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Tau factor polymers are similar to paired helical filaments of Alzheimer's disease

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Tau factor, upon urea treatment, is able to polymerize in vitro. These polymers are composed of tau factor as shown by immunogold staining. The structure of tau polymers is very similar to that of paired helical filaments (PHFs) of Alzheimer's disease in their dimensions as well as in their periodicity. Metal shadowing of both polymers shows a similar twisting. Also, similar peptide maps were found for tau factor and a 33 kDa protein that is the main component of our PHF preparations.

Tau factor polymer; Paired helical filaments; Morphology; Peptide mapping

1. INTRODUCTION

Alzheimer's disease (AD) is an age-related neurological disorder which results in senile dementia. A typical aspect of the disease is the appearance of specific neuropathological lesions, such as neurofibrillary tangles, neuritic plaques and extracellular amyloid deposits. The composition of these structures is currently a matter of discussion.

Neurofibrillary tangles appear to be composed of several paired helical filaments (PHFs) [1]. Recently, it has been indicated that a modified microtubule-associated protein, tau factor, is a major component of the PHFs [2], but not of amyloid deposits [3], which appear to be built by a protein, A_4, coded by a gene located on chromosome 21 [4–6]. On the other hand, it has been discussed whether this protein A_4 is a component of PHFs [7,8].

One way to clarify the problem is to test whether the putative components have the capacity for self-assembling into the same structures found in the brain of AD patients. Since it has been reported that modified tau protein may assemble into polymers [9], we have compared these polymers with AD PHFs. Our results indicate a high similarity between both types of polymers.

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2. MATERIALS AND METHODS

2.1. *Polymer preparation*

Tau polymers were prepared from purified porcine tau factor [10] as described previously. Briefly, tau protein at a concentration of at least 1 mg/ml was subjected to dialysis against 8 M urea in buffer A (1 mM $MgCl_2$, 2 mM EGTA, 0.1 M Mes, pH 6.4) for at least 3 days at room temperature. Then it was subjected to dialysis against buffer A, tested for polymers and negatively stained with 2% uranyl acetate as previously indicated [9]. They appear to have different morphology depending on dialysis and storage time. Storage time increases the frequency of appearance of polymers. When untreated tau, in buffer A, was incubated in the same conditions we were never able to observe polymers.

PHFs were prepared by the method of Wischik et al. [11] or Iqbal et al. [12] and negatively stained as above.

Tau polymers were immunostained with monospecific anti-tau antibody [13] as previously described for microtubules [14].

2.2. *Metal shadowing*

Tau polymers and PHFs prepared as described previously were metal shadowed after being adsorbed on carbon-coated electron microscope grids. They were allowed to air-dry and shadowed with 20 Å of Pt/C at 20° elevation angle. Stabilization of the replica was obtained by a carbon layer 100 Å thick.

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2.3. *Peptide mapping*

Tau and 33 kDa proteins were labelled with ^{125}I by the chloramine T method as previously indicated for tau [13] and extensively digested with endoproteinase Arg-C as described by Schenkein et al. [15]. The samples were lyophilized, resuspended in 0.1% trifluoroacetic acid, applied to a reverse-phase HPLC column (NOVA PAK C-18) and fractionated using a gradient of 0–80% acetonitrile in the same buffer.

3. RESULTS AND DISCUSSION

Five different preparations of porcine purified tau factor were extensively dialyzed against 8 M urea (see section 2) resulting in the appearance of polymers in three of the cases but they showed different morphologies; these differences are primari-

