

CRYSTAL STRUCTURE OF ELONGATION FACTOR P FROM *Thermus thermophilus* HB8

Genetic information encoded in messenger RNA is translated into protein by the ribosome, which is a large ribonucleoprotein complex. It has been clarified that diverse proteins called translation factors and RNAs are involved in genetic translation. Translation elongation factor P (EF-P) is one of the translation factors and stimulates the first peptidyl transferase activity of the ribosome [1]. EF-P is conserved in bacteria, and is essential for their viability. Eukarya and Archaea have an EF-P homologue, eukaryotic initiation factor 5A (eIF-5A).

We succeeded in the determination of the crystal structure of EF-P from *Thermus thermophilus* HB8 at 1.65 Å resolution, using beamline **BL45PX** [2]. The crystallographic asymmetric unit contains two nearly identical EF-P monomers (Fig. 1(a)). EF-P is a β -rich protein containing 16 β -strands, and is composed of three β -barrel domains (domains I, II, and III) (Fig. 1(b)). These domains are arranged in a string with an approximately 95° bending at domain II, to form a unique L-shaped structure of EF-P (Fig. 1(c)).

The most noteworthy finding in the present study is a similarity in the overall shape between EF-P and tRNA molecules (Figs. 1(a,b,c)). In general, overall shapes and sizes of the tRNA molecules are well conserved, as they must bind to the same binding site(s) on the ribosome. The shape and the size of the EF-P molecule are comparable to those of tRNA

molecules. Several proteins were found to possess domain(s) similar to a portion of tRNA, by which they interact with the ribosome. The C-terminal domain of elongation factor G (EF-G) has a shape similar to that of the anticodon-stem loop in the elongation factor Tu (EF-Tu)•tRNA•GDPNP ternary complex. Release factor 2 (RF2) and ribosome recycling factor (RRF) were also found to possess a protruding domain, by which they are believed to bind to the ribosome A site [3]. In contrast with these factors, the entire structure of the EF-P mimics the overall shape of the tRNA molecule (Fig. 2). In addition, it is notable that EF-P is an acidic protein and most of its surface is negatively charged. The overall tRNA-like shape of the EF-P molecule and the charge distribution seem to be suitable for this protein to bind to the ribosome by spanning two subunits. Therefore, EF-P may bind to the tRNA-binding site(s) on the ribosome by mimicking the tRNA shape.

It is interesting that domains II and III of EF-P share a remarkable structural similarity to each other; they are superposed on each other with rms of 1.2 Å for 31 C α atoms (Fig. 3). The crystal structure of the eukaryotic/archaeal EF-P homologue, eIF-5A, was reported. It is shorter than EF-P in its amino-acid sequence; therefore, it consists of only two domains (the N- and C-domains) (Fig. 4). The N-domain of eIF-5A corresponds to EF-P domain I, while the

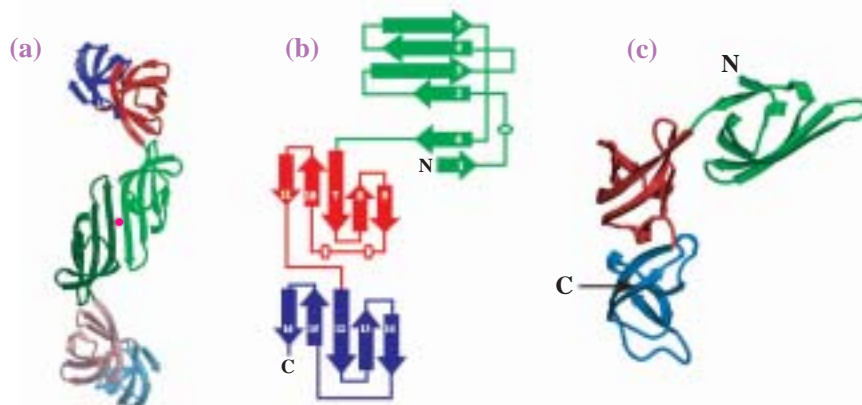


Fig. 1. Structure of *T. thermophilus* EF-P; (a) Ribbon diagram showing twofold dimer presents in asymmetric unit. (b) Topology diagram of *T. thermophilus* EF-P structure. The arrows represent β sheets and the ellipses 310 helices. Domains I, II and III are shown in green, red, and blue, respectively. (c) Ribbon presentation of *T. thermophilus* EF-P crystal structure.

