Biochemical and Biophysical Research Communications 375 (2008) 124–128

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Crystal structure of ST2348, a CBS domain protein, from hyperthermophilic archaeon *Sulfolobus tokodaii*

Preethi Ragunathan [a,1], Thirumananseri Kumarevel [b,1], Yoshihiro Agari [b], Akeo Shinkai [b], Seiki Kuramitsu [b,c], Shigeyuki Yokoyama [b,d,e,*], Karthe Ponnuraj [a,*]

[a] *Centre of Advanced Study in Crystallography and Biophysics, University of Madras, Guindy Campus, Chennai 600 025, India*
[b] *RIKEN SPring-8 Center, Harima Institute, 1-1-1 Kouto, Sayo, Hyogo 679-5148, Japan*
[c] *Department of Biological Sciences, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan*
[d] *Systems and Structural Biology Center, Yokohama Institute, RIKEN, 1-7-22 Suehiro-cho, Tsurumi, Yokohama 230-0045, Japan*
[e] *Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*

ARTICLE INFO

Article history:
Received 21 July 2008
Available online 6 August 2008

Keywords:
ST2348
Crystal structure ST2348
S. tokodaii CBS domain
Signal transduction
β-Bulges

ABSTRACT

The crystal structure of a hypothetical protein ST2348 (GI: 47118305) from the hyperthermophilic bacteria *Sulfolobus tokodaii* has been determined using X-ray crystallography. The protein consists of two CBS (cystathione β synthase) domains, whose function has been analyzed and reported here. PSI-BLAST shows a conservation of this domain in about 100 proteins in various species. However, none of the close homologs of ST2348 have been functionally characterized so far. Structure and sequence comparison of ST2348 with human AMP-kinase γ1 subunit and the CBS domain pair of bacterial IMP dehydrogenase is suggestive of its binding to AMP and ATP. A highly conserved residue Asp118, located in a negatively charged patch near the ligand binding cleft, could serve as a site for phosphorylation similar to that found in the chemotatic signal protein CheY and thereby ST2348 can function as a signal transduction molecule.

Thermostable enzymes are good candidates for many industrial applications because of their relatively high stability at elevated temperatures both in aqueous medium and in organic solvents. These enzymes are excellent subjects for investigation since they provide information on the thermostability of proteins as well as characteristics of cells living in an acidic environment.

Sulfolobales, the hyperthermophilic archaea inhabit sulfur-rich hot acid springs and grow optimally at pH 2–3 and at a temperature of 80 °C. The *Sulfolobus tokodaii* strain 7, a representative of Sulfolobales, is known to oxidize H_2S to sulfate intracellularly and has been used to treat industrial waste water [1]. Here, we report the crystal structure of a hypothetical protein ST2348 from *S. tokodaii* strain 7 at a resolution of 2.10 Å. ST2348 (15.1 kDa) has several close homologs, all of which contain "CBS domains" (cystathionine β synthase). CBS domains are small motifs that are ubiquitous in all kingdoms of life [2]. The 60-residue domain usually occurs as tandem pairs (Bateman pairs) and associate via hydrophobic interactions between homologous β-sheets and often part of a larger protein. The interface between the two CBS domains forms a cleft that is a potential ligand binding site [3]. Crystallographic studies show that a pair of CBS domain from AMP-activated protein kinase, IMP dehydrogenase-2, and chloride channel CLC2 bind adenosyl moieties such as AMP, ATP, or *S*-adenosyl methionine (SAM) and the ligands are bound at the cleft region [3–6]. Point mutations in CBS domains have been associated with several hereditary diseases in humans, including homocystinuria, retinitis pigmentosa, congential myotonia, idiopathic generalized epilepsy, hypercalciuric nephrolithiasis, classic Bartter syndrome, and Wolff–Parkinson–White syndrome [2]. Despite their clinical relevance, the precise function of CBS domains is still unclear. Recent reports provide insight into the general function of CBS domains pairs as cellular energy status sensors [2]. Although several CBS domain containing proteins have been identified in archaea, none of them were structurally and/or functionally characterized. This is the first report of structure based functional characterization of a CBS domain protein from an archaeal source.

