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Crystal structures of a subunit of the formylglycinamide ribonucleotide amidotransferase, PurS, from *Thermus thermophilus*, *Sulfolobus tokodaii* and *Methanocaldococcus jannaschii*

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The crystal structures of a subunit of the formylglycinamide ribonucleotide amidotransferase, PurS, from *Thermus thermophilus*, *Sulfolobus tokodaii* and *Methanocaldococcus jannaschii* were determined and their structural characteristics were analyzed. For PurS from *T. thermophilus*, two structures were determined using two crystals that were grown in different conditions. The four structures in the dimeric form were almost identical to one another despite their relatively low sequence identities. This is also true for all PurS structures determined to date. A few residues were conserved among PurSs and these are located at the interaction site with PurL and PurQ, the other subunits of the formylglycinamide ribonucleotide amidotransferase. Molecular-dynamics simulations of the PurS dimer as well as a model of the complex of the PurS dimer, PurL and PurQ suggest that PurS plays some role in the catalysis of the enzyme by its bending motion.

1. Introduction

Formylglycinamide ribonucleotide amidotransferase (FGAR-AT) is the enzyme which catalyzes the ATP-dependent amide-transfer reaction in the fourth step of the purine-biosynthetic pathway, in which *N*-formylglycinamide ribonucleotide (FGAR), glutamine and ATP are converted to *N*-formylglycinamide (FGAM; Fig. 1; Melnick & Buchanan, 1957; Mizobuchi & Buchanan, 1968). In some Gram-negative bacteria and in eukaryotes, FGAR-AT is a single polypeptide chain, called large PurL, composed of three domains. In contrast, in Gram-positive bacteria and archaea FGAR-AT is composed of four subunits, PurQ, (small) PurL and two PurS subunits in a dimeric form (Ebbole & Zalkin, 1987; Saxild & Nygaard, 2000), which correspond to the counterparts of the three domains of large PurL. In this work, we focused on FGAR-AT composed of four subunits.

PurQ produces an ammonia molecule by converting glutamine to glutamate, and PurL transfers the ammonia molecule to FGAR to form FGAM in an ATP-dependent reaction. One of the characteristics of FGAR-AT is that it requires a third

Table 1
Crystallization conditions.

Protein	<i>TtPurS</i>	<i>TtPurS</i>	<i>MjPurS</i>	<i>StPurS</i>
PDB code	2cuw	2dgb	2yx5	2zw2
Protein sample				
Buffer	20 mM Tris-HCl pH 8.0, 1 mM DTT	20 mM Tris-HCl pH 8.0, 1 mM DTT	20 mM Tris-HCl pH 8.0, 150 mM NaCl, 1 mM DTT	20 mM CAPS pH 9.0, 5 mM β -mercaptoethanol, 150 mM NaCl
Concentration (mg ml ⁻¹)	5.64	7.18	12.74	2.1
SeMet	Yes	Yes	No	No
Cocrystal	No	No	No	No
Reservoir solution	25% PEG 300, 0.2 M NaCl, 0.1 M sodium acetate pH 4.1	0.2 M KNO ₃ , 20% PEG 3350 pH 6.7	25%(v/v)1,2-propanediol, 10%(v/v) glycerol, 5%(w/v) PEG 3000, 0.1 M phosphate-citrate pH 4.9 (Cryo 1 No. 41)	0.1 M HEPES-Na, 0.8 M potassium sodium tartrate tetrahydrate pH 7.5 (Crystal Screen No. 29)
Crystallization	Vapour diffusion, hanging drop, 293 K	Vapour diffusion, sitting drop, 293 K	Vapour diffusion, sitting drop, 293 K	Vapour diffusion, hanging drop, 293 K
Cryoprotectant				20% glycerol

structural component, PurS, for activity (Hoskins *et al.*, 2004). Like other amidotransferases, FGAR-AT is proposed to have an ammonia channel in the PurL structure (Mullins & Raushel, 1999). PurS is thought to be essential for the activity of FGAR-AT (Hoskins *et al.*, 2004). Crystal structures of PurS from *Methanobacterium thermoautotrophicum* (*MtPurS*; PDB entry 1gtg; Batra *et al.*, 2002), *Bacillus subtilis* (*BsPurS*; PDB entries 1t4a and 1twj; Anand, Hoskins, Bennett *et al.*, 2004) and *Thermotoga maritima* (*TmPurS*; PDB entry 1vq3; Mathews *et al.*, 2006) have been determined. The crystal structure of *TmPurS* has also been determined in a complex with PurL and PurQ (PDB entry 3d54; Morar *et al.*, 2008) and, furthermore, the structure of the PurS domain of a large PurL from *Salmonella typhimurium* has also been determined as part of the large PurL structure (PDB entry 1t3t; Anand, Hoskins, Stubbe *et al.*, 2004). However, the role of PurS in the catalytic activity of FGAR-AT is still unknown.

