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The NMR solution structure of AIM2 PYD domain from *Mus musculus* reveals a distinct α2–α3 helix conformation from its human homologues

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ABSTRACT

The inflammasome is a key component of the innate immune system providing the initial defense against invading organisms. Failure of inflammasome formation is the main reason for many innate and acquired immune diseases. Cytosolic protein absent in melanoma 2 (AIM2) has been reported to play an essential role in double-stranded DNA (dsDNA) sensing and inflammasome formation in response to viruses or bacteria infection. The N-terminal pyrin domain (PYD) of AIM2 interacts with the ASC PYD domain, and then recruits downstream proteins to assemble the AIM2 inflammasome. The molecular mechanisms of PYD mediated signaling remain elusive as limited structural information on PYD family. Herein, we characterized the solution structure of mouse AIM2 PYD domain by NMR spectroscopy, and compared it with the crystal structures of its two human homologues. The comparison shows mAIM2 PYD adopts a unique α2–α3 helix conformation distinct from its human homologues, but similar to the pyrin domain of human NLRP10/PYNOD, which belongs to another family. In addition, the aggregation of mAIM2 PYD domain, with the increased salt concentration, reveals that both the charge surface and hydrophobic interaction play important roles in the self-association of mAIM2 PYD.

1. Introduction

Inflammasomes are signaling platforms which sense a diverse range of microbial products and also a number of stress and damage associated endogenous signals. Inflammasomes are typically composed of an upstream sensor/receptor molecule, an adapter protein known as ASC (apoptosis-associated speck-like protein containing a caspase-activation and recruitment domain (CARD)), and an effector molecule caspase-1 (and/or caspase-11). Upon formation, inflammasomes trigger proteolysis of caspase-1, which subsequently leads to a potent inflammatory response through the maturation and secretion of IL-1 family cytokines, and can then be accompanied by an inflammatory cell death termed pyroptosis [1–3].

Based on the distinct domain architecture, molecule sensors of inflammasomes can be grouped into two families: NOD-like receptors (NLRs) and absent in melanoma 2 (AIM2)-like receptors (ALRs). Most NLRs contain an N-terminal interaction domain (PYD, CARD, or BIR) that interacts with downstream signaling molecules to trigger and regulate inflammatory responses [3], followed by a nucleotide-binding and oligomerization domain (NACHT or NBD), and a C-terminal leucine-rich repeat (LRR) responsible for autoinhibition [4]. ALRs, including AIM2 and interferon-inducible protein 16, can form an inflammasome scaffold without the oligomerization domain in NLRs by clustering upon multiple binding sites in the ligand, dsDNA. AIM2 mainly locates in the cytoplasm of a cell, with a PYD domain at the N terminal and an HIN domain at the C terminal. When the dsDNA from microbes—such as DNA viruses and bacteria—enter the cytoplasm, the HIN domain of AIM2 will recognize and bind to dsDNA, and the PYD domain will bind to the PYD domain of the ASC protein inducing the inflammasome formation and the following immune response [5–7]. AIM2 has been documented to be an important factor in the immune responses against DNA viruses and bacteria, such as the vaccinia virus, mouse cytomegalovirus, *Francisella tularensis* and *Listeria monocytogenes*, as well as a significant role in autoimmune disorders such as psoriasis [8–13].

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PYD belongs to the death domain (DD) superfamily, which also includes two other subfamilies—CARD and death effector domain (DED) [14]. Many PYDs oligomerize and interact with other PYD-containing proteins to assemble oligomeric multiprotein signaling complexes, such as inflammasomes [3]. The molecular mechanisms of PYD-mediated signaling events are not clearly, mainly because of the lack of structural information on PYD–PYD complexes.

PYD domains tend to aggregate at high concentrations. Two crystal structures of the human AIM2 (hAIM2) PYD domain have been reported and overcome the aggregation problem by fusing it with a large maltose binding protein (MBP) tag or making a F27G mutant [15,16]. Here, we present the solution structure of wild type (WT) mouse AIM2 (mAIM2) PYD domain with just an N-terminal His tag. Although the mAIM2 PYD shares a highly similar amino acid sequence with hAIM2 PYD of 56% sequence identity, the solution structure of mAIM2 PYD shows a unique α2–α3 helix conformation distinct from those two crystal structures; and this unique conformation may be related to its functional interaction with ASC.

2. Materials and methods

2.1. Construct design, protein expression and purification

The DNA fragment encoding *Mus musculus* $AIM2^{PYD}$ (residues 1–95) was cloned into the pET28a(+) vector (Novagen) with N-terminal His_6-tag, and expressed in *E. coli* Rosetta (DE3) strain (Novagen) with 0.4 mM isopropyl β-D-1-thiogalactoside (IPTG) induction when OD600 reached 1.0, followed by 8hr incubation at 37 °C. Protein was purified with Ni-NTA Sepharose (QIAGEN) under denaturing condition, followed by renaturing through diluting rapidly into an ice-cold buffer (45 mM citric acid, 50 mM Na_2HPO_4, 10% (v/v) glycerol, at pH 3.7). For the preparation of the NMR samples, M9 minimal media and isotopes (^{13}C-glucose and $^{15}NH_4Cl$) were used during the cell culture. All NMR samples were prepared in 20 mM HAc/NaAc buffer (pH 4.0) with 0.01% NaN_3 including 90% H_2O/10% D_2O.

