Effect of DNA on Filament Formation of Tau Microtubule-Binding Domain: Structural Dependence of DNA

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To examine whether or not DNA accelerates the paired helical filament (PHF) formation of tau, the effect of various types of DNAs on filament formations of three-repeated and four-repeated microtubule-binding domains (3RMBD and 4RMBD, respectively) of tau protein was investigated by monitoring the change of thioflavin S fluorescence intensity, that is parallel to the filament formation. Consequently, the followings were clarified: 1) the structurally rigid double-stranded DNA such as poly(dG-dC) or calf thymus DNA has the high potency of promoting the filament formations of 3RMBD and 4RMBD, 2) the filament formation of 3RMBD was more promoted than that of 4RMBD, due to the intermolecular dimer formation of 3RMBD, 3) the DNA-promoted filament formations of these MBDs were temperature-dependent, and the single-stranded DNA such as poly(dA) or poly(dT) reversely protected 4RMBD from the molecular assembly at 20 °C. These are the first report on the function of DNA for the PHF formation of tau protein.

Key words tau; microtubule-binding domain; DNA; filament; promotion; protection

Tau protein is normally found on axonal microtubules (MTs) and plays an important role in the regulation of MT formation and stabilization.[1] This MT-associated tau is a highly soluble protein and hardly shows any tendency to assemble under physiological conditions. However, it dissociates from MT and aggregates to form insoluble paired helical filament (PHF) fibers in the brain of Alzheimer's disease (AD) patients.[2—4] Because these fibers are toxic to neurons owing to the damage they cause to the cell interior, many studies have been performed *in vivo* as well as *in vitro* to clarify tau PHF formation mechanism[5—9] and to find the inhibitor of tau fibrillization.[10—12] However, further studies are requested to gain the concrete results, because the PHF structure of tau at the atomic level is not available now and the mechanism of the filament formation has not yet been established satisfactorily.

The PHF formation of tau protein, though it usually takes a long period, is accelerated up to an experimentally allowable time with the help of polyanion such as heparin or other sulfated glycosaminoglycans,[13,14] RNA,[15] or polyglutamic acid.[16] On the other hand, the fluorescence of dye such as thioflavin S (ThS) or thioflavin T permits the quantitative monitoring of the PHF formation of tau.[16] By combining these two findings, thus, it is possible to examine the process of tau PHF formation.

As for the DNA–tau interaction, it has been reported that tau stabilizes the structure of DNA,[17,18] protects DNA from radical attacking,[19] and dissociate the double-stranded DNA (dsDNA) by binding to one of two strands with a sequence-specific fashion.[20] However, there is no report about the effect of DNA on tau PHF formation, despite the fact that it is a constitute of cytosolic polyanions and locates at the nucleolar organizer region in which tau protein also locates.[21] In order to clarify whether or not DNA promotes the PHF formation of tau protein, therefore, we investigated the effect of various DNAs on the filament formation using the tau microtubule-binding domain (MBD) (Fig. 1), because the MBD which consists of three or four imperfect 31—32-residue repeats (abbreviated as 3RMBD or 4RMBD, respectively) is known to constitute the core structure of tau PHF[22—24] and self-assemble itself into the filament similar to the PHF of full-length tau *in vitro*.[25] Also, in order to examine the effect of DNA structure (dsDNA or ssDNA) and its temperature dependence on the filament formation as accurately as possible, the experiment was performed at 20 °C and 30 °C, because (i) the self-assembly of MBD is meaningfully promoted with the raise of temperature and (ii) the structural fluctuation of DNA is increased at a higher temperature.

Results and Discussion

The DNA structure-dependent changes of the ThS fluorescence intensities of 3RMBD and 4RMBD at 30 °C are shown in Fig. 2. The PHF formation could be monitored by the interaction with the fluorescent thioflavin dye,[16] in which the

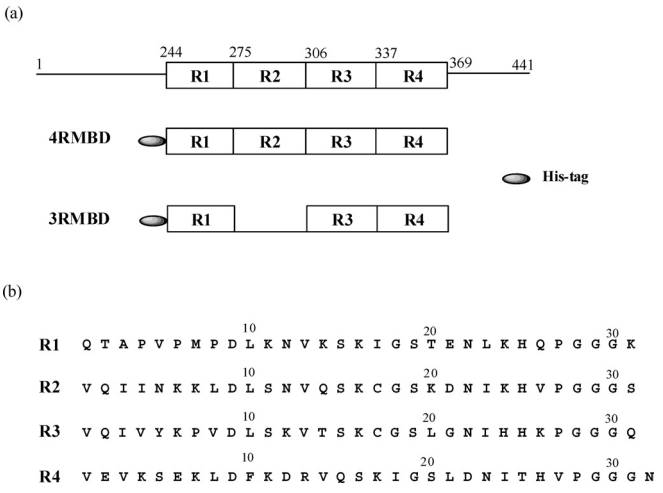

Fig. 1. (a) Schematic of Human Full-Length Tau Protein Including Four-Repeated MBD and His-Tagged 4RMBD and 3RMBD Used in This Work, and (b) Amino Acid Sequences of R1—R4 Repeat Peptides

