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Isolation and Crystallization of Heterotrimeric Translation Initiation Factor 2 from *Sulfolobus solfataricus*

E. A. Stolboushkina*, O. S. Nikonov, and M. B. Garber

Institute of Protein Research, Russian Academy of Sciences, 142290 Pushchino, Moscow Region, Russia; fax: (495) 632-7871; E-mail: esmail@vega.protres.ru

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Abstract—The structure of the intact heterotrimeric translation initiation factor 2 (e/aIF2) is of great interest due to its key role in the initiator tRNA delivery to the ribosome and in translation initiation regulation in eukaryotes and archaea. We have chosen aIF2 from the hyperthermophilic archaeobacterium *Sulfolobus solfataricus* (SsoIF2) as an object for crystallization and structural investigations. Genes of the SsoIF2 subunits α, β, and γ were cloned and superexpressed. A method for heterotrimer SsoIF2αβγ purification was elaborated with at least 95% purity. Highly ordered crystals of the full-sized SsoIF2, reflecting X-rays at the resolution up to 2.8 Å, were obtained for the first time.

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Key words: translation initiation, e/aIF2, heterotrimer, *Sulfolobus solfataricus*, SsoIF2 crystals

The translation initiation factor 2 (IF2) plays a key role in the initiation of polypeptide chain synthesis on the ribosome. The translation initiation factor 2 complexed with GTP delivers the initiator methionyl-tRNA (in bacteria it is formyl-methionyl-tRNA) into the P site of the small ribosomal subunit. Bacterial IF2 is a big six-domain monomeric protein. It exists within the cell as three functionally active isoforms with molecular mass from 80 to 100 kD. Structures of only some domains of bacterial IF2 are presently known [1-4].

In Eukarya and Archaea, the translation initiation heterotrimeric factor 2αβγ carry out the same function (eIF2 and aIF2, respectively). Eukaryal eIF2 and archaeal aIF2 are homologous to each other (40% homology for subunit α, 27% for subunit β, and 50% for subunit γ), but neither of these subunits has homology to bacterial IF2.

Functional investigations of isolated e/aIF2 subunits have shown that γ-subunit forms a heterotrimer core by interaction with α- and β-subunits, which do not contact with each other [5-7]. The isolated γ-subunit complexed with GTP binds initiator methionyl-tRNA (Met-$tRNA_i^{Met}$) but much more weakly than within the complete translation initiation factor 2. The archaeal αγ-dimer was shown to bind Met-$tRNA_i^{Met}$ with affinity equal to that of complete aIF2 [6, 7]. α-Subunit has RNA-binding properties [6] and, evidently due to direct interaction with tRNA, it stabilizes the complex γ-subunit with Met-$tRNA_i^{Met}$. However, in eukaryotes the βγ-dimer is responsible for binding to Met-$tRNA_i^{Met}$ [8, 9], while the main function of the α-subunit of eIF2 is regulation of translation initiation by its specific phosphorylation/dephosphorylation [10, 11]. Recently a system for specific phosphorylation of the α-subunit of aIF2, analogous to that for phosphorylation of α-subunit of eukaryal translation initiation factor 2, was found in the archaeobacterium *Pyrococcus horikoshii* [12]. Probably, the α-subunit of aIF2 in Archaea is also involved in protein biosynthesis regulation at the stage of initiation.

β-Subunit of archaeal IF2 is smaller by half in size compared to subunit β of eukaryotic IF2 and is homologous only to its C-terminal part [13, 14]. The N-terminal part of eIF2β interacts with other two translation initiation factors, eIF2B and eIF5, that are necessary for functioning of complete eIF2 [15-17]. No eIF2B and eIF5 homologs and analogs have been found in Archaea. The role of β-subunit of archaeal translation initiation factor

Abbreviations: DTT, 1,4-dithiothreitol; e/aIF2, eukaryotic/archaean heterotrimeric translation initiation factor 2; PEG, polyethylene glycol; PEGMME, polyethylene glycol monomethyl ether; SsoIF2, aIF2 from the hyperthermophilic archaeobacterium *Sulfolobus solfataricus*.

