New insights into the interactions of the translation initiation factor 2 from Archaea with guanine nucleotides and initiator tRNA

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Summary

Heterotrimeric translation initiation factor a/eIF2 $\alpha\beta\gamma$ delivers charged initiator tRNA to the small ribosomal subunit. In this work, we have determined the structures of aIF2 γ from the archaeon *Sulfolobus solfataricus* in the nucleotide-free and GDP-bound forms. Comparison of the free, GDP and Gpp(NH)p-Mg²⁺ forms of aIF2 γ reveals a sequence of conformational changes upon GDP and GTP binding. Our results show that the affinity of GDP to the G-domain of the γ -subunit is higher than that of Gpp(NH)p. Analyzing a pyrophosphate molecule binding to the domain II of the γ -subunit we could find a cleft, which is very suitable for the acceptor stem of tRNA accomodation. It allows suggestion of an alternative position for Met-tRNA_i^{Met} on the $\alpha\gamma$ intersubunit dimer, at variance with a recently published one. In the model reported here, the acceptor stem of the tRNAi is approximately perpendicular to that of tRNA in the ternary complex EF-Tu-Gpp(NH)p-tRNA. According to our analysis, the elbow and T-stem of MettRNA_i^{Met} in this position should make extensive contact with the α -subunit of aIF2. Thus, this model is in good agreement with experimental data, which show that the α subunit of aIF2 is necessary for the stable interaction of aIF2 γ with Met-tRNA_i^{Met}.

Keywords: crystal structure; archaea; a/eIF2; GDP; GTP; Met-tRNA_i^{Met}

Introduction

Initiation of translation requires binding of Met-tRNA_i^{Met} (tRNAi) to the small ribosomal subunit and its positioning on the start codon of mRNA. Heterotrimeric archaeal aIF2 resembles the eukaryal eIF2, but is distinct from the monomeric bacterial IF2⁻¹. In both domains of life a/eIF2 are composed of three subunits: α , β and γ ⁻². The largest γ -subunit forms the core of the heterotrimeric ^{3 4 5} and interacts with both, the α - and β -subunits, whereas the α - and β -subunits do not interact with each other ⁴. Experiments performed with individual aIF2 subunits, intersubunit dimers and the complete heterotrimeric factor from *Sulfolobus solfataricus* (Sso) showed that the factor binds tRNAi and that nucleotide-dependent tRNAi binding requires at least the aIF2 $\alpha\gamma$ -dimer ⁵.

The structures of the individual aIF2y subunits of *Pyrococcus abyssi*, *Sulfolobus solfataricus* and Methanococcus jannaschii, that of the aIF2 $\alpha\gamma$ dimer of Sulfolobus solfataricus as well as that of the aIF2 $\beta\gamma$ dimer of *Pyrococcus furiosus* have been solved ^{4 6 7 8}. The aIF2 γ subunit consists of three domains, which are similar to those found in EF-Tu or eEF1A 9 10 11. The first domain (G domain) contains the guanine nucleotide-binding site and binds to the N-terminal helix and central helix-turn-helix domain of the aIF2 β subunit ⁸. Domains II and III of aIF2 γ are β barrels. Domain II of aIF2 γ interacts with the C-terminal domain of aIF2 α ⁷. The aIF2 α subunit is also comprised of three domains. Domains I and II of $aIF2\alpha$ form a rigid body linked to a mobile third domain, which enables the binding of aIF2 α to aIF2 γ . Recent studies revealed that the aIF2 α subunit greatly increased the affinity of aIF2 γ for tRNAi⁵, and that a complex of aIF2 γ and domain III of aIF2 α exhibits full tRNAi binding capacity, identical to that observed with the intact aIF2 heterotrimer ¹². The β -subunit consists of three parts: the N-terminal α -helix, the central helix-turn-helix domain and the C-terminal zinc-binding domain ⁸. In the aIF2 $\beta\gamma$ heterodimer, the β -subunit shows significant differences from its free structure ¹³. In aIF2, the α and γ -subunit are important for tRNAi binding ⁷, whereas in eIF2 the β - and γ -subunits are responsible for this function ³.

