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Research Article

Molecular dynamics study of TMPA mediated dissociation of Nur77-LKB1 complex

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ABSTRACT

LKB1 protein is involved in the regulation of cell polarity by phosphorylating the AMPK under energetic stress conditions. LKB1 protein is expressed in both cytoplasm and nucleus. In the nucleus, LKB1 interacts with orphan nuclear receptor protein Nur77. It is reported that the interaction of LKB1 with Nur77 is disrupted by the small molecular ligand TMPA (ethyl 2-[2,3,4-trimethoxy-6-(1-octanoyl)phenyl]acetate), such that the LKB1 is enabled to play its role in cytoplasm and further to regulate/reduce the blood glucose level. In the present study, atomistic molecular dynamics simulations are performed to understand the dissociation mechanism of Nur77-LKB1 complex. The present study reveals that TMPAs induce an open-close motion of Nur77 which further decrease the stability of Nur77-LKB1 complex. As a consequence, the interface region in LKB1-Nur77 complex is more exposed for solvation and further releases the interactions existing between Nur77 and LKB1. Altogether, this study explains the TMPAs mediated Nur77-LKB1 complex dissociation.

1. Introduction

Nur77 is an orphan nuclear receptor, which involves in apoptosis and glucose metabolism by augmenting or modulating insulin sensitivity and hence Nur77 is an attractive therapeutic target for the treatment of type-2 diabetes (Fu et al., 2007). Nur77 localizes LKB1 in the nucleus and this complex assembly regulates gluconeogenesis. It is reported that, when this interaction is disrupted by the ligand, ethyl 2-[2,3,4-trimethoxy-6-(1-octanoyl)phenyl]acetate (TMPA), LKB1 is trafficked to cytoplasm and phosphorylates as well as activates the AMPKα, which in turn lowers or blocks the gluconeogenesis and serves as the therapeutic treatments for type 2 diabetes (Zhan et al., 2012). Structurally, the orphan nuclear receptor, Nur77 has DNA binding domain (DBD) and Ligand binding domain (LBD) as well (Huang et al., 2010). It has been reported that ligand binding domain (LBD) of Nur77 interacts with LKB1 and two TMPAs. While forming complex, the TMPAs interact with the ligand binding domain (LBD) of Nur77 at two sites (referred as site A and site B). The site A is a shallow cleft, which is formed by the helices αH4, αH11 and αH12, while the site B is formed by the helices αH1, αH5, αH7 and αH8. At site A, the side chains of residues Arg515 and Glu445 interact with the carbonyl moiety of TMPA and also both main as well as side chains of residue Thr595 interact with the oxygen atoms of the trimethoxy group of TMPA. At site B, the carbonyl oxygen of TMPA forms H-bond with the side chain of Lys456, while the side chain of His372 interacts with oxygen atom in the trimethoxy group of TMPA. It is also experimentally reported that LKB1 interacts with Nur77 near site A and the residue Thr595 serves as an important residue for the formation of Nur77-LKB1 complex (Zhan et al., 2012).

It is highly important to understand the complex formation of LKB1 with Nur77 and role of TMPAs in the dissociation of the Nur77-LKB1 complex, from the perspective of LKB1 localization in the nucleus. Molecular dynamics (MD) simulations are routinely used to investigate dynamic properties of biomolecular systems (Choudhury et al., 2014; Durrant and McCammon, 2011; Hertig et al., 2016; Karplus and Kuriyan, 2005; Karplus and McCammon, 2002; Karplus and Petsko, 1990). MD has also been used efficiently to study the protein-protein interaction dynamics (Gohlke et al., 2003; Gupta et al., 2016; Lu et al., 2016; Rungsung and Ramaswamy, 2017; Zhang et al., 2016) and is used in this present study to investigate the Nur77-LKB1 interaction. With the available knowledge on the domain organization and interaction of Nur77 and LKB1, the main objective of this present study is to focus on the dynamics of Nur77-LKB1 complex in the presence and absence of TMPAs. The proposed objective is expected to highlight the dissociation mechanism of Nur77-LKB1 complex, which would form the basis to understand the direct role of Nur77 as an attractive therapeutic target for the treatment of type-2 diabetes. The TMPAs mediated dissociation of Nur77- LKB1 complex is studied using atomistic simulations.