* To whom correspondence should be addressed.

2 as well as that of C-terminal part of eukaryotic eIF2β is still unknown. However, it is reasonable to assume that they are involved in the start codon recognition, probably via interaction with mRNA [18].

The structure of intact heterotrimeric translation initiation factor 2 is of great interest due to the importance of its function. Active structural investigations of this heterotrimeric protein have been under way for over five years: by now already known are structures of isolated subunits [14, 19-25], intersubunit dimers αγ [26] and βγ [27], and recently the structure of truncated heterodimeric aIF2 was determined in which the first and second domains of α-subunit were removed from the recombinant protein [28]. Up to the present moment, the structure of the full-sized heterotrimeric translation initiation factor 2 remained unknown because of the high interdomain mobility of subunits, making it impossible to obtain large highly ordered crystals of this object.

We have chosen as an object of investigations the thermostable archaeal translation initiation factor 2 from *Sulfolobus solfataricus* (SsoIF2). This work deals with cloning genes of all three SsoIF2 subunits, obtaining subunit superproducing strains, developing a method for isolation of a homogeneous preparation of SsoIF2 heterotrimer, and obtaining full-size SsoIF2 crystals suitable for X-ray analysis.

MATERIALS AND METHODS

Materials. All chemical reagents were from Sigma (USA), Serva (Germany), and Merck (Germany). Enzymes Vent-DNA polymerase, site-specific restriction endonucleases *Nde*I, *Nco*I, and *Bam*HI from Sibenzyme (Russia), T4 DNA ligase from Fermentas (Lithuania), and synthetic oligonucleotide primers from Syntol (Russia) were used. Plasmids pET11c and pET11d (Novagen, USA), cells of *Escherichia coli* strains XL1-Blue and BL21(DE3) (Novagen), C41(DE3) (Imaxio, France), and chromatographic resins S-Sepharose, Heparin-Sepharose, and Superdex-200 (all from Pharmacia (Sweden)) were used in this work. In addition, isopropyl β-D-thiogalactoside (IPTG) from Takara (Japan) and ampicillin from Biokhimik (Russia) were used.

Cloning the genes of α-, β-, and γ-subunits of SsoIF2. Genes of α-, β-, and γ-subunits (*sso_α*, *sso_β*, and *sso_γ*, respectively) were amplified by polymerase chain reaction (PCR) using as templates plasmid DNA containing base sequences of genes of these subunits together with sequences for oligohistidine "tails" [7]. The following oligonucleotide primers contain at their ends sites for recognition of site-specific restriction endonucleases *Nco*I (*Nde*I in the case of cloning α-subunit) and *Bam*HI. Forward primers: F *sso_α*, 5′-GGAATTCCATATGATTTACAGTAGAAGCAAA-3′; F *sso_β*, 5′-CATGCCATGGGTAGTTCAGAAAAAGAATAC-3′; F *sso_γ*, 5′-CATACCATGGCATGGCCTAAAGTTCAACC-3′. Reverse primers: R *sso_α*, 5′-CGGGATCCTCATTTCTTAACCACACTTATA-3′; R *sso_β*, 5′-CGGGATCCTCATAGTGGTTTCACTGGTGTT-3′; R *sso_γ*, 5′-CGGGATCCTTAGATCTCTACTAAACCCCATC-3′.

PCR was carried out in a volume of 100 μl. The reactive mixture contained 10 μl of tenfold PCR buffer (200 mM Tris-HCl, pH 8.8, 100 mM $(NH_4)_2SO_4$, 20 mM $MgSO_4$, 100 mM KCl, and 1% Triton X-100), a mixture of deoxyribonucleotides (dATP, dCTP, dGTP, and dTTP, 0.2 mM each), primers (100 pmol each), 20 ng plasmid DNA, and 5 activity units of Vent-DNA polymerase. The DNA of the gene was amplified over 35 cycles. Each cycle included three steps: DNA denaturation at 95°C for 30 sec, primer annealing for 60 sec at 55°C, DNA synthesis for 2 min at 72°C. To complete formation of double-stranded PCR products, the reactive mixture was additionally heated for 4 min at 72°C. Reaction results were analyzed by electrophoresis in 1% agarose gel.

