Journal of Molecular Graphics and Modelling 75 (2017) 340–346

Contents lists available at ScienceDirect

Journal of Molecular Graphics and Modelling

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

Investigating the effect of different transducer stiffness values on the contactin complex detachment by steered molecular dynamics

Parnian Kianfar[a,*], Nabiollah Abolfathi[a], Navid Zarif Karimi[b]

[a] Department of Biomedical Engineering, Amirkabir University of Technology, Tehran 158754413, Iran
[b] Department of Industrial Engineering, Università di Bologna, Bologna 40126, Italy

ARTICLE INFO

Article history:
Received 18 January 2017
Received in revised form 25 April 2017
Accepted 22 May 2017
Available online 2 June 2017

Keywords:
Steered molecular dynamics simulations
CNTN-PTPRG complex
Transducer stiffness
Pulling velocity
Loading rate

ABSTRACT

This study investigated the adhesion behavior of Contactin4 (CNTN4), a member of Immunoglobulin Super Family (Ig-SF) of cell adhesion molecules. Contactin4 plays a crucial role in the formation, maintenance, and plasticity of neuronal networks. Contactin in its complex configuration with protein tyrosine phosphatase gamma (PTPRG) was selected for simulation. By utilizing Steered Molecular Dynamics (SMD), the uniaxial force was applied to induce unbinding of the complex, and the force-induced detachment of complex components was probed. Three sets of simulations with three values of transducer stiffness and five pulling speeds were designed. Our results showed the dependence of unbinding force on both accessible parameters of pulling speed and spring stiffness. By increasing the stiffness value and pulling speed the rupture force increased. Accordingly, the dissociation rates due to the Bell's theory based on rupture forces and loading rates were calculated.

1. Introduction

Studying mechanical properties of biomolecules by using single-molecule measurement techniques, such as atomic force microscopy (AFM) [1], and laser optical tweezers (LOT) [2] received remarkable attentions, and they have significantly advanced our knowledge about biomolecules. Nevertheless, obtaining the atomistic level insight of underlying events is not achievable without utilizing computational modeling. Furthermore, the rare events which have high energy barriers, such as the unbinding process of the protein complex [3], unbinding of complex polymers [4], will not occur in accessible times to computational simulations unless an external stimulus is applied. In the last decade, steered molecular dynamics (SMD) as a complementary method by applying external forces to a variety of biological molecules accelerates the molecular phenomena that are too slow to be modeled by computational modeling [5,6]. SMD has been applied to a variety of biological systems such as protein-protein unbinding phenomena [7], protein unfolding [8], as well as protein-protein interaction [9] in the last decade.

Above all, the resulting pathway induced by external force differs from other types of unbinding such as spontaneously unbinding, chemically or temperature-induced types [10,11]. Unbinding forces induced by external forces are associated directly to the protein complex attractive potential energy, required energy for reaching the critical distance [12]. SMD is particularly useful to cell adhesion molecules, whose major duty is to resist forces from their environment [13]. Literature that investigated the unbinding and unfolding process in protein complexes with SMD simulations, probed several protein complexes; the most recognized complex are Streptavidin-Biotin [10], CD2-CD58 [11], E2020-Acetylcholinesterase [12], Insulin Dimer Dissociation [13], TCR-pMHC [14], NCAM ig12/ig12 [9], Barnase-Barstar [15], MCP-1-Immunoglobulin G (IgG) antibody [16], XMod-Doc:Coh [17]. In addition, some studies focused on protein domains, for instance the unfolding of Ig2 and Ig3 of CNTN4 [18] and unfolding of FNIII domain of Contactin-1 [19] have been carried out.

