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Research Article

In-silico approach to investigate death domains associated with nano-particle-mediated cellular responses

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ABSTRACT

The current research is based on computational study of the Death Domain (DD) superfamily of proteins involved in the cytoplasmic inflammasome complexes associated with apoptosis and inflammation in response to the nanoparticle treatment. The PYD domains of the DD superfamily proteins, NALP3 and ASC, were chosen for investigation as they are found to be primarily involved in the regulation of innate immunity and are associated with apoptosis and inflammation. The in-vitro studies of these proteins have proven to be a challenge as the proteins have a tendency to aggregate under laboratory conditions. The interactions between PYD-PYD domains of NALP3 & ASC proteins as well as PYD-PYD domains of NALP3 and ASC2 proteins were studied using the computational tools. In our study, the protein structures were taken from Protein Data Bank, and molecular dynamics simulation was performed using NAMD software followed by molecular docking studies using HADDOCK. The generated protein models were validated using PROCHECK and then the protein-protein interactions were analyzed using the molecular visualization tool CHIMERA. We noticed that the affinity between PYD of NALP3-ASC complex is better when compared to the NALP3-ASC2 complex based on their binding energies and docking scores. In the case of NALP3-ASC complex, seven key amino acids of ASC-PYD protein interface interact with four key amino acids of NALP3-PYD protein interface. However, in the case of NALP3-ASC2 complex, six key amino acids of ASC-PYD protein interact with four key amino acids of NALP3-PYD protein. Although, there is not much difference in the number of interacting amino acid residues between the two protein complexes, we understand that the NALP3-ASC exhibits better binding interaction and stability than the NALP3-ASC2 protein complex because the number of hydrogen bonding between them is more when compared to the latter. The hydrogen bonds between the interacting amino acids of the NALP3-ASC protein interface are 12, whereas it is 6 in the case of NALP3-ASC2 protein interaction. The protein-protein interaction seems to be dominated by the energetically significant hydrogen bonding followed by the electrostatic interaction in the NALP3-ASC protein interface, whereas both hydrogen bonding and the interaction between charged residues appears to play a significant role at the NALP3-ASC2 protein interfaces.

1. Introduction

Nanotechnology has been helping improve our environment as well as our health. For well over 10 years, nanotechnology has shown much promise towards targeted drug therapy. Our immune system is designed to detect foreign substances in the body and it is comprised of NOD-like receptors (NLRs) which form cytoplasmic complexes called inflammasomes. It is these inflammasomes that detect the foreign agents and activate the apoptotic and inflammatory pathways. The nano-drugs have been found to activate the NALP3 inflammasome (Sun et al., 2013; Peeters et al., 2013; Yazdi et al., 2010). The activation is carried out by the assembly of three proteins-NALP3, ASC and Caspase-1. NALP3 associates with ASC through the PYD-PYD interaction and its expression is restricted to immune cells and chondrocytes (Chu et al., 2001; Faustin et al., 2007; Agostini et al., 2004). The main reason behind the formation of a NALP3 inflammasome is the fact that NALP3, being the best characterized protein out of the 14 NLR proteins containing pyrin domains, is an important sensor of cellular damage or stress (Ferrero-Miliani et al., 2007a; Abderrazak et al., 2015; Lamkanfi and Kanneganti, 2010). Thus, in stressed cells it assembles with an adaptor protein and the protease caspase 1 to form the inflammasome complex. NALP3 responds to signals like ATP, *S.aureus* and *L.monocytogenes*, RNA and uric acid crystals from dying cells (Ferrero-Miliani et al., 2007b).

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Proteins exhibit their roles in various pathways or processes through their interactions with each other as well as with other regulatory molecules, such as DNA. The crucial roles of these proteins contribute to protein interactions, which ultimately determine a healthy or a diseased state of a being. The diseased states such as cancers, cystic fibrosis, Alzheimer's, etc, usually arise due to specific gene mutations which results in the formation of the mutated proteins. Mutated proteins have features such as changes in their active or binding sites or changes in the structural or biochemical properties (Gonzalez and Kann, 2012). It is thus believed that an in-depth investigation of how these proteins interact could yield information about the disease at a nano-scale level. The information thus obtained could prove to be beneficial in the development of effective diagnosis and treatment of diseases like cancers, neurodegenerative diseases and immunological disorders.

Our study aims at investigating the DD superfamily, as well as the apoptotic and inflammatory signaling pathways they are involved in. Both apoptosis and inflammation have been found to be associated with human disease which has led to an uncontrolled in-flux of researches aimed at investigating the structural and biochemical properties of the proteins involved in bringing about apoptosis and inflammation.

The NALP3 inflammasome complex is formed as a result of interaction between PYD domain of NALP3 and PYD domain of ASC in addition to the interaction between CARD domains of both NALP3 and ASC. NALP3 being the most studied inflammasome complex, has a high resolution crystal structure available but the lack of enough structural information has been a limitation to many studies. We chose to study the PYD domains of the DD superfamily since in-vitro studies have proven to be a challenge as the proteins have a tendency to aggregate under such conditions. As a result, we chose in-silico studies to ascertain the structure of PYD domains. A comparison of structural similarities between PYD domains was carried out for two reasons. The primary aim is to ascertain the relevance of the information and if their interactions contribute towards formation of the inflammasome complex; and to determine the conserved surface sequences involved in the interaction and assembly. Together, they help determine the molecular basis of the formation of the inflammasome complex (Park et al., 2007; Hiller et al., 2003; Chu et al., 2015; Vajjhala et al., 2014; Shiohara et al., 2002; Schmidt et al., 2016).

