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 \( \alpha\beta\gamma \) carry out the same function (eIF2 and aIF2, respectively). Eukaryal eIF2 and archaeal aIF2 are homologous to each other (40% homology for subunit \( \alpha \), 27% for subunit \( \beta \), and 50% for subunit \( \gamma \)), but neither of these subunits has homology to bacterial IF2.

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

\( \beta \)-Subunit of archaeal IF2 is smaller by half in size compared to subunit \( \beta \) of eukaryotic IF2 and is homologous only to its C-terminal part [13, 14]. The N-terminal part of eIF2\( \beta \) 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 \( \beta \)-subunit of archaeal translation initiation factor...
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 NdeI, NcoI, and BamHI 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 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 NcoI (NdeI in the case of cloning α-subunit) and BamHI. Forward primers: F sso _α, 5‘-GGATCCCATGATTTACAGTAGAAGC-3‘; F sso _β, 5‘-CATGCCATGGTAGTTTCAGAATAAC-3‘; F sso _γ, 5‘-CATACCATGGCAGCTAAAGTTCACC-3‘. Reverse primers: R sso _α, 5‘-CGGGATCCATGAATTTAACCACACTTATA-3‘; R sso _β, 5‘-CGGGATCCATGATTTAACCACACTTATA-3‘; R sso _γ, 5‘-CGGGATCCATGATTTAACCACACTTATA-3‘. Reverse primers: R sso _α, 5‘-CGGGATCCATGAATTTAACCACACTTATA-3‘; R sso _β, 5‘-CGGGATCCATGATTTAACCACACTTATA-3‘; R sso _γ, 5‘-CGGGATCCATGATTTAACCACACTTATA-3‘.

PCR was carried out in a volume of 100 µl. The reaction mixture contained 10 µl of tenfold PCR buffer (200 mM Tris-HCl, pH 8.8, 100 mM (NH₄)₂SO₄, 20 mM MgSO₄, 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 NcoI and BamHI. Plasmid pET11c and the α-subunit gene were treated with restriction endonucleases NdeI and BamHI. The resulting fragments were purified by electrophoresis with subsequent elution from 1% agarose. Ligation was carried out in a volume of 20 µl. The ligation mixture contained 50 ng vector and 50 ng PCR fragment. The *E. coli* strain XL1-Blue cells were transformed by ligation 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 (A₅₇₀) 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 5000g 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-