Life Science : Structural Biology

C-domain is similar to either domain II or III of EF-P. This implies that the two homologous domains II and III of EF-P might be the results of internal domain duplication. On the other hand, it is possible that the C-domain of eIF-5A is formed by a deletion of an EF-P portion ($\beta 10 - \beta 14$), which is missing in eIF-5A.

Many questions about EF-P still remain. How does EF-P interact with the ribosome? How can it activate the peptidyltransferase of the ribosome? To answer these questions, further functional and structural studies are required.

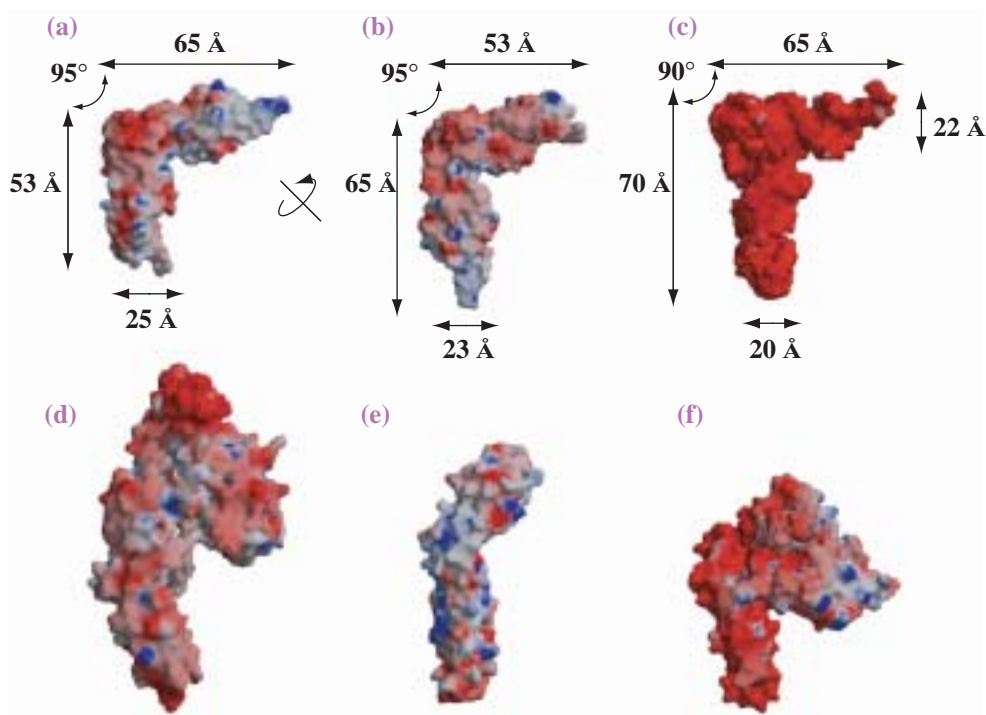


Fig. 2. Structure comparisons of tRNA (c) with EF-P (a and b), EF-G (d), RRF (e) and RF2 (f).



Fig. 3. Superimposition of backbone atoms of domains II and III of *T. thermophilus* EF-P. Domain II is in red and domain III in blue.

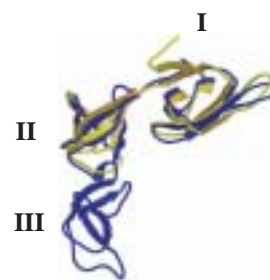


Fig. 4. Structure comparison of EF-P and eIF-5A. *T. thermophilus* EF-P is in blue and *M. jannaschii* eIF-5A in yellow.

Kyoko Hanawa-Suetsugu^{a,*}, Shun-ichi Sekine^{a,b,c},
Mikako Shirouzu^{a,b} and Shigeyuki Yokoyama^{a,b,c}

- (a) RIKEN Genomic Sciences Center
- (b) SPring-8 / RIKEN
- (c) Department of Biophysics and Biochemistry,
The University of Tokyo

*E-mail: suetsugu@gsc.riken.go.jp

References

- [1] M.C. Ganoza *et al.*: Microbiol. Mol. Biol. Rev. **66** (2002) 460.
- [2] K. Hanawa-Suetsugu, S. Sekine, H. Sakai, C. Hori-Takemoto, T. Terada, S. Unzai, J.R. Tame, S. Kuramitsu, M. Shirouzu and S. Yokoyama: Proc. Natl. Acad. Sci. USA **101** (2004) 9595.
- [3] R.V. de Brodersen: Nature Struct. Biol. **10** (2003) 78.