In the present study, four crystal structures of PurS from three prokaryotes, *Thermus thermophilus* (*TtPurS*), *Sulfolobus tokodaii* (*StPurS*) and *Methanocaldococcus jannaschii* (*MjPurS*), were determined and their roles in the enzymatic reaction were discussed based on their structural characteristics.

2. Materials and methods

2.1. Expression and purification of *TtPurS*

The *TtPurS* gene (TTHA1516) was cloned into the pET-11a vector (Novagen). Selenomethionine-derivatized protein was produced in the methionine-auxotrophic *Escherichia coli* strain B834(DE3). The harvested cell pellet of 31.2 g in total was resuspended in 20 mM Tris-HCl pH 8.0, 50 mM NaCl. After heat treatment at 70°C for 10 min, the insoluble fraction was removed by centrifugation. After centrifugation, the supernatant was applied sequentially to Resource ISO 6 ml (GE Healthcare), Resource Q 6 ml (GE Healthcare), Hydroxyapatite CHT5-I (Bio-Rad), HiLoad 16/60 Superdex 75 pg (GE Healthcare) and finally HiPrep 26/10 Desalting (GE Healthcare) columns to obtain 14.8 mg in total of purified protein sample. The purity of the sample was verified using SDS-PAGE.

2.2. Expression and purification of *MjPurS*

The *MjPurS* gene (MJ1593) was cloned into the pET-21a vector (Novagen). The protein was produced in the *E. coli* Rosetta(DE3) strain (Novagen). The harvested cell pellet of 8.7 g was resuspended in 20 mM Tris-HCl pH 8.0, 50 mM

Figure 1
The reaction catalyzed by FGAR-AT.

Table 2

Crystal data and refinement statistics.

Protein	<i>TtPurS</i>	<i>TtPurS</i>	<i>MjPurS</i>	<i>StPurS</i>
PDB code	2cuw	2dgb	2yx5	2zw2
SeMet	Yes	Yes	No	No
Ligands	—	—	—	—
Beamline	BL26B1	—	BM22, APS	BL38B1
Detector	Jupiter 210	R-Axis IV	MAR CCD 225	Jupiter 210
Wavelength (Å)	0.97944, 1.00000, 0.97979	1.5418	0.97901	1.000
Oscillation angle (°)	1	1	1	1
Oscillation range (°)	270	360	180	180
Crystal-to-detector distance (mm)	150	150	200	150
Space group	<i>C</i> 2	<i>P</i> 2 ₁	<i>P</i> 6 ₄ 22	<i>P</i> 2 ₁ 2 ₁ 2 ₁
Unit-cell parameters				
<i>a</i> (Å)	59.72	40.69	96.25	35.82
<i>b</i> (Å)	49.38	90.13	96.25	68.39
<i>c</i> (Å)	43.75	51.51	48.26	68.40
α (°)	90	90	90	90
β (°)	133.56	111.51	90	90
γ (°)	90	90	120	90
Solvent content (%)	51.40	47.58	62.97	36.80
<i>V</i> _M (Å ³ Da ⁻¹)	2.60	2.35	3.32	1.95
No. of protomers in asymmetric unit	1	4	1	2
No. of water molecules	35	168	11	200†
Resolution range (Å)	32.72–1.64 (1.70–1.64)	42.31–2.01 (2.08–2.01)	40.00–2.30 (2.38–2.30)	50.0–1.55 (1.61–1.55)
Completeness (%)	98.4 (96.4)	100.0 (100.0)	99.9 (100.0)	97.3
<i>R</i> _{merge} (%)	5.0 (22.7)	5.4 (15.2)	5.1 (35.5)	3.0 (32.9)
$\langle I/\sigma(I) \rangle$	19.3 (4.1)	30.0 (13.4)	90.7 (10.0)	17.4 (3.3)
Multiplicity	5.5	7.1	20.7	6.9
Resolution range used for refinement	32.55–2.30 (2.44–2.30)	42.31–2.10 (2.23–2.10)	31.54–2.30 (2.44–2.30)	24.74–1.55 (1.65–1.55)
No. of reflections	4124	20214	6000	23650
<i>R</i> factor (%)	23.1 (26.0)	20.9 (21.3)	24.0 (33.0)	21.2 (26.7)
<i>R</i> _{free} (%)	27.5 (30.1)	24.4 (28.0)	28.4 (31.4)	24.2 (28.4)
Deviations from stereochemical ideality				
Bond lengths (Å)	0.006	0.005	0.011	0.005
Bond angles (°)	1.4	1.3	1.5	1.3
Dihedrals (°)	23.3	23.5	24.9	23.70
Impropers (°)	0.72	0.84	0.93	0.68
Ramachandran plot (%)				
Most favoured regions	95.8	93.7	94.5	91.0
Additional allowed regions	4.2	6.3	4.1	9.0
Generously allowed regions	0	0	1.4	0
Disallowed regions	0	0	0	0
MR model	—	2cuw	1t4a	1vq3

