Structure of the PRYSPRY-domain: Implications for autoinflammatory diseases

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Received 18 November 2005; revised 22 November 2005; accepted 23 November 2005

Available online 9 December 2005

Edited by Hans Eklund

Abstract We determined the first structure of PRYSPRY, a domain found in over 500 different proteins, involved in innate immune signaling, cytokine signaling suppression, development, cell growth and retroviral restriction. The fold encompasses a 7-stranded and a 6-stranded antiparallel β-sheet, arranged in a β-sandwich. In the crystal, PRYSPRY forms a dimer where the C-terminus of an acceptor molecule binds to the concave surface of a donor molecule, which represents a putative interaction site. Mutations in the PRYSPRY domains of Pyrin, which are responsible for familial Mediterranean fever, map on the putative PRYSPRY interaction site.

Keywords: PRYSPRY; Crystal structure; FMF diseases; Multiple wavelength anomalous dispersion

1. Introduction

In 1997, sequence alignment studies [1] revealed a protein domain present in many phyla, from yeast to human. This domain was named SPRY because it occurs in a Dictyostelium discoideum dual-specificity kinase termed spkA and in ryano-dine receptor subtypes. Homologous sequences to the SPRY domain were also discovered independently and the family was named B30.2-like domain family [2].

Currently there is no structural information available for this domain although it was found in more than 500 different proteins that can be classified into five families (Fig. 1). The SPRY domain is present for example in butyrophilin-related transmembrane glycoproteins (BTN) [3] and in stonustoxin SNTX, a cytolytic secreted protein [4]. Another set of proteins containing a SPRY domain are SSB proteins (SPRY domain associated with a SOCS box) belonging to the family of suppressors of cytokine signaling (SOCS) [5]. Recently it was shown that SSBs bind target proteins and mediate their ubiquitination which is followed by degradation [6]. Another large and diverse family of proteins containing RING-finger motifs, zinc-binding β-box motifs and α-helical coiled-coil domains, known as the RBCC family, was found to carry a C-terminal SPRY domain [7,8]. Proteins from the RBCC family are generally putative transcription factors involved in cell growth regulation and differentiation [9,10]. They can be divided into several subfamilies. One of these subfamilies is the tripartite motif (TRIM) protein family [11]. Through a systematic expression analysis of TRIM genes it was found that the common function shared by these proteins is the identification of specific cell compartments by means of homo-multimerization. One family member, TRIM5α, has been identified to be involved in HIV-1 restriction [12]. Very recently, it was shown that TRIM5α from rhesus monkey can restrict HIV-1 replication [13] and that a single amino acid substitution located on the SPRY domain of human TRIM-5α can also confer this ability [14].

In the last years evidence accumulated that a newly identified family of genes, called the PYRIN family, is involved in several hereditary diseases, among them the familial Mediterranean fever syndrome (FMF) [15]. Periodic fever syndromes are autoinflammatory disorders, characterized by recurrent attacks of systemic inflammatory reactions without an infection or autoimmune. In 1997 the gene responsible for FMF was identified and named MEFV [16]. It codes for the protein called Pyrin (Marenostrin), which belongs to the RBCC family and contains a PRYSPRY-domain [7]. It could be shown that mutations in the PRYSPRY-domain are responsible for the FMF syndrome.

What is generally referred to as the SPRY domain in the literature is in fact a PRY segment of 61 amino acids followed by a 139 amino acid-long SPRY segment (UniProt, [17]). We will use here the name “PRYSPRY-domain” for the approximately 200 amino acid-long homologous sequences found in all above mentioned proteins. The presence of the PRYSPRY-domain in functionally unrelated proteins may indicate that the domain serves different functions. Although the sequence identity between PRYSPRY-domains in the described protein families is low (around 20%) secondary structure prediction methods suggest that the domain consists of 15 β-strands and has an immunoglobulin-like fold [7].

Since this domain plays a role in innate immune signaling we decided to elucidate its structure. The human gene of the PRYSPRY-19q13.4.1 domain, here simply referred to as the PRYSPRY-domain, is located on chromosome 19 between the NALP9 and NALP11 genes. In this paper, we describe the structure of this domain, its relationship to other homologous domains and we discuss where mutations responsible for FMF and HIV restriction map in PRYSPRY homology models of Pyrin and TRIM5α.
2. Materials and methods

2.1. Protein expression and purification

The human PRYSPRY-domain of the gene 19q13.4.1 (Est number CA454993, residues 1–201) was expressed in *Escherichia coli* BL21(DE3) cells. Selenomethionine-labeled GST tagged PRYSPRY was prepared in M9 media supplemented with specific amino acids [18] at 37 °C. 20 min before induction with 1 mM of IPTG, L-selenomethionine (50 mg/l) was added and the cells were grown for an additional 24 h at 20 °C. The protein was extracted from the soluble fraction of lysed cells using a glutathione sepharose column (Amerham Biosciences) and then incubated overnight at room temperature with recombinant/GST-tagged PreScission Protease (Amersham Biosciences) to cleave the GST moiety. The protein was further purified using ion exchange chromatography which yielded highly pure PRYSPRY.