Plasmid pET11d and amplified genes of β- and γ-subunits, purified using the QIAquick® PCR Purification Kit (Qiagen), were treated with restriction endonucleases *Nco*I and *Bam*HI. Plasmid pET11c and the α-subunit gene were treated with restriction endonucleases *Nde*I and *Bam*HI. The resulting fragments were purified by electrophoresis with subsequent elution from 1% agarose. Ligation was carried out in a volume of 20 μl. The ligase mixture contained 50 ng vector and 50 ng PCR fragment. The *E. coli* strain XL1-Blue cells were transformed by ligase mixture and plated onto agarized LB medium with 100 μg/ml ampicillin. Plasmid DNA was isolated from grown colonies and analyzed for the insert by PCR with gene-specific primers. The gene sequence was detected by sequencing (Institute of Protein Research, Russian Academy of Sciences) using universal T7 primers.

Obtaining superproducing strains and production of SsoIF2 subunits α, β, and γ. To obtain superproducing strains, Studier's system [29] was used. The *E. coli* cells of BL21(DE3) strain were transformed by the recombinant plasmids pET11c-*sso_α* and pET11d-*sso_β*, containing genes of α- and β-subunits, respectively, under control of T7 promoter. Plasmid pET11c-*sso_γ* containing the gene of γ-subunit was used to transform *E. coli* cells strain C41(DE3). Transformed cells were plated onto LB medium with 100 μg/ml ampicillin and grown at 37°C under intensive mixing (200 rpm) till absorption (A_{600}) 0.8 optical unit/ml, IPTG was added till final concentration 1 mM, and incubation continued under the same conditions for 3 h. Then cells were pelleted by centrifugation at 5000*g* for 20 min at 4°C. The level of SsoIF2 subunit production was estimated by electrophoresis in 15% polyacrylamide gel in the presence of SDS according to Laemmli [30]. Gels were stained with Coomassie G250.

Intact SsoIF2 heterotrimer was isolated and purified as described previously [26] with significant modifica-

tions. Cells of *E. coli* strains superproducing each of the three SsoIF2 subunits were resuspended separately in buffer A (0.01 M Hepes-KOH, pH 7.5, 0.1 M NaCl, 0.1 mM EDTA, 10 mM β-mercaptoethanol) and sonicated. Disintegrated cell walls and membranes were removed by centrifugation for 20 min at 14,000*g* and 4°C. The supernatants were heated at 65°C for 20 min and then centrifuged again under the same conditions to remove thermolabile *E. coli* proteins. Supernatants, each of which contained one of three SsoIF2 subunits, were combined and applied onto a column (25 ml) with S-Sepharose, equilibrated in buffer A, at a rate of 30 ml/h. The column was washed with the same buffer overnight at a rate of 12 ml/h. The resin-bound reconstructed complete heterotrimer SsoIF2 was eluted with 2 × 200 ml linear gradient of NaCl concentration (0.1-0.4 M) in 0.01 M Hepes-KOH buffer, pH 7.5, containing 0.1 mM EDTA and 10 mM β-mercaptoethanol. Elution rate was 60 ml/h, fraction volume 10 ml. Absorption (A_{280}) of each fraction was measured. The absence of RNA from fractions was detected by the absorption spectrum. RNA-free fractions were analyzed by SDS-PAGE. Fractions from the central peak region, containing all three SsoIF2 subunits (α, β, γ), were combined and concentrated on a VivaSpin-10 concentrator (VivaScience, GB) till absorption (A_{280}) of 20-25 optical units/ml and subjected to further purification. This protein solution was diluted 20 times with buffer B (0.01 M Tris-HCl, pH 8.0, 0.1 M KCl, 0.01 M $MgCl_2$, 1 mM EDTA, 10 mM β-mercaptoethanol) and applied onto a column (25 ml) with Heparin-Sepharose, equilibrated with the same buffer, at a rate of 60 ml/h. The column was washed with buffer B (250 ml), the resin-bound SsoIF2 protein was eluted with 2 × 250 ml linear gradient of KCl concentration (0.1-0.5 M) in 0.01 M Tris-HCl, pH 8.0, containing 0.01 M $MgCl_2$, 1 mM EDTA, and 10 mM β-mercaptoethanol. Elution rate was 60 ml/h, fraction volume 4.5 ml. The SsoIF2 protein fractions from the central part of the peak were combined and concentrated on a VivaSpin-10 concentrator till concentration of 20-25 mg/ml. The same procedure was done with fractions from slopes of this peak. Protein solution was transferred to 1.5 ml microcentrifuge tubes, frozen in liquid nitrogen, and kept at −70°C. Concentration of complete SsoIF2 heterotrimer was determined spectrophotometrically at 280 nm using molar extinction coefficient ($\varepsilon = 107{,}820\ M^{-1}\cdot cm^{-1}$), calculated as the sum of extinction coefficients of all three subunits.

