Biochemical and Biophysical Research Communications xxx (2016) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Conformation switching of AIM2 PYD domain revealed by NMR relaxation and MD simulation

Haobo Wang [b], Lijiang Yang [b, c, **], Xiaogang Niu [a, b, *]

[a] Beijing Nuclear Magnetic Resonance Center, Peking University, Beijing, China
[b] College of Chemistry and Molecular Engineering, Peking University, Beijing, China
[c] Biodynamic Optical Imaging Center, Peking University, Beijing, China

ARTICLE INFO

Article history:
Received 19 March 2016
Accepted 27 March 2016
Available online xxx

Keywords:
AIM2
PYD
NMR
MD
Conformation exchange

ABSTRACT

Protein absent in melanoma 2 (AIM2) is a double-strand DNA (ds DNA) sensor mainly located in cytoplasm of cell. It includes one N terminal PYD domain and one C terminal HIN domain. When the ds DNA such as DNA viruses and bacteria entered cytoplasm, the HIN domain of AIM2 will recognize and bind to DNA, and the PYD domain will bind to ASC protein which will result in the formation of AIM2 inflammasome. Three AIM2 PYD domain structures have been solved, but every structure yields a unique conformation around the α3 helix region. To understand why different AIM2 PYD structures show different conformations in this region, we use NMR relaxation techniques to study the backbone dynamics of mouse AIM2 PYD domain and perform molecular dynamics (MD) simulations on both mouse and human AIM2 PYD structures. Our results indicate that this region is highly flexible in both mouse and human AIM2 PYD domains, and the PYD domain may exist as a conformation ensemble in solution. Different environment makes the population vary among pre-existing conformational substrates of the ensemble, which may be the reason why different AIM2 PYD structures were observed under different conditions. Further docking analysis reveals that the conformation switching may be important for the autoinhibition of the AIM2 protein.

1. Introduction

Absent in melanoma 2 (AIM2) is a major cytosolic dsDNA sensor protein. In response to the invasion of bacteria and virus DNA, AIM2 forms an inflammasome with the apoptosis-associated speck-like protein (ASC) and procaspase-1 to activate proinflammatory cytokine processing. AIM2 is composed of a N-terminal PYD domain and a C-terminal HIN domain. The AIM2 mediated formation of inflammasome is initiated by the recognition of bacteria and virus DNA by its HIN domain, followed by the binding of the PYD domain of AIM2 to the PYD domain of the ASC protein. Subsequently, the inflammasome multiprotein oligomer can elicit a cascade of immune reactions [1–3]. As such, AIM2 inflammasome is essential for the hose defense against DNA viruses and bacteria, such as vaccinia virus, mouse cytomegalovirus, *Francisella tularensis* and *Listeria monocytogenes*, therefore plays an important role in autoimmune disorders such as psoriasis [4–9].

PYD belongs to the death domain (DD) superfamily, which also includes two other subfamilies—the caspase-activation and recruitment domain (CARD) and the death effector domain (DED) [10]. Most PYD domains oligomerize, and interact with other PYD-containing proteins to assemble into oligomeric multiprotein signaling complexes such as inflammasomes [11]. Recently, Jin et al. reported that AIM2 PYD domain could also interact with AIM2 HIN domain to form an autoinhibited intramolecular PYD-HIN complex in absence of dsDNA [12,13]. More than ten PYD domain structures have been solved by now, all of which have a unique short α3 helix that is absent in other DD superfamily members [13].

Three AIM2 PYD domain structures have been solved, including crystal structures of wild type human AIM2 PYD domain (hPYD)

Abbreviation: AIM2, absent in melanoma 2; PYD, pyrin domain; NMR, nuclear magnetic resonance; ASC, apoptosis-associated speck-like protein containing a caspase-activation and recruitment domain (CARD); CARD, caspase-activation and recruitment domain; DED, death effector domain; mPYD, mouse PYD domain; hPYD, human PYD domain; MD, molecular dynamics; PCA, Principal Component Analysis; NVT, constant volume simulation; NPT, constant pressure simulation; PCs, principal components; RMSD, root-mean-square deviation.

* Corresponding author. Beijing Nuclear Magnetic Resonance Center, Peking University, Beijing, China.
** Corresponding author. College of Chemistry and Molecular Engineering, Peking University, Beijing, China.
E-mail addresses: yanglj@pku.edu.cn (L. Yang), niuxg@pku.edu.cn (X. Niu).

http://dx.doi.org/10.1016/j.bbrc.2016.03.131
0006-291X/

and its F27G mutant [13,14], and NMR solution structure of mouse AIM2 PYD domain (mPYD) [15]. Significant structural differences were observed around the α3 helix region between the NMR structure of mPYD and the crystal structures of hPYD [15]. Although a regional amino acid sequence (residue 32~45) identity of 50% between mPYD and hPYD is noticed, the structural differences around α3 helix region should not merely come from the sequence differences because significant differences were also observed between crystal structure of hPYD and its F27G mutant, which share 100% sequence identity in the same region. The high B-factors (up to 120 $Å^2$) of this region in hPYD crystal structure indicated that this segment of polypeptide is highly flexible, which would very likely contributes to the remarkable structure differences [13,14]. Since AIM2 PYD domains tend to oligomerize under neutral pH, all the above structures of AIM2 PYD were determined at relatively low pH environments (pH 4~5). The molecular mechanisms of PYD-mediated signaling events of AIM2 were not fully understood for a long time because of the lack of structural information on PYD-PYD complexes. Recently, a cryo-electron microscopic structure of AIM2 PYD filament was determined at physiological pH environment (pH 8). The structure of AIM2 PYD domain in filament was similar to its crystallography structures except for some minor differences around α2-α3 helix region, which indicated that the structural plasticity around α2-α3 helix of AIM2 PYD domain is important in the PYD−PYD interaction [16].