2.2. Biochemical and biophysical characterization

2.2.1. Investigation of the solution state of PRYSPRY. Dynamic light scattering measurements at 25 and 4 °C on a DynaPro Titan (Wyatt Technology) revealed for a concentrated PRYSPRY-solution (25 mg/ml) a hydrodynamic radius of 2.6 nm with a polydispersity of 20–25%. For a diluted sample (2 mg/ml) the hydrodynamic radius and the polydispersity decreased to 2.3 nm and 17%, respectively.

### Table 1

Statistics for MAD data collection and structure determination of PRYSPRY (P4122)

<table>
<thead>
<tr>
<th></th>
<th>Peak</th>
<th>Inflection</th>
<th>Remote</th>
<th>“Native”</th>
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<tr>
<td><strong>Data collection</strong></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Wavelength (Å)</td>
<td>0.9786</td>
<td>0.9793</td>
<td>0.9001</td>
<td>0.8999</td>
</tr>
<tr>
<td>a, b, c (Å)</td>
<td>77.02, 77.02, 296.92</td>
<td>77.07, 77.07, 296.95</td>
<td>77.06, 77.06, 296.04</td>
<td>77.22, 77.22, 297.30</td>
</tr>
<tr>
<td>Resolution (Å)</td>
<td>20–2.78 (2.88–2.78)</td>
<td>20–2.78 (2.88–2.78)</td>
<td>20–2.78 (2.88–2.78)</td>
<td>34–2.52 (2.61–2.52)</td>
</tr>
<tr>
<td>R*sym</td>
<td>9.5 (26.7)</td>
<td>10.5 (28.0)</td>
<td>8.1 (41.2)</td>
<td>8.1 (50.2)</td>
</tr>
<tr>
<td>R*free</td>
<td>13.3 (7.3)</td>
<td>13.3 (6.7)</td>
<td>9.7 (3.8)</td>
<td>16.1 (3.1)</td>
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<tr>
<td>Completeness (%)</td>
<td>98.8 (99.4)</td>
<td>98.7 (99.4)</td>
<td>97.0 (98.9)</td>
<td>96.4 (99.0)</td>
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<tr>
<td>Redundancy</td>
<td>5.1 (4.7)</td>
<td>5.1 (4.9)</td>
<td>3.5 (3.5)</td>
<td>4.3 (4.3)</td>
</tr>
</tbody>
</table>

**Refinement**

|                           |        |            |          |          |
| Resolution (Å)             | 30.46–2.52 | 30329/1502 | 22.7/27.6 |          |
| No. reflections (work/test)| 775    | 243        | 0.009    | 1.8      |
| Ramachandran plot          |        |            |          |          |
| Residues in most favoured regions (%) | 83    |            |          |          |
| Residues in additional allowed regions (%) | 16    |            |          |          |
| Residues in generously allowed regions (%) | 1    |            |          |          |
| Ramachandran outliers (%)  | 0      |            |          |          |
Analytical gel filtration using a Tricorn Superdex 75 column (30 cm, Amersham Biosciences) showed the presence of monomeric (approx. 23 kDa) and dimeric (approx. 48 kDa) species. The protein was eluted with 50 mM Tris–HCl (pH 7.0), 150 mM NaCl, 1 mM EDTA, 0.5 mM TCEP at 4 °C.

2.2.2. Crystallization of PRYSPRY. Selenomethionine-incorporated PRYSPRY was crystallized by vapor diffusion at 4 °C against a well solution containing 0.1 sodium cacodylate–AcOH (pH 6.5), 0.55 M KSCN, 25% (v/v) PEG 2000 MME using the sitting drop technique. Crystals of suitable size were obtained after 7 days. The crystals belong to the tetragonal space group P4122, with cell dimensions a = b = 77.22 Å and c = 297.30 Å.

2.3. Data collection and structure determination

A multiple wavelength anomalous dispersion (MAD) dataset to 2.8 Å resolution (Table 1) was collected from a single crystal of selenomethionine-labelled PRYSPRY at the X06SA beamline of the Swiss Light Source (PSI, Villigen, Switzerland). A further dataset was collected at 2.5 Å resolution from another single crystal. Data were integrated with DENZO [19], scaled with SCALEPACK [19] and further processed using the CCP4 package [20].

