X-Ray crystal structure analysis of human initiation factor 4E S209K mutant

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Human eukaryotic initiation factor-4E (eIF-4E) is about 25kDa polypeptide that exists as the smallest subunit of eIF-4F which consists of eIF-4E, eIF-4A and eIF-4γ, and is required for the efficient binding to mRNA cap structure [m7G(5')ppp(5')N..., where N is any nucleotide] during the first stage of protein synthesis; eIF-4E also plays a key role in the regulation of translation. Elucidation of the recognition of mRNA cap structure by eIF-4E is necessary for understanding the initiation step of the protein synthetic mechanism. In order to get a plenty of eIF-4E for elucidating the biological and structural function at the atomic level, by X-ray crystal analysis, we have started X-ray crystal analysis of human eIF-4E.

Preparation.

Mutant(S209K) protein of human eIF-4E was expressed in *E.coli* according to the method of Morino et al. Protein was purified with m7GTP-Sepharose 4B column. The eluted protein was concentrated up to 8 mg/ml for crystallization.

Crystallization of eIF-4E S209K mutant

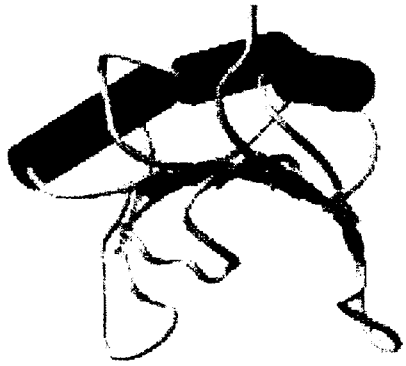
Crystallization was carried out by the hanging drop vapor diffusion method. The long needle-shaped crystals ($1.0 \times 0.15 \times 0.1$ mm^3) were obtained within a month at 15°C when 50 mM 2-(N-morpholino) ethanesulfonic acid-KOH (pH 6.5) containing 27% (w/v) polyethylene glycol 6000 and 0.2 M $(NH_4)_2SO_4$ was used as the reservoir solution.

X-Ray diffraction data collection

Crystals were cryoprotected by dialyzing them against the 10% glycerol and were flash-frozen in liquid nitrogen. Data were collected at 100K with synchrotron radiation of 1.0 Å at Spring 8. The crystals are tetragonal with the space group $P4_1$ or $P4_3$ with one molecule in the asymmetric unit, and following cell dimensions: a=b= 88.18 Å, c=38.27 Å. We collected reflection data up to 1.9 Å resolution. The data exhibited R-merge of 7.86% and completeness of 89.8%.

Determination of initial structure

Data processing and scaling were carried out using the usual method. The initial structure was determined by the molecular replacement method using the solution structure of yeast eIF-4E elucidated by NMR analysis. The correct solution of molecular replacement was obtained with diffraction data between 15 and 4 Å resolution. we are now starting the model building for structure refinement.



The initial structure of eIF-4E S209K mutant