PYD, known as an adaptor molecule, is primarily involved in mediating those interactions involved in apoptosis and inflammation and is involved in the regulation of innate immunity. Studies have demonstrated how inflammatory as well as immunodeficiency diseases are linked to genetic mutations of several NLRs, one of which is NALP3, thus proving the pivotal importance of NALP3 and other NLR proteins in mechanisms involving host defenses (Hugot et al., 2001; Fairbrother et al., 2001; Opitz et al., 2005; Manon, 2006).

In-silico studies are computational means of studying processes or phenomena within the body by either acting as a complement to in-vitro and in-vivo studies. Most commonly, the computational methods have been used to create in-vitro models so as to design advanced molecules possessing a great affinity for the target, or as an alternate to in-vitro and in-vivo studies (Palsson, 2000). For example, computational studies have been extensively done to understand the interaction between the various PYD domains as the in-vitro studies are difficult due to the aggregatory nature of the PYD proteins. However, in this case, biophysical studies may be difficult but have been previously done (Oroz et al., 2016).

Databases, docking studies, molecular visualization softwares, homology modeling, all form the different methods of in-silico studies. Further, in-silico studies helps to determine information related to physiochemical properties, structural properties, pharmacological properties and even information ranging from the atomic level, such as identification of specific atoms involved in binding and other interactions (Ekins et al., 2007).

Our study utilizes the simulation docking method to study the structural similarities between the PYD-PYD domains of the NALP3-ASC and NALP3-ASC2 proteins and the results enable us to understand the molecular mechanisms of formation of the nanoparticle associated NALP3 inflammasome complex.

2. Materials and methods

To study the protein-protein interactions, the high resolution crystal structure of human NALP3 PYD (PDB code: 3QF2; Chain B), NMR structure ASC PYD (PDB code: 1UCP; Chain A) and the NMR structure ASC2 PYD (PDB code: 2HM2; Chain Q) were used as the starting structures. Molecular dynamics (MD) was used to simulate the 3 protein models: 3QF2, 1UCP AND 2HM2. The MD simulation was performed by NAMD (version 2.9) (Phillips et al., 2005).

Using VMD (version 1.9.2) (Humphrey et al., 1996), following the generation of the Protein Structure File (PSF), solvation of the protein in a water box of 5 Å radius, was carried out. This step was followed by ionization at pH 7.0 with an ionic concentration of 0.5 mol/L. Lastly, after energy minimization of 10000 steps, the MD simulation was carried out for 1 nanosecond under constant temperature of 300 K and the ensemble being selected as NVT, i.e., constant volume.

Once simulation was completed, the frame with the lowest potential energy was selected for further analysis. The biomolecular docking program HADDOCK (High Ambiguity Driven Docking version 2.2) expert interface server was used for studying the protein-protein interactions (De Vries et al., 2010; Wassenaar et al., 2012) through the docking simulation.

Based on the previous researches, mostly mutational, NMR and/or crystallographic studies, on NALP3, ASC and ASC2 protein structures, the amino acid residues Glu-13, Lys-21, Arg-41 Asp-48 and Asp-51 were defined as active residues for the ASC and ASC2 proteins and for NALP3 protein, the amino acid residues that makes the conserved hydrophobic patches, namely Leu-17, Leu-22, Pro-33, Pro-34, His-51, Val-52, Ile-59, Gly-63, Ile-78 & Tyr-84 were defined as active residues for interaction with ASC. The passive residues were chosen by the program based on the given active residues (Vajjhala et al., 2012; Bae and Park, 2011; Natarajan et al., 2006; Liepinsh et al., 2003).

The statistics of the top 10 clusters were analyzed and the top cluster that has least binding energy and the most negative Z-score was chosen for analysis. The molecular visualization software, CHIMERA, version 1.10.1, is used for studying and comparing the interaction between the PYD domains of NALP3 – ASC proteins and NALP3 – ASC2 proteins (Pettersen et al., 2004).

The stereochemical quality of the native protein PDB files, docked protein complexes and the MD simulated proteins were compared using the program PROCHECK (Laskowski et al., 1993, 1996). The RMS deviations between backbone atoms of the docked complexes were performed to study the tightness of the protein binding by submitting to SuperPose Version 1.0 (Maiti et al., 2004).

3. Results and discussion

Study of protein-protein interactions (PPIs) is a crucial part of biology as it paves way to understand the molecular mechanisms of cellular processes and helps to investigate the functioning and regulation of proteins in the diseased as well as the normal state. As a result of their importance, there is a higher level of interest in PPIs at the industrial level, mainly for designing and developing proteins for therapy and diagnostics (Dourado and Flores, 2016, 2014).