† This structure contains two glycerol molecules.

NaCl. After heat treatment at 90°C for 10 min, the insoluble fraction was removed by centrifugation. After centrifugation, the soluble fraction was applied onto Super Q Toyopearl 650M (Tosoh Bioscience LLC), Resource Q 6 ml, Hydroxyapatite CHT10-I and finally HiLoad 16/60 Superdex 75 pg columns to obtain 31.2 mg purified protein sample. The purity of the sample was verified using SDS–PAGE.

2.3. Expression and purification of *StPurS*

The *StPurS* gene (STS178) was cloned into the pET-21a vector. The protein was produced in the *E. coli* Rosetta-gami(DE3) strain. The harvested cell pellet of 190 g in total was resuspended in 20 mM Tris–HCl pH 8.0, 50 mM NaCl. After heat treatment at 70°C for 10 min, the insoluble fraction was removed by centrifugation. After centrifugation, the soluble fraction was applied onto Resource ISO 6 ml, Resource Q 6 ml, Hydroxyapatite CHT5-I, HiLoad 16/60 Superdex 75 pg and finally HiPrep 26/10 Desalting columns to obtain 35 mg purified protein sample. It was noted that the

StPurS protein is easy to precipitate. The purity of the sample was verified using SDS–PAGE.

2.4. Crystallization, X-ray diffraction data collection and data processing of *TtPurS*

Two crystals of the *TtPurS* sample were obtained by the vapour-diffusion method at 20°C. For the first crystal (which gave the structure deposited as PDB entry 2cuw), the crystallization hanging drops were prepared manually by mixing 1 µl protein solution (5.64 mg ml⁻¹) with 1 µl reservoir solution; for the second crystal (which gave the structure deposited as PDB entry 2dgb), the crystallization sitting drops were prepared with an HTS-80 robotic system (Miyatake *et al.*, 2005) by mixing 0.5 µl protein solution (7.18 mg ml⁻¹) with 0.5 µl reservoir solution. The final crystallization conditions are shown in Table 1.

For the first crystal, X-ray intensity data used for phasing and refinement were collected on beamline BL26B1 at SPring-8. A cooled crystal was used to measure 270° of data at

three wavelengths: 0.97944, 1.00000 and 0.97979 Å. Each data frame was collected with a 1° oscillation angle. The crystal-to-detector was 150 mm and detailed conditions are shown in Table 2. The collected data were processed using *CrystalClear* (Rigaku) and the crystallographic parameters are also shown in Table 2. The crystals were found to belong to space group *C2*, with unit-cell parameters $a = 59.72$, $b = 49.38$, $c = 43.75$ Å, $\beta = 133.56^\circ$.

