Crystal Structure of the Intact Archaeal Translation Initiation Factor 2 Demonstrates Very High Conformational Flexibility in the α- and β-Subunits

Elena Stolboushkina1, Stanislav Nikonov1, Alexei Nikulin1, Udo Bläsi2, Dietmar J. Manstein3, Roman Fedorov3, Maria Garber1 and Oleg Nikonov1

1Institute of Protein Research, Russian Academy of Sciences, 142290 Pushchino, Moscow Region, Russian Federation
2Max F. Perutz Laboratories, Department of Microbiology and Immunobiology, University Departments at the Vienna Biocenter, Dr. Bohrgasse 9/4, A-1030 Vienna, Austria
3Institute for Biophysical Chemistry, Hannover Medical School, Carl-Neuberg-Strasse 1, D-30625 Hannover, Germany

Received 22 April 2008; received in revised form 22 July 2008; accepted 8 July 2008

Edited by K. Morikawa

Keywords: ribosome; a/eIF2; translation; initiation; G-protein

Introduction

In Eukarya and Archaea, translation initiation factor 2 (eIF2/aIF2), which contains three subunits (α, β, and γ), is pivotal for binding of charged initiator tRNA to the small ribosomal subunit. The crystal structure of the full-sized heterotrimeric aIF2 from Sulfolobus solfataricus in the nucleotide-free form has been determined at 2.8-Å resolution. Superposition of four molecules in the asymmetric unit of the crystallized complex and the comparison of the obtained structures with the known structures of the aIF2αγ and aIF2βγ heterodimers revealed high conformational flexibility in the α- and β-subunits. In fact, the full-sized aIF2 consists of a rigid central part, formed by the γ-subunit, domain 3 of the α-subunit, and the N-terminal α-helix of the β-subunit, and two mobile “wings,” formed by domains 1 and 2 of the α-subunit, the central part, and the zinc-binding domain of the β-subunit. High structural flexibility of the wings is probably required for interaction of aIF2 with the small ribosomal subunit. Comparative analysis of all known structures of the γ-subunit alone and within the heterodimers and heterotrimeric in nucleotide-bound and nucleotide-free states shows that the conformations of switch 1 and switch 2 do not correlate with the assembly or nucleotide states of the protein.

© 2008 Elsevier Ltd. All rights reserved.

Abbreviations used: aIF2, archaeal translation initiation factor 2; EF-1A, elongation factor 1A; eIF2, eukaryotic translation initiation factor 2; GEF, guanine exchange factor; NCS, non-crystallographic symmetry; SsoIF2, initiation factor 2 from S. solfataricus; tRNAi, initiator tRNA (Met-tRNAiMet).

*Corresponding author. E-mail address: alik@vega.protres.ru.

Received online at www.sciencedirect.com

Available online at www.sciencedirect.com

0022-2836/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
plete heterotrimeric αIF2 (the β- and γ-subunits and domain 3 of the α-subunit) has also been solved. In summary, these studies shaped the view of the structural features of αIF2 and the structure-function relationships in this heterotrimeric protein. Conformational changes in functionally important switch regions of the γ-subunit are of interest and were analyzed in all the known structures. Some differences in conformations of the switches were described and discussed in terms of correlation with the different states of the γ-subunit. However, no reliable correlation was observed when a thorough analysis of the known structures of the γ-subunit was performed. However, no reliable correlation was observed when a thorough analysis of the known structures of the γ-subunit was performed. However, no reliable correlation was observed when a thorough analysis of the known structures of the γ-subunit was performed. However, no reliable correlation was observed when a thorough analysis of the known structures of the γ-subunit was performed. However, no reliable correlation was observed when a thorough analysis of the known structures of the γ-subunit was performed.

