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Crystallization and Preliminary X-Ray Diffraction Study of Recombinant Human Eukaryotic Initiation Factor-4E

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Recombinant human eukaryotic initiation factor-4E (eIF-4E), purified by m^{7}GTP-Sepharose 4B affinity chromatography, was used for crystallization. After concentration of the eIF-4E protein (7 mg/ml), the solution was subjected to crystallization by the hanging-drop method. Transparent needle crystals complexed with m^{7}GTP were obtained from 50 mM 2-(*N*-morpholino)ethanesulfonic acid-KOH buffer (pH 6.5) containing 25% (w/v) polyethylene glycol 6000 and 0.2 M $(NH_4)_2SO_4$. The crystals belong to tetragonal space group $P4_1$ or $P4_3$, of $Z=4$, with unit-cell dimensions of $a=89.26$, $b=89.26$, and $c=38.51$ Å, and diffract beyond 2.1 Å resolution. The V_m value was calculated to be 3.07 Å^3/Da, which indicates a solvent content of 59.9%.

Key words: cap structure, complex, crystallization, eukaryotic initiation factor-4E, m^{7}GTP.

Human eukaryotic initiation factor-4E (eIF-4E) is an about 25 kDa polypeptide that is the smallest subunit of eIF-4F, which consists of eIF-4E, eIF-4A, and eIF-4γ, and is required for efficient binding to the mRNA cap structure [m^{7}G(5′)ppp(5′)N..., where N is any nucleotide] during the first stage of protein synthesis (for recent reviews, see Refs. *1-3*); eIF-4E also appears to play a key role in the regulation of translation (*4, 5*). Elucidation of the mechanism underlying recognition of the mRNA cap structure by eIF-4E is necessary for understanding the initiation step of the protein synthetic mechanism. Recently, we succeeded in the construction of a direct expression system for a synthetic gene encoding human eIF-4E under the control of the T7 promoter in *Escherichia coli* (*6*). We report here the preparation and crystallization of recombinant human eIF-4E, and the results of a preliminary X-ray diffraction study.

Recombinant human eIF-4E was expressed in *E. coli* according to Morino *et al.* (*6*). Purification of the recombinant human eIF-4E on a m^{7}GTP-Sepharose 4B affinity column (Pharmacia LKB Biotechnology) was carried out with according to the method of Rychlik *et al.* (*7*) with the following modifications. The supernatant of the expressed culture was applied to a 5 ml m^{7}GTP-Sepharose 4B column equilibrated with buffer A [20 mM HEPES-KOH (pH 7.5), 1 mM DTT, 0.1 mM EDTA, and 100 mM KCl] at the flow rate of 10 ml/h, the column was thoroughly washed with buffer A until the optical density returned to the base line, and then the bound material was eluted with buffer A containing 100 μM m^{7}GTP. The fractions containing eIF-4E were concentrated with a Centricon 10 (Amicon), about 1 ml of 7 mg/ml eIF-4E solution being prepared for crystallization.

Crystallization was carried out by the hanging-drop vapor diffusion method. Each droplet was composed of 3 μl of an aqueous solution containing 7 mg/ml protein and an equal volume of the reservoir solution. Long needle-shaped crystals were obtained within a month at 20°C when 50 mM 2-(*N*-morpholino)ethanesulfonic acid-KOH (pH 6.5) containing 25% (w/v) polyethylene glycol 6000 and 0.2 M $(NH_4)_2SO_4$ was used as the reservoir solution.

Figure 1 shows a micrograph of a complex crystal with the dimensions of $1.0\times0.15\times0.1$ mm. A single crystal together with some mother liquor was mounted in a thin-walled glass capillary, which was then sealed. X-ray diffraction data were collected with an R-AXIS IIC using the imaging plate as the area detector with Ni-filtered Cu-Kα radiation from a Rigaku rotating-anode generator,

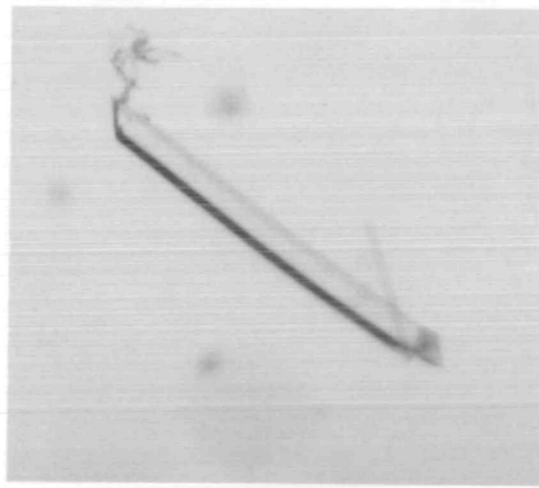

Fig. 1. **Photomicrograph of a crystal of the recombinant human eIF-4E-m^{7}GTP complex.**

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RU-300. The crystals are tetragonal with space group $P4_1$ or $P4_3$, of $Z=4$, and cell dimensions of $a=b=89.26$ Å and $c=38.51$ Å. The corresponding unit cell volume is 3.07×10^5 Å. The V_m value was calculated to be 3.07 Å^3/Da, assuming one molecule with a molecular weight of 25 kDa per asymmetric unit. This value is within the range observed by Matthews for a variety of protein crystals (*8*). The solvent content thus could be 59.9%. We collected diffraction data up to 2.1 Å resolution. The data exhibited an *R*-merge value of 7.86% and completeness of 81%. Crystal structure determination of the recombinant human eIF-4E-m^{7}GTP complex is now in progress.

REFERENCES

1. Merrick, W.C. (1992) Mechanism and regulation of eukaryotic protein synthesis. *Microbiol. Rev.* **56**, 291-315
2. Rhoads, R.E. (1993) Regulation of eukaryotic protein synthesis by initiation factors. *J. Biol. Chem.* **268**, 3017-3020
3. Sonenberg, N. (1994) Regulation of translation and cell growth by eIF-4E. *Biochimie* **76**, 839-846
4. Rychlik, W., Domier, L.L., Gardner, P.R., Helmann, G.M., and Rhoads, R.E. (1987) Amino acid sequence of mRNA cap-binding protein from human tissues. *Proc. Natl. Acad. Sci. USA* **84**, 945-949
5. Joshi, B., Cai, A., Keiper, B.D., Minich, W.B., Mendoz, R., Beach, C.M., Stepinski, J., Stolarski, R., Darzynkiewicz, E., and Rhoads, R.E. (1995) Phosphorylation of eukaryotic protein synthesis initiation factor 4E at Ser-209. *J. Biol. Chem.* **270**, 14597-14603
6. Morino, S., Yasui, M., Doi, M., Ishida, T., Ueda, H., and Uesugi, S. (1994) Direct expression of a synthetic gene in *Escherichia coli*: Purification and physicochemical properties of human initiation factor 4E. *J. Biochem.* **116**, 687-693
7. Rychlik, W., Gardner, P.R., Vanaman, T.C., and Rhoads, R.E. (1986) Structural analysis of the messenger RNA cap-binding protein. *J. Biol. Chem.* **261**, 71-75
8. Matthews, B.W. (1968) Solvent content of protein crystals. *J. Mol. Biol.* **33**, 491-497