polymer; Taupathy

1. Introduction

Tau was first isolated as a microtubule associated protein, since it co-purifies with microtubules [1,2] and stabilizes those polymers [3,4].

The binding of tau to microtubules was found to be through specific microtubule binding domains. These domains are three or four imperfectly repeated sequences of 31 or 32 residues located in the C-terminal half of the tau molecule [5–7]. Additionally, the domains could be separated into two regions, a highly conserved 18 amino acid repeat and a less conserved region (13 or 14 residues) that composes the inter-repeat domains [5,6,8,9]. The conserved repeated sequences can bind to microtubules in vitro [10–12], although the inter-repeat between repeat 1 and 2 has also a high microtubule binding ability [13].

Tau protein, in modified form [14], is also the main component of the aberrant filament structures present in Alzheimer's disease [15–18] and other diseases [19–21]. In these diseases, modified tau (see for example, [22]) binds with lower affinity to microtubules than in controls and it self-aggregates into aberrant structures, probably helped by other molecules such as heparin [23–25]. The minimal sequence for that self-interaction has also been studied and it was found that the third repeat of tau protein could be involved in that interaction [23].

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Abbreviations: FTDP-17, frontotemporal dementia and Parkinsonism linked to chromosome 17; MES, 2-(*N*-morpholino) ethanesulphonic acid; τ, Tau; PHF, paired helical filament

Recently, some mutations in the tau gene have been identified in fronto temporal dementia and Parkinsonism linked to chromosome 17 (FTDP-17) [26–29] and two of these mutations were present in the first and second tubulin binding repeats domains of tau.

In this work, we have studied the ability of the four tubulin binding repeats to self-assemble into filaments. Additionally, we have compared the previous results with those observed using the peptides containing the FTDP-17 mutations present in the tau repeats.

Our results indicate a different behavior for each repeat. Of particular interest are the data for the peptide containing the mutation P30IL, since it aggregates with a higher efficiency than its normal counterpart.

2. Materials and methods

2.1. Materials

Heparin and chondroitin sulfate B were obtained from Sigma. The synthesis and purification of the tau peptides: VKSKIGSTENLKMQPGGG, corresponding to the first repeat; VKSKIGSTENLKHQPVGG, the mutated first repeat; VQSKCGSKDNIKHVPGGG, the second repeat; VQSKCGSKDNIDHVLGGG, the mutated second repeat; VTSKCGSLGNIHHKPGGG, the third repeat and VQSKIGSLDNITHVPGGG, the fourth repeat, were performed as indicated in [30].

Expression and purification of recombinant τ protein and its fragments (3R and 2R) were performed as described in [23].

2.2. Assembly of tau peptides into filaments

Filaments were grown by vapor diffusion in hanging drops in the standard way used for protein crystallization as previously indicated [31]. In a typical experiment, 14 mg of τ peptides were lyophilized and resuspended in 10–15 μl of buffer A (0.1 M MES (pH 6.4), 0.5 mM $MgCl_2$ and 2 mM EGTA) plus 50 mM NaCl in either the absence or the presence (up to 1 mg/ml) of heparin. In other assays, a higher amount of tau was used but, usually, it was tested at a concentration of 1–10 mg/ml in the absence or presence of heparin or chondroitin sulfate B [32]. The reservoir contained 0.2 M NaCl in buffer A. Filaments were obtained after incubation for 4 days at 4°C.

Aggregation of τ peptides in presence of heparin was tested by incubation of τ at 1 mg/ml with increasing amounts of heparin (0.5–5 mg/ml) for 4 days at 4°C in a final volume of 20 μl. Samples were centrifuged for 30 min at maximal speed in an Airfuge (Beckman) and the protein present in the supernatant and pellet was analyzed by SDS-gel electrophoresis [23]. The amount of protein was quantified by densitometry of the fractionated protein after Coomassie brilliant blue staining of the gel and after comparing the results obtained with those found for known amounts of bovine serum albumin, used as a control.

2.3. Electron microscopy

Samples were incubated on a carbon-coated grid for 2 min and then stained with 2% (w/v) uranyl acetate for 1 min. Transmission electron microscopy was performed in a JEOL model 1200EX electron microscope operating at 100 kV. Electron micrographs were obtained at a magnification of 40 000 on a Kodak SO-163 film.