For the second crystal, the X-ray intensity data used for phasing and refinement were collected using a Rigaku ultraX rotating-anode X-ray generator. The anode material used was copper. A cooled crystal was used to measure 360° of data with a wavelength of 1.5418 Å. Each data frame was collected with a 1° oscillation angle. The crystal-to-detector length was 150 mm and details of the conditions are given in Table 2. The collected data were processed using *HKL-2000* (HKL Research) and the crystallographic parameters are shown in Table 2. The crystals were found to belong to space group *P2₁*, with unit-cell parameters $a = 40.69$, $b = 90.13$, $c = 51.51$ Å, $\beta = 111.51^\circ$.

2.5. Crystallization, X-ray diffraction data collection and data processing of *MjPurS*

The crystal of *MjPurS* was obtained by the sitting-drop vapour-diffusion method at 20°C. The crystallization drops were prepared with an HTS-80 robotic system by mixing 0.5 µl protein solution (12.74 mg ml⁻¹) with 0.5 µl reservoir solution. The final crystallization conditions are shown in Table 1.

The X-ray intensity data used for phasing and refinement were collected on SER-CAT beamline 22-BM at the Advanced Photon Source. A cooled crystal was used to measure 180° of data. Each data frame was collected with a 1° oscillation angle. The crystal-to-detector distance was 200 mm and detailed conditions are shown in Table 2. The collected data were processed using *HKL-2000* (HKL Research) and the crystallographic parameters are also shown in Table 2. The crystals were found to belong to space group *P6₄22*, with unit-cell parameters $a = b = 96.25$, $c = 48.26$ Å.

2.6. Crystallization, X-ray diffraction data collection and data processing of *StPurS*

Crystallization of *StPurS* was performed by the hanging-drop vapour-diffusion method by mixing 1 µl protein solution (2.1 mg ml⁻¹) with an equal volume of reservoir solution at 20°C, as shown in Table 1.

The X-ray intensity data used for phasing and refinement were collected on beamline BL38B1 at SPring-8. Diffraction data were collected to a resolution of 1.55 Å on a Jupiter CCD detector. All measurements were carried out on crystals cryoprotected with 20% (v/v) glycerol and cooled to 100 K in a stream of nitrogen gas; other conditions are given in Table 2. The collected data were processed using *HKL-2000* (HKL Research) and the crystallographic parameters are also shown in Table 2. The crystals were found to belong to space group *P2₁2₁2₁*, with unit-cell parameters $a = 35.82$, $b = 68.39$, $c = 68.40$ Å.

2.7. Structure determination of *TiPurS*

The first crystal structure of *TiPurS* was solved by the multiwavelength anomalous diffraction (MAD) method using *SOLVE/RESOLVE* (Terwilliger, 2000, 2003; Terwilliger & Berendzen, 1999). *CNS* (Brünger *et al.*, 1998) was used for refinement cycles. Atomic models were fitted into the electron-density map using the graphics program *Coot* (Emsley & Cowtan, 2004). According to *PROCHECK* (Laskowski *et al.*, 1993) in the *CCP4* suite (Winn *et al.*, 2011), 95.8% of the residues in the final model are in the most favoured region of the Ramachandran plot. Data-collection statistics and processed data statistics are presented in Table 2. The coordinates are available in the Protein Data Bank under accession code 2cuw.

The second crystal structure of *TiPurS* was solved by the molecular-replacement (MR) method using *MOLREP* (Vagin & Teplyakov, 2010) in the *CCP4* suite by using the first structure of *TiPurS* (PDB entry 2cuw) as a model. *CNS* and *Coot* were used for refinement cycles. According to *PROCHECK*, 93.7% of the residues in the final model are in the most favoured region of the Ramachandran plot. Data-collection statistics and processed data statistics are presented in Table 2. The coordinates are available in the Protein Data Bank under accession code 2dgb.

2.8. Structure determination of *MjPurS*

The crystal structure was solved by the molecular-replacement method using *MOLREP* with the crystal structure of *BsPurS* (PDB entry 1t4a) as a model. Model refinement was carried out using *CNS* and *Coot*. 94.5% of the residues in the final model are in the most favoured region of the Ramachandran plot. Data-collection statistics and processed data statistics are presented in Table 2. The coordinates are available in the Protein Data Bank under accession code 2yx5.