Materials and methods

Cloning, expression, and purification of ST2348. The gene encoding the hypothetical protein (ST2348) from *S. tokodaii* strain 7 was amplified from genomic DNA using PCR. The PCR fragment was digested with NdeI and BamHI and cloned into the pET21a (+)

* Corresponding authors. Fax: +81 45 503 9195 (S. Yokoyama), +91 44 2220 0122 (K. Ponnuraj).
E-mail addresses: yokoyama@biochem.s.u-tokyo.ac.jp (S. Yokoyama), pkarthe@hotmail.com (K. Ponnuraj).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.07.140

expression vector. The plasmid was transformed into the *Escherichia coli* BL21-CodonPlus (DE3)-RIL-X (Stratagene) strain, and the selenomethionine-containing ST2348 protein was overexpressed at mid-log phase by addition of 1 mM IPTG. Harvested cells (15.8 g/3 L) were lysed by sonication in 20 mM Tris–HCl, pH 8.0, 50 mM NaCl, 5 mM β-mercaptoethanol, 1 mM phenylmethylsulphonyl fluoride (PMSF). The sonicated lysate was heat-treated at 90 °C for 13 min to denature most of the contaminant proteins and centrifuged at 15,000 rpm for 30 min at 4 °C to remove cell debris. The supernatant was applied onto a pre-equilibrated HiPrep desalting column, and eluted with 20 mM Tris–HCl buffer (pH 8.0). The protein-containing solutions were pooled and loaded onto a SuperQ Toyopearl 650 M column (Tosoh). The bound proteins were eluted in a linear sodium chloride gradient (0–0.4 M), and the fractions were analyzed by SDS–PAGE. The sample-containing fractions were pooled, desalted, and exchanged with buffer (0.02 M Tris–HCl, pH 8.0). The equilibrated protein was run on a ResourceQ column (GE Healthcare Biosciences), and the bound sample was eluted with a sodium chloride gradient (0.0–0.3 M). The sample-containing fractions were pooled, desalted, and exchanged with buffer (0.01 M potassium phosphate, pH 7.0). The equilibrated protein was applied to a hydroxyapatite column (CHT10-I, Bio-Rad), and the bound sample was eluted with a potassium phosphate gradient (0.01–0.5 M). The eluted sample was concentrated and loaded on a gel filtration column (Superdex 75, GE Healthcare Biosciences), which was pre-equilibrated with 20 mM Tris–HCl, pH 8.0, and 0.2 M NaCl. The homogeneity of the final purified protein was over 99%, as determined by SDS–PAGE. In total, 24.4 mg of protein was purified from a 3 L culture and concentrated to 19.6 mg/ml for crystallization studies.

Crystallization and data collection. Initial crystals of ST2348 were obtained at 20 °C by the sitting drop vapor diffusion method. Crystals were grown by adding 1 μl of protein solution to 1 μl of reservoir solution that contained 16% polyethylene glycol (PEG) 3350, 0.16 M ammonium chloride, pH 6.3. Refinement of this condition yielded diffraction quality crystals within 3 weeks period. Complete multiple anomalous dispersion (MAD) data sets were obtained at 100 K on the RIKEN structural genomics beamline I (BL26B2) at the SPring-8, Hyogo, Japan. The crystals belong to the orthorhombic $P2_12_12$ space group and diffracted to 2.10 Å. Data sets were processed using the HKL 2000 suite [7]. The crystal parameters are given in Table 1.

