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EXPERIMENTAL AND ATOMISTIC COMPUTATIONAL STUDIES OF MECHANO-CHEMICAL TRANSPORT PROCESSES INVOLVED IN NEURODEGENERATIVE DISEASES

Simona PATRICHE[1], Mihaela BANU[1], Shinji MATSUSHITA[2], Sung-Woong HAN[2]

[1]Department of Manufacturing Science, Robotics and Welding, "Dunarea de Jos" University of Galati 111 Domneasca St., 800201 Galati, Romania
[2]Department of Biomechanics, Research Center for Nano Medical Engineering, Institute for Frontier Medical Sciences, Kyoto University, Sakyo, Kyoto, Japan 606-8507
simona.patriche@ugal.ro

ABSTRACT

One of the most studied neurodegenerative diseases is Alzheimer's disease (AD), which consists of axonal degeneration caused by no correctly reaching of neurotransmitters to the synapse. This deficiency is because binding of kinesin heads onto microtubules (MTs) is hindered by the attachment of tau proteins on MTs near (or between) binding sites. Thus, early detection of changes in the kinesin-MT interaction can be used to predict the presence of an incipient stage of AD. To predict changes in mechano-chemical behavior, a computational model for the kinesin-MT system was constructed. In this paper, molecular dynamics simulations and experiments were performed to identify the micro-mechanical properties of the components of the model, e.g., the behavior of the MT region plus the neck linker structure under fluctuating forces and characteristics of kinesin transport on MT for a healthy MT.

KEYWORDS: kinesin-MT system, neurodegenerative diseases, molecular dynamics simulations, optical tweezers technique

1. INTRODUCTION

Mechano-chemical transport processes involved in neurodegenerative diseases represent the affected displacement mechanism of kinesin along microtubules. This mechanism, known as the processivity represents the ability of kinesin to move along these cytoscheletal filaments without dissociating from them, carrying various cargoes inside of cell [1, 2, 3, 4]. Neurodegenerative diseases, such as Alzheimer's disease, appear when the processivity of kinesin is affected by presence of different obstacles, like: tau protein. This molecule has capacity to attach on MTs near (or between binding sites) and thus, the displacement mechanism of kinesin on microtubules is disrupted [5, 6, 7, 8]. There are two key features of kinesin processivity: microtubule binding region plus neck linker from kinesin structure and processivity parameters: run length and velocity. Thus, early detection of changes in the kinesin-MT interaction can be used to predict the presence of an incipient stage of AD. To predict changes in mechano-chemical behavior, a computational model for the kinesin-MT system was constructed. In this paper, molecular dynamics simulations and experiments were performed to identify the micro-mechanical properties of the components of the model, e.g., the behavior of MT region plus the neck linker structure under fluctuating forces and the characteristics of kinesin transport on MT for a healthy MT.

2. ATOMISTIC COMPUTATIONAL PROCEDURE

The behavior of MT region plus the neck linker structure from conventional kinesin molecule under fluctuating forces, was studied through ABF method and steered molecular dynamics (SMD) performed using NAMD 2.8b3 [9] and VMD 1.9 [10] with CHARMM parameters [11] for proteins and lipids. In our simulations, we used a crystal structure

of monomeric conventional kinesin from Protein Data Bank (PDB ID: 1MKJ). This structure is composed from a heavy chain of 349 amino acids and neck linker region, which has an important role in displacement mechanism of kinesin and force producing, being defined between 325-337 residues. This short structure (13 amino acids) connects the central catalytic domain of conventional kinesin and helicoidal domain, which has an important role in stabilizing of dimeric molecular motors and leads to forming of globular domain involved in binding process of celular cargo [12]. Another important feature in kinesin processivity is binding region to microtubules defined between 255-285 residues (MT region) (Fig. 1).