It has been shown that the conformation of EF-Tu in the active GTP-bound state differs from that in the inactive GDP-bound state. The conformational changes emerge from the relative motion between domain I and domains II and III of the protein, as well as in coordinated motion of two flexible regions called switch 1 and switch 2^{14 15 16}. By contrast, in both nucleotide-free and nucleotide-bound structures of aIF2 γ , of the aIF2 $\alpha\gamma$ dimer, as well as of the aIF2 $\beta\gamma$ heterodimer the relative orientation of the three domains of the γ -subunit remains identical, and the structure of the aIF2 γ -subunit resembles the active EF-Tu-Gpp(NH)p complex rather than the inactive EF-Tu-GDP one ^{47 8}.

Here, we report the structures of the aIF2 γ -subunit from *S. solfataricus* (Sso-aIF2 γ) in the nucleotide-free and nucleotide-bound states at 2.9 Å and 2.65 Å resolution, respectively. In the latter case co-crystallization was performed using a preparation of Gpp(NH)p, which contained an impurity of GDP. In spite of excess of Gpp(NH)p, only GDP molecules were found in the nucleotide-binding pockets of both non-crystallographic symmetry related γ -subunits whereas one Gpp(NH)p molecule was observed outside the nucleotide-binding pocket in one of the two γ -subunits in the asymmetric unit. This suggests that the affinity of GDP to this specific site of the protein is much higher than that of the non-cleavable GTP analogue Gpp(NH)p. No Mg²⁺ ions were observed in the electron density map near nucleotides (possibly Mg-phosphate precipitated in crystallization mixture). The comparison of the Sso-aIF2 γ structures in the nucleotide-free and GDP-bound forms with the structure of Sso-aIF2 $\alpha\gamma$ in the GTP-bound form ⁷ revealed a sequence of conformational changes upon the nucleotide binding. At variance with another report ⁷ we describe here an alternative position for Met-tRNA_i^{Met}, which should make extensive contacts with the α -subunit of aIF2.

Results and Discussion

Overall structure of the Sso-aIF2y subunit

The overall domain organization of the Sso-aIF2 γ subunit is similar to that in the earlier determined homologous structures ^{7 6 4}. The molecule consists of three consecutive domains (Figure 1): domain I (G) is packed onto domains II and III. Domain I corresponds to the guanine nucleotide binding site. It encompasses amino acid residues 1-206 and contains six β -strands and five α -helices. The connectivity scheme of this domain is $\beta\alpha\beta\beta\alpha\beta\alpha\beta\alpha\beta\alpha$. Domain I contains the so-called P-loop ¹⁷ and two very flexible regions, switch 1 and switch 2 (Figure 1). Domains II and III encompass regions 207-322 and 323-415, accordingly. Both domains are β -barrels. The relative orientations of domains II and III are identical in all known aIF2 γ structures, whereas the orientation of domain I relative to domains II and III are slightly different: in particular, the structures of the present nucleotide-free and nucleotide-bound aIF2 γ forms have slightly more open conformations.

Although co-crystallization of the nucleotide bound form of Sso-aIF2 γ was performed with Gpp(NH)p and the search model contained this nucleotide in the nucleotide-binding pocket, GDP molecules were observed in the guanine nucleotide-binding sites of both Sso-aIF2 γ molecules in the asymmetric unit of the crystal. However, one of the two γ -subunits of the asymmetric unit contained a Gpp(NH)p molecule in the pocket formed by strands β 8, β 11, β 14 and a part of switch 1 (Figure 1). This results in space group $P3_1$ for these crystals instead of $P3_121$ for that of the nucleotide-free form (Table 1).

Differences between the structures of Sso-aIF2 γ in nucleotide-free and nucleotide-bound forms are restricted to local conformational changes around the guanine nucleotide-binding pocket and the regions of switch 1 and switch 2. Superposition of C α atoms of both structures excluding these flexible regions yields an rms deviation of 0.39 Å. Since the model of SsoaIF2 γ -GDP was determined with a higher resolution, hereafter we refer to this structure unless stated otherwise.

Two molecules of Sso-aIF2 γ -GDP in the asymmetric unit of the crystal are slightly different in some loops and switch regions: superposition of C α atoms of these molecules with the same exceptions as in the previous case yielded the rms deviation equal to 0.40 Å. The molecules are related by the 2-fold symmetry axes and contact each other through the so-called γ L1 loop (residues 222-236) and the region, containing amino acid residues 301-305 of strand β 14. It is interesting to note that the same loop contacts the aIF2 α subunit in the aIF2 $\alpha\gamma$ heterodimer ⁷.