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3. Results

3.1. Polymerization of the peptides containing the different tau repeats

It has been previously shown that a peptide containing the sequence of the third tubulin binding domain (of the longest human tau isotype [6]) is able to polymerize at a relative low protein concentration in the presence of sulfated glycosaminoglycans, such as heparin or chondroitin sulfate [23,25,32]. In order to complement the previous study, we have analyzed the ability for self-aggregation of the other three tau repeats. Fig. 1 shows that the peptide (3r) containing the third repeat is the one showing a higher capacity for self-assembly. Additionally, the peptide containing the sequence for the second repeat (2r) could also aggregate into polymers, in a very low proportion (Fig. 2). No polymers were observed when the peptides containing the sequences corresponding to the first (1r) and fourth (4r) tau repeats were tested (Fig. 1, Fig. 2).

3.2. The role of tau repeats in the formation of tau polymers

Previous results suggest that the peptide containing the third repeat of tau is mainly involved in the formation of tau polymers and that the second repeat could also help, in a lower proportion, to that aggregation. Thus, we wanted to know if the first and fourth repeats may have any role in tau aggregation.

In a previous study [23], it was observed, in a qualitatively way, that the whole tau and tau fragments 3R (containing the three repeats 1r, 3r, 4r of the smaller tau isotype), 2R (containing the two first repeats (1r, 3r) of the same tau isotype) and a peptide containing only the third repeat (3r), were all able to polymerize into fibrillar aggregates. Now, we have performed a quantitative analysis of the ability for self-polymerization in the presence of heparin or chondroitin sulfate of these different peptides (3R, 2R and 3r). When the same molar concentration was taken for the 3R, 2R and 3r samples, we found that a much higher proportion of 3r peptide was polymerized in comparison with the assembly of the other peptides (Fig. 3). It suggests that the adjacent regions of 3r peptide could play an inhibitory role in the ability of 3r peptide for self-assembly and that upon deletion of those regions that ability for 3r peptide polymerization increases. Since we observed (see Fig. 1) that neither 1r peptide or 4r peptides were able to self-aggregate, we suggest an inhibitory role of those peptides (mainly 4r peptide) in tau aggregation (see Fig. 3), although it cannot be excluded that the inhibitory role is, alternatively, due to the presence of the interrepeat sequences.

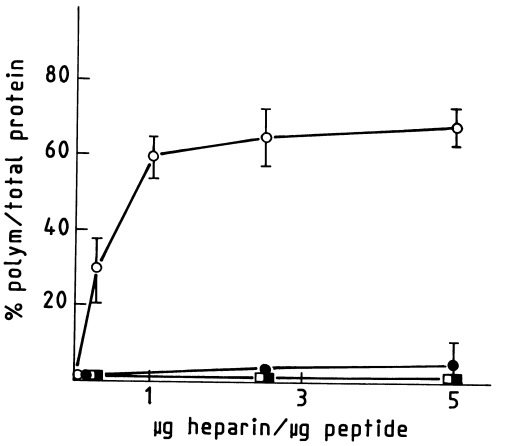

Fig. 2. Aggregation of tau peptides in the presence of increasing amounts of heparin. Tau peptides (1 mg/ml) containing the sequences for the first (■), the second (●), the third (○) and the fourth (■) tau repeats were incubated with increasing amounts of heparin. Polymerized peptides were quantified by centrifugation of the mixtures and measurement of the amount of peptide present in supernatant and pellet was done as indicated in Section 2. The average of three experiments is shown.