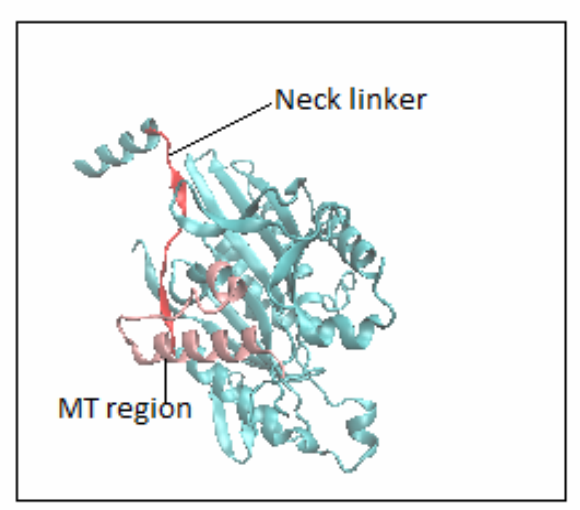

Fig. 1: Monomeric conventional kinesin structure with neck linker (325-337 residues) and MT region (255-285 residues) represented in VMD editor

Monomeric conventional kinesin structure of dimensions (60 x 68 x 45 $Å^3$) was placed into a water box of dimensions (80 x 88 x 65 $Å^3$) and the water layer of 15 Å, in which it was used TIP3P model for water molecules and there were added Na^+ and Cl^- counter ions at a concentration of 30 mM. Periodic boundary conditions are defined to consider all interactions between the two structures and environment. After this proteic structure is introduced in water box, the total system contains: 42741 atoms, 5306 MT region atoms, 37435 water atoms, 36 Na^+ ions and 33 Cl^- ions.

The van der Waals interactions were calculated with a cutoff of 12 Å and the electrostatic interactions were calculated using the Particle Mesh Ewald method [13]. A multiple time-stepping algorithm [14] used a 2 fs step. NAMD simulations were made in an NPT ensemble (pressure = 1atm, temperature 300 K), in which the pressure was controlled by the hybrid Nosé - Hoover Langevin piston method [15, 16] and the temperature was controlled using Langevin dynamics. A free dynamics simulation for entire system was performed for 500ps (monomeric conventional kinesin) to obtain the equilibrium structure [17, 18, 19].

2.1. ABF method

By applying different external forces on this structure, we could evaluate the free energy evolution using ABF method [20] done for unfolding process of monomeric kinesin in the gas phase. There were defined two reaction coordinates: **AtomDistance** (interatomic distance between the carbon atoms pertaining to the first and the last carbonyl moietes of the peptide chain (C-255 from MT region)) and respectively, last carbon atoms from last residue (C-349 from last residue of conventional kinesin); **COMDistance** (separation between two groups of atoms formed by the Cα atom and its aliphatic hydrogen in the first and the last residues of the peptide chain).

2.2. Steered molecular dynamics (SMD)

Steered molecular dynamics were done applying a constant force to a SMD atom. In our simulations, we keep fixed the group of atoms from MT binding region (255-285 residues) and applied constant forces in range of 700-1400 pN to SMD atom represented by Cα atom of the last residue (349). The constant force was applied in direction defined by the vector which connects the fixed region and SMD atom (Fig. 2.).

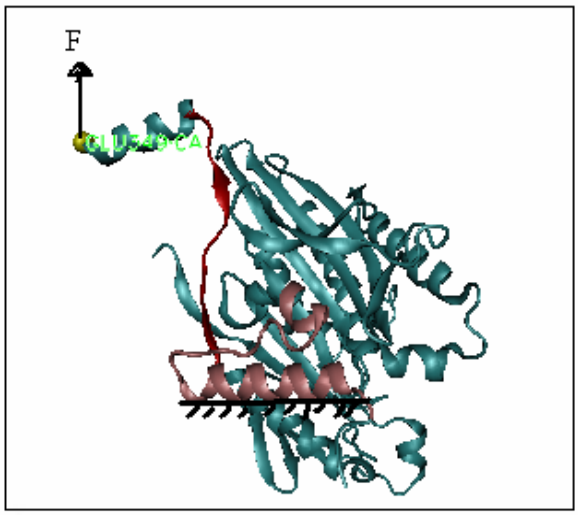

Fig. 2: Monomeric conventional structure with applied force in direction defined by the vector which connects the fixed region (225-285 residues) and SMD atom (GLU 349: CA)