The structure of PRYSPRY was solved with SHELXS [21] and Auto-Sharp [22]. The four molecules in the asymmetric unit were manually completed and rebuilt using the program O [23]. The model was refined with CNS [24] to a final resolution of 2.5 Å with Rwork/Rfree = 22.9/27.9%. Model geometry and stereochemistry were checked with PROCHECK [25]. The coordinates were deposited at the Protein Data Bank (PDB ID: 2FBE).

2.3.1. Alignment and mutation models. Sequences were aligned using ClustalX [26]. Model structures of the Pyrin and the TRIM5α PRYSPRY-domain were created with MODELLER [27] using the PRYSPRY structure as a template and the alignment described above.

3. Results and discussion

3.1. Structure determination of PRYSPRY

The structure of PRYSPRY was solved by MAD and refined at 2.5 Å resolution with Rwork/Rfree values of 22.9% and 27.9%, respectively. Crystallographic statistics are given in Table 1.

The asymmetric unit contains four molecules that form two nearly identical pairs of dimers. Nine residues of the C-terminus of the first PRYSPRY bind to the surface of a second PRYSPRY molecule (Fig. 3A). The final model contains...
Fig. 3. (A) Stereo view of a ribbon and surface diagram of two interacting PRYSPRY domains. The acceptor molecule appears in blue and the donor molecule in orange. (B) Ribbon diagram of the PRYSPRY dimer in comparison with pea lectin showing the position of a bound mannose (in CPK orange) in the binding site (PDB code 1RIN). Pea lectin is represented in the same orientation as the acceptor PRYSPRY-domain. (C) Stereo view of the interaction of the donor C-terminal tail with the acceptor molecule. (D) Stereo view of the interaction between Trp 122 and Pro 120 of the acceptor molecule with Pro 2, Tyr 144, Val 146, Gly 149 and Phe 8 of the donor molecule.
residues 1 to 188/190 for acceptor molecules 1 and 3 and residues 1 to 198/199 for donor molecules 2 and 4. The unbound C-terminal residues 189/191–201 of molecules 1 and 3 as well as the residues 199/200 to 201 of molecules 2 and 4 are not visible in the electron density map.

3.2. Fold and overall structure of PRYSPRY

The PRYSPRY-domain has a jellyroll β-sandwich fold consisting of a convex 7-stranded antiparallel β-sheet and a concave twisted 6-stranded antiparallel β-sheet packed against each other. The two β-sheets form a central flat and elongated hydrophobic core. The 13-stranded β-sandwich structure represents one compact domain and is not divided into a N-terminal PRY domain and a C-terminal SPRY domain (UniProt, [17]).

The PRY segment of the structure starts with a long loop of 22 residues and continues with three β-strands (β1 to β3 of the β-sandwich, Fig. 2). The SPRY segment consists of 10 β-strands continuing the β-sandwich fold. The strands are connected by loops of varying length. For a donor molecule the C-terminal part of PRYSPRY continues in a loop-like fashion and enters the binding site of an acceptor molecule with residues 1 to 188/190 for acceptor molecules 1 and 3 and residues 1 to 198/199 for donor molecules 2 and 4. The unbound C-terminal residues 189/191–201 of molecules 1 and 3 as well as the residues 199/200 to 201 of molecules 2 and 4 are not visible in the electron density map.

The binding site is located on the concave side of the β-sandwich of the acceptor molecule. It is formed by the concave twisted 6-stranded antiparallel β-sheet and is further defined by the end of loop2 (residues 50–53, PRY) and loops 5–7 and 12 (SPRY, Figs. 2A, 3A). The interactions with the C-terminal tail of the donor molecule B are mainly mediated by main chain–main chain hydrogen bonds (Fig. 3C). In addition Pro 120 and Trp 122, located at the entrance of the binding pocket, interact with a hydrophobic region of the donor molecule (Pro 2, Phe 8, Tyr 144, Val 146 and Gly 149, Fig. 3D). The complementarity of the interacting surfaces is 70% (surface area of 701 Å² for the donor and 812 Å² for the acceptor molecule) corresponding to interactions seen in antibody/antigen complexes [28]. Thus, PRYSPRY is probably capable to interact with putative target proteins offering a donor strand with a different sequence.

These interactions affect the entire structure of the donor molecule. Superposition of the donor and acceptor molecule reveals an RMSD of 1.7 Å. Most of the differences are located around the binding site. Upon peptide binding the β-strands 2 (PRY) and 13 (SPRY) slightly rearrange opening the hydrogen bond between Ser 34 N (PRY segment) and Asn 177 C–O (SPRY segment).

