

STRUCTURE NOTE

Crystal structure of *Arabidopsis* translation initiation factor eIF-5A2

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INTRODUCTION

The protein eukaryotic translation initiation factor 5A (eIF-5A) is a highly conserved eukaryotic translation initiation factor (eIF) found in eukaryotes and archaea.^{1–3} Biochemical and molecular studies revealed that eIF-5A is the sole protein that contains a modified amino acid residue hypusine (Nε-(4-amino-2-hydroxybutyl)lysine).⁴ The hypusination modification is made by two sequential reactions catalyzed by deoxyhypusine synthase (*EC* 1.1.1.249) and deoxyhypusine hydroxylase (*EC* 1.14.99.29).^{5–7}

eIF-5A was originally purified and identified from immature red blood cells.⁸ However, unlike the traditional translation initiation factors, eIF-5A is not essential for global protein synthesis^{8,9} but might be involved in mRNA translocation across the nuclear envelope.^{10,11} The hypusinated yeast eIF-5A was recently found to promote translation elongation.¹² Moreover, hypusine of the yeast eIF-5A has been found to be required for the sequence-specific interaction with RNA.¹³ To help clarify these diverse and even somewhat controversial functions, seven structures of eIF-5A from various organisms have been solved (*Methanococcus jannaschii*, PDB codes: 1eif and 2eif¹⁴; *Pyrobaculum aerophilum*, PDB code: 1bkb¹⁵; *Pyrococcus horikoshii*, PDB code: 1iz6¹⁶; *Leishmania braziliensis*, PDB code: 1 × 6o; *Leishmania mexicana*, PDB code: 1 × td; *Homo sapiens*, PDB code: 3cpf; *Saccharomyces cerevisiae*, PDB code: 3er0). They all share an overall structure of two domains, both of which resemble the nucleic acid binding fold.¹⁴

The plant *Arabidopsis thaliana* encodes three isoforms of eIF-5A: AteIF-5A1, 2, and 3 (GenBank Accession Numbers AF296082, BE039424, and AV526594). As the best investigated one, eIF-5A2 has been found to play a crucial role in plant growth and development by controlling cell proliferation and senescence.¹⁷ Here, we report the crystal structure of eIF-5A2 at 2.3 Å resolution, which represents a novel dimerization pattern specifically conserved in all plants.

METHODS

Construction, expression, and purification of eIF-5A

The coding sequence of *eIF-5A* gene was amplified by PCR using the cDNA library of *A. thaliana* as the template. An additional sequence coding for a six-histidine tag was introduced at the 5' end of the gene during PCR amplification. Then, the PCR product was cloned into a pET28a-derived vector and expressed at 37°C using the transformed *Escherichia coli* BL-21 (DE3) strain and 2 ×

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YT medium (OXOID LTD.) supplemented with 30 µg/mL kanamycin. When the cell culture reached an OD_{600 nm} of 0.6, protein expression was induced with 0.2 mM IPTG (BBI), and the cells were grown for a further 20 h at 16°C. Cells were collected by centrifugation, resuspended in 30 mL buffer containing 200 mM NaCl and 20 mM Tris-HCl, pH 7.5. Cells were lysed by three cycles of freezing/thawing followed by sonication. His-tagged proteins were purified using a Ni²⁺ affinity column. Eluted protein was further purified by gel filtration using a SuperdexTM 75 column (GE Healthcare Bioscience) equilibrated in 200 mM NaCl, 20 mM β-mercaptoethanol, and 20 mM Tris-HCl, pH 7.5. The purity of the pooled fractions was checked by SDS-PAGE.

Crystallization, data collection, and processing

Crystals of eIF-5A2 were obtained by the hanging drop vapor diffusion method at 16°C. In each drop of crystallization, 1 µL protein sample at 10 mg/mL in the buffer of 20 mM NaCl, 20 mM Tris-HCl, pH 7.5, and 20 mM β-mercaptoethanol was mixed with 1 µL reservoir solution (25% ethylene glycol) and equilibrated against 0.5 mL reservoir solution. Typically, crystals appeared in 1–2 days and reached the maximum size of 300 × 400 × 0.05 µm in 1 week. The crystal was flash frozen at 100 K in a stream of nitrogen gas. Images of diffraction were collected using a MAR345dtb detector (MarResearch, Germany), with wavelength of 1.5418 Å and oscillation of 1°. X-ray crystallographic data were processed with MOSFLM¹⁸ and scaled with SCALA.¹⁹

Structure solution and refinement

The structure was determined by molecular replacement method with the program PHASER,²⁰ using the structure of eIF-5A from *L. braziliensis* (PDB code 1 × 6o) as the search model. The initial model was refined by using the maximum likelihood method implemented in REFMAC5²¹ as part of CCP4²² program suite and rebuilt interactively by using the σ_A-weighted electron density maps with coefficients 2mFo-Fc and mFo-Fc in the program COOT.²³ The final model was validated with the programs PROCHECK²⁴ and MOLPROBITY.²⁵ Structure factors and coordinates have been deposited in the Protein Data Bank (PDB <http://www.rcsb.org/pdb>) under the accession code of 3HKS. The final statistics and refinement parameters were listed in Table I. All the structure figures were prepared using the program PyMol (<http://pymol.sourceforge.net/>).²⁶