Before crystallization experiments, an aliquot of protein solution (100 μl) was thawed and gel filtered on Superdex-200 equilibrated with buffer C (0.01 M Tris-HCl, pH 8.0, 0.2 M KCl, 0.01 M $MgCl_2$, 0.3 mM EDTA, 2 mM DTT). Total volume of the resin was 24 ml, filtration rate 24 ml/h, and fraction volume 0.5 ml. The SsoIF2-containing fractions were combined and concentrated on a VivaSpin-10 concentrator to 20-25 mg/ml. Homogeneity of the protein preparation was also analyzed by electrophoresis in 13.5% polyacrylamide gel under non-denaturing conditions as described previously [6].

Full-size SsoIF2 was crystallized at 22°C by vapor diffusion in a hanging drop [31]. Initial experiments on crystallization were carried out using the Clear Strategy Screen I kit (Molecular Dimensions Limited, GB), on the SsoIF2 protein preparation purified by ion-exchange chromatography on S-Sepharose: 1 μl protein was mixed with 1 μl reagent and equilibrated against a vessel containing the same reagent from the kit. The first microcrystals of SsoIF2 were found in two similar conditions, No. 6 B5 and No. 18 B5. Improvement of the quality of protein preparation and conditions of its crystallization produced large perfect crystals of the full-size SsoIF2: 2 μl protein with concentration of 20-24 mg/ml were mixed with 2 μl No. 18 B5 (0.1 M Tris-HCl, pH 8.5, 0.72 M sodium formate, 9% PEG 8000, 9% PEG 1000) and 1 μl 5% PEGMME 5000 as an additive. The counter-solution volume was 400 μl. Crystals appeared 2-3 days later and grew during a week to dimensions of 600 × 200 × 40 μm. Fifteen percent ethylene glycol was chosen as cryoprotector for freezing crystals in liquid nitrogen.

RESULTS AND DISCUSSION

Construction of superproducing strains of α-, β-, and γ-subunits of SsoIF2. Plasmids containing genes of α-, β-, and γ-subunits of SsoIF2 were kindly given by Dr. U. Blasi (Vienna Biocenter, Austria). These plasmids appeared to be unsuitable for our purposes because subunits produced from such plasmids contained oligohistidine "tails" at their C-termini, which may interfere during crystallization of the protein. Owing to this, genes of α-, β-, and γ-subunits of SsoIF2 were re-cloned without the sequence, encoding the oligohistidine "tail" into expression vectors pET11c and pET11d under control of T7 promoter. The absence of mutations and correct orientation of nucleotide sequence of the SsoIF2 subunit genes in expression vectors were checked by sequencing.

The genes of the α-, β-, and γ-subunits of SsoIF2 were expressed in cells of *E. coli* strains BL21(DE3) and C41(DE3) (for γ-subunit) and analyzed by electrophoresis in 15% SDS-polyacrylamide gel. It was found (Fig. 1) that the level of γ-subunit production was very low compared to those for the other SsoIF2 subunits. To increase the expression efficiency of the SsoIF2γ encoding gene, we have tested different expression strains of *E. coli*. The synthesis of γ-subunit was observed only in the strain C41(DE3) intended for the toxic protein gene expression. Probably, toxic effect is associated with the RNA-binding activity of the γ-subunit of SsoIF2. Earlier a French group [19] also encountered the problem of toxicity for host cells of the produced γ-subunit of aIF2 from *Pyrococcus abyssi*.

