Biochemical and Biophysical Research Communications 466 (2015) 486–492

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Effects of lysine residues on structural characteristics and stability of tau proteins

Myeongsang Lee [1], Inchul Baek [1], Hyunsung Choi, Jae In Kim, Sungsoo Na*

Department of Mechanical Engineering Korea University, Seoul 136-701, Republic of Korea

ARTICLE INFO

Article history:
Received 8 August 2015
Accepted 11 September 2015
Available online 14 September 2015

Keywords:
Amyloid proteins
Tau protein
Lysine mutation
Molecular dynamics
Size effects

ABSTRACT

Pathological amyloid proteins have been implicated in neuro-degenerative diseases, specifically Alzheimer's, Parkinson's, Lewy-body diseases and prion related diseases. In prion related diseases, functional tau proteins can be transformed into pathological agents by environmental factors, including oxidative stress, inflammation, Aβ-mediated toxicity and covalent modification. These pathological agents are stable under physiological conditions and are not easily degraded. This un-degradable characteristic of tau proteins enables their utilization as functional materials to capturing the carbon dioxides. For the proper utilization of amyloid proteins as functional materials efficiently, a basic study regarding their structural characteristic is necessary. Here, we investigated the basic tau protein structure of wild-type (WT) and tau proteins with lysine residues mutation at glutamic residue (Q2K) on tau protein at atomistic scale. We also reported the size effect of both the WT and Q2K structures, which allowed us to identify the stability of those amyloid structures.

1. Introduction

Alzheimer's, Parkinson's, Lewy-body, and prion-related disease are representative neuro-degenerative diseases caused by the accumulation of amyloid proteins [1–4]. Accumulated amyloid proteins originate from monomer structures that are the product of cleavage of the amyloid precursor proteins by activation of secretases [5]. These amyloid monomer proteins are transformed from a substantially unstable status, and the monomers from several types of to aggregates, including oligomeric, fibrillar and plaque aggregates. These structures are located near the neuron and synapse, disrupting normal cell functions. Oligomeric, fibrillar, and plaque structures are insoluble under physiological conditions; their inherent stability is due to the high proportion of β-sheets, since β-sheets are sustained by hydrogen bonds, act like glue. However, amyloid proteins implicated to neuro-degenerative disease; functional tau proteins are also agents involved in neuro-degenerative diseases.

Generally, tau proteins stabilize the microtubules located near the axons of neuron [6]. Oxidative stress, inflammation, Aβ-mediated toxicity, and covalent modifications of tau proteins lead to hyperphosphorylation of the proteins. Tau proteins then detach from the microtubule leading to microtubule structural instability [7–9]. Subsequently, neuronal cell death results from the destabilization of the microtubules by hyperphosphorylated tau proteins. Additionally, the detached tau proteins can form as the paired helical filaments (PHF), the contents of which are rich in contents of β-sheets [10]. According to S. Bloom and co-worker revealed that the detached full length of tau protein grow as the PHF structures with β-strands characteristics [8]. Experimental studies have revealed that the repeat regions of full-length human tau proteins are composed of six isoforms rich in proline residues. Except the N- and C- terminal regions, four repeated isoform regions (VQIVYK and VQIINK) are the critical segments for the formation of PHF structures. These repeat regions are microtubule binding sites [11,12]. Berriman et al., revealed the fibril formation of tau proteins through kinetic and imaging experimental studies [13]. Also, Lew and co-worker found that the VQIVYK and VQIINK segments mediate the possible generation of oligomeric structures [12]. Similar computational results were found by Cheon et al. They revealed potential oligomeric formations of tau proteins through the replica exchange molecular dynamics (REMD) method [14]. Also, Shea group reported the formation of tau proteins with β-sheet characteristic after deleting of the 287th lysine residue with additional residues [15]. From several experimental and computational studies, tau

* Corresponding author.
E-mail address: nass@korea.ac.kr (S. Na).
[1] M.L. and I.B. made equal contributions of this work.

http://dx.doi.org/10.1016/j.bbrc.2015.09.056
0006-291X/

fibrillar structures share common β-sheets characteristics with amyloidogenic proteins such as Aβ, α-synuclein, and β_2-microglobulin. Thus, stable fibrillar structures from tau segments are implicated to neuro-generative disease.

Developed fibrillar tau proteins from microtubules involved in amyloid proteins have been recognized as toxic agents, which contribute to several neuro-degenerative diseases. Oligomeric amyloid proteins, including tau protein structures have more toxic characteristic than fibrillar structures [16]. Spefecifically, oligomeric amyloid proteins, including Aβ, tau and amylin proteins have been recognized as toxic agents due to the easy permeation of membrane, formation of ion channel in membrane and deletion of lipid bilayers of membrane [17]. These toxic oligomeric amyloid proteins are formed through a repetitive process of elongation and fragmentation, generated by internal flow, thermal fluctuation, pH and ionic strength values within the cell. Oligomeric amyloid proteins are also related to the growth of fibrillar structures [18]. Recent studies have reported that the oligomer amyloid proteins mediate the fibrillar growth of amyloid proteins through the experiment techniques. Oligomeric amyloid proteins, including prefibrillar structures of different sizes, are not only generated by the fragmentation and elongation, but also formed by the interaction between growth seeds and additional amyloid monomers. Several research groups have revealed the oligomeric stability of amyloid proteins through molecular dynamics (MD) simulations under different oligomer protein structures. Kahler et al., revealed the one and two fold structures of $A\beta_{1\text{-}42}$ through geometry information and the protein's interaction energies [19]. Nussinov and co-worker constructed the polymorphic $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}40}$ proteins, which are polymorphic structures constructed by the specific residue interactions to reveal the role of the turn region [20,21]. Similarly, Shea group investigated the stability of polymorphic $A\beta_{9\text{-}40}$ fibrils according to the stacking and facing directions of subunit fibrils structures based on subunit fibrils [22]. For tau proteins, Cheon et al., investigated the diverse kinds of generated tau protein and their thermodynamic stabilities through the REMD methods [14].

