# Crystallization and Preliminary Crystallographic Analysis of Human Eukaryotic Translation Initiation Factor 5A (eIF-5A)

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**Abstract:** Eukaryotic translation initiation factor 5A (eIF-5A) is universally found in all eukaryotic cells. It is the only protein in nature known to contain the unusual amino acid hypusine, a post-translationally modified lysine. Recombinant human eIF-5A was crystallized by the hanging-drop vapor diffusion method. Crystals were grown at 291K using $(NH_4)_2SO_4$ as precipitant. Diffraction data were obtained to a resolution of 2.7Å from a single frozen crystal belonging to space group *C2*, with unit-cell parameters a=147.1Å, b=60.4Å, c=76.4Å, β=92.4°. There are more than three molecules per asymmetric unit.

## 1. INTRODUCTION

Eukaryotic translation initiation factor 5A (eIF-5A) is universally found in eukaryotes [1] and archaebacteria [2]. It has been reported that, in mammalian cells, eIF-5A is present both in the cytoplasm and nucleus [3]. The reason that eIF-5A is named as an initiation factor comes from earlier observations that it can be isolated from the ribosome-bound fraction and is considered to be involved in the first step peptide bond formation in translation [4]. It is the only protein in nature known to contain a hypusine residue. This unusual amino acid is formed via a two-step post-translational modification which is carried out by deoxyhypusine synthase and deoxyhypusine hydroxylase [1, 5]. It has been reported that hypusine formation is closely associated with the G1-S stage of the cell cycle [6], and that inhibition of the deoxyhypusine synthase activity leads to growth arrest [7-8], tumor differentiation [9] or cell death [10].

Recent reports have indicated that eIF-5A may serve as a cofactor for the Rev protein of human immunodeficiency virus type 1 (HIV-1) [11] and the Rex protein of human T-cell leukemia virus type 1 (HTLV-1) [12]. Nevertheless, experimental data has suggested a lack of direct evidence for the interaction between eIF-5A and viral proteins [13].

Although eIF-5A is essential for cell survival and proliferation, its precise physiological function remains unclear. The role of eIF-5A in translation initiation has been doubted because of the absence of any obvious correlation between eIF-5A and general protein synthesis [14-16]. The findings that eIF-5A can bind to RRE and U6 [17] and that eIF-5A recognizes synthetic RNA in a sequence-dependent manner [18] suggest that eIF-5A may function as an RNA-binding protein. This suggestion is further supported by two X-ray diffraction studies of the archaeal eIF-5A precursor, in which it is revealed that the archaeal eIF-5A contains two domains: one contains the positively-charged hypusine residue and the other is homologous with the cold shock domain of CspA. Both domains have the potential to bind the nucleic acids [19-20].

Here, we have cloned the human eIF-5A gene encoding the homolog of eIF-5A protein by polymerase chain reaction (PCR) amplification method. In the present study, we report the crystallization and preliminary crystallographic studies of human eIF-5A in order to understand the function and properties of eIF-5A proteins more clearly.

## 2. MATERIALS AND METHODS

### 2.1. Protein Expression and Purification

The human eIF-5A gene was amplified using the polymerase chain reaction (PCR) method. Two PCR primers, 5‘- ATGGCAGACGACCTGGAC -3’ and 5‘- TTATTTTGCCATGGCCTT -3’ were designed. The PCR product was restricted with EcoRI and XhoI, purified and ligated into the EcoRI and XhoI restriction sites of the pGEX-6p-1 vector (Amersham Biosciences). The recombinant plasmid was transformed into *Escherichia coli* strain BL21 (DE3). Transformed cells were then cultured at 310K in LB medium containing 50μg/ml Ampicillin resistance. When the culture density reached $A_{600}$=0.6-0.7. Induction with 1 mmol $l^{-1}$ isopropyl−β-D-thiogalactopyranoside (IPTG) was performed and cell growth continued for 12-14 h at 289K. Cells were harvested by centrifugation and stored at 253 K.

The bacterial cell pellet was resuspended by 1×PBS buffer (140 mM NaCl, 2.7 mM KCl, 10 mM $Na_2HPO_4$, 1.8mM $KH_2PO_4$, pH 7.3) and was homogenized by sonication. The lysate was centrifuged at 20,000 g for 30min to remove the cell debris. The supernatant was applied to Glutathione Sepharose 4B matrix column and the contaminant protein was washed off with the 1×PBS buffer. The fusion eIF-5A protein was then cleaved by GST-rhinovirus 3C protease at 277K for more than 12 hours. The following day, the target eIF-5A protein was eluted with

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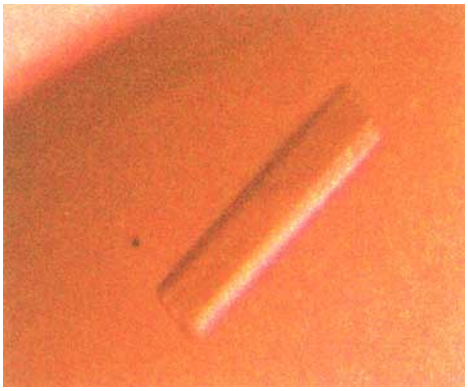