Here, we describe for the first time the crystal structure of the full-sized heterotrimeric initiation factor 2 from *S. solfataricus* (SsoIF2) at 2.8Å resolution. Superposition of the four molecules in the asymmetric unit and comparison of the determined structures with the known ones reveal very high conformational flexibility in the α- and β-subunits, which is probably required for the functioning of αIF2. For the first time, the complete structures of the switch regions of the γ-subunit have been solved at once for four molecules of αIF2 in the asymmetric unit of the crystal, allowing accurate analysis of their conformations. The conformations of the switch regions in the individual γ-subunits were found to differ. Moreover, a comparative analysis of all the known structures of the γ-subunit alone and within the heterodimers and heterotrimer in nucleotide-

**Fig. 1.** Stereoview of the intact αIF2αβγ heterotrimer from *S. solfataricus* and the subunit sequences displaying secondary structure topology. (a) Amino acid sequences of the α-, β-, and γ-subunits. The numbering and secondary structure elements are indicated. Strictly conserved residues are shown in green. Switch 1 is boxed in orange, and switch 2 is boxed in yellow. (b) Stereo representation of the intact SsoIF2 molecule. The α-subunit is shown in magenta; the β-subunit, in blue; the γ-subunit, in green; switch 1, in orange; and switch 2, in yellow. (c) Close-up view of the superposition of the switch 1 (orange) and switch 2 (yellow) regions of the heterotrimers in the asymmetric unit of the crystal. Switch 1 of heterotrimer 2 (Table 1) is omitted since its structure is incomplete.
bound and nucleotide-free states shows that the conformations of switch 1 and switch 2 do not correlate with the assembly or nucleotide states of the protein.

Results

Overall structure of the aIF2 heterotrimer

A stereoview of the intact aIF2αβγ heterotrimer from *S. solfataricus* and its sequence and topology are shown in Fig. 1. The trimeric aIF2 has an L-shaped conformation, with the α-subunit comprising most of the long arm, the γ-subunit situated in the angle, and the β-subunit extending the short arm. The G-domain of aIF2γ contacts helix α1 and the C-terminal region of the β-subunit, whereas aIF2α domain 2 interacts with domain 3 of the α-subunit. There is no contact between the α- and β-subunits. This result is in good agreement with all available biochemical data. The most flexible and functionally important regions of aIF2γ, switch 1 and switch 2, are located on the inside of the corner between the α- and β-subunits. There are four independently refined intact aIF2 heterotrimers in the asymmetric unit of the crystal (Table 1), but only

Fig. 1. (legend on previous page)
two of them contain the central part and distorted zinc-binding domain of the β-subunits, whereas the N-terminal α-helices of all these subunits are visible in all heterotrimers. In the crystal, heterotrimers 1 and 2 (Table 1) are situated in alternative layers with heterotrimers 3 and 4. The β-subunits of one layer contact the α- and γ-subunits of the neighboring layers (Fig. 2). It is interesting to note that the α- and γ-subunits of the heterotrimers located in the same layers are related by non-crystallographic symmetry (NCS) 2-fold axes, whereas these axes intersect the β-subunit in the N-terminal region of helix α4.

**The α-subunit**

The overall domain organization of the α-subunit in the trimeric aIF2 is similar to that described for the isolated α-subunit and for that in the aIF2γ heterodimer. In the crystal, the four α-subunits in the asymmetric unit have essentially the same structures, with an r.m.s.d. between all Cα atoms of less than 1.5 Å. Loop β3–β4 (residues 43–62) is the most flexible region of the α-subunit. The relative positions of domains 1 and 2 are the same in all SsoIF2 molecules, whereas the position of domain 3 deviates slightly (∼2.0 Å). Domain 3 retained its position relative to the γ-subunit in all NCS-related structures of SsoIF2. This domain interacts with domain 2 of the γ-subunit through loops β6–αα and β7–β8. Both loops are buried in the cavities on the surface of aIF2γ. The superposition of regions 186–200 and 218–225 contacting the γ-subunit shows their close similarity, with an r.m.s.d. of less than 0.7 Å in all four α-subunits.