Why AIM2 PYD domain exhibits different conformations under different conditions? Is this structural plasticity relevant to its biological functions? To address these questions, we herein use NMR relaxation and MD simulations to study the dynamic properties of AIM2 PYD domain. Our results indicate that AIM2 PYD domain undergo significant conformation exchange around the α3 helix region, and the PYD domain may exist as a conformation ensemble in solution. The populations of pre-existing conformational substrates of the ensemble may shift in response to the environment changes. This conformation switching makes AIM2 PYD domain adopts proper conformations to facilitate the transition from the autoinhibited state to the oligomeric state.

2. Materials and methods

2.1. NMR experiments and data processing

Uniformly ^{15}N-enriched *Mus musculus* AIM2 PYD domain was expressed and purified as described previously [15]. The final NMR sample was concentrated to 0.2 mM in a buffer containing 20 mM HAc/NaAc (pH 4.0), 0.01% NaN_3, 90% H_2O and 10% D_2O. NMR relaxation experiments were carried out on a Bruker Advance 700 MHz spectrometer equipped with cryo-probes. The backbone ^{15}N relaxation parameters, including the longitudinal relaxation rates (R_1), transverse relaxation rates (R_2), and steady-state heteronuclear ^{1}H-^{15}N NOE values of mPYD were measured using NMR relaxation experiments collected at 20 °C. The NMR spectra were processed by NMRpipe and analyzed by CcpNmr Analysis [17,18]. The relaxation rate constants were obtained by fitting the peak intensities to a single exponential function using the nonlinear least squares method by CcpNmr Analysis. The ^{1}H-^{15}N NOE experiments were recorded in the presence and absence of a 3 s proton presaturation period prior to the ^{15}N excitation pulse and using recycle delays of 2 and 5 s, respectively. The data were analyzed using the Lipari-Szabo model-free formalism to extract the dynamic parameters [19,20]. Briefly, the ^{15}N relaxation data were interpreted in terms of motion of the NH bond by fitting with five models with increasing complexity. The models include model M1 (S^2), model M2 (S^2, τ_e), model M3 (S^2, R_{ex}), model M4 (S^2, τ_e, R_{ex}), and model M5 (S^2, τ_e, S^2_f), where S^2 is the squared order parameter, τ_e is a correlation time describing the internal motion on ps-ns time scales, S^2_f is a second squared order parameter describing the fast internal motion on ps-ns time scales, and R_{ex} is the chemical exchange contribution to R_2. Standard errors of the dynamic parameters were obtained by Monte Carlo simulations.

2.2. All-atom molecular dynamics simulations

All molecular dynamics (MD) simulations were performed using the Amberff12SB force field with Amber12 [21]. The initial atomic coordinates for mPYD and hPYD were obtained from the protein data bank (PDB codes: 2N00 and 3QF2, respectively). Both models were embedded in a pre-equilibrated truncated octahedron TIP3P explicit water box [22]. Sodium counterions were added to neutralize the system. Periodic boundary condition was applied. The systems were then minimized using steepest descent and then conjugated gradient minimization. Next, the simulation systems were slowly heated from 0 to 300 K over 40 ps of constant volume simulation (NVT), and then allowed to equilibrate with constant pressure simulation (NPT) using PMEMD. 1200 ns of production dynamics was calculated for mPYD and 1000 ns for hPYD. The B-factor analysis and Principal Component Analysis (PCA) were all carried out by CPPTRAJ [23]. PCA is based on diagonalization of the covariance matrix of atomic fluctuations to obtain orthogonal eigenvectors (also called principal modes) and the corresponding eigenvalues [24]. The eigenvalue indicates the magnitude in the direction of the principal mode. The principal components (PCs) are the projections of a trajectory on the principal modes, of which usually the first few ones are largely responsible for the most important motions.

2.3. Computational molecular docking

To evaluate the HIN domain binding affinities to different conformations of AIM2 PYD domain, we used two AIM2 PYD models with identical amino acid sequences as the first docking partners. One model (model A) was the crystal structure of human AIM2 PYD, and the other (model B) was built by SWISS-MODEL web server [25] using the amino acid sequence of human AIM2 PYD and the NMR structure of mouse AIM2 PYD. The structure of human HIN domain (PDB code: 3RN2) without the GB1 tag and DNA parts was used as the second docking partner. The active residues of HIN domain were defined as basic residues responsible for DNA binding (K160, K162, K163, K204, R244, K309, R311, K335), and the active residues of PYD domain were defined as acidic residues (D19, D20, E21, D23) which involved in HIN-PYD interaction in previous mutational analysis [13]. The docking was performed with HADDOCK 2.1 package and followed the standard docking protocol [26]. The final structures were clustered using the pairwise backbone root-mean-square deviation (RMSD) at the interface. A cluster is defined as an ensemble of at least two conformations displaying the backbone RMSD at the interface smaller than 7.5 Å.

3. Results and discussion

3.1. Backbone dynamics of mouse AIM2 PYD domain and model-free analysis

To get detailed information on the dynamic properties of AIM2 PYD domain, we performed backbone ^{15}N relaxation measurements on mouse AIM2 PYD, including R_1, R_2 and ^{1}H-^{15}N NOE values (Fig. 1). R_1, R_2 and NOE values of 66 residues of total 95 residues were calculated, and data of other 29 residues were discarded mainly because of peak overlaps in the NMR spectra. R_1 values were reasonably constant throughout the whole sequence of mPYD, and

Please cite this article in press as: H. Wang, et al., Conformation switching of AIM2 PYD domain revealed by NMR relaxation and MD simulation, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.03.131