Structure determination and refinement. The ST2348 structure was solved by MAD phasing using three different wavelength data sets collected at the Se edge. The Se sites were determined using the program SOLVE [8]. The initial phases and their figure of merit (FOM) obtained from SOLVE were input into RESOLVE [8] for solvent flattening and auto-building. Partial model derived from RESOLVE was further improved using the program ARP/wARP [9]. Manual model building was carried out using the program Coot [10]. Refinement of the structure was carried with CNS [11] which involved steps of energy minimization, simulated annealing and individual *B*-factor refinement. The final refinement cycle gave an overall *R* factor of 0.22 (R_{free} = 0.26) for all data up to 2.1 Å resolution. The final model contained two monomers (256 residues) in the asymmetric unit. Final refinement statistics are summarized in Table 1.

Table 1
Data collection and refinement statistics of ST2348

Data collection			
Space group	$P2_12_12$		
Cell dimensions (Å)	a = 51.42, b = 135.27, c = 41.23		
No. of molecules/asu	2		
Wavelength (Å)	0.97897 (peak)	0.97929 (edge)	0.90000 remote (high energy)
Resolution range (Å)	50.0–2.00 (2.07–2.00)[a]	50.0–2.00 (2.07–2.00)	50.0–2.00 (2.07–2.00)
Unique reflections	19618 (1577)	19408 (1441)	19843 (1694)
Redundancy	6.3 (4.50)	6.3 (4.1)	12.7 (12.1)
Completeness (%)	97.1 (79.7)	95.6 (72.1)	96.5 (75.0)
R_{merge} (%)	0.070 (0.273)	0.062 (0.294)	0.065 (0.246)
Refinement statistics			
Resolution range (Å)	39.44–2.10		
Reflections used in the refinement	17,453		
Total number of reflections used for working set	15,719		
R (%)	0.22		
Total number of reflections used for R_{free}	1734		
R_{free} (%)	0.26		
No. of protein atoms	1950		
No. of water molecules	134		
Rmsd bond lengths (Å)	0.006		
Rmsd bond angles (°)	1.2		
Average *B*-factor (Å^2)	38.3		
Ramachandran statistics			
Most favored regions	91.5		
Allowed region	8.5		
PDB ID	2EF7		

[a] Values in parentheses are for the highest resolution shell.

Results and discussion

Protein sequence analysis

A PSI-BLAST query of ST2348 against the non-redundant protein database identified 100 candidates, with identities ranging from 72% to 26%. The 10 closest related proteins were from *Sulfolobus sulfataricus* (GI: 46560096), *Sulfolobus acidocaldarius* (GI: 68566501), *Metallosphaera sedula* (GI: 145701035), *Pyrobaculum islandicum* (GI: 119871520), *Methanocaldococcus jannaschii* (GI: 6626255), *Fulvimarina pelagi* (GI: 116496329), *Mesorhizhobium sp., Pyrobaculum arsenaticum* (GI: 145590267), *Hyperthermus butylicus* (GI: 124026906), *Halothermothrix orenii* (GI: 89160859), *Candidatus cryptofilum* (GI: 170173707), *Thermoproteus neutrophilus* (GI:171184485), and *Bacillus* sp. (GI:189913531). Most of these proteins including the 10 closest homologs, were annotated as either hypothetical or unknown indicating that their biological function is yet to be determined. However, all these belong to a discrete family of proteins called CBS domain proteins (Pfam entry PF00571), which are known to contain a conserved "CBS domain". In ST2348, residues 14–76 constitute one CBS domain whereas residues 1–13 and 77–128 form another CBS domain. The sequence comparison of ST2348 with its closest homologs shows there are nine residues namely Gly36, Gly48, Asp54, Glu52, Arg53, Ala89, Met93, Gly112, and Asp118 that are highly conserved in all species.

Overall fold

The folding of ST2348 molecule is shown in Fig. 1A. It folds into two domains (CBS1 and CBS2) of approximately equal size (64 residues). CBS1 is made up of three helices and a β-sheet composed of four strands of topology 1↑ 2↑ 3 ↓ 4↑. Similarly CBS2 has three α-helices and a β-sheet with strands 5↓ 6↓ 7↑. The CBS1 and CBS2 domains are structurally very identical and superpose with an rms deviation of 2.10 Å on Cα atoms. A cleft measuring approximately 16 Å in length and 10 Å in depth observed between CBS1 and a CBS2 domain constitutes the putative ligand binding region. The cleft is formed by the β-sheets of both CBS1 and CBS2 domains and is composed of hydrophobic and charged residues.