<table>
<thead>
<tr>
<th>Heterotrimer</th>
<th>α-Subunit Chain</th>
<th>Residues</th>
<th>ADP*</th>
<th>β-Subunit Chain</th>
<th>Residues</th>
<th>ADP</th>
<th>γ-Subunit Chain</th>
<th>Residues</th>
<th>ADP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C</td>
<td>1–266</td>
<td>95.5</td>
<td>K</td>
<td>2–129</td>
<td>148.3</td>
<td>A</td>
<td>2–415</td>
<td>105.2</td>
</tr>
<tr>
<td>2</td>
<td>D</td>
<td>1–266</td>
<td>102.4</td>
<td>L</td>
<td>2–22</td>
<td>120.6</td>
<td>B</td>
<td>2–35, 46–336, 341–415</td>
<td>104.8</td>
</tr>
<tr>
<td>3</td>
<td>G</td>
<td>1–266</td>
<td>93.1</td>
<td>M</td>
<td>2–139</td>
<td>145.7</td>
<td>E</td>
<td>2–415</td>
<td>105.5</td>
</tr>
<tr>
<td>4</td>
<td>H</td>
<td>1–265</td>
<td>101.7</td>
<td>N</td>
<td>2–31</td>
<td>135.8</td>
<td>F</td>
<td>2–415</td>
<td>105.2</td>
</tr>
</tbody>
</table>

* ADP (Å²) is an atomic displacement parameter. Its value is close to that of the usual B-factor.

Fig. 2. Arrangements of four heterotrimers in the asymmetric unit of the crystal. Heterotrimers 1 and 2 lie in alternative layers with heterotrimers 3 and 4 (Table 1). The β-subunits make inter-layer contacts. The aIF2γ heterodimers in the same layer are related by local 2-fold axes, whereas helix α4 of the two β-subunits lies on these axes. In heterotrimers 1 and 3, the α-subunits are shown in gray; the β-subunits, in dark blue; and the γ-subunits, in green. In heterotrimers 2 and 4, the α-subunits are shown in magenta; the β-subunits, in pale blue; and the γ-subunits, in gold.
The net of hydrogen bonds between the α- and γ-subunits is mainly formed by main chain oxygen and nitrogen atoms in two regions separated by more than 12 Å. The first of the regions contains three hydrogen bonds, which result in the formation of a continuous four-stranded anti-parallel β-sheet, including β6, β7, and β8 of the α-subunit and β7 of the γ-subunit. The second one contains two hydrogen bonds formed by the N-terminus of helix α6 and loop β13–α6 of the α-subunit and loop β14–β15 of the γ-subunit. Possibly, these two regions constitute sites on the aIF2α and aIF2γ surfaces needed for their recognition and binding.

The β-subunit

Only two of four heterotrimers in the asymmetric unit of the crystal contained the intact β-subunit; in the two other molecules, only the N-terminal α-helices of the aIF2β were visible. As observed previously in solution and in crystal structures, this subunit consists of an N-terminal α-helix connected by a flexible linker to the central α–β domain, which is followed by the C-terminal zinc-binding domain. The whole polypeptide chain was observed only for one β-subunit. The atomic displacement parameters for both subunits are higher than those for the α- and γ-subunits (Table 1). The β-subunit has a flexible structure with regard to all its parts. The superposition of the N-terminal regions (residues 5–17) produces an r.m.s.d. of 0.73 Å, and the central domains (residues 35–102) can be superimposed with an r.m.s.d. of 3.36 Å, whereas the C-terminal parts of the two β-subunits have different conformations. The zinc-binding pocket in both aIF2β structures is distorted, and no electron density for the Zn ion is detectable. The mutual arrangements of four cysteine residues, which could be incorporated into this zinc-binding pocket, are also different in both well-detectable β-subunits. In chain M, residue Cys109 makes a disulfide bridge with Cys127 (Fig. 3), whereas in chain K, Cys109 forms a hydrogen bond with Glu25.