To apply an external force to SMD atom, there are defined x, y and z-components of the normalized direction between the fixed region and the SMD atom. The values of the normalized vector of applied

constant forces are calculated using the following equation:

$$n_x = \frac{x_2 - x_1}{\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2 + (z_2 - z_1)^2}}$$
$$n_y = \frac{y_2 - y_1}{\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2 + (z_2 - z_1)^2}} \quad (1)$$
$$n_z = \frac{z_2 - z_1}{\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2 + (z_2 - z_1)^2}}$$

where, n_x, n_y, n_z = normalized vector components of applied constant force
x_1, y_1, z_1 = coordinates of the fixed region
x_2, y_2, z_2 = coordinates of the Cα atom from last residue (349)

3. EXPERIMENTAL PART

The characteristics of kinesin transport on microtubules for a healthy MT were studied using optical tweezers technique, which is a useful method to identify micro-mechanical properties of molecular motors.

3.1. Experimental set-up

The set-up used in optical tweezers experiments, contains an inverted microscope on which is placed the flow cell and its objective converges the two laser beams. The stage of flow cell can be controlled manually and by a high-resolution piezo element (nanometer precision). First laser (an infrared ND:YVO_4 laser with a wavelength of 1064 nm) is used to trap the bead. The second laser (a red HeNe laser with a wavelength of 633 nm) is used together with a quadrant-photodiode detector (QPD, sample rate 30 kHz) to calibrate the stiffness of the trap. To calculate the value of stiffness trap, k, it was measured the power spectrum of a trapped bead close to the surface [21, 22, 23]. In our experiments, the laser power used to trap beads was set to 0.2 W and the measured stiffness was around 0.01 pN/nm.

The displacement of conventional kinesin along axonemes (bundles of microtubules) was studied without an optical trap. Video tracking is started after a kinesin motors – coated polystyrene bead is placed on a horizontal axoneme using the optical trap. The laser beam is then turned off and the bead starts to walk. The motion of the bead is recorded over time. A sketch of the experiment performed using optical tweezers technique is shown in Fig. 3.

To measure the displacement of this motor proteic, a conventional kinesin – coated polystyrene bead (2μm diameter) is placed on a horizontal axoneme using the optical trap.

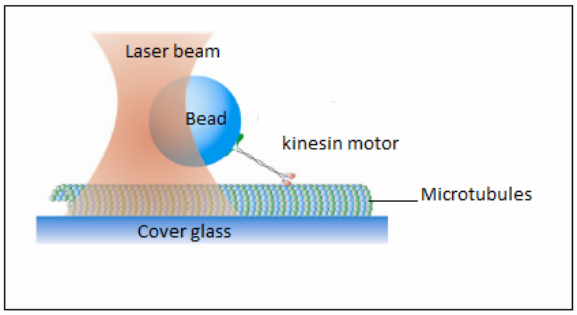

Fig. 3: Sketch of the experiment using optical tweezers technique [24].

4. RESULTS AND DISCUSSION

To perform our molecular dynamics simulations, a structure consists of MT region and neck linker from monomeric kinesin was used. A steered molecular dynamics was performed for 100ps by applying a constant force in range of 700-1400 pN. Deformation of neck linker structure under fluctuating forces was identified using the evolution in time of end-to-end distance (Fig. 4). This is defined between the Cα atom of the first residue (325) from neck linker structure and the one of the last residue (337). As previous researches on the neck linker molecule [10], from figure 4 it can be observed that the behavior of this structure is characterized by rotations and contractions during the movement of conventional kinesin along microtubules. Even if there was applied an increased force (F = 1400 pN) on the analyzed structure, the neck linker presents a compression of its initial length from 36.2 Å to 31.8 Å. All these events are due to significant thermal fluctuations during transport inside of the cell.