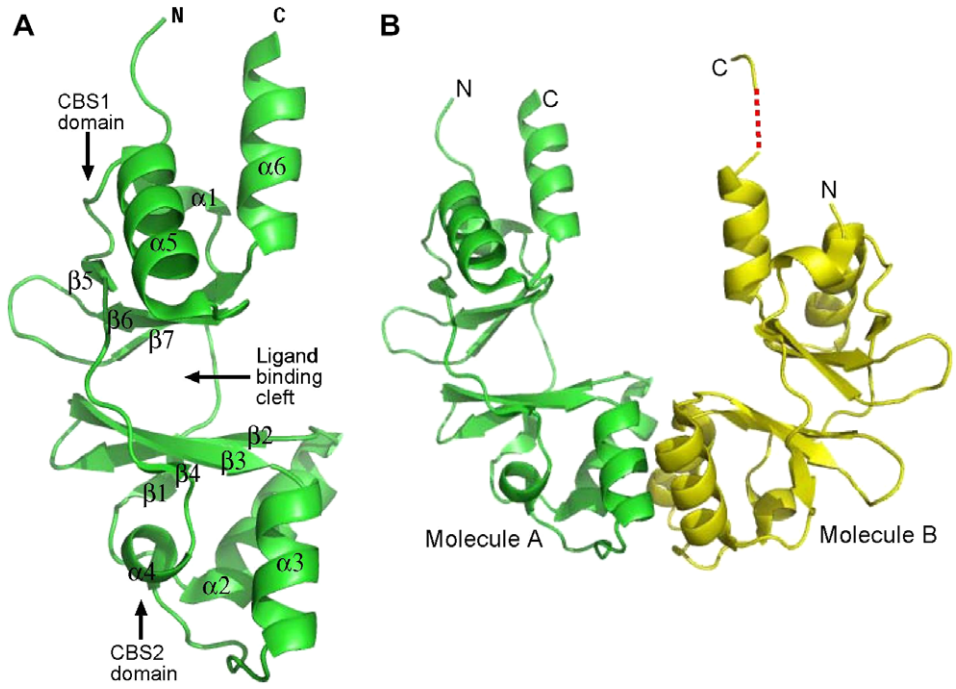

Fig. 1. Structure of ST2348. (A) Ribbon representation of ST2348 monomer. The molecule has two domains namely CBS1 and CBS2. The α-helices and β-strands in the two domains are numbered. (B) Ribbon representation of dimer formation of ST2348 monomers (denoted molecules A and B) in the asymmetric unit of the crystal. The missing region is indicated by red dotted lines. Diagrams were generated using PyMOL (http://www.pyMOLsourceforge.net/). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

The structure contains three β-bulges: two anti-parallel classic β-bulges formed by residues (Val40, Val47, and Gly48) and (Val103, Lys111, and Gly112). A single anti-parallel G1-β-bulge is formed by residues Asp105, Gly108, and Asn109. These β-bulges, at the β-strands 2, 3, and 7 are located within the cleft region and orient the charged side chains towards the interior of the cleft. Formation of these β-bulges provides a slight bend in these strands and could perhaps help the ligand to fit more snuggly in the cleft region.

