MEMBRANE INITIATED GELSOLIN AMYLOID FORMATION

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KEYWORDS

Gelsolin, amyloid, amyloid formation, DMPC membrane, molecular dynamics.

ABSTRACT

Molecular dynamics method is applied to simulate amyloid formation process.

Mutated gelsolin amyloidogenic fragments 173-243 (G173-243) and 173-202 (G173-202) cause Finnish familial amyloidosis disease (FAF). The mechanism of possible amyloid formation involving gelsolin G173-202 fragments and dimyristoyl-phosphatidylcholine (DMPC) lipid was investigated by means of molecular dynamics (MD). G173-202 fragment adhered to the DMPC membrane in 100 ps, retaining β -sheet structure, suggesting that a lipid membrane could bind G173-202 fragments and serve as a germ for amyloidogenesis.

Afterwards one more amyloidogenic fragment G173-202 was placed in the vicinity of G173-202 adhered to the DMPC membrane. After 700 ps of MD, the newly added amyloidogenic fragment G173-202 was interacting with the membrane attached fragment G173-202, indicating the possible mechanism of membrane initiated amyloidosis.

INTRODUCTION

Molecular dynamics method is useful computer calculations method to simulate interactions of molecules on a time scale thus simulating physiological and pathophysiological processes. In this work molecular dynamics is applied to simulate gelsolin amyloid formation process. Molecule interactions as

the sum of all atom pair interactions (pair-wise approximation) are described in the same manner as in classic molecular mechanics of empirical force fields (Leach, 1996):

$$E = E_{bond} + E_{angle} + E_{tor} + E_{el} + E_{vdW} + E_{H-bond} \quad (1),$$

where E_{bond} is the bond-stretching energy, E_{angle} is the valence-angle-bending energy, E_{tor} is the torsional energy (which arises because of intrinsic barriers to rotation about the bonds), E_{el} is the electrostatic energy, E_{vdW} is the energy of van der Waals interactions, and E_{H-bond} comprises E_{vdW} between the protons and electronegative atoms that can form hydrogen bonds with them (it is treated separately, because usually different atom-type-dependent parameters are required than those for non-hydrogen-bonding atom pairs). In the present work the flexible valence geometry force field AMBER was used:

$$E_{bond} = \sum_{bonds} \frac{k_{di}}{2} (d_i - d_{ei})^2 \quad (2)$$

where d_i is the actual length of the i bond, d_{ei} is the equilibrium length (the length of a non-strained bond), and k_{di} is the force constant.

$$E_{angle} = \sum_{bond\ angles} \frac{k_{\theta_i}}{2} (\theta_i - \theta_{ei})^2 \quad (3)$$

where θ_i is the actual value of the i th valence angle, θ_{ei} is the equilibrium value, and k_{θ_i} is the force constant. Electrostatic energy is described as a sum of the interaction of charges localized on atomic nuclei,

$$E_{el} = 332 \sum_{\substack{i < j \\ i, j \text{ not bonded}}} \frac{q_i \cdot q_j}{\epsilon \cdot r_{ij}} \quad (4)$$

The energy of van der Waals interactions is described by the Lennard-Jones potential,

$$E_{vdW} = \sum_{\substack{i < j \\ i, j \text{ not bonded} \\ \text{and not 1,3-nonbonded} \\ \text{and not hydrogen-bonded}}} \frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^6} \quad (5)$$

where A_{ij} and B_{ij} are constants depending on the types of interacting atoms.

$$E_{H-bond} = \sum_{\substack{\text{hydrogen} \\ \text{bonding} \\ \text{pairs}}} \frac{C_{ij}}{r_{ij}^{12}} - \frac{D_{ij}}{r_{ij}^{10}} \quad (6)$$

The torsional energy, E_{tors} , is expressed by

$$E_{tor} = \sum_{\substack{\text{torsion} \\ \text{angles}}} \frac{V_{1i}}{2} (1 + \cos \varphi_i) + \frac{V_{2i}}{2} (1 - \cos 2\varphi_i) + \frac{V_{3i}}{2} (1 - \cos 3\varphi_i) \quad (7)$$