The tightest contact between the β- and γ-subunits in the heterotrimer is formed by the N-terminal α-helix of aIF2β and the region of aIF2γ containing loop β5–α4, helix α4, loop α4–β6, and strand β6. This contact includes a hydrophobic core and some hydrogen bonds. In addition, there are stacking interactions between side chains of βTyr77 and γTyr163. The great majority of non-polar residues of the inter-subunit core are conserved in all known eIF2/aIF2β structures (Fig. 1a). Several hydrogen bonds stabilize the mutual arrangement of the N-terminal α-helix of the β-subunit and the corresponding region of aIF2γ, but only one of them, formed by the carbonyl oxygen atom of βMet10 and the ND2 atom of γAsn190, is strongly conserved and inaccessible to the solvent. The C-terminal part of one β subunit (chain M, residues 134–139) contacts the N-terminal part of switch 1 and loop β2–β3 of the γ-subunit. The inter-subunit hydrophobic core and hydrogen bonds stabilize this contact. The overall surface of interaction between two subunits is 1135 Å², with the C-terminal part of the β-subunit contributing 376 Å². Like in other published structures, the contacting surface formed by the C-terminal region of the β-subunit accounts for about 35% of the total interface. In addition, in the crystal, the C-terminal parts of both β-subunits interact with switch 1 of the symmetry-related molecules arranged in the same layers (Fig. 2).

Fig. 3. Arrangement of cysteine residues in the distorted zinc-binding domain of the β-subunit (chain M). The electron density is contoured at the 1σ level. Cys109 and Cys127 make a disulfide bridge. Cys130 belonging to the β-subunit zinc finger is located apart.
The area of contact between the C-terminal parts of the β-subunit and the γ-subunit in the heterotrimer is small compared with the contact area between αIF2β and symmetry-related heterotrimers. In the crystal, the N-terminal and central parts of the β-subunit interact with all domains of the α- and γ-subunits of the symmetry-related heterotrimers. Relatively small movements of these domains in the four heterotrimeric structures could result in the configuration flexibility of the central and C-terminal parts of both β-subunits. In the present structure, the crystal contacts fix the β-subunit relative to the γ-subunit in a position that differs from that in other known β-γ heterodimers. As both well-resolved β-subunits occupy the special positions on the local 2-fold symmetry axes, which relate αIF2γ heterodimers (Fig. 2), it is impossible to arrange the two other β-subunits in the same manner. Although crystal packing allows the presence of four complete heterotrimers in the asymmetric unit, it is likely that contacts formed by two β-subunits are insufficient to stabilize their positions.

Comparison of the intact αIF2 structures with other structures containing β-γ heterodimers revealed that only the inter-subunit contact formed by the N-terminal α-helix of the αIF2β is retained. This helix mainly consists of conserved residues and is the most conserved part of the αIF2 structure. Based on these data, we suggest that the β-subunit recognizes the γ-subunit by virtue of its N-terminus. It is interesting to note that in the solution structure of the isolated αIF2β, the N-terminus is disordered. A similar re-organization was observed, for example, for the N-terminus of ribosomal protein L25 in complex with SS rRNA.

The γ-subunit

The γ-subunit forms the core of the heterotrimeric αIF2. The structure of this subunit in the intact heterotrimeric factor is close to that described previously. Four of the NCS-related αIF2γ molecules in the asymmetric unit of the crystal can be superimposed to one another, with an approximately identical r.m.s.d. of 0.9 Å for 348 compared Cα atoms. There is no obvious displacement between the G-domain and domains 2 and 3. The most flexible regions of the γ-subunit are switch 1 (residues 31–51), switch 2 (residues 93–113), loop β1–α1 (residues 17–21), loop β15–β16 (residues 338–344), and loop β19–β20 (residues 399–405). All flexible regions are grouped around the N-terminus of switch 2, and their conformations vary with each molecule.