Oligomeric state of the protein

The asymmetric unit of the crystal contains two ST2348 molecules (denoted A and B) related by a twofold non-crystallographic symmetry (Fig. 1B). Although the biological oligomerization state of ST2348 is unknown, crystal packing suggests that ST2348 might be a tetramer. The crystallographic twofold axis perpendicular to the non-crystallographic symmetry generates the other two monomers (C and D) of another asymmetric unit such that the four monomers (A, B, C, and D) form the tetrameric structure. This tetrameric assembly can be described as an asymmetrically shaped 'doughnut' with dimensions of $84 \times 66 \times 27$ Å (Fig. 2A). In this packing arrangement, two types of intermolecular interactions are observed. In the first type, molecules A and B interact with each other through the exposed residues on helices α2 and α3 of their respective CBS2 domains. In the second type, molecules A and D interact via (i) helices α5 and α6 of their respective CBS1 domains and (ii) through their N- and C-terminal ends.

The dimer in the asymmetric unit (A and B) is stabilized by a combination of hydrogen bonds, ionic, and hydrophobic interactions. The hydrogen bonds at the interface are formed between the following pairs of residues (1) Glu52A (OE2) and Arg53B (NE), (2) Arg53A (NE) and Glu52B (OE2), and (3) Arg53A (NH2) and Glu52B (OE1). The hydrophobic interactions are contributed by residues Ala27, Val56, Ile59, and Leu65 of molecule A with Ala27, Leu23, Ile59, Val56, and Gly60 of molecule B. There are 64 non-bonded contacts (less than 4 Å) between monomers A and D as against 84 contacts between A and B, indicating that the interactions between A and D (or B and C) are less substantial when compared to those between monomers A and B of the asymmetric unit. Calculations of accessible surface area indicate that approximately 1216 and 906 A^2 are buried between monomers A and B (or C and D) and A and D (or B and C), respectively. The 'doughnut' shaped molecular assembly creates a channel structure (Fig. 2A) that passes through center of the tetramer. The central hole has a diameter varying from 12 to 41 Å. The inner surface of the channel is predominately positively charged contributed by arginine and histidine residues that line the channel. The electrostatic surface representation of the ST2348 tetramer is shown in Fig. 2B.

Structure comparison

Proteins with a structure homologous to ST2348 monomer were identified by DALI search [12]. This search revealed 30 candidates that show a *z* score above 1.5. The most structurally conserved proteins were uncharacterized CBS domain proteins such as *Methanococcus jannaschii* MJ0922, (2p9m, *z* score = 17.2, rmsd = 2.5), *Mycobacterium tuberculosis* Rv2626C (1y5h, z score 17.1, rmsd = 2.3), *Bacillus subtilis* BSU14130 (1yav, *z* score = 16.4 and rmsd = 2.5), and *Vibrio cholerae* ACUB (2o16, *z* score = 16.2, rmsd = 3.0). Among the few functionally characterized CBS domain proteins found in the DALI list, the ST2348 monomer closely matches with the AMP-bound CBS domain pair from the regulatory γ1 subunit of human AMPK [13] and bacterial inosine-5′-monophosphate dehydrogenase (IMPDH) [5]. ST2348 monomer and AMPK γ1 subunit (143 residues) superpose with an rms deviation of 1.9 Å for 92 common Cα atoms. Similar comparison with bacterial IMPDH showed an rms deviation of 1.9 Å for 73 common Cα atoms. In both AMPK γ1 subunit and IMPDH, the nucleotide-binding site is located between the two CBS domains and the ligand AMP binds in a pocket with the adenine ring sandwiched between the side chains of hydrophobic residues such as Ile, Val, Pro, Phe, or