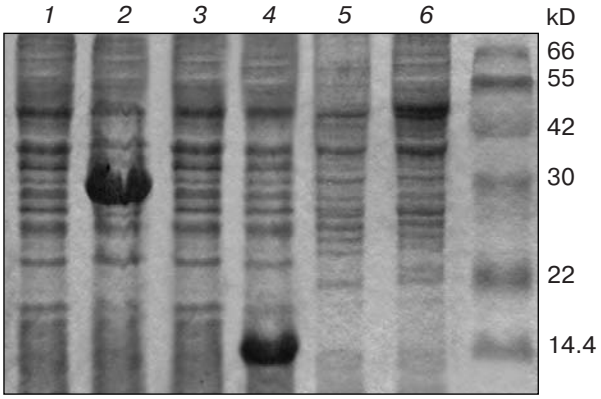

Fig. 1. Electrophoretic analysis in 15% SDS-polyacrylamide gel of production of the recombinant α-, β-, and γ-subunits of SsoIF2 in *E. coli* cells. *1*) Cell lysate of *E. coli* strain BL21(DE3)/pET11c-*sso_α* before induction; *2*) cell lysate of *E. coli* strain BL21(DE3)/pET11c-*sso_α* in 3 h after induction by IPTG; *3*) cell lysate of *E. coli* strain BL21(DE3)/pET11d-*sso_β* before induction; *4*) cell lysate of *E. coli* strain BL21(DE3)/pET11d-*sso_β* in 3 h after induction by IPTG; *5*) cell lysate of *E. coli* strain C41(DE3)/pET11c-*sso_γ* before induction; *6*) cell lysate of *E. coli* strain 41(DE3)/pET11c-*sso_γ* in 3 h after induction by IPTG (figures on the right show molecular mass values of marker proteins).

The problem was solved by spontaneous replacement of the unique amino acid residue G235D. However, it became clear later that such mutation does not allow γ-subunit to bind Met-tRNA$_i^{Met}$ (U. Blasi, unpublished).

Production of a homogeneous preparation of SsoIF2 heterotrimer. The thermostable 92 kD protein of SsoIF2 consists of three subunits: α (271 amino acid residues), β (142 residues), and γ (422 residues). One of the methods tested by us for production of homogeneous preparation of the subunit protein reduced to isolation of individual recombinant subunits from superproducing strains with subsequent *in vitro* reconstruction of SsoIF2 heterotrimer and its purification by gel filtration. The shortcoming of this approach is its multiphase character and a low yield of purified protein, insufficient for a broad search of conditions for SsoIF2 crystallization.

Recently the description of a different procedure for SsoIF2 heterotrimer isolation has appeared [26]: cells of each of three hyperproducing strains were mixed in equal amounts, disintegrated, cell debris were removed by centrifugation, and the cell lysate was heated with the following removal of precipitated denatured proteins of the host cells. Then the protein solution was fractionated by chromatography on S-Sepharose and gel filtration on Superdex-75. An attempt to use this method without modifications did not give the desired result. In the final variant the developed procedure for SsoIF2 isolation reduced to the following: the biomass of each of three producers was disintegrated separately (the amount of biomass of the γ-subunit of SsoIF2 superproducer was taken in threefold excess due to the low production of this protein), cell debris was removed by centrifugation, cell lysates were heated with subsequent removal of precipitated denatured host cell proteins, supernatants (each containing one of three subunits) were combined, and then the reconstructed SsoIF2 was purified by chromatography on S-Sepharose and Heparin-Sepharose and then by gel filtration on Superdex-200.

