Crystal structure study on human S100A13 at 2.0 Å resolution

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Abstract

The S100 protein family is the largest group of calcium-binding protein families, which consists of at least 25 members. S100A13, which is widely expressed in a variety of tissues, is a unique member of the S100 protein family. Previous reports showed that S100A13 might be involved in the stress-induced release of some signal peptide-less proteins (such as FGF-1 and IL-1α) and also associated with inflammatory functions. It was also reported that S100A13 is a new angiogenesis marker. Here we report the crystal structure of the Ca2+-bound form of S100A13 at 2.0 Å resolution. S100A13 is a homodimer with four EF-hand motifs in an asymmetric unit, displaying a folding pattern similar to other S100 members. However, S100A13 has the unique structural feature with all α-helices being amphiphilic, which was not found in other members of S100s. We propose that this characteristic structure of S100A13 might be related to its ability to mediate the release of FGF-1 and IL-1α.

Keywords: S100A13; Crystal structure; Calcium-binding protein; Amphiphilic helix

Calcium-binding proteins with EF-hand motifs are widely present and have many functions within the cell. The S100 proteins are acidic proteins with low molecular weight of 10–12 kDa, forming the largest group of calcium-binding protein families. At least 25 members have been identified so far [1]. Most S100 members present as dimers in solution, and each monomer contains two distinct EF-hand motifs [2,3]. S100 proteins are expressed in vertebrates exclusively and are completely absent in invertebrates [4]. Multiple sequence alignments of S100 proteins revealed four major subgroups in mammals [5]. Crystal structures of S100 members belonging to the first three subgroups have been reported [6–14]; however, no crystal structure for the fourth subgroup member has been presented yet.

Human S100A13 was first described by Wicki et al. at 1996 [15]. It belongs to the fourth subgroup of the S100 protein family and is widely expressed in various types of tissues and cells [16]. S100A13 can bind Ca2+, Zn2+, and Cu2+ ions. Previous results indicated that S100A13 is involved in the nonclassical secretion of signal peptide-less proteins fibroblast growth factor I (FGF-1) and interleukin 1α (IL-1α) [17–20]. It may use Cu2+ to facilitate the formation of a multiprotein complex aggregate with FGF-1 that enables FGF-1 to release in response to stress [19,20]. It was also reported that some anti-allergic drugs bind to S100A13 [21], leading to the conclusion that S100A13 is related to inflammatory functions. Moreover, S100A13 was proved to be a new angiogenesis marker, implicating the association between S100A13 and tumourigenesis [22,23]. Recent data showed that the level of S100A13 is altered during brain development [24,25], suggesting it may play a role in nervous system function.

S100A13 has unique features compared with other members of the S100 family. The last 11 C-terminal amino acids contain six lysine and two arginine residues; this positively charged C-terminal region could potentially be involved in the interactions with its target protein. It was reported that a S100A13 deletion mutant lacking its basic residue rich C-terminus fails to bind FGF-1 [18,26].
order to better understand the biochemical function and mechanism of S100A13, we solved the crystal structure of recombinant human S100A13 at 2.0 Å resolution.

Materials and methods

Materials. Escherichia coli M15 cells and pQE-30 plasmid were purchased from Qiagen. Ni-NTA Chelating Sepharose Fast Flow resin, Resource ISO column and Superdex 75 column were obtained from GE Healthcare. Mastoparan from Polistes jadwagae was purchased from Sigma.

Protein expression and purification. The gene sequence encoding S100A13 was amplified by polymerase chain reaction (PCR) and then cloned into the pQE-30 vector with BamHI and HindIII restriction sites. S100A13 was then expressed in E. coli strain M15 after induction with 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 6 h at 37 °C. The cells were harvested by centrifugation at 5000 rpm for 10 min and were lysed by an ultrasonic cell disrupter. The soluble fraction was loaded onto affinity chromatography on a Ni-NTA Chelating Sepharose Fast Flow resin. Proteins eluted from the resin were further purified by hydrophobic interaction chromatography on a Resource ISO column and subsequently by size exclusion chromatography on a Superdex 75 column. The purified S100A13 protein is about 11.5 kDa as measured by SDS–PAGE. S100A13 protein was concentrated to approximately 20 mg/ml in 20 mM Tris–HCl (pH 8.0) for the crystallization trials.

Crystallographic data collection. Crystallographic trials were set up at 277 K using the Hampton Research Index Screen Kit (96 conditions), applying the hanging drop vapor diffusion technique with drops of 1 μl protein solution and 1 μl of crystallization reagent, which were allowed to equilibrate against 500 μl of crystallization reagent in the well reservoir. Conditions number 54 and 55 of the Index Screen Kit delivered multiple small crystals within 2 weeks. Crystals suitable for diffraction studies were obtained using optimized conditions (25% PEGmme550, 10 mM CaCl2, 0.1 M Na-Cacodylate, pH 6.0).