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2. Methodology

2.1. Molecular modelling of full-length LKB1 protein

The knowledge on the complete structure of LKB1 is necessary to analyze the site specific recognition of Nur77 while forming complex with LKB1. In this present work, the full length of LKB1 protein structure was modelled using the Homology modelling suite from SWISS-model (Biasini et al., 2014; Guex et al., 2009) as the N-terminal (residues Met1-Arg42) and C-terminal (residues Asp348-Gln433) regions are missing from the available crystal structure. The crystal structure of LKB1 kinase domain (PBD ID: 2WTK at a resolution of 2.65 Å) was used as the template for the homology modelling (Zeqiraj et al., 2009). The full length of LKB1 was generated using the automated SWISS model server using the experimentally reported crystal structure (PDBID:2WTK) as the template. This crystal structure lacks the N-terminal (Met1– Arg42) and C-terminal (Asp348 – Gln433) residues for which the experimental data are not available. Therefore, these regions were generated as random coils. The quality of the modelled full LKB1 is validated using RAMPAGE (Lovell et al., 2003), which showed 79.3% in favored region, 14.2% in allowed region and 6.5% in outlier regions. The residues that are found in the outlier region such as residues from N-ter coil region (Met11, Glu14, Thr24, His27, Pro38, Leu45), β1-β2 loop (Gly58), αD-αE loop (Glu145-Lys146, Pro149), β7-β8 loop (Gly187), β9′-αEF loop (Thr212, Gln220), αEF- β9″ loop (Thr230), αG-αH loop (Tyr272, Gly279), and C-ter coil region (Asp330, Met335, Glu357, Thr363, Glu376, Glu396, Leu400, Ala406, Pro413, Arg425, Leu427, Ala429) belong to the coil and loop regions of LKB1 from both modeled regions and the crystal structure as well. The modelled complete LKB1 structure was allowed to relax for 30 ns and subsequently, the 30 ns LKB1 was docked at the site A of Nur77 using HADDOCK server (de Vries et al., 2010).

2.2. Molecular modeling of Nur77-LKB1 complex based on binding energy

The crystal structure of Nur77 (PDB ID: 3V3Q at a resolution of 2.22 Å) containing two TMPAs at site A and site B was taken as the starting structure (Zhan et al., 2012). The structure of Nur77 and LKB1 complex has not been solved yet. However, the experimental study shows that LKB1 is likely to bind with Nurr77 at the site A (Zhan et al., 2012), whereas the binding site of Nur77 on LKB1 has not been elucidated. Zhan et al. (2012) have demonstrated that the N-lobe of LKB1 is the probable binding site of Nur77 through *in silico* approach (Zhan et al., 2012). As the specific binding site of Nur77 on LKB1 was not known, three regions of LKB1 are specified for docking and are: (i) N-lobe, (ii) C-lobe and (iii) both N- and C-lobes together. HADDOCK applies Ambiguous Interaction Restraints (AIRs) to generate the best possible conformations for the protein-protein complex. The amino acid residues of LKB1 such as Ala43-Ile46, GLy215-Ser216 and Arg74, Phe204, respectively are defined as the active site for the binding of Nur77 during docking. Similarly, the N-lobe residues (Tyr36-Arg42 and Gly47-Asp53), C-lobe residues (Arg211-Gln214 and Ile260-Leu263) and the combined N- and C-lobe residues (Arg106-Ile111 and Glu130-Cys132) were defined as the passive residues while docking. On the other hand, the residue Thr595 from site A was specified as active residue and the residues Leu444-Glu445, Arg515, Leu556, Leu570 and Ile591 of Nur77 were specified as passive residues while generating the three complexes. Here after, the complex in which Nur77 binds to the N-lobe of LKB1 will be referred as Nur77-LKB1 (N-lobe) in the text. Similarly, the complexes Nur77-LKB1 (C-lobe) and Nur77-LKB1 (N-&C-lobes) will be used to indicate the complexes formed by the binding of Nur77 at the C-lobe and N-&C-lobes of LKB1, respectively.