2.9. Structure determination of *StPurS*

The crystal structure was solved by the molecular-replacement method using *AMoRe* (Navaza, 1994) in the *CCP4* suite with the crystal structure of *TmPurS* (PDB entry 1vq3) as a model, followed by model reconstruction using *ARP/wARP* (Langer *et al.*, 2008). Model refinement was carried out using *REFMAC* (Murshudov *et al.*, 2011), *Coot* and *CNS*. 91.0% of the residues in the final model are in the most favoured region of the Ramachandran plot. Data-collection statistics and processed data statistics are presented in Table 2. The coordinates are available in the Protein Data Bank under accession code 2zw2.

2.10. Comparison of *PurS* structures

For crystal structures for which only one protomer was found in the asymmetric unit, a dimeric form was prepared based on their crystallographic symmetry using *UCSF Chimera* (Pettersen *et al.*, 2004). The root-mean-square deviation (r.m.s.d.) of C α positions between two *PurS* monomers was calculated using the *MatchMaker* function of *UCSF*

Chimera. The tertiary structures of PurS dimers were superimposed by *UCSF Chimera* with the manually produced multiple sequence alignments based on the crystal structures.

2.11. Molecular-dynamics simulations of PurS dimers

To perform the molecular-dynamics (MD) simulations, a dimeric model without any missing residues was prepared for each PurS. For *TiPurS* (PDB entries 2cuw and 2dgb), the missing Met1 was added and the conformation of Pro2 was modified so that Met1 is located outside the molecule and the proper peptide bond between Met1 and Pro2 is maintained. SeMet residues were then replaced by Met residues. For PDB entry 2cuw, the dimer was produced according to the crystallographic symmetry. For PDB entry 2dgb, subunits *A* and *D* were used as a dimer. For *MjPurS*, the missing Gly49 was inserted and the dimer was produced according to the crystallographic symmetry. Met1, Ser2 and Lys3 were added at the N-terminus of *StPurS*, and Asp89, Ser90, Ser91 and Asn92 were added at the C-terminus. To locate the additional residues outside the molecule and retain the proper peptide bonds, the conformations of Met4 and Glu88 were modified. Additions and modifications of residues were performed using *UCSF Chimera*.

An MD simulation was performed with *AMBER12* (Case *et al.*, 2012). In the preparation steps, the total charge was neutralized by adding sodium ions or chloride ions and the protein was surrounded by TIP3 water molecules in a box with a buffer distance between the wall of the box and the closest atom in the solute of 9.0 Å. The ff94 force field was used. An

energy minimization in 1000 iterations was applied to the system in the presence of positional restraints for the protein molecules with a force constant of 10.0. The temperature of the system was increased to 300 K in 10 ps (10 000 steps) and a constant-pressure simulation was then performed for 50 ps to equilibrate the density of the system, with a force constant of 5.0 for the positional restraints. The force constant for the positional restraints was decreased to 0.5 and a constant-volume simulation in 50 ps was performed. Finally, a productive simulation at constant volume without positional restraints was performed for 10 ns (10 000 000 steps). Rectangular periodic boundary conditions and the particle-mesh Ewald procedure for long-range electrostatic interactions were used, and the nonbonded cutoff was 9 Å. All simulations were performed by the *sander.MPI* module. The trajectory of the productive simulation was processed by *ptraj* in the *AMBER* suite. The positional fluctuations were shown as the *B* factor by multiplying the squared fluctuations by $8\pi^2/3$ using the *ptraj* function.

2.12. Modelling of the active complex and molecular-dynamics simulation of *TiPurS*, *TiPurL* and *TiPurQ*

A homology model of *T. thermophilus* PurQ (*TiPurQ*) was manually prepared using *UCSF Chimera* based on the structure of the PurQ subunit in the complex of PurS, PurL and PurQ from *T. maritima* (PDB entry 3d54; Morar *et al.*, 2008). For *T. thermophilus* PurL (*TiPurL*; PDB entry 3viu; Suzuki *et al.*, 2012), the missing loop with 20 residues was inserted into the structure based on the structure of *T. maritima* PurL

Figure 2

Dimeric structures of PurS. (a) *TiPurS* (PDB entry 2cuw), (b) *TiPurS* (PDB entry 2dgb), (c) *MjPurS* (PDB entry 2yx5), (d) *StPurS* (PDB entry 2zw2). One of the two subunits is coloured cyan in each dimer. For *TiPurS* (PDB entry 2cuw) and *MjPurS* (PDB entry 2yx5), dimeric structures were prepared based on the crystallographic symmetry. For *TiPurS* (PDB entry 2dgb), one of the two dimers is shown.