The brief heating of the cell lysate at 65°C with following chromatography on S-Sepharose (Fig. 2a) allowed us to obtain the SsoIF2 protein preparation free of RNA contaminants, with 90-92% purity (according to SDS-PAGE) (Fig. 2b). Minor contaminants and aggregates were removed from the SsoIF2 preparation by affinity chromatography on Heparin-Sepharose and gel filtration on Superdex-200, respectively. Final purity of the SsoIF2 homogeneous preparation (according to SDS-PAGE) reached approximately 95-98% (Fig. 3). The above-described technique allows us to obtain up to 30-40 mg of

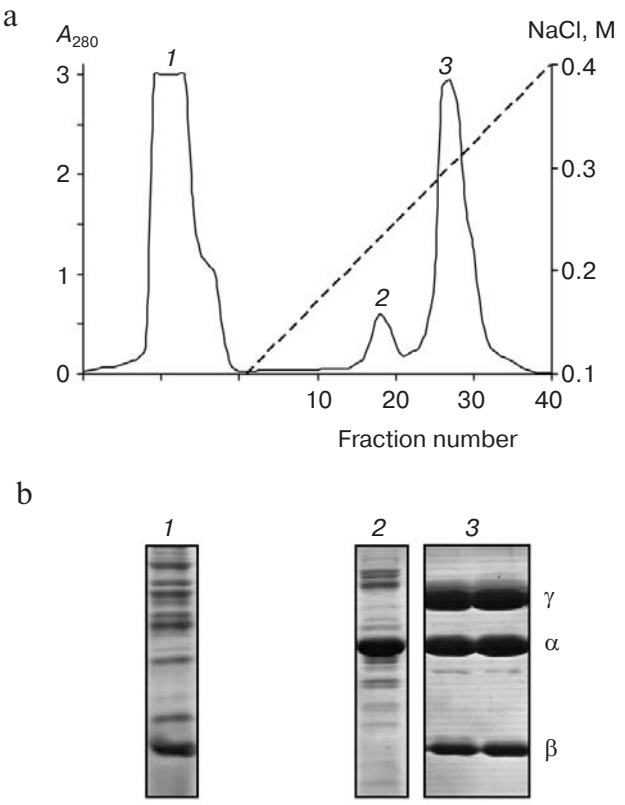

Fig. 2. a) Chromatography on S-Sepharose of the combined after heating cell lysates of strains superproducing α-, β-, and γ-subunits of SsoIF2. *1*) Elution profile of proteins not bound to the resin (including the excessive amount of the β-subunit of SsoIF2) and nucleic acids; *2*) elution profile of excessive amount of the α-subunit of SsoIF2; *3*) elution profile of the SsoIF2αβγ heterotrimer; dashed line marks linear gradient of NaCl concentration. b) Electrophoretic analysis of eluates in 15% SDS-polyacrylamide gel. Numbers on electrophoregrams correspond to numbers on elution profiles.

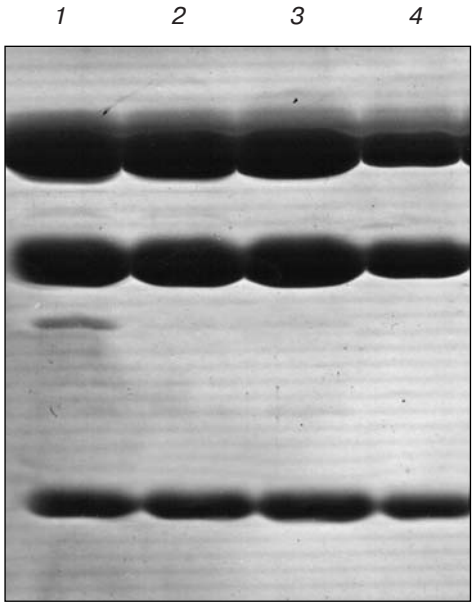

Fig. 3. Electrophoretic analysis (in 15% SDS-polyacrylamide gel) of the purity of SsoIF2 preparation obtained at different stages of chromatographic purification. *1*) Preparation after chromatography on S-Sepharose; *2*) preparation of combined fractions from slopes of the peak obtained upon chromatography on Heparin-Sepharose; *3*) preparation of combined central fractions of the peak obtained upon chromatography on Heparin-Sepharose; *4*) preparation after gel filtration on Superdex-200.