In molecular dynamics, successive configurations of the system are generated by integrating Newton's equations. The result is a trajectory that specifies how the positions and velocities of the atoms in the system vary with the time. The trajectory is obtained by solving the Newton's equation: ($F=ma$) for the particle with mass m_i moving along the coordinate x_i being driven by force F_{xi} :

$$\frac{d^2 x_i}{dt^2} = \frac{F_{xi}}{m_i} \quad (14)$$

similarly

$$\frac{d^2 y_i}{dt^2} = \frac{F_{yi}}{m_i} \quad (15)$$

$$\frac{d^2 z_i}{dt^2} = \frac{F_{zi}}{m_i} \quad (16)$$

where x_i , y_i and z_i are the Cartesian coordinates at i th atom, F_{xi} , F_{yi} , F_{zi} are the components of the forces acting on that atom and m_i is the mass of i th atom.

The forces are derivatives of energy E , described in (1).

$$F_{xi} = \frac{\partial E}{\partial x_i} \quad (17)$$

The molecule system described by above mentioned method consists of protein molecule fragments - amyloidogenic fragments of gelsolin, and of a membrane bilayer fragment.

Gelsolin is a six-domain dynamic actin-filament binding, severing and capping protein capable of severing and nucleating of the actin cytoskeleton (Kwiatkowski, 1999). By modulating actin network via actin assembly and disassembly gelsolin regulates cell shape and motility (Kwiatkowski, 1999; Sun et al., 1999; Robinson et al., 1999; Laine et al., 1998) in the cytoplasm and cleans blood from stray actin filaments (Lee et al., 1992). In addition to actin regulatory function, gelsolin affects cell growth through the

regulation of apoptosis (Sun et al., 1999). Cellular actin scaffold is continuously reorganized in response to a variety of signals. Apoptosis promotes dismantling the actin cytoskeleton, growth factor stimulation induces actin filament assembly at the plasma membrane, which changes cell shape and regulates cell movement. Gelsolin is a calcium-activated regulator of the actin cytoskeleton (Kwiatkowski, 1999; Robinson et al., 1999). Elevated calcium ion concentration activates actin filament severing and capping activities of gelsolin, which results in shorter actin filaments. Another type of gelsolin activation is performed by caspase-3, which cleaves the Ca^{2+} -insensitive N-half from the Ca^{2+} -dependent C-half (Kothakota et al., 1997). Ca^{2+} opens up gelsolin by inducing conformational changes in the C-half (Kwiatkowski et al., 1989; Patkowski et al., 1990), to expose actin binding sites of the N-half; thus the C-half acts as a regulatory domain to impose calcium regulation on the N-half. Caspase-3 acts cleaves the regulatory C-half, thus giving the N-half calcium uncontrolled actin-severing activity.

Human gelsolin is expressed as an 81 kDa protein in the cytoplasm and an 84 kDa protein in the plasma (Kwiatkowski et al., 1986a,b). The secreted protein differs by a signal sequence required for export and a 25-residue N-terminal extension. Human gelsolin has 87% sequence similarity to horse gelsolin (Koeppf & Burtinck, 1998; McLaughlin & Gooch, 1992)).

Natural gelsolin exists in two forms – cytoplasmic and plasmic, which are derived from the same gene by alternative splicing (Kwiatkowski et al., 1986a,b). The cytoplasmic or intracellular gelsolin is a 84 kDa protein responsible for the assembly of actin fragments during cytoskeletal formation and disassembly of actin filaments during cytoskeletal rearrangement. The cytoplasmic form of gelsolin modulates the actin cytoskeleton and plays a role in the cell motility and apoptosis (Kwiatkowski, 1999; Azuma et al., 1998; Kothakota et al., 1997). Plasma gelsolin is a scavenger that degrades stray actin filaments, thrown out from apoptotic cells into the blood plasma, and retrieves actin monomers for intracellular use. The cysteines at positions 188 and 201 (of human gelsolin) form a disulfide bond in the plasma gelsolin, whereas the cytoplasmic gelsolin form remains reduced (Wen et al., 1996). The actin severing function enables gelsolin to reduce the viscosity of blood and blood clots (Vasconcellos et al., 1994) or cystic fibrosis sputum (Davoodian et al., 1997).