RESULTS AND DISCUSSION

Overall structure

The crystal structure of eIF-5A2 was refined to the resolution of 2.3 Å, with two subunits (A and B) in one

Table I

Crystal Parameters, Data Collection and Structure Refinement Statistics

Data processing	
Space group	<i>P2₁2₁2₁</i>
Unit cell (Å, °)	<i>a</i> = 56.56, <i>b</i> = 79.83, <i>c</i> = 94.95, α = β = γ = 90.00
Resolution range (Å)	94.92–2.23 (2.28–2.23) ^a
Unique reflections	20,393 (1,293)
Completeness (%)	99.5 (96.4)
⟨ <i>I</i> /σ(<i>I</i>)⟩	22.1 (8.1)
<i>R</i> _{merge} ^b (%)	5.6 (23.9)
Average redundancy	5.7 (5.7)
Refinement statistics	
Resolution range (Å)	94.92–2.23
<i>R</i> -factor ^c / <i>R</i> -free ^d (%)	20.82/24.26
Number of protein atoms	2,358
Number of water atoms	169
RMSD ^e bond lengths (Å)	0.011
RMSD bond angles (°)	1.248
Mean B factors (Å ²)	22.15
Ramachandran plot^f (residues, %)	
Most favored (%)	97.41
Additional allowed (%)	2.59
Outliers (%)	0
PDB entry	3HKS

^aThe values in parentheses refer to statistics in the highest bin.

^b $R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of an observation and $\langle I(hkl) \rangle$ is the mean value for its unique reflection; Summations are over all reflections.

^c $R\text{-factor} = \sum_h |F_o(h) - F_c(h)| / \sum_h F_o(h)$, where F_o and F_c are the observed and calculated structure-factor amplitudes, respectively.

^d*R*-free was calculated with 5% of the data excluded from the refinement.

^eRoot-mean square-deviation from ideal values.

^fCategories were defined by Molprobability.

asymmetric unit. The majority of residues are well fitted in the electron density map, except for the N-terminal His-tag and residues Met1-Ala15 in both subunits, and the last two residues Gly158 and Lys159 in subunit B. The side chains of Arg87, Asp106, and Asp135 in subunit A, and those of His52, Glu132 in subunit B are fitted in the electron density map at a lower occupancy, using the program PHOENIX.²⁷ At the surface, six molecules of ethylene glycol are well fitted in the flat but slightly longer pieces of electron density [Fig. 1(A)]. The final refinement and validation statistics are listed in Table I.

The structure of eIF-5A2 comprises two distinct domains of antiparallel β-sheet that resembles the classic architecture of eIF-5A [Fig. 1(A)]. The highly conserved N-terminal domain has an SH3-like barrel motif,¹⁶ composed of strands β1–β6 and a ₃10 helix. Strands β3–β6 forms a distorted semiopen β-barrel. The hypusine modification site Lys51 is located at the protruding loop between strands β3 and β4. The C-terminal domain resembles the oligonucleotide-binding fold (OB fold)³⁰, and it contains strands β7–β12 and helix α1.

Comparative structural analysis with the DALI server (http://ekhidna.biocenter.helsinki.fi/dali_server)³¹ indicates that both the N- and C-terminal domains have the nucleic acid binding fold as previous reports.^{14,16} The

code:2eif), the two $\beta 3$ strands interact with each other via six hydrogen bonds to form a continuous six-stranded antiparallel β -sheet, leading to the two active site loops pointing to the opposite directions.¹⁴ In human eIF-5A dimer (PDB code: 3cpf), the two chains are linked via a disulfide bond. The dimeric structure of eIF-5A2 formed between subunits A and B was significantly different than those observed in other structures. The two subunits form a dimer in parallel via an interface of 400 Å² between the N-terminal domains, and one of 280 Å² between the C-terminal domains, respectively [Fig. 1(A)]. In fact, a small fraction of eIF-5A2 also exists as homodimer in solution, as detected by gel filtration (data not shown).

To have a better view, the two interfaces between the pairs of N- and C-terminal domains are shown in Figure 1(C). The N-terminal interface is stabilized by the surrounding residues via three hydrogen bonds: Ser22A-N ~ Gln21B-N ϵ ; Gln21A-N ϵ ~ Pro20B-O; and Pro20A-O ~ Tyr19B-O ϵ [Fig. 1(C)]. The smaller C-terminal interface possesses two main chain hydrogen bonds of Ser107A ~ Thr110B and Thr110 ~ ASer107B [Fig. 1(C)].

The structure of eIF-5A2 represents the first structure of plant eIF-5A, indicating a rather distinct dimerization pattern from the previous eIF-5A of known structure. After performing a multiple sequence-alignment by the programs MultAlin²⁸ and ESPript,²⁹ we noticed that the residues Tyr19 (substituted by Phe in several cases), Pro20, and Gln21 are highly conserved in all species of plant [Fig. 1(D)]. All these three residues are crucial to stabilize the N-terminal interface [Fig. 1(C)]. The residues contributing to the C-terminal interface are also conserved [Fig. 1(D)]. Thus, we proposed that this dimerization pattern is unique in plants. Further biochemical and biophysical analyses are needed to prove whether this form of dimer could facilitate the RNA binding.

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