The polypeptide chains of switch 1 and switch 2 in all the four γ-subunits occupy approximately the same region, but the conformations of these chains differ (Fig. 1c). The most remarkable differences are observed in the N-terminal parts of both switches. Analysis of crystal packing shows that these conformational changes are a result of interactions of switch regions with different mobile parts of their own and symmetry-related heterotrimers.

The regions of domains 2 and G responsible for interactions with the α- and β-subunits are structurally conserved. In all NCS-related molecules, superposition of the Cα atoms constituting the corresponding regions yields a maximum r.m.s.d. of 0.65 Å. Our analysis shows that similar values are typical for superposition of other known αIF2γ structures from different organisms in the nucleotide-free and nucleotide-bound forms.

Discussion

Conformations of the switch regions

The structure of the eIF2/αIF2 γ-subunit closely resembles the structure of translation elongation factor 1A (EF-1A) in its “active” GTP-bound state. In EF-1A, the conformations of the switch regions correlate with transition from the active GTP-bound state of the protein to the inactive GDP-bound one. During the transition, domains 2 and 3 move relative to the G-domain and the conformation of the protein changes from “switch on” to “switch off.” Both switch regions attain new conformations, and switch 1 undergoes the most dramatic structural modifications.

In the γ-subunit of αIF2, switch 1 is located on the subunit surface mainly in the groove formed by the G-domain and domain 2, while switch 2 penetrates into the hole comprised by all three domains and bridges the gap between domains G and 3. This hole has a diameter of about 10 Å. Contrary to EF-1A, the “switch off” conformation was not observed in αIF2γ and the groove and hole retain their shapes in all states, thus limiting the conformational changes of both switch regions. The polypeptide chains of switch 1 occupy close positions on the surfaces of αIF2γ in the nucleotide-free, GDP-bound, and GTP-bound forms in all known structures (Fig. 4a), excluding the position in the GDP-bound form of the incomplete heterotrimeric αIF2. In this structure, switch 1 interacts with both switch 2 and the P-loop and its conformation is probably stabilized by a phosphate ion near the nucleotide-binding site. In the absence of such a ligand, switch 1 in αIF2 in the GDP-bound and nucleotide-free forms can adopt close conformations (Fig. 4a). Unfortunately, the complete structures of switch 1 in the GTP-bound form of αIF2 have not yet been determined. Nevertheless, the two incomplete structures of this switch from Pyrococcus abyssi and S. solfataricus with Gpp(NH)p demonstrate different conformations. The different contacts between switch 1 and the C-terminal parts of the β-subunit observed in αIF2βγ heterodimer and heterotrimers suggest that conformational changes of the β-subunit could affect tRNA binding.

Despite the fact that the γ-phosphate of GTP competes with the N-terminal part of switch 2 and
restricts a number of its possible conformations, the superposition of the switch regions of all known structures and the present aIF2γ structure shows no obvious correlation between their conformations and the states of the γ-subunit (Fig. 4b). Indeed, at least the γ-subunit from Methanococcus jannaschii and one of the four γ-subunits of the present structure demonstrate that the N-terminal parts of switch 2 can occupy in the nucleotide-free state the same area that they occupy in the GTP-bound state (Fig. 4b). These data also show that switch 2 has the ability to attain spontaneous conformations that permit GTP binding. Nevertheless, as noted previously, such spontaneous GTP binding cannot occur before loop P and switch 2 attain the suitable conformations. In Eukarya, eIF2B possibly stabilizes suitable conformations of switch 2 and thus gives GTP a preference in the competition with GDP.