Gelsolin folding occurs in the cytoplasm, where calcium ion concentration is normally low, and folding produces probably the inactive native protein, which can be activated by an increase in calcium concentration. Folding of gelsolin plasma form could take place in the endoplasmic reticulum, where the free calcium ion concentration is 100-1000 M (Meldolesi & Pozzan, 1998), which should be sufficient to generate the active, relaxed form (Zapun et al., 2000).

Plasma gelsolin is implicated in the familial amyloidosis-Finnish type (FAF), inherited disease. A point mutation in the S2 gelsolin domain causes an

amyloidosis with neurological, ophthalmological and dermatological symptoms. This mutation does not affect the cytoplasmic form, while the plasma form is proteolysed, possibly both in the secretory pathway and in the extracellular medium (Kangas et al., 1996, 1999). Thus in addition to its role of actin binding, gelsolin domain S2 is involved in the pathogenesis of familial amyloidosis-Finnish type caused by inherited mutations D187N and D187Y in domain S2 of gelsolin (Maury et al., 1990). At the genetic level, FAF is caused by a single nucleotide substitution in the genomic DNA sequence of gelsolin where guanine 654 is replaced with adenine or thymine (De la Chapelle et al., 1992a,b). This causes replacement of an aspartate residue Asp¹⁸⁷ with asparagine or tyrosine, which makes gelsolin susceptible to aberrant trypsin-like protease cleavage site between residues Arg¹⁷² – Ala¹⁷³ (Kangas et al., 1996). Structural changes unmask an aberrant proteolysis site at Arg¹⁷²-Ala¹⁷³ leading to a trypsin-protease sensitive molecule (Kiuru, 1998, Kiuru et al. 1999; Kiuru-Enari et al., 2002). The hydrolysis at the Arg¹⁷²-Ala¹⁷³ site results in the formation of a 68 kDa C-terminal fragment, which is further digested at the Met²⁴³ residue, forming an amyloidogenic 8.1 kDa peptide containing residues 173-243 (G173-243) of gelsolin domain S2 (Maury et al., 1997, 1994). The peptides G173-243 or its sequent cleavage fragment Ala¹⁷³-Gly²⁰², spontaneously associate into amyloid fibrils. The three-strand peptide Ala¹⁷³-Gly²⁰² has a greater propensity to form amyloid fibrils than the amyloidogenic fragment G173-243 comprising four β -strands and an α -helix (Weeds & Maciver, 1993, Burtneck et al., 1997). The structure of the Ala¹⁷³-Gly²⁰² fragment consists of an antiparallel β -sheet that is stabilized by a disulphide bond, from Cys¹⁸⁸ to Cys²⁰¹ (Burtneck et al., 1997). Amyloid fibrils are deposited mainly in the facial regions, in the cornea and cranial nerve. As a result of deposition and accumulation of amyloid fibrils the FAF phenotype is characterized by corneal lattice dystrophy, cranial neuropathy, hyperelastic skin, facial muscle weakness, and renal complications (Kiuru, 1998). Mutated gelsolin has defective actin severing activity (Weeds & Maciver, 1993), but FAF disease phenotype arises from the accumulation of extracellular amyloid tissues rather than the loss of gelsolin functions.

The mechanism of amyloidogenesis is not clear. In our previous work (Liepina et al., 2004) we started to investigate the mechanism of amyloid formation. In this work, using molecular dynamics (MD), we investigate interactions of gelsolin amyloidogenic fragments G173-202 [cleaved from the x-ray structure of gelsolin (1DON)] with dimyristoylphosphatidylcholine (DMPC) membrane bilayers.

METHODS

Method of molecular dynamics with AMBER force field was used.