However, recently, amyloid proteins have been utilized as the basic platform of bio-functional materials [23,24]. For example, for the utilization of amyloid nanowires, Scheibel et al. developed conductive functional nanowires using Sup35 amyloid proteins [25]. Knowles et al., developed nanostructure films using β_2-lactoglobulin amyloid proteins, the material properties of amyloid nanostructured film occupied up to ~10 GPa [26]. In similar manner, Chen et al. exploited the switchable biofilm conjugated with CsgA amyloid film with gold nano particles [27]. Li et al. used the fibrillar tau proteins as a template for bio-functional materials in order to capture the carbon dioxide [28,29]. Under high pressure and temperature conditions, fibrillar tau proteins successfully capture carbon dioxides while sustaining their own structures. To effectively capture carbon dioxide, they mutated the glutamic acid residue to lysine residues, which can capture more carbon dioxide than the wild-type structures, while sustaining with high structural stability [28]. Further, they selectively designed peptide segments for capturing the carbon dioxide using additional amyloid proteins such as α-synuclein, Aβ and insulin [29]. From their study using tau and Aβ fibrils, lysine residue mutation significantly increased the capturing the carbon oxides while sustained their own structures stabilities [28]. Despite the high rate of capturing carbon dioxides on wildtype and lysine mutated amyloid proteins, the basic structural characteristic of the lysine residue mutation has not been investigated yet.

In present study, we report the structural characteristics of oligomeric prefibrillar tau protein that successfully captures carbon dioxide under harsh environment conditions. We measured the structural characteristics of fibrillar tau proteins in various size conditions on an atomic scale using MD simulations. Further, we describe the structural characteristics of 5, 10, 15, and 20 layers VQIVYK segment and mutated VQIVYK segments through the mutation glutamic acid residue to yield of lysine residues. On atomistic scale, mutated lysine residues increased the interaction energies (i.e. non-bonded energy) and lowered the number of hydrogen bonds, causing the instability of VKIVYK fibrils. In addition, we found structural instabilities of 20 layers of the VKIVYK structures during equilibrated 50 ns. Through an analysis of geometric information and structural characteristic parameters, we found a size effect of oligomeric tau proteins with lysine residues.

2. Material & method

To construct the prefibrillar types of oligomeric tau structures, we use amyloid forming VQIVYK repeat segments from human tau proteins. This VQIVYK segment was identified with a 1.51 Å resolution X-ray diffraction method (Protein Data Bank ID: 2ON9) [30]. Next, we mutated the amino acid residues of the basic VQIVYK segments to discover the effects of charged amino acids on the stability of the prefibrillar tau structures. We mutated the glutamine (Q) residues of the VQIVYK segment to lysine (K) residues [31]. The mutation process was conducted by the mutator plugin of VMD 1.9.2 version [32]. We then constructed four tau amyloid models of different lengths, 5, 10, 15 and 20 layers for each wild-type VQIVYK model (Fig. 1 a) and mutated VKIVYK model (Fig. 1 c) to investigate effect of fibril size on stability of the prefibrillar tau structures. Here, we denoted the wild-type tau structure as the WT and the mutated model (VKIVYK) as Q2K. For the different sizes of tau prefibrillar structures (5, 10 15 and 20 layers) we notated each layer as L.

All eight tau amyloid fibril models were simulated using GROMACS 4.6.5 software [33] with a CHARMM27 force field [34]. We made a 3 nm water box using the TIP3P model [35] and surrounded the protein structure all directions. The simulation box was neutralized by adding a salt concentration of ~0.15 mol/liter using Na^+ ion and Cl^- ions. After constructing the simulation box, the energy of the system was minimized using a steepest descent algorithm [36]. To constraint the prefibrillar tau structure within the surrounding system, we restrained the backbone of protein structure and set the system temperature to 310 K using a velocity rescaling method with a stochasticity term [37] and 1 bar pressure using the Parrinello–Rahman method [38,39]. Subsequently, equilibration simulation of the entire system was performed using a leap-frog algorithm with 2 fs time step [40]. The total simulation elapsed 100 ns, including WT and Q2K models per each layer models. During the simulation, the particle mesh Ewald (PME) method was applied to calculate the long-range electrostatic interactions [41,42]. All bonds were constrained using a linear constraint solver [43].

3. Results

3.1. Lysine residue effects on structure composition

During the 50 ns equilibration simulation of WT and Q2K model for each, we observed a lysine residue mutation effect on the prefibrillar tau protein. From the equilibrated simulation results, six different types of tau amyloid fibrils, including the 5, 10 and 15 layer structures, were stable and had a twisted shape. From the Fig. 1, shows the representative twisted shape of both 10 layers of WT and Q2K models. Remaining models such as 5 and 15 layer structures both WT and Q2K models were stable after the 50 ns equilibrated simulations, while 20 layer structures of the WT and Q2K models were unstable as shown in Supplementary Fig. 1. Specifically, the Q2K-20L model fractured after a 10 ns simulation due to size effects and an increment in potential energy. The WT models such as 5, 10, 15, and 20 layer WT models showed the twisted shapes compared