**Figure 1.** A single crystal of eIF-5A. The size of the crystal is about 0.5×0.1×0.05mm.

1×PBS buffer. The protein was further purified by gel filtration on a Superdex200 HR 10/30 (Amersham Pharmacia, USA) column run in 20 m*M* HEPES pH 7.5, 0.15 *M* NaCl. The pooled fractions were loaded onto a Resource Q (Amersham Pharmacia, USA) ion-exchange chromatography column run in 20 m*M* HEPES pH 7.5 and developed with a 0-500 m*M* NaCl gradient. The purity of eIF-5A was estimated to be greater than 95% by SDS-PAGE.

### 2.2 Crystallization

The purified eIF-5A protein was concentrated to ~10mg/ml in 150 m*M* NaCl, 20 m*M* HEPES pH7.5 and 5m*M* DTT. Crystallization was performed by the hanging-drop vapor-diffusion method at 291K in 16-well plates. Each drop contained 1.5 μl protein solution and 1.5 μl reservoir solution. Initial screening used the sparse-matrix (Jancarik & Kim, 1991) reagent kits from Hampton Research Crystal Screen Kits I and II (Riverside, CA, USA). The optimized reservoir solution consisted of 1.8-2.0 *M* ammonium sulfate and 0.02M DTT in 0.1 *M* HEPES pH 7.0. Crystals were obtained within two days (Fig. **1**).

### 2.3 X-Ray Crystallographic Studies

A set of diffraction data were collected from a single eIF-5A derivative crystal at the Beijing Synchrotron Radiation Facility (BSRF) using a MAR Research CCD detector and radiation of wavelength λ=1.00Å. 0.5 *M* tri-sodium citrate dehydrate was added to the mother liquor as a cryoprotectant. Crystals were first immersed into the cryoprotectant for 5-10 sec, picked up with a loop and then flash-frozen in a stream of nitrogen gas cooled to 100 K. The crystal diffracted to 2.7 Å. Data processing and scaling was performed in HKL and SCALEPACK [21].

**Table 1. Data collection and processing statistics**

| Space group | C2 |
|---|---|
| Unit-cell parameters | a =147.1 Å , b =60.4 Å, c = 76.4Å, β=92.4° |
| Resolution range (Å) | 30-2.7 |
| Total reflections | 45870 |
| Unique reflections | 16397 |
| Redundancy | 2.9(2.9) |
| Average I/σ (I ) | 6.0(2.1) |
| $R_{merge\ (\%)}$ | 12.4(34.4) |
| Data completeness $_{(\%)}$ | 88.1 (99.3) |

$R_{merge} = 100\Sigma|I_i - \langle I \rangle|/\Sigma I_i$ , where $I_i$ is the intensity of the observation.

## 3. RESULTS AND DISCUSSION

Initially, although we obtained dozens of eIF-5A crystals using a solution containing 1.8-2.0 *M* ammonium sulfate and 0.1 *M* HEPES pH7.0, they were unsuitable for X-ray diffraction. It seems likely that too many crystals in one single drop have influenced the quality of them. Therefore, further crystallization optimization was performed by adding additives to the reservoir solution in order to reduce the amount of the crystals in a single drop. Fortunately, there are a few additives especially 0.02M DTT that have an obvious effect on increasing the crystal quality. Crystals grown from the optimized reservoir solution (0.1 *M* HEPES pH 7.0, 1.8-2.0 *M* ammonium sulfate, 0.02M DTT) were found to be more suitable for X-ray diffraction and diffracted to 2.7 Å. A set of data were subsequently collected from one single crystal in BSRF (Beijing Synchrotron Radiation Facility) (Fig. **2**). The crystal belongs to space group C2, with unit-cell parameters a=147.1Å, b=60.4Å, c= 76.4Å, β=92.4°.