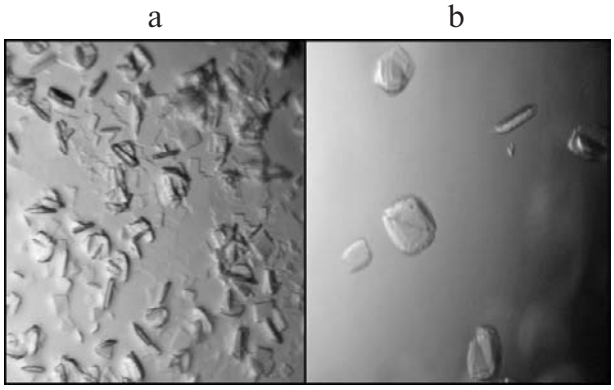

Fig. 4. Microcrystals of SsoIF2: a) crystals obtained in the presence of 0.05 M Tris-HCl, pH 8.5, 0.36 M sodium formate, 4.5% PEG 8000, 4.5% PEG 1000 (conditions No. 18 B5); b) crystals obtained in the presence of 0.05 M Tris-HCl, pH 8.5, 0.36 M sodium formate, 11.25% PEGMME 2000 (conditions No. 6 B5).

homogeneous SsoIF2 heterotrimer from 5 liters of culture (1 liter of culture of SsoIF2α superproducing strain, 1 liter of culture of SsoIF2β superproducing strain, and 3 liters of culture of SsoIF2γ superproducing strain) and it does not decrease protein activity as shown by experiments on binding to initiator methionyl-tRNA (data not shown). It is important to note that our isolated SsoIF2 protein remained stable without undergoing natural proteolysis, unlike SsoIF2 preparations isolated by the French group [28].

Crystallization of full-size heterotrimeric SsoIF2. We carried out the initiating search for crystallization conditions using the SsoIF2 protein preparation purified by the first chromatography on S-Sepharose and obtained two forms of microcrystals (Fig. 4). However, this result was not reproducible. Analysis of factors responsible for the crystal non-reproducibility showed that the protein in solution undergoes rapid aggregation, and the admixture of even a small amount of aggregates inhibits crystallization (Fig. 5). It was possible to remove aggregates by gel filtration and obtain a homogeneous protein preparation that began to form crystals, but after a short period aggregates appeared again in the protein solution. We supposed that the SsoIF2 aggregation is caused by partial oxidation of the protein SH-groups and formation of intermolecular S–S bonds. Owing to this, at all stages of the SsoIF2

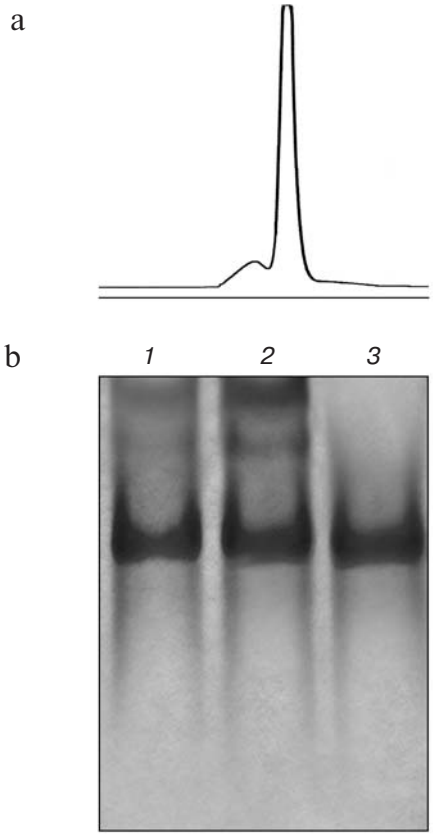

Fig. 5. Testing SsoIF2 protein preparation homogeneity by gel filtration on Superdex-200 (a) and electrophoresis under non-denaturing conditions (in 13.5% polyacrylamide gel) (b). a) Elution profile of SsoIF2 protein; b) electrophoretic analysis of SsoIF2 protein preparation: *1*) preparation before gel filtration; *2*) preparation of combined fraction from the left slope of the peak obtained upon gel filtration; *3*) preparation of combined central fractions of the peak obtained upon gel filtration. Preparations *1* and *2* exhibited no ability to crystallize.

trimer isolation we added β-mercaptoethanol or DTT to buffer solutions, and after purification the protein preparation either was immediately transferred into crystallization conditions or was quickly frozen in liquid nitrogen and kept at −70°C.