(*TmPurL*; PDB entry 3d54), in which the structure of the corresponding loop is defined. A structure of the complex of *TiPurS*, *TiPurL* and *TiPurQ* was prepared based on the complex from *T. maritima*; each subunit was superimposed by matching the main-chain atoms of conserved residues using *UCSF Chimera*. The ligand-binding model was prepared as described by Suzuki *et al.* (2012).

An MD simulation was performed as described above except that the productive simulation was performed for 100 ns. The system consists of four subunits with 1120 amino-acid residues in total, an FGAR, two ATP molecules, two Mg^{2+} ions, a glutamine, 40 Na^+ ions and 39 719 water molecules. Positional restraints were used for the protein molecules and all ligands including Mg^{2+} , with a force constant of 10.0 during the preparation steps. The preparative simulations were performed by the *sander.MPI* module and the productive simulation was performed by the *pmemd* module. The trajectory of the productive simulation was processed by *ptraj* in the *AMBER* suite.

3. Results

3.1. Structure determination of *TiPurS*, *StPurS* and *MjPurS*

TiPurS was crystallized in two crystal forms: *C2* and *P2₁*. For the crystal in the *C2* form, the structure was determined to a highest resolution of 2.3 Å using the MAD method (PDB

entry 2cuw). The crystal contained one protomer in the asymmetric unit, and the dimeric structure as a biological unit was obtained by a symmetry operation on the monomer as shown in Fig. 2(a). For the crystal in the *P2₁* form, the structure was determined to a highest resolution of 2.01 Å by the MR method using the previously determined structure as a search model. The *P2₁* crystal contained two dimers (four protomers) in the asymmetric unit (PDB entry 2dgb). One of the two dimers is shown in Fig. 2(b). The two *PurS* structures were almost identical to one another, with an *C α* r.m.s.d. of 0.617 Å for 166 atoms. *TiPurS* has one disordered residue at the N-terminus in each crystal structure (PDB entries 2cuw and 2dgb).

For the crystal of *MjPurS* in space group *P6₄22*, the structure was determined to a highest resolution of 2.30 Å by MR using PDB entry 1t4a as a search model and was deposited as PDB entry 2yx5. The crystal contained one protomer in the asymmetric unit and the dimer was obtained by a symmetry operation as shown in Fig. 2(c). The dimeric structure is essentially the same as that of *TiPurS*. A residue (Gly49) connecting a β -strand and an α -helix of *MjPurS* was disordered.

For the crystal of *StPurS* in space group *P2₁2₁2₁*, the structure was determined to a highest resolution of 1.55 Å by MR using PDB entry 1vq3 as a search model and was deposited as PDB entry 2zw2. The crystal contained one dimer (two protomers) in the asymmetric unit and the dimer structure is shown in Fig. 2(d). The crystal data and refinement statistics are summarized in Table 2. The N-terminal three residues and the C-terminal four residues were disordered in the *StPurS* structure. It is noted that the amino-acid sequence of *StPurS* has been replaced and that Met4 is the first residue in the current sequence data.

3.2. Structures of *PurS*

Each monomer contains three antiparallel β -strands (β 1– β 3) and two helices (α 1 and α 2), as shown in Fig. 3. The overall structure of the monomer has a boomerang-like shape which bends in the central part of the molecule. Helices α 1 and α 2 are located on the outside of the bent structure. On one side of the blade of the boomerang, helix α 2 is located on the three β -strands, while on the other side of the blade helix α 1 is located on the β 2 strand. The dimer structure is formed by the interaction between the β 2 strands of two monomers with an antiparallel direction. The six-stranded antiparallel β -sheet in

Figure 3
Topology diagram of the *PurS* dimer. One of the two subunits is coloured grey.

Figure 4
Sequence alignment based on the tertiary structures. All *PurS*s for which structures have been determined were used in the alignment. Secondary-structure elements identified for all structures are shown at the top. Conserved residues are coloured red. Semi-conserved residues are coloured orange.

the dimer forms a half barrel. In general, the four structures of PurS are similar to each other, with a C α r.m.s.d. of 0.7–1.3 Å (Supplementary Table S1), as well as to the previously determined structures of *BsPurS* (Anand, Hoskins, Bennett *et al.*, 2004), *MtPurS* (Batra *et al.*, 2002) and *TmPurS* (Mathews *et al.*, 2006). In *StPurS*, the $\alpha 1$ helix was slightly disordered because of the insertion of an amino-acid residue, as described below.