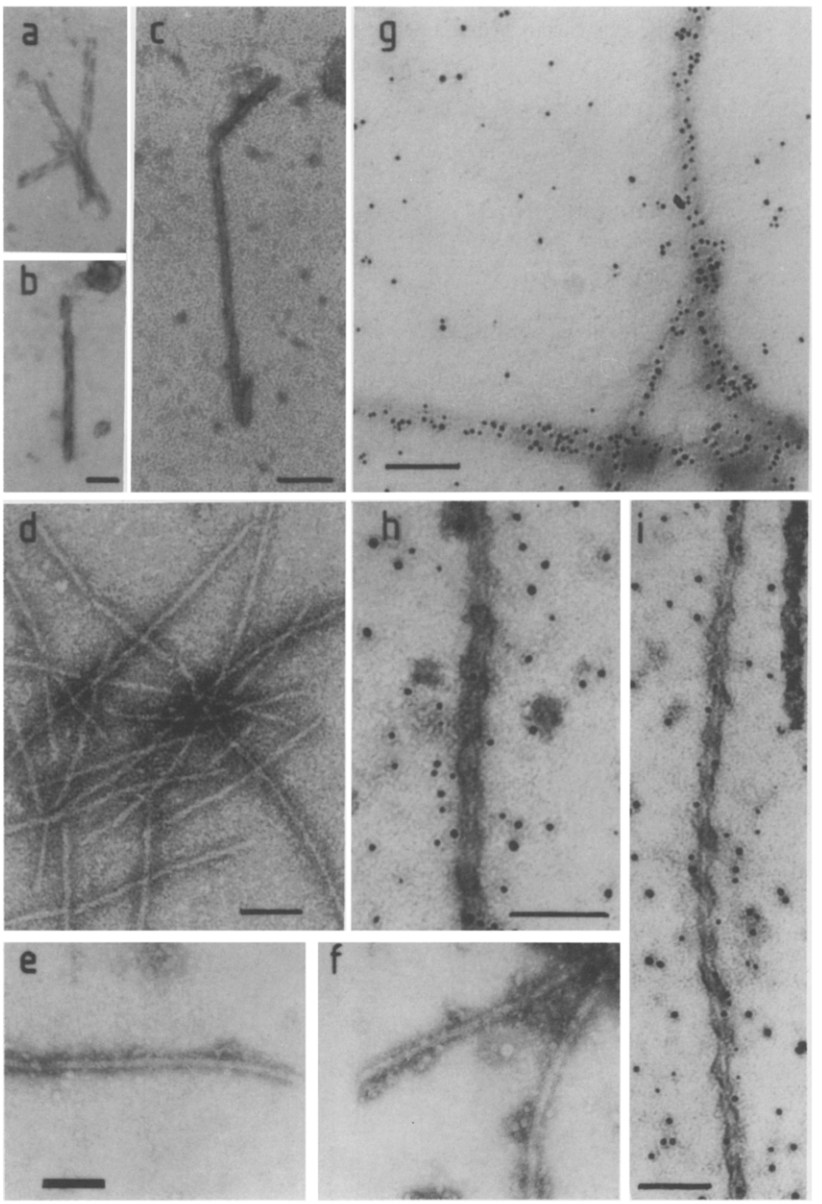

Fig.1. Tau polymers and paired helical filaments. Paired helical fragments (a–c) and tau factor polymers (d–f) were prepared as indicated and stained with 2% uranyl acetate. Tau polymers were also stained with protein A conjugated with colloidal gold in the presence (g) or the absence (h,i) of anti-tau factor antibody. Inset in i shows a PHF purified by the method of Iqbal et al. Bars indicate 0.1 μm.

ly dependent on the dialysis and storage time. Examples of tau polymers with different morphologies are indicated in fig.1d–f. Fig.1 also shows that these polymers indeed contain tau protein, as determined by their immunostaining with a monospecific tau antibody, followed by incubation with protein A conjugated with colloidal gold particles in the presence (g) or absence (h,i) of tau antibody. Monoclonal YL1/2 tubulin antibody did not react with the polymerized protein (not shown).

Some of the tau polymer (fig.1d,h,i) and the PHF preparations (fig.1a–c), negatively stained with uranyl acetate showed that both samples have a similar appearance, with a morphology compatible with the helical arrangement of subunits. The diameter of both filaments ranges from 10 to 20 nm, with the characteristic modulation described for PHFs with a period of ~70 nm (average of 30 measurements).

Unidirectionally shadowed material shows two populations of tau polymers, one corresponding to