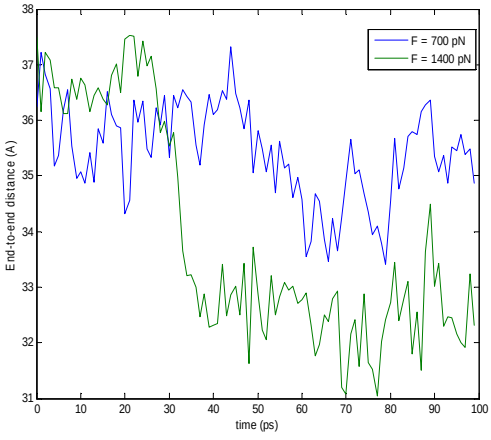

Fig. 4: The evolution in time of the end-to-end distance under the two applied constant forces (F_1 = 700 pN, F_2 = 1400 pN) for neck linker structure

To evaluate the free energy stored inside of the region from monomeric conventional kinesin defined

between first residue of MT region (255) and last residue from molecule, we applied a constant force of 700 pN, during 100 ps. For a free energy of 18.23 kcal/mol (**AtomDistance** reaction coordinate) and respectively, 23.65 kcal/mol (**COMDistance** reaction coordinate), approximately all hydrogen bonds are broken (Fig. 5). Thus, conventional kinesin becomes more processive and can advance on microtubules performing a correct transport of different cargoes inside of cell.

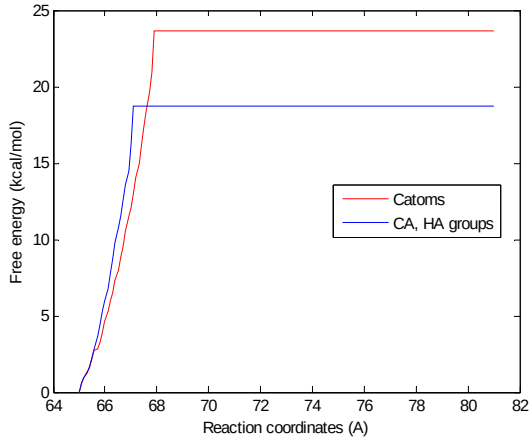

Fig. 5: Free energy profile delineating the reversible unfolding of monomeric conventional kinesin in the gas phase, using two distinct reaction coordinates and applying a constant force of 700 pN (10 kcal/mol/ Å).

The characteristics of kinesin transport on microtubules for a healthy MT was studied using two processivity parameters (the average velocity of molecular motors displacement along axonemes and their run length), which were measured during optical tweezers experiments. There were analyzed four samples with different concentrations: c = 2.5 mg/ ml, c = 0.25 mg/ ml, c = 0.125 mg/ ml, c = 0.083 mg/ ml. In case of samples with high concentrations, the values of average velocity were ranged between 20 nm/s and 122 nm/s. These values correspond to multiple motors behavior. For sample with low concentration (c = 0.083 mg/ ml), the values of average velocity were ranged between 367.36 nm/s and 659.299 nm/s and run length was equal to 0.1 µm.

These results are a signature that in this solution we had a single motor behavior. All these experimental data are in agreement with values obtained by Svoboda and Block [25, 26] for processivity parameters of single kinesin molecules studied with optical tweezers.

By plotting average velocity of molecular motors as function of motor concentration, it can be observed that the single kinesin walks only few steps along axonemes, meanwhile multiple kinesin takes more steps. Therefore, the value of average velocity decreases with increasing of motor concentration, while the run length of these molecular motors increases with decreasing of motor protein concentration (Fig. 6, 7).