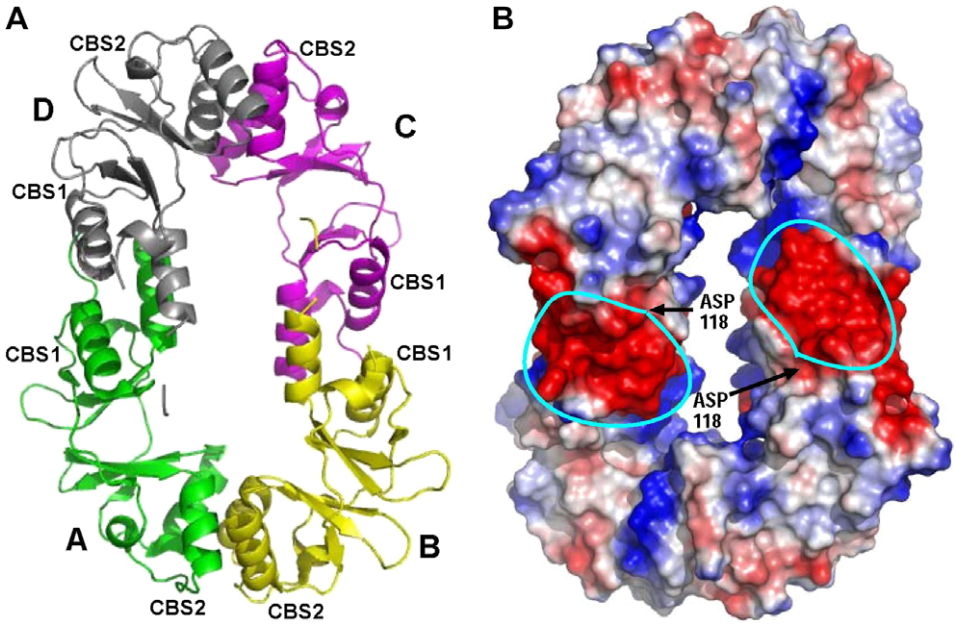

Fig. 2. Tetrameric structure of ST2348. (A) Monomers A, B, C, and D are shown in green, yellow, magenta and gray, respectively. The molecular assembly generates a channel in the middle of the tetramer. (B) Electrostatic surface representation of the tetramer assembly created using Coot [10]. Positively charged regions represented in blue, negatively charged regions in red, and both polar and nonpolar regions are in white. Note that the interior of the channel in predominately positively charged. The position of the highly conserved residue Asp118 is indicated by an arrow and the negatively charged patch near to Asp118 is circled. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

Tyr [3,13]. Further in both complex structures, the hydroxyls of ribose moiety and the phosphate oxygen atoms of the AMP molecule makes several hydrogen bonds with side chains of charged residues like His, Asp, Lys, and Arg and hydroxyl group of Ser that line the surface of the ligand binding region. In ST2348 monomer, cleft between the CBS domains has a similar hydrophobic pocket formed by residues Val14, Ile39, Ile49, Leu110, and Leu113. It also has charged residues such as Lys11, Arg99, and His100. In addition, a novel motif [(Gly-h-x-Ser/Thr-x-Ser/Thr-Asp), where x is any amino acid and h is hydrophobic] for the recognition of ribose phosphate moiety was found in the structural characterization of AMPK γ1 subunit-AMP complex [14]. In ST2348, a similar motif formed by residues Gly112-Ile113-Ile114-Ser115-Ile116-Arg117-Asp118 is located well within the cleft region of the CBS domains. This observation together with the finding of the hydrophobic pocket and a cluster of charged residues at the cleft region of ST2348 strongly suggests that the CBS domains in ST2348 may also be involved in AMP/ATP binding. To find out the possible binding mode of adenosyl molecule in the cleft region of ST2348 monomer, insilico docking was carried out with ATP using the program PATCHDOCK [15]. In the ATP docked ST2348 molecule, the adenosine ring and ribose moiety of the ligand is positioned at one end of the cleft and the phosphate groups are oriented to the other end of the cleft (Fig. 3).

Functional hypothesis

ST2348 monomer is made up of two domains. It has already been reported that a CBS domain pair binds to AMP/ATP and acts as an energy sensor [2]. In general, ATP binding to a protein has two implications, either allosteric control or covalent modification [16]. In ST2348, allosteric mechanism is not very evident due to the absence of other subunits or domains in the protein. In addition, the classification of many structural homologs of ST2348 as putative signal transduction proteins suggest that AMP/ATP binding could be involved in covalent phosphorylation of the protein.