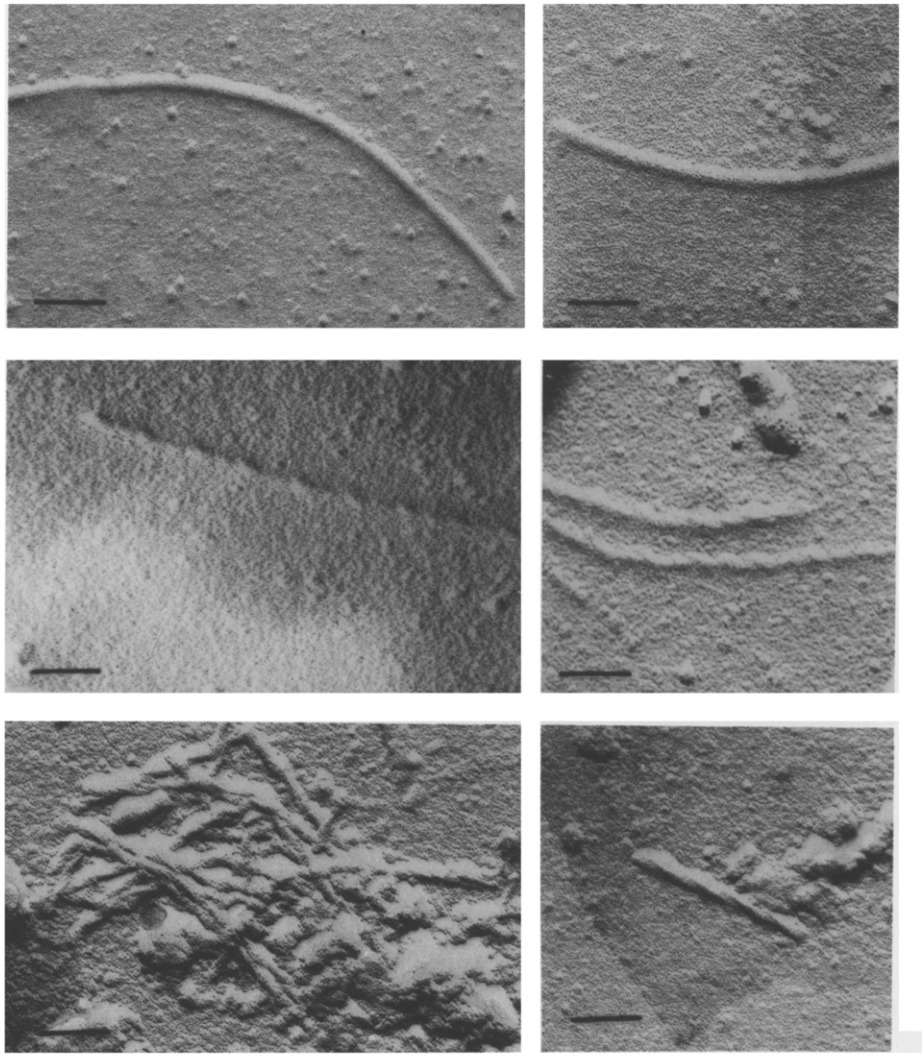

Fig.2. Metal shadowing of tau factor polymers and paired helical filaments. Tau factor polymers corresponding to fig.1e,f (upper) and 1 (middle) preparations and paired helical filaments (lower) were metal shadowed as indicated in the text and observed. Bars indicate 0.1 μm.

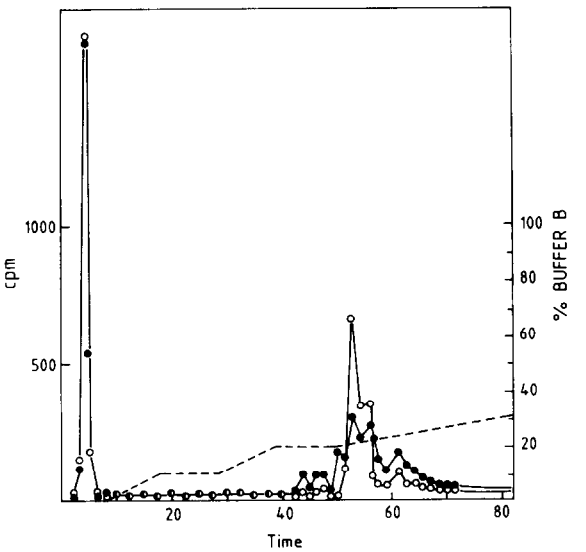

Fig.3. Peptide mapping of urea modified tau and 33 kDa protein. Tau protein and 33 kDa protein were prepared, radiolabelled and digested as indicated in the text, with endoproteinase Arg-C. Then, they were fractionated by reverse-phase HPLC in a Nova-Pak C-18 column with a gradient of 0–80% acetonitrile and 0.1% trifluoroacetic acid.

that shown in fig.1d and the other to that shown in fig.1e,f. The first population (fig.2, upper) shows no structure, while the second one (fig.2, middle) shows a similar surface structure (a right-handed twist of two paired filaments) to that of PHFs (fig.2, lower). It was also possible to detect some left-handed twisting among these PHFs in addition to the right-handed twisted filaments previously described by Wischik et al. [11], suggesting some flexibility in the twisting step during the formation of these polymers.

Porcine tau protein, upon urea treatment, has the capacity to assemble into paired helical filaments resembling those found in AD. This result is compatible with the suggestion that modified tau protein is not a PHF-associated protein, but the main component of such polymers, in other words, the protein which builds them up. To test this, we have compared the urea-modified tau protein with the major component of purified PHFs, that was highly purified by sedimentation, following a method based on the procedure of Wischik et al. [11]. This method includes a treatment with detergent to remove proteins associated to PHFs (Nieto et al., to be published elsewhere). Highly purified PHFs contain a major 33 kDa protein which accounts for more than 80% of total protein, as determined by Coomassie blue staining by gel electrophoresis (Nieto, A., unpublished).

Endoproteinase Arg-C digestions of urea modified tau and 33 kDa protein gave similar peptide maps (fig.3). The similarities in the dimensions of PHFs and tau polymers and, in the structures of their components are compatible with the suggestion that tau may be the main component of PHFs.

APPENDIX 1

The procedure of PHF isolation will be described in more detail in another paper, in which the effect of the concentration of salt and detergents will be indicated. For review purposes we indicate briefly in this appendix the procedure. Paired helical filaments were obtained following the method of Wischik et al. [11]. This preparation was treated with SDS, β-mercaptoethanol and afterwards 5% NP-40 was added. After that, sucrose was added to the mixture to a final concentration of 0.32 M and centrifuged at 40000 × g. The pellet thus obtained shows the appearance of a main band of 33 kDa.

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