2.2. NMR spectroscopy and structure determination

All NMR spectra were acquired at 25 °C on Bruker 700 MHz and 600 MHz NMR spectrometer both equipped with cryo-probes. The $^{15}N/^{13}C$-labeled AIM2 PYD domain protein samples were prepared in NMR buffer to a final sample volume of 450 μl and a final concentration of about 0.5 mM. The 2D and 3D NMR experiments including $^{1}H–^{15}N$ HSQC, HNCO, HNCACB, CBCA(CO)NH, TOCSY-HSQC, CC(CO)NH, HC(CO)NH, (H)CCH-TOCSY, and HCCH–COSY were utilized for backbone and side chain resonances assignments [17]. NOE distance restraints were obtained from ^{15}N-edited NOESY-HSQC and ^{13}C-edited NOESY-HSQC spectra (120 ms mixing time). All NMR data were processed with the software NMRPipe [18] and analyzed by the software CcpNmr Analysis [19].

Structure calculation used the distance restraints from $^{15}N–$ and ^{13}C-edited NOESY spectra, and the dihedral angle restraints generated by TALOS + based on chemical shifts of the backbone ^{1}H, ^{15}N, C_α, $C_\beta$and CO atoms [20]. The highly flexible N-terminal His_6-tag was excluded in the structure calculation because there was almost no cross peaks of this part observed in the NOESY spectra. The initial structure was calculated by Cyana 2.1 [21] and further refined by CNS 1.3 [22]. One hundred structures were calculated in this process, and an ensemble of the 20 best structures with the lowest energy was obtained. The final structures were analyzed with PROCHECK [23] and visualized using MOLMOL [24].

The 1D ^{1}H spectra of unlabeled mAIM2 PYD with water suppression were acquired on Bruker 700 MHz spectrometer at a concentration of about 0.1 mM. After first measurement of mAIM2 PYD in 20 mM HAc/NaAc, pH4.0 buffer, 20 μl of 2M NaCl stock was added to the 400 μl sample, which made the NaCl concentration to 100 mM. The first 1D ^{1}H spectrum was recorded immediately, and the final one measured after 20 h. Spectra were processed with Bruker® TopSpin® 3.2.

3. Results and discussion

3.1. Solution structure of mAIM2 PYD

The structure of mAIM2 PYD was determined using 1388 experimental NMR restraints, including 1264 NOE-derived distance restraints and 124 dihedral angle restraints. A summary of the structural statistics for these 20 structures is given in Table 1. Analysis of a Ramachandran plot for the ensemble of 20 structures indicates that all the residues are in the allowed region. The averaged root-mean-square deviation (RMSD) value of the 20 structures to the average structure was 0.84 ± 0.03 Å for the heavy atoms of residues from 1 to 92 (Fig. 1A). The atomic coordinates and NMR-derived restraints of mAIM2 PYD have been deposited in the PDB with accession code 2n00. The structure adopts a six-helix bundle which is conserved in the DD fold superfamily (Fig. 1B).

3.2. Structure comparison between mAIM2 PYD and hAIM2 PYD domains

The secondary structure distribution is very similar to the solution structure of mAIM2 PYD and the crystal structure of its two human homologues [15,16]. The overall RMSD of the mAIM2 PYD solution structure to crystal structure of WT hAIM2 PYD is 1.9 Å and 2.0 Å to the crystal structure of F27G hAIM2 PYD. Superimposed with these two crystal structures, the significant local structural difference was observed at the α2–α3 helix (Fig. 2B). Interestingly, it is also the main difference between the two crystal structures of hAIM2 PYD. In the solution structure of mAIM2 PYD, as shown in Fig. 2C, the α3 helix is close to the N terminal of the α2 helix, while the α3 helix is close to the C terminal of α2 helix in the crystal structure of F27G hAIM2 PYD, while the α3 helix of the crystal structure of WT hAIM2 PYD is located in between of the above two.

Structure comparison shows that the solution structure of mAIM2 PYD adopts a unique conformation of α2–α3 helices distinct from its human homologues. Is this conformation special in the PYD family? We looked through other PYD domains, and found that the α3 helix of the PYD domain in human NLRP10 is also close to the N terminal of its α2 helix [25].

Table 1
Structural statistics for the final 20 simulated annealing structures of mAIM2 PYD.

	mAIM2 PYD domain
NOE distance restraints	
All	1264
Short range (|i-j| ≦ 1)	692
Medium range (1 <|i-j|< 5)	369
Long range (|i-j| ≧ 5)	203
Dihedral restraints	124
Averaged RMSD from the average coordinate (Å)[a]	
Backbone atoms (N, C_α, C_β,C′, O)	0.40 ± 0.05 Å
Heavy atoms	0.84 ± 0.03 Å
Ramachandran plot (%)	
Residues in most favored regions	92.3
Residues in additional allowed regions	7.7
Residues in generously allowed regions	
Residues in disallowed regions	

[a] Calculation of RMSD used residues 1–92.