Nucleotide-binding pocket

GDP-bound form of Sso-aIF2 γ

The nucleotide-binding pocket of Sso-aIF2 γ -GDP is formed by the two conserved ¹⁴⁹NKVD₁₅₂ and ¹⁸⁴SALH₁₈₇ regions, which surround the nucleotide base, and by the P-loop, which interacts with the phosphate moiety (Figure 1). The P-loop containing consensus motif GxxxxGK $^{1}T/S^{1}$ (¹⁶GHVDHGKT₂₃ in Sso-aIF2 γ) was found in all GTPases ¹⁷. Both conserved regions and His20 of the P-loop are connected through the hydrogen bonds formed by strictly conserved residue Asn149. The angle, which the nucleoside forms with the phosphate moiety, is approximately 90°. The base and the phosphates are located in cavities on the aIF2 γ surface, whereas the ribose ring is exposed to the solvent.

The sides and bottom of the nucleotide base cavity are formed by side chains of Lys150, Val153, Asp152, Ser184, His187 and Leu186. Some of these residues make hydrogen bonds with nucleotide base polar atoms (Table 2) whereas the side chains of Lys150 and Leu186 stack with the base and together with side chains of Leu186 and Val153 form a hydrophobic patch on the surface of the protein with a deep cavity in its center. The nucleotide base is immersed in this cavity. Hydrogen bonds formed by N1, O6 and N7 of the base are inaccessible to the solvent and the hydrogen-bonding capacity of these atoms is fully saturated (Table 2).

The α - and β -phosphates of GDP are located in the cavity limited by the main chain amide groups of the P-loop (residues 18-23), switch 2 (residues 96-97), hydroxyl group of Thr23 and the side chain of strictly conserved Lys22. It is interesting to note that most of the main chain amide groups of the residues surrounding the phosphate moiety are approximately parallel to the axis of the GDP phosphate tail. As a result, no hydrogen bonds can be formed between the main chain nitrogen atoms of these residues and the oxygen atoms of the GDP phosphates. There are only three oxygen atoms of the phosphate moiety (O1A, O1B and O2B) inaccessible to the solvent and only two of them form single hydrogen bonds with the main chain nitrogen atoms of residues 19 and 23 (Table 2). Therefore, the hydrogen-bonding capacity of charged atoms of the GDP phosphates is not fully saturated and the interaction of this part of GDP with the protein is predominantly electrostatic. Residues ₉₃DAPG₉₆ of switch 2 (Figure 1) are part of the conserved DxxG motif, which exists in many different proteins that hydrolyse GTP or ATP. This motif is known to undergo major conformational changes upon GTP hydrolysis ¹⁸.

Conformational changes upon GDP-binding

The nucleotide ring cavities in nucleotide-free and nucleotide-bound forms of Sso-aIF2y have the same conformations, whereas the conformations of the P-loops are different (Fig. 2a). In the nucleotide-free form of Sso-aIF2 γ the P-loop and switch 2 approach each other and the main chain carbonyl group of Gly96 and the amide group of Asp19 are connected by hydrogen bonding. The distance between the C α atoms of these two residues is 3.2 Å. The strictly conserved Lys22 forms two hydrogen bonds with the main chain oxygen atom of Ala94 and OE1 of Glu98. The P-loop in such position closes the phosphate-binding cavity (Fig. 2a). To open this cavity residues 19-21 of the P-loop must be displaced in the direction of strand β4 and occupy positions unique for nucleotide-bound forms of the γ -subunit. Upon GDP binding the hydrogen bond Gly96 N – Asp19 O is broken, whereas the hydrogen bond formed by Lys22 with Ala94 is retained. The distance between C α atoms of Gly96 and Asp19 increases up to 7.14 Å. The C α atom of His20 undergoes the most significant displacement (7.55 Å), and the main chain nitrogen atom of this residue forms a hydrogen bond with side chain of Asn149. The phi and psi torsion angles of P-loop residues 17-22 differ in the nucleotide-free and nucleotide-bound forms, whereas region 93-97 of switch 2 retains its position and the torsion angles of its amino acid residues are practically unchanged. Conformations of the rest of switch 2 (Fig. 2b) and switch 1 are slightly different in the free and GDP-bound forms, but these regions are unstable and do not take part in nucleotide binding. Thus, only the P-loop changes its conformation spontaneously or during nucleotide binding.