Conformational flexibility of aIF2

Contrary to EF-1A, there is no visible conformational flexibility in the aIF2γ structure. Moreover, superposition of aIF2αγ and aIF2βγ heterodimers and the present intact aIF2 structures shows that domain 3 of aIF2α and the N-terminal helix of aIF2β retain their positions relative to aIF2γ in both nucleotide-free and GDP-bound forms of aIF2. The conformational flexibility of aIF2 that is probably required for its function can be achieved through movement of domains 1 and 2 of aIF2α (Fig. 5) and
the central part and the zinc-binding domain of aIF2β (Fig. 6).

In eukaryotes, the eIF2α subunit plays a key role in translational regulation and is a target of specific kinases in response to several stress conditions. Amino acid residue Ser51 in eIF2α has been identified as a phosphorylation site for these kinases. It was shown that phosphorylation of Ser51 converts eIF2 into a competition inhibitor of eIF2B, which results in strong inhibition of translation initiation. This serine residue is conserved in all eukaryal IF2α sequences but not in archaeal IF2α. Nevertheless, it was shown that an aIF2-specific kinase from Pyrococcus horikoshii can phosphorylate Ser48 within the

---

**Fig. 5.** Mutual arrangement of the α- and γ-subunits. (a) Front view. The α-subunit from the present structure is shown in magenta; the isolated aIF2α from _P._ abyssi, in green; the α-subunit from SsoIF2αγ heterodimer, in light blue; and the human α-subunit, in yellow. Residue Ser48 of aIF2α (Ser51 of eIF2α) responsible for phosphorylation is indicated by balls. (b) Same as (a) but rotated by 90°. The γ-subunit from the presented heterotrimer is shown in gray.
Fig. 6. Mutual arrangement of the β- and γ-subunits. The β-subunit from the present structure is shown in dark blue; the β-subunit from P. furiosus αIF2βγ heterodimer,16 in orange; and the β-subunit from the incomplete heterotrimer SsoIF2,17 in magenta. The γ-subunit from the present structure (chain E) is shown in gray. Hairpins β2–β3 are shown in green to show mutual arrangements of central domains from different crystal structures of the β-subunits relative to the γ-subunit.

Yatime et al.15 suggested an indirect effect of αIF2γ on the affinity of the γ-subunit for tRNAi. Superposition of domain 3 of the isolated human eIF2α11 and that of the present intact αIF2 structure (Fig. 5) showed that the long C-terminus of eIF2α (residues 273–302) can contact switch 1 of eIF2γ and may thus affect tRNAi binding to eIF2. However, in Archaea, such a function of the C-terminal part of αIF2 domain 3 is not conceivable: the α-subunit of αIF2 is shorter (only 266 amino acid residues in SsoIF2) and its domain 3 has no unfolded part and forms one tight contact with the γ-subunit. Moreover, domains 1 and 2 of the α-subunit of αIF2 do not interact with the γ-subunit in all observed mutual positions. Based on these data, it is reasonable to conclude that only direct interactions between the α-subunit and tRNAi can account for the role of this subunit in tRNAi binding.3,14 A model for the αIF2αγ–tRNAi complex has been proposed in our previous publication.14 This model demonstrates how the third domain of the α-subunit can be involved in an extensive direct interaction with tRNAi. Moreover, this model shows how the binding specificity for tRNAi could be achieved through the interaction of the αIF2γ with a unique ridge formed by two bulged nucleotides on the tRNAi surface. Discrimination against all aminoacylated tRNAs except tRNAi is a key feature of the IF2. It would be very difficult to explain this feature through an indirect role of the α-subunit.

The αIF2γ regions responsible for αIF2β binding (residues 152–200 of SsoIF2γ) were superimposed and the obtained matrices were used to find the corresponding αIF2β16 positions to determine the mutual arrangements of the β- and γ-subunits in different αIF2 structures. Figure 6 clearly shows that only the N-terminal α-helical parts of αIF2β (residues 5–17) retain their positions in all known structures. The conformations of the central parts and zinc-binding domains of αIF2β vary in each structure. It is interesting to note that the central part of αIF2β16 contacts the G-domain of αIF2γ in the P. furiosus αIF2βγ heterodimer, whereas the zinc-binding domain interacts with close parts of the αIF2γ G-domain in the structures of the truncated αIF2β.
and full-sized (this work) heterotrimeric αIF2 from S. solfataricus.