As a consequence the C-terminus of the acceptor molecule rearranges so that it cannot bind to a third molecule. The binding molecule itself cannot accept a further molecule because the conformations of the N- and C-termini are fixed. Thus the aggregation stops at the dimer state.

We further investigated the quaternary structure of PRYSPRY in solution by dynamic light scattering and gel filtration. Both methods reveal monomeric and dimeric species for high protein concentrations (25 mg/ml), and only monomers at low concentrations (2 mg/ml) showing that PRYSPRY forms dimers in solution, in a concentration-dependent manner.

3.3. Fold comparison with other proteins

A DALI [29] search revealed that the protein fold of PRYSPRY is most similar to the carbohydrate recognition domain of the ER folding chaperone p58/ERGIC-53 [30], belonging to the agglutinin family (Table 2). In spite of the low sequence identity, usually below 10%, PRYSPRY and the proteins of the top DALI hits share the same core β-sandwich architecture, that of a jelly roll topology (CATH, [31]). Interestingly, in pea lectin [30], a homologue of the CRD of p58/ERGIC-53, an extended binding pocket is located on the concave side of the β-sheet as we see in the PRYSPRY-domain (Fig. 3B).

3.4. Alignment and mutation models of Pyrin and TRIM5α

A ClustalX alignment [26] of 15 PRYSPRY sequences showed that the PRYSPRY-domain can be found in several protein families (Fig. 4). These families can be divided into five groups depending on sequence motifs present before or after the PRYSPRY-domain. Families 2–4 share the highest identity to human PRYSPRY-19q13.4.1 both in the PRY and in the SPRY segments. The PRY segment of TRIM5α is only distantly related to the PRY segment of PRYSPRY and SSB2 contains no PRY segment at all. They are similar only in the SPRY segment. The average identity for the SPRY segment in all families is 20%.

Based on the PRYSPRY structure we created models of the PRYSPRY domains of Pyrin and of TRIM5α to locate mutations causing the FMF disease (involving Pyrin mutations, http://fmf.igh.cnrs.fr/ [32]) and HIV-1 restriction (involving a single mutation in TRIM5α, [12]). Mutations responsible for
Fig. 4. Multiple sequence alignment of proteins containing a SPRY segment (generated with ClustalX [26]). Proteins are grouped by family. Accession numbers: PRYSPRY (human, EST database no. CA454993), xlNF7 (Xenopus laevis, UP: Q92021), huPyrIN (human, UP: O15553), huRFP (human, UP: P14373), prA33 (Plaurodeles waltlii, UP: Q02084), hu30RFP (human, UP: Q01UDY6), huEBBP (human, UP: O95361), huEF (human, UP: Q14258), huTRIM5 (human, UP: Q9CO35), huBTN (human, UP: Q13410), boBTN (bovine, UP: P18892), cpENT1 (Cavia porcellus, UP: Q99PR5), shSNTXa (Synanceia horrida, UP: Q98989), shSNTXb (Synanceia horrida, UP: Q91453) and moSSB2 (mouse, UP: O88838). Sequence alignment was colored according to the properties of the amino-acids using the default values of CHROMA (for chromatic representation of multiple alignments) [40]. Conserved residues appear in the same color.

Table 3

<table>
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<tr>
<th>Residue</th>
<th>591</th>
<th>653</th>
<th>675</th>
<th>680</th>
<th>680</th>
<th>681</th>
<th>692</th>
<th>694</th>
<th>694</th>
<th>694</th>
<th>694</th>
<th>695</th>
<th>726</th>
<th>744</th>
<th>761</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation</td>
<td>I→T</td>
<td>R→H</td>
<td>S→N</td>
<td>M→I</td>
<td>M→L</td>
<td>T→I</td>
<td>Missing</td>
<td>M→I</td>
<td>M→V</td>
<td>Missing</td>
<td>K→R</td>
<td>V→A</td>
<td>A→S</td>
<td>R→H</td>
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</tr>
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</table>

Consensus/60%...
the FMF disease (Table 3, Fig. 5B), mapped onto the Pyrin-PRYSPRY model, surprisingly cluster in loops belonging to the putative PRYSPRY-binding site, affecting binding to unknown target proteins (Fig. 5A and B). This supports our proposal of a ligand-binding function for the previously defined interaction site of PRYSPRY (Fig. 3A, C, and D). In contrast, the mutation leading to HIV-1 restriction in TRIM5α is located outside the putative binding pocket of the PRYSPRY-domain, namely in the PRY segment. Furthermore, the PRY segment of TRIM5α and PRYSPRY are only distantly related, suggesting that the interaction mechanism of TRIM5α is different.

Acknowledgement: We thank the beamline scientist Dr. Clemens Schulte-Briese of the SLS (Villigen) for excellent technical support and the Swiss National Science Foundation for financial support.

References