Sequence comparison analysis of ST2348 and its homologs was used to identify the possible sites of phosphorylation. In this analysis, two highly conserved residues Asp54 and Asp118 were observed, indicating that they could be the active sites for other kinases. A similar aspartyl phosphorylation is observed in the chemotactic signal protein CheY of the two-component regulatory system in *Salmonella typhimurium* and *E. coli.* [17] where a highly conserved Asp57 gets phosphorylated by an upstream kinase and regulates its activity. However, in ST2348, the neighboring ORF gene products are annotated as either hypothetical or unknown, indicating the fact that there are no similar upstream kinases in

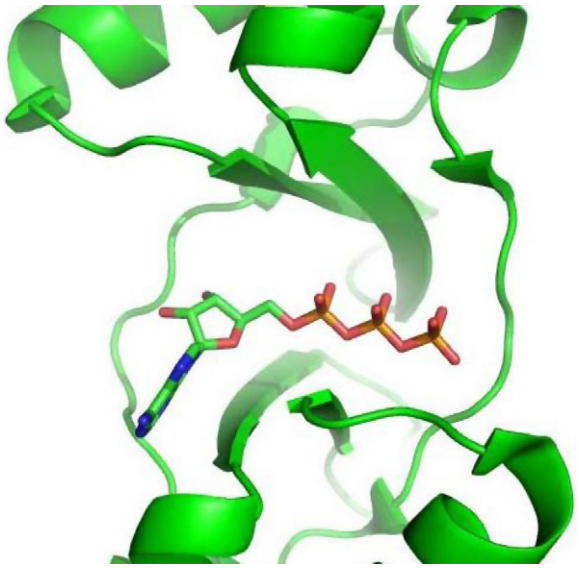

Fig. 3. Docking of ATP with ST2348. Close up view of the cleft region between the CBS1 and CBS2 domains showing the likely ligand-binding mode. The ATP molecule is shown in sticks and the ST2348 molecule is represented in ribbons.

the operon. In CheY, the conserved aspartate is located an acidic patch surrounded by aspartates and is known to serve as a site of phosphorylation [18]. Interestingly in ST2348, the conserved Asp118 is located near the ligand binding cleft and also close to a highly acidic patch suggesting that it could function similar to CheY (Fig. 2B). There are already lines of evidence for the possible existence of a two-component signal system in microbes from the archaeal kingdom [19].

Hence, we hypothesize that ST2348, being an archaeal protein, does not form a part of the regulatory operon as in bacteria. However, it could be involved in phosphotransfer reactions and function as a signal transduction molecule.

Acknowledgments

The authors thank Y. Fujimoto and C. Kuroishi for cloning of the *ST2348* gene; M. Manzoku and S. Baba for crystallization and data collection, respectively. This work was supported by the RIKEN Structural Genomic/Proteomics Initiative (RSGI), the National project on Protein Structural and Functional Analyses, Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

[1] Y. Kawarabayasi, Y. Hino, H. Horikawa, K. Jin-no, M. Takahashi, M. Sekine, S. Baba, A. Ankai, H. Kosugi, A. Hosoyama, S. Fukui, Y. Nagai, K. Nishijima, R. Otsuka, H. Nakazawa, M. Takamiya, Y. Kato, T. Yoshizawa, T. Tanaka, Y. Kudoh, J. Yamazaki, N. Kushida, A. Oguchi, K. Aoki, S. Masuda, M. Yanagii, M. Nishimura, A. Yamagishi, T. Oshima, H. Kikuchi, Complete genome sequence of an aerobic thermoacidophilic crenarchaeon, *Sulfolobus tokodaii* strain7, DNA Res. 8 (2001) 123–140.

[2] Bruce E. Kemp, Bateman domains and adenosine derivatives form a binding contract, J. Clin. Invest. 113 (2004) 182–184.

[3] Robert Townley, Lawrence Shapiro, Crystal structures of the adenylate sensor from fission yeast AMP-activated protein kinase, Science 315 (2007) 1726–1729.