Conformational changes upon GTP-binding

Superposition of the β -sheets of Gpp(NH)p⁷ and GDP forms of Sso-aIF2 γ yielded the rms deviation of 0.44 Å. The most significant changes are found in switch 1 and switch 2. The additional γ -phosphate in the GTP analogue results in a displacement of region 93-97 of switch 2 in direction of domain III, whereas the P-loop practically retains its position. Hydrogen bonds formed by strictly conserved Lys22 with amino acid residues of switch 2 are broken and instead Lys22 forms two hydrogen bonds with the oxygen atoms of β - and γ -phosphates and the main chain oxygen atom of His17. In the GTP form of Sso-aIF2 γ switch 2 dramatically changes its conformation when compared with its nucleotide-free and GDP-bound forms. Major conformational changes occur in the conserved ${}_{93}DAPG_{96}$ motif and adjacent residues. It is possible to suggest that an inverse motion of this unstable region of aIF2 γ can promote GTP hydrolysis.

Sequence of conformational changes upon GDP and GTP binding

Superposition of all known aIF2 γ structures shows the very close positioning of the nucleotide bases in their G-domains, as well as the similarity of conformations of amino acid residues interacting with these bases. The deep cavity in the G-domain assigned to nucleotide base pocket is open in all aIF2 γ structures. Most of the protein-nucleotide hydrogen bonds inaccessible to the solvent are also formed by the base of the guanine nucleotide and amino acid residues located on the bottom of the G-domain cavity (Table 2). Therefore, it seems plausible to suggest that the initial step of recognition and binding of GDP or GTP is realized through contacts between the base and corresponding part of the nucleotide-binding pocket of aIF2 γ .

To bind GDP, conformational changes of the P-loop of the nucleotide-free form are required (Fig. 2a), whereas switch 2 predominantly retains its position. To bind GTP both the P-loop and switch 2 must change their conformations (Fig. 2b). It seems difficult to predict whether the conformational changes of the P-loop and switch 2 occur spontaneous, or promoted by nucleotide binding. In the first case, $aIF2\gamma$ has a higher probability to bind GDP than GTP.

In addition to GDP, we found a single molecule of Gpp(NH)p in the Sso-aIF2 γ -GDP structure (Fig. 3). Its location in one of two aIF2 γ subunits in the asymmetric unit of the crystal coincides with position of A76 of tRNA in the docking model for aIF2 $\alpha\gamma$ -Gpp(NH)p-tRNA⁷. The non-specific Gpp(NH)p pocket is formed by strands β 7, β 8, β 11, β 14, loop β 11- β 12 and residues 40-45 of switch 1. The Gpp(NH)p molecule makes up some hydrogen bonds with domain II of aIF2 γ , whereas there are only one hydrogen bond with Glu40 of switch 1. In another γ -subunit, there are conformational changes in this region, which impede binding of the

nucleotide at that position. In this case the binding pocket is closed by Met45, which forms hydrogen bond with Lys225, and together with Phe221, Val223 and Val237 constitutes a hydrophobic patch on the protein surface. Both Sso-IF2 γ molecules are not involved in crystal contacts through these regions and it seems therefore likely that conformational changes in these nucleotide-binding regions occur spontaneously and one of possible switch 1 conformations is suitable for binding of Gpp(NH)p . Our results show that the affinity of GDP to the G-domain of the γ -subunit is higher than at least that of Gpp(NH)p.

Possibly, the position of switch 2 induced by GTP binding can stabilize a conformation of switch 1, which is preferred to tRNAi binding. Thus, in the Sso-aIF2 $\alpha\gamma$ -Gpp(NH)p-Mg²⁺ structure ⁷ a visible part of switch 1 takes the conformation different from that in the Sso-aIF2 γ -GDP.