After establishment of conditions for isolation and keeping the crystallized protein preparations, we began our work on optimization of crystallization conditions. The most promising for obtaining large crystals were conditions with the precipitating reagent consisting of sodium formate and a mixture of polyethylene glycols. It is important to note that the emergence of crystals is strongly pH-dependent. The protein crystallized only at pH 8.5, near its isoelectric point. The protein concentration in the drop of 10-12 mg/ml and temperature 22°C were optimal for the SsoIF2 crystal growth, but a crucial role in the emergence of big monocrystals appeared to belong to addition to the crystallization medium of PEGMME 5000 to final concentration of 1%.

After each chromatographic stage, the SsoIF2 preparation was tested in crystallization experiments. The best quality crystals were obtained from combined fractions from the peak slopes upon chromatography on the column with Heparin-Sepharose (Fig. 6). Just these crystals reflected X-rays up to 2.8 Å and were then used to collect diffraction data. However, from one experiment to another, protein preparations differed in their ability to form large perfect crystals. Later we were able to grow large and well-ordered crystals after complete solubilization of the drop-precipitated small crystals or from seed crystals. These distinctions in the ability of protein preparations to crystallize can be considered as the result of SsoIF2 conformational heterogeneity caused by the high conformational flexibility and interdomain mobility within the structure of α- and β-subunits (unpublished data on the SsoIF2 structure).

To collect diffraction data (DESY synchrotron; Hamburg, Germany) from SsoIF2 crystals, ethylene glycol was added to the preserving solution as the cryoprotector. The final cryosolution contained 0.1 M Tris-HCl, pH 8.5, 0.72 M sodium formate, 9% PEG 8000, 9% PEG 1000, and 15% ethylene glycol.

The SsoIF2 αβγ structure was determined by the method of molecular substitution using the PHASER program [32], which is the most powerful tool for this method. The earlier determined by us structure of the γ-subunit of SsoIF2 was chosen as the starting model [25]. Electron density calculated on the basis of γ-subunit allowed us to find positions and to build the models for α-subunit and N-terminal α-helix of β-subunit. The solution was obtained in the $P2_12_12$ space group. We failed in obtaining electron density for the complete β-subunit at this stage despite numerous attempts to solve this problem by changing resolution, repeated processing of diffraction data sets, and other approaches. Further detailed analysis of experimental data has shown that the SsoIF2 crystals

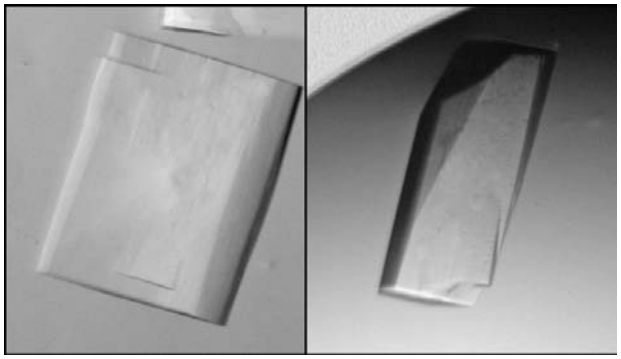

Fig. 6. SsoIF2 protein crystals obtained in the presence of 0.05 M Tris-HCl, pH 8.5, 0.36 M sodium formate, 4.5% PEG 8000, 4.5% PEG 1000, 1% PEGMME 5000. Crystals dimensions 600 × 200 × 40 μm.

should be included into a different space group P2(1), in which crystal pseudohemihedral twinning is found. The solution in this group was also found using the PHASER program for the model of αγ-dimer obtained at the first stage of the SsoIF2 structural investigations. Later the PHENIX program [33], making possible efficient work with this kind of twinning data, was used for structure calculation refinement. As a result, electron density was obtained for the whole β-subunit and the structure of the full-size archaeal translation initiation factor 2 was determined and now is in preparation for publication.

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