[4] M. Janosik, V. Kery, M. Gaustadnes, K.N. Maclean, J.P. Kraus, Regulation of human cystathionine beta-synthase by *S*-adenosyl-L-methionine: evidence for two catalytically active conformations involving an autoinhibitory domain in the C-terminal region, Biochemistry 40 (2001) 10625–10633.

[5] R. Zhang, G. Evans, F.J. Rotella, E.M. Westbrook, D. Beno, E. Huberman, A. Joachimiak, F.R. Collart, Characteristics and crystal structure of bacterial inosine-5′-monophosphate dehydrogenase, Biochemistry 38 (1999) 4691–4700.

[6] C.P. Ponting, CBS domains in ClC chloride channels implicated in myotonia and nephrolithiasis, J. Mol. Med. 75 (1997) 160–163.

[7] Z. Otwinowski, W. Minor, Processing of X-ray diffraction data collected in oscillation mode, in: C.W. Carter, R.M. Sweet (Eds.), Methods in Enzymology, Macromolecular Crystallography, Part A, vol. 276, Academic Press, New York, 1997, pp. 307–326.

[8] T.C. Terwilliger, J. Berendzen, Automated MAD and MIR structure solution, Acta Crystallogr. D Biol. Crystallogr. 55 (1999) 849–861.

[9] A. Perrakis, R. Morris, V.S. Lamzin, Automated protein model building combined with iterative structure refinement, Nat. Struct. Biol. 6 (1999) 458–463.

[10] P. Emsley, K. Cowtan, Coot: model-building tools for molecular graphics., Acta Crystallogr. D Biol. Crystallogr. 60 (2004) 2126–2132.

[11] A.T. Brunger, P.D. Adams, G.M. Clore, W.L. DeLano, P. Gros, R.W. Grosse-Kunstleve, J.S. Jiang, J. Kuszewski, N. Nilges, N.S. Pannu, R.J. Read, L.M. Rice, T. Simonson, G.L. Warren, Crystallography and NMR system (CNS): a new software system for macromolecular structure determination, Acta Crystallogr. D Biol. Crystallogr. 54 (1998) 905–921.

[12] L. Holm, C. Sander, Mapping the protein universe, Science 273 (1996) 595–603.

[13] P. Day, A. Sharff, L. Parra, A. Cleasby, M. Williams, S. Hörer, H. Nar, N. Redemann, I. Tickle, J. Yon, Structure of a CBS-domain pair from the regulatory [gamma] 1 subunit of human AMPK in complex with AMP and ZMP, Acta Crystallogr. D Biol. Crystallogr. 63 (2007) 587–596.

[14] J. Adams, Z. Chen, B.J.W. Van Denderen, C.J. Morton, M.W. Parker, L.A. Witters, D. Stapleton, B.E. Kemp, Intrasteric control of AMPK via the gamma1 subunit AMP allosteric regulatory site, Protein Sci. 13 (2004) 155–165.

[15] D. Schneidman-Duhovny, Y. Inbar, R. Nussinov, H.J. Wolfson, PatchDock and SymmDock: servers for rigid and symmetric docking, Nucleic Acids. Res. 33 (2005) 363–367.

[16] Peter J. Kennelly, Archaeal protein kinases and protein phosphatases: insights from genomics and biochemistry, Biochem. J. 370 (2003) 373–389.

[17] Gudrun S. Lukat, Benjamin H. Lee, James M. Mottonen, Ann M. Stock, Jeffry B. Stocks, Roles of the highly conserved aspartate and lysine residues in the response regulator of bacterial chemotaxis, J. Biol. Chem. 266 (1991) 8348–8354.

[18] A. Stock, J. Mottonen, J. Stock, C. Schutt, Three dimensional structure of CheY, the response regulator of bacterial chemotaxis, Nature 337 (1989) 745–749.

[19] G. Osorio, C.A. Jerez, Adaptive response of the archaeon *Sulfolobus acidocaldarius* BC65 to phosphate starvation, Microbiology 142 (1996) 1531–1536.