Binding of tRNAi to aIF2

Docking of tRNAi onto the Sso-aIF2 $\alpha\gamma$ -Gpp(NH)p heterodimer ⁷ showed that the terminal A76 base interacts with a pocket formed by strands β 11 and β 14 of aIF2 γ domain II. In this model, there is no direct contact between tRNAi and the α -subunit. However, it has been shown that domain III of the α -subunit is required for high affinity binding of tRNAi to aIF2¹²⁵. To account for this role of domain III of aIF2 α Yatime *et al.* (2006) ⁷ suggested that the participation of the α -subunit in tRNAi binding is indirect, and that the aIF2 α -subunit helps aIF2 γ to reach and maintain the switch conformations induced by Gpp(NH)p.

To check this hypothesis, we superimpose the $aIF2\alpha\gamma$ -Gpp(NH)p structure ⁷ on the nucleotide free and $aIF2\gamma$ -GDP ones (this work). Neither considerable conformational changes between the regular parts of these structures nor contacts between the $aIF2\alpha$ subunit and switch regions were observed.

The alternative possibility is that the positions of tRNAi are different in the Sso-aIF2-GTP-tRNA and EF-Tu-Gpp(NH)p-tRNA structures. In the Sso-aIF2 γ -GDP structure three pyrophosphates per molecule of the subunit were found. One of them is located in a cleft formed by strand β 10, loops β 10- β 11 and β 14- β 15 on the surface of domain II (Figure 1). Docking of Met-tRNA_i^{Met 19} in this cleft was done (Fig. 4). The acceptor stem of tRNAi in this model is approximately perpendicular to that of tRNAi in the model, obtained by superposition of EF-Tu-Gpp(NH)p-tRNA on the aIF2 $\alpha\gamma$ heterodimer ⁷. In our model the methionyl residue esterified to tRNAi can be accommodated in a hydrophobic pocket formed by Ile47, Val99, Leu100, Ala 102 and Trp405. This explains the effects of mutations in the strand β 8 and switch regions altering the tRNA binding capacity of the aIF2 factor ^{12,8}. The acceptor and T stems of tRNAi in this

position make extensive contacts with the α -subunit in the aIF2 $\alpha\gamma$ heterodimer. For instance, residues R186 and R226 of the α subunit can form hydrogen bonds with O1P of Ade73 and O2' of Ade72 of tRNAi, respectively. The last hydrogen bond is partly inaccessible to the solvent and substitution R226A⁷ is capable to lower the binding affinity of aIF2 to tRNAi. An additional binding specificity may be achieved through the interaction of a ridge formed by nucleotides 17 and 17A with the surface of the α -subunit. The ridge is unique for Met-tRNA_i^{Met} and contains two uracils exposed to the solvent. In the model proposed here tRNAi forms extensive contacts with both, the α - and γ -subunits, and thus supports the observation that the aIF2 $\alpha\gamma$ heterodimer is necessary and sufficient for the stable interaction with tRNAi⁵¹².

Materials and Methods

Cloning and expression of the Sso-aIF2γ gene and purification of the γ-subunit

The gene encoding Sso-aIF2y was cloned into the NcoI/BamHI sites of plasmid pET11d (Novagen) by means of PCR. The resulting plasmid was verified by DNA sequencing and introduced into E. coli strain C41(DE3) (Imaxio). Bacterial culture (5 1) was grown in LB medium containing 100µg ml⁻¹ ampicilin. Synthesis of the protein was induced by addition of isopropyl-β-D-thiogalactopyranoside to 1 mM, and the cells were harvested 3 h later. The cells were suspended in 100 ml of 50 mM Tris-HCl, pH 8.0, 250 mM MgCl₂, 1 M NaCl, 5 mM EDTA and disrupted by sonication. Cellular debris and ribosomes were removed by centrifugation for 20 min at 14,000 g and for 1h at 150,000 g, respectively. Host proteins were removed by heating at 70°C for 20 min and by subsequent centrifugation for 20 min at 14,000 g. Ammonium sulfate was added to the supernatant (1.7 M final concentration), which was loaded onto a Butyl-Toyopearl column. After washing the column with 1.5 M ammonium sulfate and 1M NaCl solution, the protein was eluted with a linear gradient of ammonium sulfate concentration (1.5-0 M) and NaCl concentration (1.0 - 0.1 M) in 50 mM Tris-HCl buffer, pH 8.0. The fractions containing the γ -subunit were pooled and concentrated. The protein was diluted with 50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 10 mM β-mercaptoethanol and loaded onto a CM-Sepharose column. After washing the column with the same buffer, the protein was eluted with a linear gradient of 0.05-1.0 M NaCl in 50 mM Tris-HCl, pH 7.5. The fractions containing homogeneous $aIF2\gamma$ protein (as judged by UV spectral analysis and SDS-PAGE) were pooled and concentrated to 40-60 mg ml⁻¹.