Our analysis revealed that the intact αIF2 structure can be considered as consisting of three parts: a rigid central part and two mobile “wings.” The rigid part contains the γ-subunit, domain 3 of the α-subunit, and the N-terminal α-helix of the β-subunit. The mobile wings include domains 1 and 2 of the α-subunit from one side and practically the entire β-subunit from the opposite side. It appears that inter- and intra-subunit interactions in αIF2 cannot ensure its stable conformation. Interactions with external partners are needed to fix the positions of the mobile parts. In crystals, the symmetry-related molecules manifest themselves as such partners. As a result, different crystallization procedures not only produce different crystal forms of αIF2 but also can change the shape of mobile parts of the molecule.

Materials and Methods

Overproduction of the α-, β-, and γ-subunits and purification of the intact SsoIF2αβγ

The genes encoding the α-, β-, and γ-subunits of S. solfataricus αIF2 were amplified by PCR and cloned in vectors pET11c (for the α-subunit gene) and pET11d (for the β- and γ-subunit genes). The resulting plasmids were introduced into Escherichia coli strain BL21(DE3) (Novagen; for production of the α- and β-subunits) or C41(DE3) (Imaxio; for production of the γ-subunit). The bacterial strains were grown in LB medium containing 100 μg ml⁻¹ of ampicillin. The synthesis of each subunit was induced by addition of IPTG (final concentration = 1 mM), and the cells were harvested 3 h later. The purification of the full-sized Sso-αIF2αβγ was performed with some modifications of the procedure described in Ref. 15. The E. coli cells were resuspended in buffer A [10 mM Hepes, pH 7.5, 100 mM NaCl, 0.1 mM ethylenediaminetetraacetic acid (EDTA), and 0.1 mM 2-mercaptoethanol] and disrupted by sonication. Cellular debris was removed by centrifugation for 20 min at 14,000g. After centrifugation, the supernatants were heated for 20 min at 65 °C. After removal of unstable proteins by centrifugation, the supernatants were mixed and directly loaded onto an S-Sepharose column equilibrated in buffer A. The assembled heterotrimer Sso-αIF2αβγ was eluted with a linear gradient of NaCl (100–400 mM) in 10 mM Hepes, pH 7.5, 0.1 mM EDTA, and 10 mM 2-mercaptoethanol. The fractions were analyzed by SDS–gel electrophoresis. The fractions containing all three subunits were pooled and concentrated. The protein solution was diluted with buffer B (10 mM Tris–HCl, pH 8.0, 100 mM KCl, 10 mM MgCl₂, 1 mM EDTA, and 10 mM β-mercaptoethanol), loaded onto a heparin-Sepharose column equilibrated with buffer B, and then eluted with a linear gradient of KCl (100–500 mM) in 10 mM Tris–HCl, pH 8.0, 10 mM MgCl₂, 1 mM EDTA, and 10 mM β-mercaptoethanol. Fractions containing pure Sso-αIF2αβγ were collected; the protein solution was concentrated to a small volume and finally loaded onto a Superdex 200 column (16 mm × 60 cm; Amersham) equilibrated with buffer C (10 mM Tris–HCl, pH 8.0, 200 mM KCl, 0.3 mM EDTA, and 2 mM 1,4-dithiothreitol). Fractions containing homogeneous Sso-αIF2αβγ protein (as judged by UV spectral analysis and non-denaturing PAGE) were pooled and concentrated.