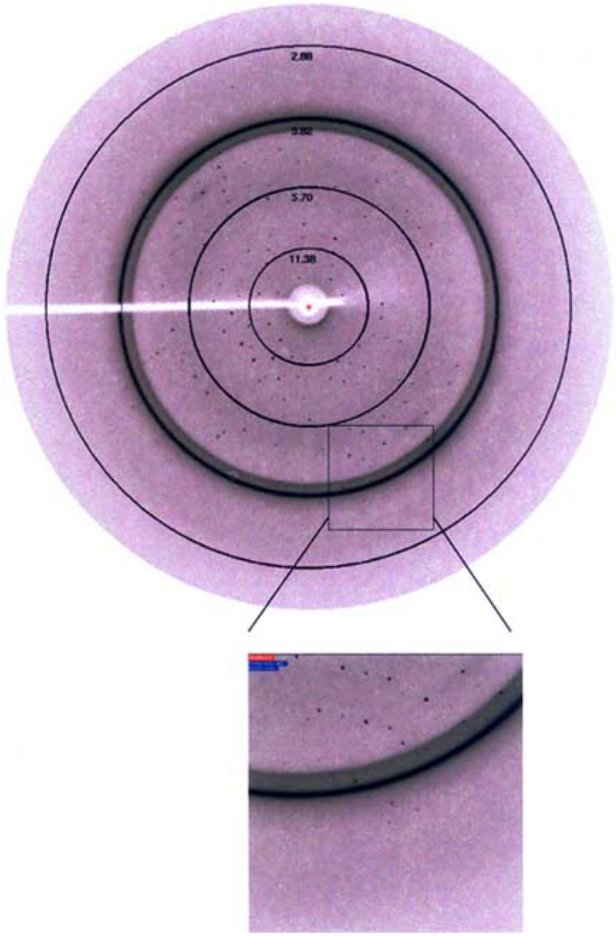

**Figure 2.** A typical diffraction pattern of eIF-5A crystals. The exposure time was 300 seconds, detector distance was 140mm and oscillation range per frame was 1°.

There are more than three molecules per asymmetric unit. Complete data-collection statistics are given in Table **1**.

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## REFERENCES

[1] Park, M. H., Wolff, E. C. and Folk, J.E. (**1993**) *Biofactors, 4*, 95-104.
[2] Schumann, H. and Klink, F. (**1989**) *System. Appl. Microbiol., 11*, 103-107.
[3] Bevec, D., Klier, H., Holter, W., Tschachler, E., Valent, P., Lottspeich, F., Baumruker, T. and Hauber, J. (**1994**) *Proc. Natl. Acad. Sci. USA, 91*, 10829-10833.
[4] Kemper, W. M., Berry, K. W. and Merrick, W. C. (**1976**) *J. Biol. Chem., 251*, 5551-5557.
[5] Park, M. H., Lee, Y. B. and Joe, Y. A. (**1997**) *Biol. Signals, 6*, 115-123.
[6] Hanauske-Abel, H.M., Paek, H., Hanauske, A.R., Popowicz, A.M., Lalande, M. and Folk, J.E. (**1994**) *Biochim. Biophys. Acta, 22*, 115-124.
[7] Jakus, J., Wolff, E.C., Park, M.H. and Folk, J.E. (**1993**) *J. Biol. Chem., 268*, 13151-13159.
[8] Park, M. H., Wolff, E. C., Lee, Y. B. and Folk, J. E. (**1994**) *J. Biol.Chem., 269*, 27827-27832.
[9] Chen, Z.P., Yan, Y.P., Ding, Q.J., Knapp, S., Potenza, J.A., Schugar, H.J. and Chen, K.Y. (**1996**) *Cancer Lett., 105*, 233-239.
[10] Tome, M.E., Fiser, S.M., Payne, C.M. and Gerner, E.W. (**1997**) *Biochem. J., 328*, 847-854.
[11] Ruhl, M., Himmelspach, M., Bahr, G. M., Himmerschmid, F., Jaksche, H., Wolff, B., Aschauer, H., Farrington, G. K., Probst, H., and Bevec, D. (**1993**) *J. Cell Biol., 123*, 1309-1320.
[12] Katahira, J., Ishizaki, T., Sakai, H., Adachi, A., Yamamoto, K., and Shida, H.J. (**1995**) *J. Virol., 69*, 3125-3133.
[13] Mattaj, I. W. and Englmeier, L. (**1998**) *Ann. Rev. Biochem., 67*, 265-306.
[14] Duncan, R. F., and Hershey, J. W. (**1986**) *J. Biol. Chem., 261*, 12903-12906.
[15] Gordon, E.D., Mora, R., Meredith, S.C. and Lindquist, S.L. (**1987**) *J. Biol. Chem., 262*, 16590-16595.
[16] Kang, H.A. and Hershey, J.W.B. (**1994**) *J. Biol. Chem., 269*, 3934-3940.
[17] Liu, Y.P., Nemeroff, M., Yan, Y.P., Chen, K.Y. (**1997**) *Biol. Signals, 6*, 166-174.
[18] Xu A. and Chen, K.Y. (**2001**) *J. Biol. Chem., 276*, 2555-2561.
[19] Kim, K.K., Hung, L-W., Yokota, H., Kim, R. and Kim, S-H. (**1998**) *Proc. Natl. Acad. Sci. USA, 95*, 10419-10424.
[20] Peat, S.T., Newman, J., Waldo, G.S., Berendzen, J. and Terwiliger, T.C. (**1998**) *Structure, 6*,1207-1213.
[21] Otwinowski, Z. and Minor, W. (**1997**) *Meth. Enzymol., 276*, 307-326.

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