Crystallization

Crystallization experiments for the Sso-aIF2 y-subunit in nucleotide-free and nucleotidebound form were performed using the hanging drop vapor diffusion technique. All drops were set up on siliconized cover slides by mixing 1.0 μ l of the protein solution (50 mg ml⁻¹ Sso-aIF2 γ dialyzed into 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM β-mercaptoethanol) with 2.0 µl of precipitation solution (5% natrium malonate dihydrate, 16.7 mM glycine, pH 4.5) and 9.0 µl 1.4 mM CdCl₂ as an additive. The drops were equilibrated with 0.3 ml of the final precipitation solution: 10% natrium malonate dihydrate, 33.3 mM glycine, pH 4.5. The commercial preparation of Gpp(NH)p (Sigma) used for co-crystallization with Sso-aIF2y contained up to 30% GDP impurity by thin-layer chromatography analysis. 13 mM solution of this Gpp(NH)p preparation in the presence of 13 mM MgCl₂ was added to the protein solution to reach 10 times molar excess of the nucleotide over the protein. Then this mixture was used for crystallization as described above. Trigonal crystals of both nucleotide-free and nucleotide-bound forms of SsoaIF2y were obtained within 2 days at 22°C. The crystal size was up to 450 x 100 x 100 µm. Crystals of nucleotide-free aIF2 γ belong to space group P3₁2₁ and diffract to 2.9Å resolution. They contain one molecule per asymmetric unit. Crystals of the nucleotide-bound $aIF2\gamma$ belong to space group P3₁, diffract to 2.6Å resolution and contain two molecules per asymmetric unit (Table 1). Cryoprotection for both types of the crystals was achieved by adding ethylene glycol to the final concentration of 15% (v/v).

Data collection, structure determination and refinement

X-ray diffraction data were collected employing synchrotron radiation at the X12 beamline, DESY (Hamburg, Germany) using MAR CCD detector. Data were processed and merged with the XDS program suit ²⁰. A molecular replacement solution was obtained with program PHASER ²¹. A search model was derived from the aIF2 $\alpha\gamma$ heterodimer (PDB code 2AHO) by using the structure of the aIF2 γ in the case of the nucleotide-free subunit and aIF2 γ -Gpp(NH)p-Mg²⁺ in the case of its nucleotide-bound form. The unique solutions were found and were used to calculate initial maps.

Both structures were subjected to several rounds of computational refinement and map calculation with CNS ²² and manual model inspection and modification with O ²³. A free R-factor calculated from 5% of reflections set aside at the outset was used to monitor the progress of refinement. The initial anisotropic overall B-factor was replaced successively with per-residue B-factors, separate per-residue B-factors for main chain and side chain atoms and, finally, restrained individual atomic B-factors. The model bias present in the initial molecular-replacement solutions was tackled using composite omit cross-validated σ A-weighted maps

implemented in CNS. When the R-factor value reached 30%, water molecules were placed into 3σ peaks in Fo-Fc maps, when they were within a suitable hydrogen-bonding distance of the existing model. After refinement, water molecules, whose positions were not supported by the electron density at 1σ contouring in a σ A-weighted 2Fo-Fc map, were deleted. The additional Gpp(NH)p molecule and pyrophosphates in the nucleotide-bound form of Sso-aIF2 γ were found by using of Fo-Fc maps. The final models of nucleotide-free and nucleotide-bound forms were refined at 2.90 Å and 2.65 Å resolution, respectively. The quality of the electron density map of aIF2 γ in both forms was sufficient to rebuild the entire model. In addition to two γ -subunits the asymmetric unit of the crystal of the nucleotide-bound form contained six pyrophosphates, one Gpp(NH)p and 196 water molecules. Data collection and refinement statistics are given in Table1.