S100A13 crystals were cooled to 100 K using well solution as a cryoprotectant for data collection. Data were collected utilizing a Rigaku R-AXIS IV image plate detector in the National Laboratory of Biomacromolecules, Institute of Biophysics, CAS. Diffraction data to 2.0 Å resolution were integrated and scaled with DENZO and SCALEPACK [27]. The results showed that the crystal belongs to space group P212121 with deposited in the Protein Data Bank with Accession No. 2H2K.

Structure determination and refinement. The structure was solved by molecular replacement with MolRep [28] in the CCP4 suite [29]. The search model S100A6 (PDB Code: 1K96) [7] displays 30% sequence identity to S100A13. Throughout refinement, the agreement between the model and the observed data was monitored by calculating Rmerge based on 10% of the reflections. The initial model was manually rebuilt with the program O [30] and refined by ARP/wARP [31] and refmac5 [32]. The cofactor calcium ions were fitted into difference electron density maps when both the R and Rfree values were about 30%. The new model was further refined with CNS [33] and rebuilt with O [30]. The R and Rfree values dropped to 21.3% and 24.9% between 50 and 2.0 Å in the final model. The stereochemical quality of the refined structure was checked with the program PROCHECK [34]. A summary of the data collection and structure refinement statistics is given in Table 1.

The atomic coordinates of the S100A13 crystal structure have been deposited in the Protein Data Bank with Accession No. 2H2K. Figs. 1–3 were generated using the program PyMol (DeLano Scientific, LLC).

Table 1

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<th>Data collection and refinement statistics of S100A13</th>
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Binding was evaluated over a range of MP-PJ concentrations (160–10 μM) at a continuous flow of 30 μl/min at 25 °C. The eluent contained 150 mM NaCl, 50 mM Hepes (pH 7.4), 1 mM CaCl2 and 0.005% Tween 20. Kinetic parameters were further determined using BIACore evaluation 4.1 software.

Results and discussion

The S100A13 monomer

Human S100A13 is a 98 amino acid residue protein. The monomer consists of four α-helices (H1–H4). The N-terminal EF-hand consists of H1 (residues 8–25), loop1 (residues 26–33) and H2 (residues 34–42). The C-terminal EF-hand consists of H3 (residues 57–67), loop2 (residues 68–71) and H4 (residues 72–92). The hinge region (residues 43–56) joins the two EF-hand motifs (Fig. 1A). The Cα2+-ligands of N-terminal EF-hand are mostly main chain carbonyl O atoms (Ala24 (O), Glu27 (O), Arg29 (O), Ser32 (O), Glu37 (OE1, OE2) and a water molecule (W101)). The Cα2+- ion in the C-terminal EF-hand is largely coordinated by side chain carboxyl groups (Asp64 (OD1), Asn66 (OD1), Asp68 (OD1), Glu75 (OE1, OE2), Glu70 (O) and a water molecule (W102)). The water molecule (W101) forms a hydrogen bond with the side chain of Glu70, thus providing a potential link for cooperativity between the two sites (Fig. 1B), which is similar to the structures of S100A6, A11 and A12 [7,10,11].

A hydrophobic core is formed by three aromatic residues, Phe20, Phe73, and Tyr76, which is conserved in other S100 protein structures. These aromatic residues, along with some hydrophobic residues, such as Val16, Val17, Phe23, Leu33, Phe38, Leu41, Val42, Leu46, Leu50, Leu56, Met60, Leu63, Leu71, and Leu79, form a hydro-
phobic cluster, which is the central core of S100A13 monomer and stabilizes its structure (Fig. 1C).

The S100A13 dimer

The current atomic model contains residues −1 to 91 (number −1 and 0 for the residues encoded by the expression vector) and 3 to 95 for two S100A13 molecules in the crystallographic asymmetric unit, respectively. The N- and C-termini of the protein are flexible and disordered in the crystal structure, which is consistent with the NMR structures of human and mouse S100A13 [26,35].

Comparison between the crystal structure and solution structure of human S100A13 (PDB Code: 1YUT) [35], based on a superposition of the backbone of molecule A, was performed to investigate the differences between the two structures. The results showed that relative to molecule A, molecule B in the crystal structure is tilted about 20° compared with the NMR structure, which might be due to crystal packing.