3.3. Structural comparison among PurSs

A sequence alignment of *TtPurS*, *StPurS* and *MjPurS*, as well as the three previously determined structures, was obtained with *ClustalW* v.1.83 on the website of the DNA Data Bank of Japan and the sequence identity of each pair of PurS structures was obtained (Supplementary Table S2). The sequence identity among *TtPurS*, *MjPurS* and *StPurS* is 22–38% and the sequence identities of *TtPurS*, *StPurS* and *MjPurS* to the other three PurSs (*BsPurS*, *MtPurS* and *TmPurS*) are 27–32, 32–45 and 21–29%, respectively. The highest identity of 45% was observed between *MjPurS* and *MtPurS*, and the lowest was 21% between *StPurS* and *BsPurS*. Despite the variety in sequence identities, the tertiary structures of PurSs are quite similar to one another, indicating that the structure is important for the function of PurS.

Based on the tertiary structures of six PurS proteins, *TtPurS*, *MjPurS*, *StPurS*, *BsPurS*, *MtPurS* and *TmPurS*, a sequence alignment was produced by *ClustalW* and manually modified as

Figure 5
Comparison of six PurS structures. One of the two subunits is coloured cyan in each dimer. (a) The three conserved residues are coloured red in one subunit. (b) Side chains of the conserved Pro in the $\alpha 1$ helix. (c) Side chains of the conserved Leu and Asn residues in the loop between the $\alpha 2$ helix and the $\beta 3$ strand.

shown in Fig. 4; the position of the gap at the 3'-terminus of the $\alpha 2$ helix was modified for *TtPurS*. It was found that three residues, Pro in the $\alpha 1$ helix and Leu-X-Asn in the loop between the $\alpha 2$ helix and the $\beta 3$ strand, were conserved. As shown in Fig. 5, the side chains of the conserved Pro and Asn residues adopt a similar conformation and face towards the outside of the molecule. On the other hand, the conserved Leu faces the inside of the molecule, probably forming a hydrophobic core. Residues showing amino-acid similarities are indicated in orange in Fig. 4. These residues are predominantly located in the $\beta 1$ strand and the loop between the $\alpha 2$ helix and the $\beta 3$ strand. It is noted that the Pro residue in the $\alpha 1$ helix and the Asn residue in the loop between the $\alpha 2$ helix and the $\beta 3$ strand are highly conserved among PurS sequences, with 97 and 98% conservation, respectively, among 701 sequences in the Pfam database (Finn *et al.*, 2016). The Leu in the loop between the $\alpha 2$ helix and the $\beta 3$ strand is also well conserved (94% in the Pfam database), with some replacement by Ile or Phe.

3.4. MD simulation of the PurS dimer

Figs. 6(a) and 6(b) show the atomic fluctuations for each residue during the MD simulation. If one subunit of the dimer

Figure 6
Structural fluctuations of *TtPurS* (PDB entry 2cuw). (a) Atomic fluctuations for each residue during the MD simulation calculated by the superposition of one subunit of the dimer. The blue curve indicates the fluctuation of the superimposed subunit and the grey line indicates the fluctuation of the other subunit. The blue arrow indicates that a subunit corresponding to the blue curve is superimposed. (b) Atomic fluctuations on the superposition of one half of the dimer, as indicated by the arrows (residues 3–12 and 42–84 of one subunit and 19–41 of the other subunit). (c) Superposition of ten structures during the MD simulation by half of the molecule as defined in (b); the right half of the molecule was superimposed.