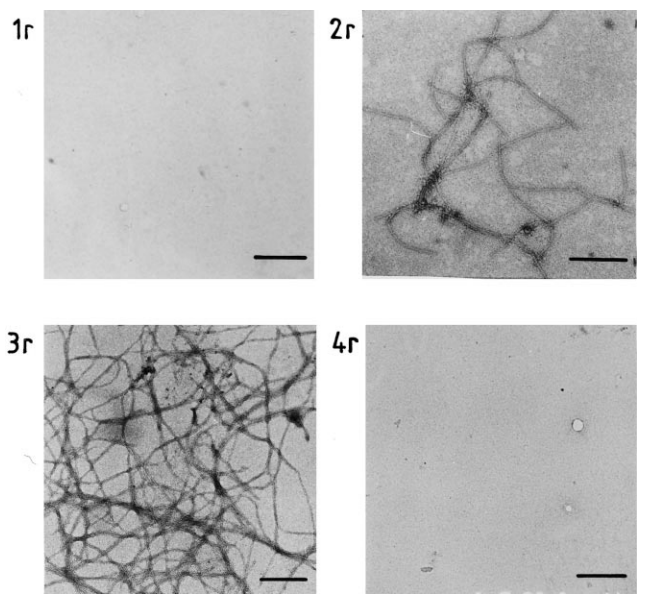

Fig. 1. Filaments assembled in vitro from the peptides containing the sequences for the first (1r), second (2r), third (3r) and fourth (4r) tau repeats (see Section 2). Bars indicate 200 nm.

3.3. Influence of FTDP-17 mutations in the polymerization of tau peptides

Recently, two mutations in the tubulin binding domain of

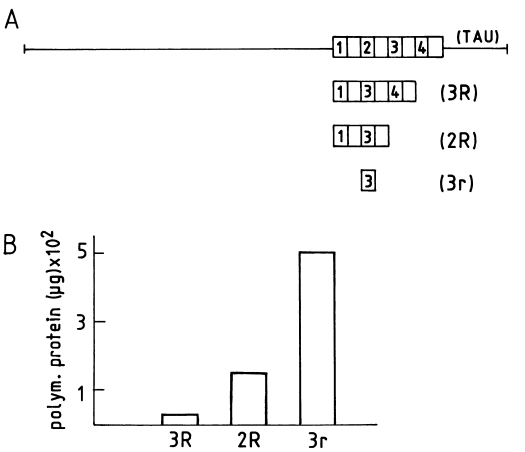

Fig. 3. (A) Schematic diagram of the whole tau molecule, the tau fragment containing the three repeats (and interrepeats) present in the smallest tau isoform (3R), the tau fragment containing the first and second repeats (and interrepeats) (2R) and the tau fragment containing only the third repeat (3r). (B) The aggregation of tau fragments 3R, 2R and 3r (at 1 mg/ml) was measured in the presence of heparin (1 mg/ml) as indicated in Fig. 2 and the result of that measurement is indicated.

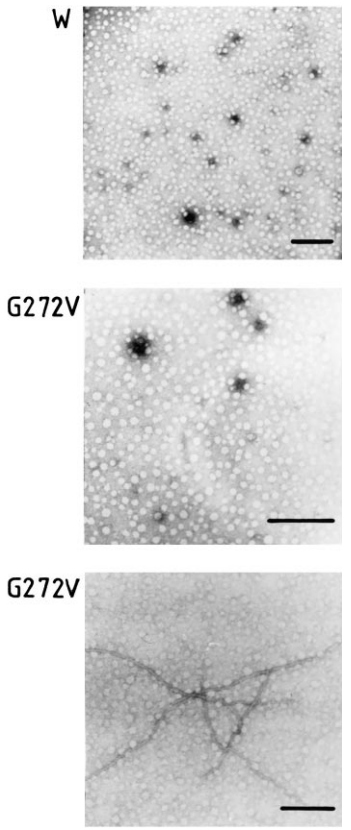

Fig. 4. Analysis for the assembly of tau peptides containing the sequence for the first repeat, in the wild type from (W), or with the mutation G272V found in a FTDP-17 family. Bars indicate 200 nm.

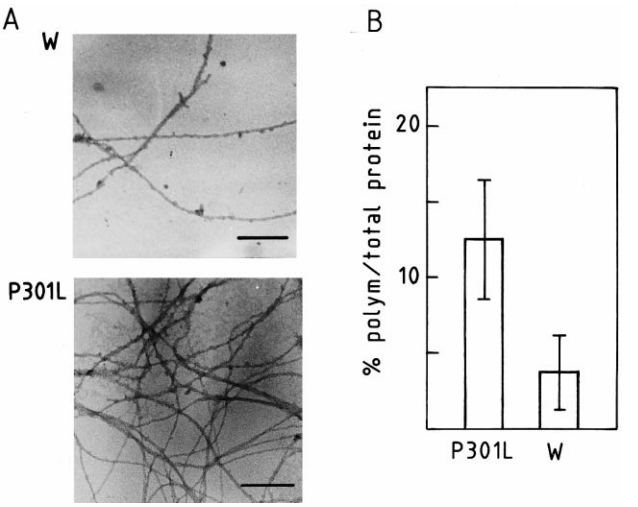

Fig. 5. (A) Filaments assembled from tau peptides containing the sequence for the second repeat, in the wild type form (W), or with the mutation P310 L found in a FTDP-17 family. Bars indicate 200 nm. (B) The aggregation of the two peptides was measured as indicated in Fig. 2 using a peptide concentration of 1 mg/ml and a heparin:peptide ratio of 5:1 (weight:weight). The average of three experiments is shown. A similar result was obtained when chondroitin sulfate B was added to tau peptides at a ratio chondroitin:peptide of 1:1 (weight:weight).