Protein Data Bank accession numbers

The coordinates and structure factors for the crystal structures of $aIF2\gamma$ in nucleotide-free and GDP-bound forms have been deposited in the Protein Data Bank under ID codes 2PLF and 2PMD, respectively.

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Data set	aIF2γ	aIF2 ₇ +GDP	
Data collection			
Beamline	X12 DESY	X12 DESY	
Wavelength, Å	1.0	1.0	
Space group	<i>P</i> 3 ₁ 21	<i>P</i> 3 ₁	
Unit cell, Å ,°	94.84, 94.84, 166.43	95.06, 95.06, 165.67	
	90.0, 90.0, 120.0	90.0, 90.0, 120.0	
Unique reflections	19 821	45642	
Resolution, Å	20.0-2.90	20.0-2.65	
	(3.00-2.90)	(2.81-2.65)	
Completeness, %	98.1 (97.0)	94.3 (85.1)	
Redundancy	12.0 (10.5)	3.2 (3.2)	
$I/\sigma(I)$	18.2 (4.4)	11.6 (2.6)	
Rmerge, %	8.5 (47.1)	7.6 (45.8)	
Refinement			
Resolution range, Å	20.0-2.90	20.0-2.65	
R_{work}/R_{free} , %	22.2/28.8	22.6/28.0	
Number of atoms (protein	1,		
water, others)	3212/106/0	6424/204/142	
rmsd bond length,			
/angles, ° 0.0091/1.65		0.0083/1.47	
Average B factor, Å2	58.8	63.9	

Table 1. Data collection and refinement statistics

Values in parentheses are for the highest resolution shell.

 R_{free} factors were calculated for 5.0 % randomly selected test sets that were not used in the refinement.

nucleotide base	protein polar	residue	Length	Accessibility
polar atom	atom	name	of H-bond, Å	to the solvent
N1	OG1	Ser184	3.16	no
O6	Ν	Ala185	2.52	no
O6	Ν	Leu186	3.20	no
N7	ND2	Asn149	3.16	no
O4'	NZ	Lys150	3.18	yes
O2B	Ν	Asp19	2.72	no
O1B	Ν	Thr23	3.07	no

Table 2. Hydrogen bonds between GDP and $aIF2\gamma$

Figure legends

Fig. 1.

a. Amino acid sequence of aIF2y from S. solfataricus

Residues, conformations of which are changed in free and GDP forms of $aIF2\gamma$ are boxed. The two conserved regions, which surround the nucleotide base are shown in magenta. The P-loop is shown in green. Switch 1 and switch 2 are shown in brown and blue, respectively.

b. Stereo representation of one of the two $aIF2\gamma$ -GDP molecules.

Numbered C α atoms are shown as gold spheres. Pyrophosphates are shown in magenta, Gpp(NH)p is shown in green and GDP is shown in red.

- Fig. 2. Conformational changes upon GDP and GTP-binding.
 - a. Conformations of the P-loop in nucleotide-free, GDP and Gpp(NH)p-bound ⁷ forms. The nucleotide-free form is shown in red, the GDP form in dark blue and the Gpp(NH)p form in light blue, respectively.
 - b. Conformations of switch 2 in nucleotide-free, GDP and Gpp(NH)p-bound ⁷ forms. The colors are the same as in (a).

Fig. 3. Stereoview showing the Gpp(NH)p molecule in the non-specific binding pocket of one of two aIF2 γ subunits in the asymmetric unit of the crystal. The molecule forms hydrogen bonds with side chain nitrogen atom of Arg280 and main chain oxygen atom of Asp222 (shown by dotted lines). The map is contoured at 1 σ level.

Fig. 4. Docking of tRNAi to the Sso-aIF $2\alpha\gamma$ structure.

The α - and γ -subunits are shown in grey and blue, respectively, tRNAi is in green and Gpp(NH)p is in red. The 3' end of tRNAi in this model and the acceptor stem of tRNA in the the SsoaIF2 $\alpha\gamma$ -Gpp(NH)p-tRNA model ⁷ occupy the same pocket on the aIF2 γ surface. They are approximately perpendicular to each other.



Fig. 1a





Fig. 2a







Fig. 3



Fig. 4