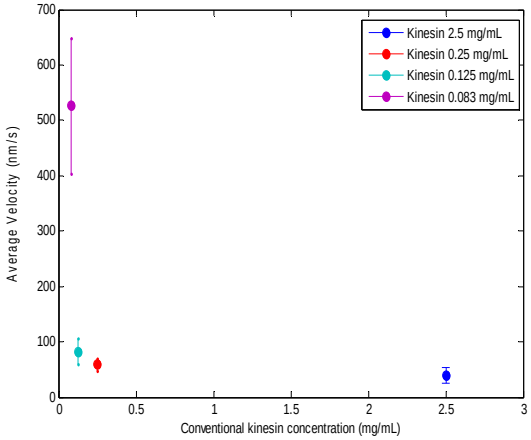

Fig. 6. Conventional kinesin average velocity as function of the motor concentration. Error bars indicate the standard deviation of average velocity

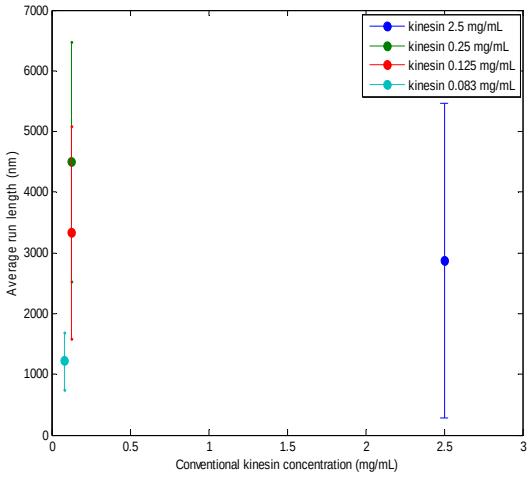

Fig.7. Conventional kinesin average run length as function of the motor concentration. Error bars indicate the standard deviation of average run length

One explanation of low values of average velocity during the movement of multiple conventional kinesins along axonems consists of the presence of both different obstacles and some free molecular motors bound on the axonemes structure.

To avoid these obstacles, first molecular motors stall a while and then due to the cooperation between them will continue the walking process across axonemes. This behavior is similar to the one present in case of Alzheimer's disease, which is characterized by the attachment of tau proteins on MTs near (or between) binding sites.

5. CONCLUSIONS

Studies performed in this article helped us to identify structural properties of kinesin-MT system and respectively, to identify the characteristics of kinesin transport on microtubules for a healthy MT. The conformational changes of this molecular motor were identified by free energy evaluation and deformation of neck linker structure from conventional kinesin molecule under fluctuating forces. Experimental studies performed using optical tweezers technique showed that at high concentration of molecular motors, the processivity of beads which have attached these proteic motors is reduced, meanwhile velocity and run length values are slower than the ones identified to low concentration. Both mechanical and structural properties contribute to understand in detail the biomimetics of kinesin-MT system used to develop different bio-sensors with essential applications in treatment of neurodegenerative diseases.

In conclusion, molecular dynamics simulations and optical tweezers experiments performed in this paper, were used to identify the micro-mechanical properties of the components of mechano-chemical transport processes involved in neurodegenerative diseases. These mechano-chemical transport processes represent the affected displacement mechanism of kinesin along microtubules by presence of different obstacles, such as: tau protein in case of Alzheimer's disease.

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Studii experimentale si numeric-atomistice ale proceselor mecano-chimice ale transportului implicat in bolilele neurodegenerative

Rezumat

Una dintre cele mai studiate neurodegenerative boli este boala Alzheimer (AD), care consta in degenerarea axonala cauzata de neajungerea corecta a neurotransmitatorilor la sinapsa. Aceasta deficienta este datorata faptului ca atasarea capetelor kinezinei la microtubuli este impiedicata de atasarea proteinelor tau la microtubuli (MTs) langa (sau intre) zonele de atasare. Astfel, detectarea din timp a schimbarilor din interactiile kinezina-MT poate fi utilizata pentru a prezice prezenta unui stadiu initial al bolii Alzheimer. Pentru a prezice schimabrile din comportamentul mecano-chimic, un model numeric pentru sistemul kinezina-MT a fost conceput. In aceasta lucrare, simulari de dinamica moleculara si experimente au fost realizate pentru a identifica proprietatile micro-mecanice ale componentelor modelului, e.g. comportamentul regiunii MT plus structura neck-linker-ului sub acitunea fortelor fluctuante si caracteristicile transportului kinezinei de-a lungul microtubulilor pentru un microtubul MT neafectat.