The interaction between two monomers of S100A13 is largely hydrophobic, which is mainly provided by H4 of one monomer with H1 and H4' helices of another. Six aromatic residues, Phe21, Phe73, Trp77, Phe21', Phe73', and Trp77', line at the monomer-monomer interface and form a hydrophobic pairs. In addition, residues Leu9, Ile13, Val16, Val17 in H1/H1', and Leu79, Ile80, Leu83, Ala84 in H4/H4' further strengthen the inter-monomer hydro-

![Fig. 1](image-url)

![Fig. 2](image-url)
The reader is referred to the web version of this paper. For interpretation of the references to color in this figure legend, the target binding of the S100 family of proteins. [36], suggest that this hydrophobic patch is important for structure of the complex of S100B with a p53 fragment.

The Ca²⁺-S100A13 dimer has mostly polar residues at its surface

Biochemical and structural evidence indicated that the intra-monomer hydrophobic cluster, which is buried in apo-S100 proteins, is partly exposed to solvent upon Ca²⁺ binding and forms a hydrophobic patch at the molecular surface [9]. X-ray structures of complexes of S100A10/annexin II [13], S100A11/annexin I [11], and the NMR structure of the complex of S100B with a p53 fragment [36], suggest that this hydrophobic patch is important for the target binding of the S100 family of proteins.

Ca²⁺-S100A13 dimer has mostly polar residues at its surface, which is a unique surface charge distribution compared with other S100 members. As shown in Fig. 2, one face of the S100A13 molecule displays an extended and continuous positive patch composed of basic residues located in helices H4 and H4’ (Fig. 2A), whereas on the opposite side a dense and continuous negative patch composed of acidic residues located in helices H1 and H1’ is observable (Fig. 2B). This structural feature suggests that the way in which S100A13 interact with its target protein is different from that of other S100 members, it might be mainly via hydrogen bonding interaction instead of hydrophobic interaction. This hypothesis was confirmed by the experimental result that the positive charged C-terminus of S100A13 is vital to the interaction of S100A13 with FGF-1 [26].

Amphiphilic helix and biological implications

The core of the S100 structures consists of four α-helices. Interestingly, all of the four helices of S100A13 display amphiphilic characteristic with distinguishable hydrophilic and hydrophobic faces, which was not found in other members of S100s.

We noticed that the amphiphilic helix is a structural motif characteristic of antimicrobial peptides [37]. Antimicrobials kill bacteria by permeabilizing the cytoplasmic membrane, inducing the formation of transmembrane pores and causing the leakage of cytoplasmic compounds. Some of the antimicrobials are short peptides composed of hydrophobic and basic residues, and can form an amphiphilic helix under special conditions. Although the whole process is not fully understood, it was proposed that the amphiphilic helix firstly interacts with the lipid head groups by its basic residues through electrostatic effect, and subsequently inserts into the lipid bilayer, forming multimeric pores and leading to the leakage of cytoplasmic compounds [38]. It was also reported that Trp may play an important role in the interaction between antimicrobial and cytoplasmic membrane [39].

Among the four helices of S100A13, H1 and H4 have special amino acid composition. H1 comprises mostly acidic and hydrophobic residues; whereas in H4, several basic residues are clustered in the polar region, moreover, a Trp77 in the hydrophobic region is located adjacent to the basic residue cluster (Fig. 3), which implies that the mechanism by which S100A13 induces the release of FGF-1 might be similar to that of antimicrobials. Previous report showed that S100A13 can induce destabilization of membrane liposomes composed of acidic phospholipid, which might be caused by the interaction of its basic residues with pL [40]. We propose that H4 might play an important role in this process by attaching to the membrane via Trp77 and the basic-rich residue cluster and then form transmembrane pore via its amphiphilic helices and destabilizes the cytoplasmic membrane.

Previous experiments indicated that calmodulin, another calcium binding protein, can bind mastoparans with high affinity [41]. Mastoparans are a family of short peptides originally isolated from wasp venom and can induce degranulation of mast cells. Mastoparans are a typical antimicrobial and form an amphiphilic helix as its functional form. So we ask that whether S100A13 can bind mastoparans as calmodulin does? We investigated the interaction between S100A13 and mastoparan from Polistes jadwagae (MP-PJ) using surface plasmon resonance assays. Experimental results showed that S100A13 does bind MP-PJ and has a dissociation constant ($K_d$) of 13.8 µM (Fig. 4). Although the interaction sites of these two protein/peptide are not clear, from the amino acid composition, we propose that H1 of S100A13 might be
involved in interacting with MP-PJ. We also presumed that this interaction might relate to the inflammatory functions of S100A13 or mastoparans; though this hypothesis remains to be fully tested.

Acknowledgments

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References