The regions from the first repeat to the fourth repeat in MBD are named R1 to R4, respectively. The number of amino acid residues in (a) refers to the longest isoform of the human protein tau (441 residues).

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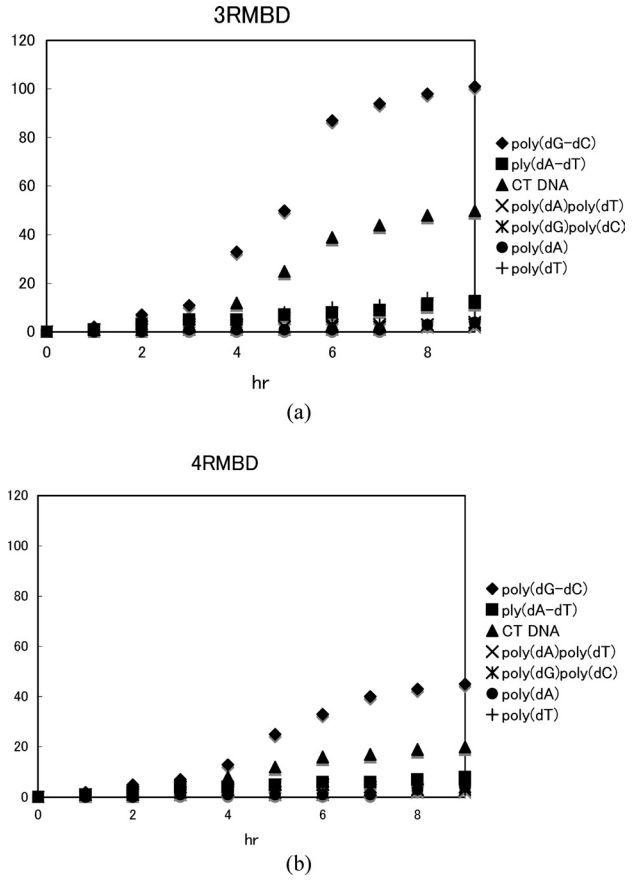

Fig. 2. DNA Structure-Dependent Change of ThS Fluorescence Intensity Accompanying Filament Formation of 3RMBD (a) or 4RMBD (b) at 30 °C

The change of ThS fluorescence intensity of 60 μM MBD in buffer A was measured as a function of time after the addition to 30 μM ssDNA [poly(dA), poly(dT)] or 15 μM dsDNA [poly(dG-dC), poly(dA-dT), CT DNA, poly(dA)·poly(dT), poly(dG)·poly(dC)]. The ThS fluorescence intensity at the starting time (t=0 h) was set to be zero and the subsequent intensity change, which was subtracted from that of the same, but DNA-excluded, sample solution, was plotted with the reaction time. The respective intensity changes were averaged from the four times-repeated experiments, and their deviations were all within ±30% of the given values.

fluorescence increases in proportion with the binding to the cross-β-structure typical fibers.[26] Since the intensity of ThS fluorescence was little changed by the coexistence of DNA alone, the increase of the intensity reflects the filament formation of MBD. The ability of DNA to accelerate the filament formation of MBD was much weak, as compared with that of heparin under the same experimental condition.[27] Thus, the increase of the fluorescence intensity continued for more than 10 h until arriving at a plateau, and the increased intensity was limited into a relatively small range. However, this figure shows that poly(dG-dC) and CT DNA promote the filament formations of 3RMBD and 4RMBD effectively, and the promoting effects of other DNAs on the filament formation of MBD are not so significant as to be remarked. This result would indicate that the rigid B-form structure of dsDNA is most effective for the promotion of filament formation of MBD; the structural stability is known to be in an order of poly(dG-dC)>CT DNA>poly(dA-dT),[28] although these take a B-form dsDNA structure in the usual neutral solution.