CNTN4 plays a crucial role in the formation, maintenance, and plasticity of neuronal networks; its gene disruption affects the developmental delay, mental retardation and Autism Spectrum Disorder (ASD) [14,15]. Rapid conduction and efficient propagation of action potential depend on Myelin sheath on axons, which is produced by Oligodendrocytes in the central nervous system (CNS) and by Schwann cells in the peripheral nervous system (PNS) [16]. Myelin sheaths around the axons are separated by nodes of Ranvier. At both sides of the Nodes of Ranvier, the paranodal loops are connected to the axolemma by a complex of two proteins, Contactin-associated proteins (Caspr) [17] and Contactins [18]. This

* Corresponding author.
E-mail address: parnian.kianfar@gmail.com (P. Kianfar).

http://dx.doi.org/10.1016/j.jmgm.2017.05.015
1093-3263/

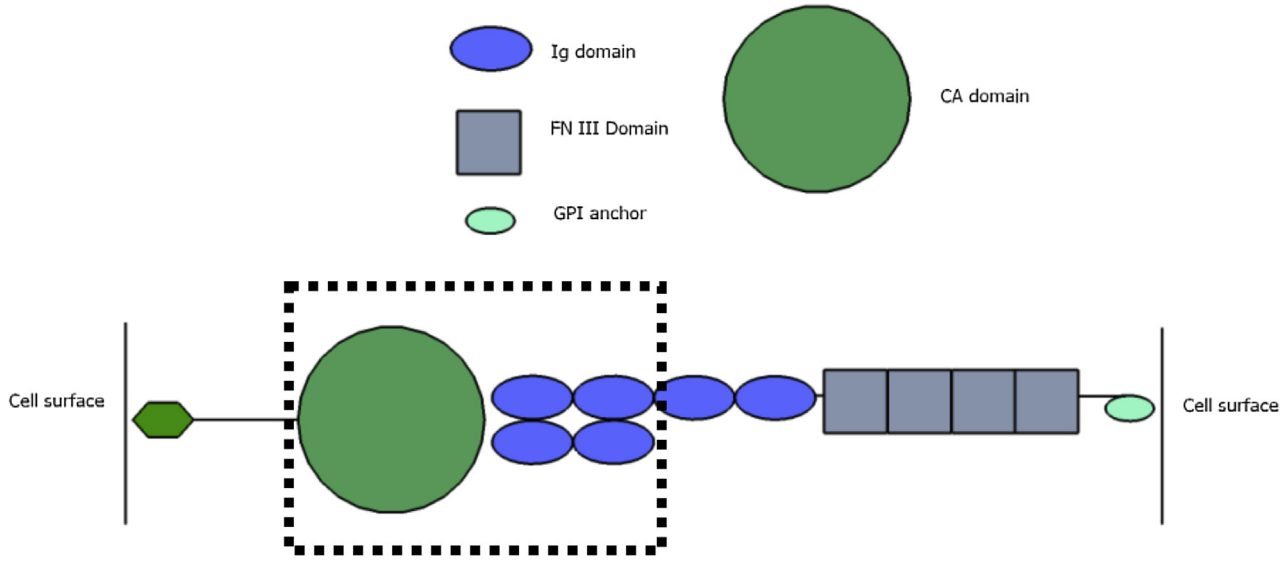

Fig.1. Schematic view of CNTN-PTPRG complex, the CA domain of PTPRG with four Ig domains of CNTN4 are indicated by a dashed rectangle, Ig2 and Ig3 are responsible for adhesive behavior in CNTN4 and make the attachment with CA domain of PTPRG through the salt bridges and H-bonds.

complex is essential for the axoglial junction, and its absence may cause a lack of the septa and an increase of the space between adjacent paranodal loops [19–21]. Reports showed that this complex is essential for the tightening of the junction of two membranes at the paranodal region [16]. The study by Kaneko-Goto et al. revealed that the CNTN4 expression specifically occurred in accessory optic system (AOS) targets. CNTN4 found abundantly in developing the nucleus of optic tract (NOT), and the minor expression was detected in the dorsal medial terminal nucleus (MTNd). Osterhout et al. revealed that the loss of CNTN4 expression brought about the perturbation in projections to the NOT by AOS-projecting RGCs; and in mice carrying mutate CNTN4, the interface of axons with AOS targets was affected. CNTN4 regulates targeting of RGC axons to the NOT. In addition, the CNTN4 mutation caused reduced complexity of arbors which affected the innervation to the target [22].