the tau gene have been described in patients of familial frontotemporal dementia linked to chromosome 17 (FTDP-17 [26–29]). Since it is known that the brains of these patients have tau filaments, we have tested if the presence of those mutations, located in the first (1r) and the second (2r) repeats, results in a higher ability for self-assembly of the peptides containing those repeats. In the case of the peptide corresponding to the first tau repeat, no main differences were observed in its capacity to polymerize either if glycine is present instead of valine (G272V) (Fig. 4). Essentially, no polymers were found in each case. However, in one experiment (out of a total of four experiments) a polymer was observed for the peptide containing the G272V mutation (the lower panel in Fig. 4). Nevertheless, we don not think that it could have an important relevance, since it appears to be an exception.

However, in the case of the second repeat, the peptide containing the mutation (P3O1L) shows a high level of polymerization (in presence of heparin or chondroitin sulfate B) when it was compared to its unmutated counterpart (Fig. 5). On the other hand, the polymers obtained with the P301L mutant are morphologically similar to those found for the peptide containing the sequence for the third repeat (3r).

4. Discussion

The longest tau isotype [6] contains four imperfectly repeated sequences of 18 residues, separated by other less conserved sequences (or interrepeats) [5]. Those repeats play a role in the binding of tau to microtubules and it has been described that the first, second and third repeat are able to bind to microtubules in vitro, whereas no binding was observed for the fourth repeat [10–13]. Moreover, Goode and Feinstein (1994) found that the tau region containing the first repeat, the interrepeat and the second repeat is probably the one that is more implicated in the binding of tau to microtubules. In a recent study, it has been indicated that a mutation in the second repeat (P30IL), present in some FTDP-17 patients, decreases the binding of tau to microtubules [33].

In this work, we have studied the role of the tau repeats in their ability for self-assembly. Our results suggest that the third repeat is mainly involved in tau aggregation. This observation is compatible with the fact that PHF-like structures could be assembled from fetal (containing 1r, 3r and 4r repeats) and adult (containing the four repeats) tau isoforms [31,34,35]. Also, we found that the second repeat (only present in adult tau) could facilitate tau assembly into polymers but in much lower proportion and it is also compatible with the data suggesting that an intronic mutation, present in some FTDP-17 patients, may favor tau aggregation [33].

Additionally, it was found that the mutation in the second repeat (P3O1L) facilitates tau aggregation, but a negligible effect on polymerization was observed when the peptide, from the first repeat, containing the mutation G272V was tested. Moreover, this peptide could probably have an inhibitory effect on the aggregation of the third repeat. Thus, a possible way to explain the consequences of this G272V mutation is to consider that the change of a glycine for a valine could result in a conformational change. A possibility could be a decrease in the capacity to form a turn in the tau molecule. If such a turn takes place, a possible intramolecular interaction, mediated by heparin, could be proposed and as a consequence of that intramolecular interaction, the capacity for intermolecular aggregation should decrease.

In summary, tau repeats play different roles. For self-assembly, the most important element appears to be the third repeat, whereas for the binding of tau to microtubules, the peptide containing the first two repeats appears to be the one that is more involved in that interaction [13]. A mutation in the second repeat may facilitate tau aggregation in a direct way and also results in a decrease in its interaction with microtubules [33]. Finally, there is not an active role of the fourth repeat in microtubule binding but it probably has an inhibitory role in tau aggregation. Thus, a hypothetic tau form in which repeat 3 is not expressing should probably retain the microtubule binding capacity and will have a very decreased capacity to form aberrant aggregates. In this way, it can be hypothesized a possible therapeutical approach in which a possible compound could specifically block the third repeat, avoiding tau aggregation without affecting the microtubule binding capacity of the other tau repeats.

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