The DNA-promoted filament formation was more effective for 3MBD than 4MBD, although the type of DNA to promote the filament formation was the same. This would be

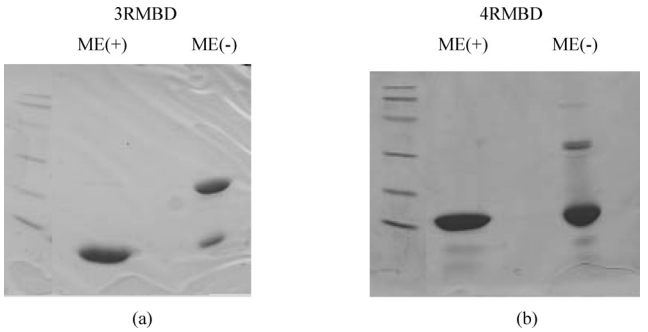

Fig. 3. SDS-PAGE of 3RMBD (a) or 4RMBD (b) in Presence (+) or Absence (−) of 5% Mercaptoethanol at 30 °C

The left side of each figure is the molecular weight marker (14.4, 21.5, 31.0, 45.0, 66.2, 97.4 kDa from the bottom, respectively), indicating that the lower and upper bands of respective PAGEs correspond to the monomeric and dimeric molecular weights of 3RMBD and 4RMBD, respectively.

due to the difference between the Cys oxidative states of 3RMBD and 4RMBD. Figure 3 shows the prominent formation of the intermolecular dimer of 3RMBD *via* the disulfide bond formation of Cys17 residue in R3 repeat under a neutral solution without containing any reducing agent. As the dimer structure of MBD is known to become a core of the filament formation,[27,29,30] this would explain the more effective DNA-promoted filament formation of 3RMBD than 4RMBD; 4RMBD tends to form an intramolecular cross-bridge *via* the disulfide formation between two Cys17 residues in R2 and R3 peptides[3] and this may be a reason why 4RMBD exhibits the resistance for the filament formation.

On the other hand, the DNA-dependent profiles of 3RMBD and 4RMBD at 20 °C are shown in Fig. 4. The significant difference was observed between the profiles of 3RMBD and 4RMBD. As for 3RMBD, all DNAs act at least as a promoter for the filament formation, although the efficiency is much less active than that at 30 °C. In contrast, in the case of 4RMBD, all DNAs showed the protective effect for the filament formation, and the significant protective effect was observed for the single-stranded poly(dT) and poly(dA). These DNA structure-dependent profiles indicate obviously the different thermal responses of 3RMBD and 4RMBD to their filament formations. The decrease of the intensity observed for 4RMBD shows that DNA, particularly ssDNA, dissociates the filamentous β-structure-assembly of MBD, indicating that the interaction of DNA with 4RMBD is energetically more advantageous rather than the promotion of DNA for the self-assembly of 4RMBD at this temperature.

The electron microscopy (EM) showed that the PHFs of 3RMBD and 4RMBD promoted by poly(dG-dT) are very similar to those by RNA[15] or heparin,[27] which have the typical PHF-like ultrastructure similar to that of full-length tau protein.[3]

As for the tau–DNA interaction, it has been reported that tau stabilizes the DNA structure, prevent and renature from the thermal denaturation, and protect from the radical attacking.[17—20] However, there is no report on the promoting affect of DNA for the filament formation of tau protein. Thus, we examined the effect by using various types of DNAs, and clarified for the first time the following important insights: 1) the structurally rigid dsDNA such as poly(dG-dC) promotes