Crystallization of the intact SsoIF2αβγ

All crystallization trials were carried out at 22 °C using the hanging-drop vapor-diffusion technique by mixing 2.0 μl of the protein solution (10–20 mg ml⁻¹ of Sso-αIF2αβγ in buffer C) with 2.0 μl of reservoir solution [100 mM Tris–HCl, pH 8.5, 720 mM sodium formate, 9% PEG (polyethylene glycol) 8000, and 9% PEG 1000] and 1.0 μl of 5% monomethyl ether PEG 5000 as an additive.

Cryoprotection of the crystals was achieved by adding ethylene glycol to the reservoir solution to a 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 at DESY (Hamburg, Germany) using the MAR CCD detector. The data were processed and merged with the XDS program suite.29 An analysis of diffraction data suggested that the crystal had an orthorhombic symmetry. A molecular replacement solution was obtained with the program PHASER30 using space group P2₁2₁2₁. The structure of αIF2 in the apo form was used as a search model [Protein Data Bank (PDB) code 2PLF]. The unique solution was found and used to calculate an initial electron density map. The quality of the map was sufficient to build the γ- and α-subunits of two αIF2 molecules in the asymmetric unit. The β-subunit was not visible. The model was subjected to several rounds of computational refinement and map calculation with Crystallography & NMR System31 as well as manual model inspection and modification with O.32 However, we could not confidently detect the β-subunit exclusive of its N-terminal α-helix and refine this model to an R-free value less than 40%. Detailed analysis of the experimental data showed that the crystals of intact αIF2 are twinned and...
that they belonged to the monoclinic symmetry system with four alF2 molecules in the asymmetric unit. The cumulative intensity distribution calculated with DETWIN of the CCP4 program suite\(^3\) indicated pseudo-hemihedral twinning in space group \(P2_1\). The twin low \(b\), \(-k\), \(-l\) describes a real space rotation about axis \(a\) of the unit cell. The twin fraction was estimated to be 0.367. The previously determined structure of alF2\(\gamma\) was used as a search model to find unique positions of all heterodimers in this space group. The unique solution was found also with the program PHASER and twinned data.

At the next stage of structure determination, the program suite PHENIX\(^1\) was used. The twin fraction calculated by this program increased noticeably and was estimated to be 0.465. The electron density calculated after several rounds of this program increased noticeably and was estimated to be 0.556. The electron density calculated after several rounds of refinement and manual model modification allowed us to build the \(\beta\)-subunits in two NCS-related molecules of the alF2 factor. These \(\beta\)-subunits occupy positions on the local 2-fold axes, which connect the alF2\(\alpha\gamma\) heterodimers in the asymmetric unit. At the final step of the refinement, the translation-liberation-screw procedure was used. The final model, refined to an \(R\)-free factor of 27.6% and an \(R\)-factor of 22.5% at 2.8-Å resolution, includes 3023 amino acid residues. Data and refinement statistics are summarized in Table 2. NCS restraints were used during the early stages of refinement, but, finally, all four alF2 molecules in the asymmetric unit were refined separately. The model bias present in the initial molecular replacement solution was tackled using composite omit, cross-validated, and \(\sigma\)-A-weighted maps implemented in Crystallography & NMR System.

PDB accession number

The coordinates and structure factors for the crystal structures of the intact alF2\(\alpha\beta\gamma\) have been deposited in the PDB under ID code 3CW2.

Acknowledgements

This research was supported by the Russian Academy of Sciences, the Russian Foundation for Basic Research (05-04-48696), the Program of Russian Academy of Sciences on Molecular and Cellular Biology, and the Program of the Russian Federation President in support of outstanding scientific schools. The research of O.N. was supported by International Association for the promotion of cooperation with scientists from the New Independent States of the former Soviet Union through grant no. 05-109-4979, and that of M.G. was supported, in part, by an International Research Scholar’s award from the Howard Hughes Medical Institute. The work in U.B.’s laboratory was supported by the Austrian Science Fund through grant no. 15334. D.M. acknowledges the Fonds der Chemischen Industrie. We thank David Hasenöhrl for his support in cloning.

References

Crystal Structure of eIF2