Dimyristoylphosphatidylcholine (DMPC) lipid bilayer containing 6 x 6 arrays of DMPC molecules was taken from a previous study (Czaplewski et al, 1999). Mutated gelsolin amyloidogenic fragment 173-202 (G173-202) was cleaved from horse gelsolin crystal structure 1DON.

The G173-202 fragment was placed 9 Å away from a DMPC bilayer at two different starting orientations (Fig.1a) – start 1, and Fig. 2a) - start2) and subjected to NTP MD simulations at elevated body temperature T=312 K for 150 ps. After 100 ps of MD, the G173-202 fragment at either initial orientation adhered to the DMPC membrane (Fig.1 b), Fig.2 b).

Afterwards one more amyloidogenic fragment G173-202 was placed in the vicinity of the G173-202 fragment adhered to the DMPC membrane (Fig.3 a)), and the new system was subjected to NTP MD simulations at T=312 K for 3744 ps.

Subsequently all systems were placed in a periodic lipid-water box and subjected to MD simulations to investigate of the stability of the structures.

Preliminary calculations on DMPC lipid interactions with amyloid fragments G173-202 were carried out with program MOE, AMBER94 force field. Afterwards the peptide-lipid systems were surrounded by water layer forming periodic lipid-water box, and submitted to AMBER 7.0 molecular dynamics (MD) equilibration to investigate the stability of the systems.

The pictures representing snapshots from molecular dynamics were created by program MOE.

RESULTS AND DISCUSSION

The gelsolin amyloidogenic fragment G173-202 was placed 9 Å away from a DMPC bilayer at two different starting orientations (Fig.1 a) – start 1, and Fig. 2 a) – start 2) and subjected to NTP MD simulations for 150 ps at the elevated body temperature T=312 K, which is believed to promote amyloid structure formations. After 100 ps of MD simulations, the G173-202 fragment at either initial orientation adhered to the DMPC membrane (Fig.1 b), Fig.2 b), retaining β -sheet structure, suggesting that a lipid membrane could bind G173-202 fragments and serve as a germ for amyloidogenesis (Fig.1, Fig.2).

Afterwards one more amyloidogenic fragment G173-202 was placed in the vicinity of G173-202 adhered to the DMPC membrane of the previous system “start-2” (Fig.3 a)), and the new system was subjected to NTP MD simulations at T=312 K for 3744 ps. After 700 ps of MD, the newly added amyloidogenic fragment G173-202 was interacting with the membrane attached fragment G173-202 and after 1465 ps they were bound with hydrogen bounds, indicating the possible mechanism of membrane initiated amyloidosis (Fig 3). Subsequently all systems were placed in a periodic lipid-water box and subjected to MD simulations to investigate of the stability of the structures.

a) 0 ps

b) 99.5 ps

c) 144.5 ps

Fig.1. Molecular dynamics of the DMPC lipid with G173-202, **start 1**, at a) 0 ps, b) 99.5 ps, c) 144.5 ps.

a) 0 ps

b) 100 ps

c) 149.5 ps

Fig.2. Molecular dynamics of the DMPC lipid with G173-202, **start 2**, at a) 0 ps, b) 100 ps, c) 149.5 ps.

a) 0 ps

d) 789 ps

b) 50 ps

e) 1110.5 ps

c) 129 ps

f) 1978 ps

Fig. 3. MD of DMPC lipid with two fragments of G173-202, at a) the start, 0 ps, b) 50 ps c) 129 ps, d) 789 ps, e) 1110.5 ps, f) 1978 ps (to be continued).

g) 2492 ps

h) 3017 ps

i) 3744 ps

Fig. 3. (continued) MD of DMPC lipid with two fragments of G173-202, at g) 2492 ps, h) 3017 ps, i) 3744 ps

CONCLUSIONS

Lipid membrane could bind G173-202 fragments and serve as a germ for amyloidogenesis.

Gelsolin fragments G173-202 could stick together forming β -structure.

Results show that such approach of molecular dynamics is reasonable to model the mechanism of amyloid formations.

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