Figure 7

Structural fluctuations of the active complex. (a, b) Atomic fluctuations for each residue of *TtPurL* and *TtPurQ*, respectively, during the MD simulation calculated by superposition of the *TtPurS* subunit or part of it. Blue, orange, grey and yellow lines indicate the fluctuations superimposed by subunit A, subunit B, α1 of subunit A and α1 of subunit B of the PurS dimer. (c) Averaged fluctuations. Grey and white bars indicate the averaged fluctuations of the A1–B1 domain (residues 1–356) of the PurL subunit and the PurQ subunit, respectively.

is superimposed (Fig. 6a, blue), the atomic fluctuation seems to be small. In contrast, as shown in Fig. 6(b), if half of the molecule is superimposed, as indicated by the coloured arrows, the other part fluctuates significantly. The fluctuation is visualized in Fig. 6(c), in which the right half of the molecule was superimposed, showing the bending motion of the PurS dimer. Similar motions were observed for *MjPurS* and *StPurS* (Supplementary Figs. S1 and S2), suggesting that this movement is required for the function of PurS.

3.5. MD simulation of the complex of *TtPurS*, *TtPurL* and *TtPurQ*

A model of the active complex of the *TtPurS* dimer, *TtPurL* and *TtPurQ* was prepared based on the structure of the complex of the *TmPurS* dimer, *TmPurL* and *TmPurQ* (Morar *et al.*, 2008) and a 100 ns MD simulation was performed. As shown in Fig. 7, significant movements of the PurL and PurQ subunits were observed when the structures were superimposed using the α1 helix of each PurS subunit (Figs. 7a and 7b). In the case of superposition by the α1 helix of the PurS monomer, the average fluctuation of the A1–B1 domain including the FGAR-binding site (residues 1–356) of PurL, including the active site, was increased over that of PurQ (Fig. 7c). In contrast, in the case of the superposition by the α1 helix of the other PurS monomer, the average fluctuation of PurQ was increased more than that of the A1–B1 domain of PurL. Thus, the results of the MD simulation suggested that PurQ is associated with the α1 helix of one PurS subunit and PurL is associated with the α1 helix of the other PurS subunit.

4. Discussion

In the present study, four crystal structures of three PurSs have been determined and their structural characteristics have

been analyzed, suggesting that the shape and dynamics of the PurS dimer are important for its function. A few residues are conserved and may be required for its dynamics or interaction with the other subunits of FGAR-AT: PurL and PurQ. In the purine-biosynthetic pathway, two further glutamine amidotransferases are known: glutamine 5-phosphoribosylpyrophosphate (PRPP) amidotransferase and GMP synthetase, which are also called PurF and GuaA, respectively (Zalkin & Dixon, 1992; Zhang *et al.*, 2008). Glutamine PRPP amidotransferase is a globular protein and the ammonia molecule can move between two active centres in the structure of the protein. In contrast, GMP synthetase is composed of two domains: a glutamine amidotransferase domain and an ATP pyrophosphatase domain. In some archaea, GMP synthetase is composed of two kinds of subunit: a glutamine amidotransferase subunit and an ATP pyrophosphatase subunit. This organization of GMP synthetase is similar to that of FGAR-AT. However, GMP synthetase does not have the third subunit/domain corresponding to PurS in FGAR-AT. Thus, PurS is unique to FGAR-AT and its function needs to be elucidated. It is also important to analyze the origin of the PurS structure. At present, we have found no structure related to PurS among known protein structures.

In all crystal structures, PurS forms a conserved dimeric structure. The same dimeric structure has also been found in the complex of *TmPurS*, *TmPurL* and *TmPurQ* (Morar *et al.*, 2008). In the case of *TtPurS* in the *P2₁* form, two dimers were found in the asymmetric unit, as in the cases of *BsPurS* in the *P2₁* form and *TmPurS*. However, the relative locations of the two dimers differ among the three structures, suggesting that the biological unit is not a tetramer. In fact, size-exclusion chromatography and static light scattering have indicated that PurS forms a dimer in solution (Mathews *et al.*, 2006).

As shown in Figs. 6 and 7, the relative location of PurL and PurQ is probably correlated to the hinge-like motion of PurS.

It is possible that this structural change of FGAR-AT is required for the reaction to control solvent accessibility to the active site, because the substrate glutamine should come from the solvent region but the obtained ammonia molecule should not contact water. Thus, it is expected that PurS regulates the relative location of PurL and PurQ to control the reaction in the two active sites as well as the movement of the ammonia molecule between the two subunits. To clarify the reaction mechanism in detail, further analyses including the determination of PurL and PurQ structures in the free form as well as in the active complex, the determination of the ligand-binding mechanism and MD analysis of ammonia-molecule transport are required.

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