CNTN4 is composed of six Ig domains and four fibronectin type III domains; CNTN4 is anchored to the plasma membrane of the cell by glycophosphatidylinositol (GPI); Ig2 and Ig3 are responsible for its adhesive character. Generally, Ig domains consist of seven to ten antiparallel β-stands which are between 2-layer sheets with a Greek-key topology [23,24]. Among immunoglobulin domains, Ig2 and Ig3 are in charge of adhesive characteristics of Contactins [23]. Contactins interact with different known ligands, including itself, [25], the adhesion molecules Nr-CAM, Ng-CAM, and neurofascin [26–28], tenascin [29,30]), and PTPR [17–21,31,32]. The members of Protein Tyrosine Phosphatase Receptor (PTPR) family are integral cell-surface proteins, which regulate the intracellular tyrosine phosphorylation activities. These receptors have extracellular carbonic anhydrase (CA) domain that can join to cell adhesion molecules due to sequence homology. Protein Tyrosine Phosphatase Receptor Gamma (PTPRG) has been shown to coordinate the function of other proteins by protein-protein interaction, [33] Studies revealed that PTPRG is a potential ligand for all member of CNTN family of IgG cell adhesion molecules due to biochemical and structural properties. PTPRG is expressed by neurons in the nervous system of mouse embryos and can be found in all sensory organs [34,35].

CNTN4 and PTPRG in their complex have an interface of 1072 A^2 with a shape complementarity coefficient of 0.67 values which is similar to values of antibody-antigen complexes. At the interface, they form 5-starnds antiparallel β-sheet in which two strands of β-hairpin of PTPRG match 3-strand β-sheet in CNTN4 with H-bonds among residues 295–299 PTPRG and residues 139–143 of CNTN4. The hydrophobic region in interface consists of VAL132, LEU142, MET220, TYR223, and LEU250 in CNTN4 and GL288, VAL296, and VAL299 in PTPRG as well as a hydrophilic region that includes ARG135, GLN138, GLU228, and the main chain of SER130 in CNTN4 and ASP294, LYS297, and GLU300 in PTPRG. Moreover, side chains of HSD226 in PTPRG connects the aliphatic compound of LYS226 in CNTN4, besides the residue LYS229 of PTPRG mediates H-bonds and salt bridges with residues GLU224 and ASN304 in CNTN4 based on updated bibliography: [35]. Ig domains of [1–4] CNTN4 have a horseshoe-like conformation. In this study, domains of Ig1 to Ig4 of CNTN4 in complex with CA domain of PTPRG were studied. This complex has 4 segments from which P1 and P2 belong to CNTN and, P3 and P4 belong to PTPRG (Fig. 1).

2. Methodology

Steered Molecular Dynamics (SMD) simulations were carried out using the NAMD program based on updated bibliography: [36] and the CHARMM22 force field based on updated bibliography: [37]. The constant pulling velocity mode of SMD (cv-SMD) was used. The crystal structure of CNTN4/PTPRG complex was taken from the 3KLD entry of the Protein Data Bank based on updated bibliography: [35]. The molecular graphics system setup and data analysis were carried out by VMD program based on updated bibliography: [38]. Initially, the original coordinates were transformed in such a way that the x-axis was placed along the joining line of N-terminal of the first residue of CNTN in complex ILE402, and C-α of LYS297 of PTPRG.

2.1. Solvate

By using the solvate 1.4 package of VMD, the protein complex was solvated in a box of explicit water (TIP3). To keep the complex solvated even after detachment and to retain the water density, a large box was selected. To obtain the box dimension, the primary constant velocity-SMD simulation without solvent was carried out. Thus, the optimized water box dimensions were achieved (180 Å, 60 Å, 40 Å). After solvating the complex in the water box, two Ca^{2+}