HADDOCK follows a three-step process to generate the complexes, namely (i) rigid body docking (ii) semi-flexible simulated annealing and (iii) refinement in explicit solvent. The generated complexes were allowed to relax using atomistic molecular dynamics simulation for a period of 5 ns. Further, for all these three simulated complexes, the binding free energy of Nur77 while interacting with LKB1 were analyzed for the last 1 ns of the trajectories using the *g_mmpbsa* method (Kumari et al., 2014a). The calculated binding free energy for all these three simulated complexes is tabulated in Table 1. Based on the calculated binding free energy, the most feasible interaction site of Nur77 on LKB1, with the minimum binding energy of -88.26 kcal, is observed to be the N-lobe region. Hence, the Nur77-LKB1 complex, interacting at the N-lobe region, was selected for further molecular dynamics simulation analyses. The Nur77-LKB1 complex was generated using the HADDOCK server. As, the HADDOCK server is capable of performing protein-protein docking, TMPA molecules, which are small chemical molecules, are not recognized. Hence, these two TMPAs were removed from the crystal structure while generating the Nur77-LKB1 complex. The two TMPAs were then docked to both sites A and B by superimposing the newly generated Nur77-LKB1 complex to the original crystal structure using the PyMol visualization tool. This newly modelled Nur77-LKB1 complex, with two TMPAs, is referred as the (TMPA-Nur77-TMPA)-LKB1complex in the manuscript.

Table 1
The binding free energy components (in kcal/mol) of calculated using the in-built tool *g_mmpbsa* in GROMACS.

Energy components	Studied Complex Systems		
	Nur77-LKB1 (N-lobe)	Nur77-LKB1 (C-lobe)	Nur77-LKB1 (N-&C-lobes)
van der Waal	−19.56 ± 1.29	−35.65 ± 2.07	−27.99 ± 1.33
Electrostatic	−113.84 ± 9.21	−84.57 ± 8.91	−40.90 ± 10.66
Polar solvation	47.16 ± 19.27	54.76 ± 6.88	54.39 ± 6.57
Non-polar solvation	−2.0 ± 0.25	−3.24 ± 0	−2.84 ± 0.34
Total binding free energy	−88.25 ± 9.43	−68.68 ± 6.81	−17.36 ± 8.56

In this present work, atomistic molecular dynamic simulations were performed on two systems; (i) Nur77-LKB1 and (ii) (TMPA-Nur77-TMPA)-LKB1 for 75 ns in order to understand the TMPAs mediated disruption of Nur77-LKB1 complex, which is implicated in the medical condition of type 2 diabetes.

2.3. Molecular dynamics simulation

Molecular dynamics simulations have been performed on (i) Nur77-LKB1 and (ii) (TMPA-Nur77-TMPA)-LKB1 complexes (identified from the docking analysis) using GROMACS 4.5 package (Pronk et al., 2013) with Gromos43a1 forcefield (Scott et al., 1999). Generally, proteins are surrounded by solvent in the cytoplasm or nucleus in the natural biological condition and this is required for its biological function. Therefore, the systems were solvated using a water model (SPC water model) to mimic the physiological environment and 4 counter ions (Na^+) were added to neutralize the systems. The systems were energy minimized using steepest descent followed by the conjugate gradient for 100 ps each. The modified Berendsen thermostat (V-rescale) (Bussi et al., 2007) and Parrinello-Rahman pressure coupling algorithm (Parrinello, 1981) were adopted for temperature and pressure coupling, respectively. Temperature and pressure were maintained at 300 K and 1 bar, respectively. Particle Mesh Ewald algorithm was used for the long range electrostatic calculation (Darden et al., 1993). All covalent bonds involving hydrogen atoms were constrained with LINCS algorithm (Hess, 2008; Hess et al., 1997). The van der Waals interactions are treated using a cut-off distance of 14 Å and the systems were equilibrated at NVT and NPT conditions for 100 ps each. After the preliminary relaxation, the systems were allowed for production run for a period of 75 ns. The MD trajectories were analyzed using GROMACS tools like *g_rms, g_rmsf, g_gyrate, g_sas, g_covar, g_anaeig, g_hbond* and *g_mmpbsa*. The domain motions have been analyzed using another

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