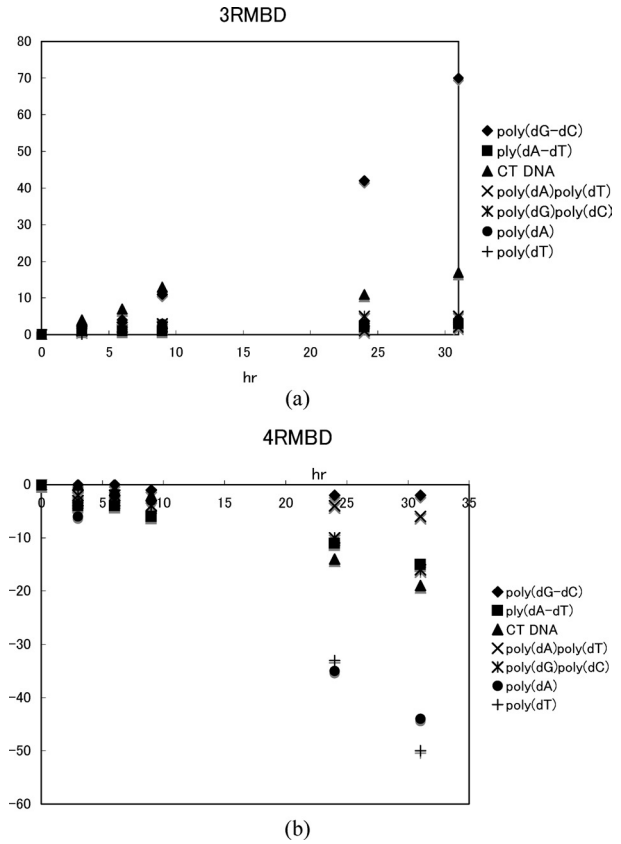

Fig. 4. DNA Structure-Dependent Change of ThS Fluorescence Intensity Accompanying Filament Formation of 3RMBD (a) or 4RMBD (b) at 20 °C

The conditions for the measurements are the same as those in Fig. 2.

the filament formation of tau MBD, 2) the more effective DNA promotion for the filament formation of 3RMBD than 4RMBD is due to the intermolecular dimer formation, and 3) the promotive effect of DNA on the filament formation of MBD is temperature-dependent and ssDNA such as poly(dA) or poly(dT) protects 4RMBD from the filament formation at 20 °C. As both of DNA and tau protein could coexist in the same organ, the present results would be useful on declaring the cause of tau PHF formation in AD partients.

Experimental

Materials Various types of DNAs [poly(dA-dT), poly(dG-dC), poly(dA)·poly(dT), poly(dG)·poly(dC), poly(dA), poly(dT), and calf thymus (CT) DNA (deproteinized by phenol extraction and precipitated with ethanol)] were purchased from Pharmacia P.L., and used without further purification. The gene expressions and purifications of His-tagged 3RMBD and 4RMBD of human brain tau (Fig. 1) were performed according to a previous paper,[27] and their purities were confirmed by SDS-PAGE analyses. ThS was purchased from Sigma Co. The other commercially available materials used were of reagent grade or higher.

Sample Preparation The DNA solution was adjusted with buffer A (50 mM Tris–HCl buffer (pH 7.6) containing 1 mM EDTA). The DNA concentration was determined using the standard molar extinction values ($M^{-1}\,cm^{-1}$) reported in the commercial documentation from P-L Biochemicals or in the literature[31,32] and was expressed in terms of moles of phosphate per liter. The concentrations of 3RMBD and 4RMBD were determined spectrophotometrically using the molar extinction coefficient ($1280\,M^{-1}\,cm^{-1}$ at 280 nm).

SDS-PAGE To investigate up to what extent the 3RMBD and 4RMBD are dimerized by the disulfide bond formation *via* Cys residues of the second and/or third repeats, their sample solutions were prepared in buffer A with or without containing 5% mercaptoethanol (ME) and were subjected into the SDS-PAGE after leaving for 12 h.

ThS Fluorescence Measurement By reference to the RNA–tau interaction,[15] the solutions of 120 μM MBD (3RMBD, 4RMBD), 60 μM ssDNA [poly(dA), poly(dT)], and 30 μM dsDNA [poly(dA-dT), poly(dG-dC), poly(dA)·poly(dT), poly(dG)·poly(dC), CT DNA] were separately prepared using buffer A. After the addition of ThS to the DNA solution, the MBD solution was then added and the final concentration was adjusted to 60 μM MBD, 30 μM ssDNA or 15 μM dsDNA, and 10 μM ThS. The fluorescence intensity with excitation at 440 nm and emission at 550 nm was measured using a JASCO FP-770F instrument with a 2-mm quartz cell maintained using a circulating water bath. The effect of DNA on the filament formation of each MBD was examined by measuring the fluorescence intensity at fixed time intervals. The fluorescence of MBD or DNA itself was subtracted when necessary. Because MBD itself self-assembles to some extent in process of time, the ThS fluorescence intensity at the starting time of the experiment ($t=0$ h) was set to be zero. The subsequent change of the intensity was measured as a function of reaction time and was subtracted from that of the same, but DNA-excluded, sample solution. The experiments were four times repeated using the newly prepared samples and were averaged.

Electron Microscopy Sixty micromolar MBD was mixed with 15 μM poly(dG-dC) in buffer A. The solution was then incubated at 37 °C for 6 h. Negative-staining electron microscopy was performed in an electron microscope (Hitachi Co.) operated at 75 kV, according to the previous paper.[27]

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