The Crystal Structure of Translation Elongation Factor P (EF-P) Complexed with an Aminoacyl–tRNA Synthetase Paralog, GenX, Reveals that EF-P Functionally Mimics Transfer RNA

We determined the crystal structures of *Escherichia coli* GenX, an aminoacyl–tRNA synthetase (aaRS) paralog, and its complex with translation elongation factor P (EF-P), a tRNA-like L-shaped protein. Based on the finding that the GenX•EF-P structure is very similar to that of an aaRS•tRNA complex, we elucidated that EF-P accepts the amino acid lysine from GenX, with a mechanism similar to that of a tRNA. Striking similarities exist in both the structures and the reactions between a nucleic acid (tRNA) and a protein (EF-P). This phenomenon appears to be analogous to "convergent evolution," in which different living organisms acquire similar shapes and behaviors through evolution.

For accurate protein synthesis, the 22 "canonical" amino acids must be joined properly, according to the genetic code. Aminoacyl–tRNA synthetases (aaRSs), enzymes that ligate a specific amino acid to its cognate tRNA, are essential components of protein synthesis. Besides the classical aaRS proteins, aaRS-related proteins lacking aminoacyl–tRNA synthesis (tRNA aminoacylation) activity exist in various species. Among the aaRS paralogs, GenX (PoxA, YjeA) is homologous to the C-terminal catalytic domain of lysyl–tRNA synthetase (LysRS). The absence of the tRNA anticodon-binding domain suggests that GenX by itself does not act as a classical aaRS. To obtain clues about the function of GenX, we crystallized *Escherichia coli* GenX in complex with a LysRS inhibitor, and determined the crystal structure at 1.9-Å resolution, using the diffraction data collected at BL-5A [1]. Superposition of the GenX structure on that of LysRS revealed that the active site residues are highly conserved with those of LysRS (Fig. 1(a)). The active site pocket of GenX is slightly wider than that of LysRS, and it has sufficient space to accommodate tRNA. Nevertheless, GenX did not ligate lysine to *E. coli* tRNAs [1].

To clarify the function of GenX, we searched for a "tRNA-like" molecule in *E. coli*, and noticed that translation elongation factor P (EF-P) assumes an L-shaped structure mimicking that of tRNA [2]. EF-P reportedly binds to the ribosome [3], and stimulates the ribosomal peptidyl transferase activity [4]. The crystallization of GenX complexed with EF-P was successfully achieved, and diffraction data of the crystals were collected up to 4 Å at BL-5A. We finally determined the crystal structure of the GenX•EF-P complex at 2.5-Å resolution, using the data collected at SPring-8 [1]. The GenX•EF-P complex forms a $GenX_2$•$EF\text{-}P_2$ heterotetramer (Fig. 1(b)). The GenX•EF-P interactions are mediated mainly by the active site loops wrapped around the EF-P domain 1. The structure resembles that of the aspartyl–tRNA synthetase (AspRS)•$tRNA^{Asp}$ complex, because a conserved lysine residue (Lys34) in the exposed loop of EF-P appears to be located at the 3´-terminal adenosine (A76) of the tRNA. We examined the GenX activity, and found that GenX ligates lysine to Lys34 of EF-P [1]. This is a novel example of a post-translational protein modification, and GenX is the first aaRS paralog that has been shown to modify a protein side chain with an amino acid. Our crystallographic and biochemical analyses suggest that the lysyl modification of EF-P by GenX mimics the aminoacylation of tRNA by an aaRS ("aaRS•tRNA mimicry") (Fig. 1(c)).

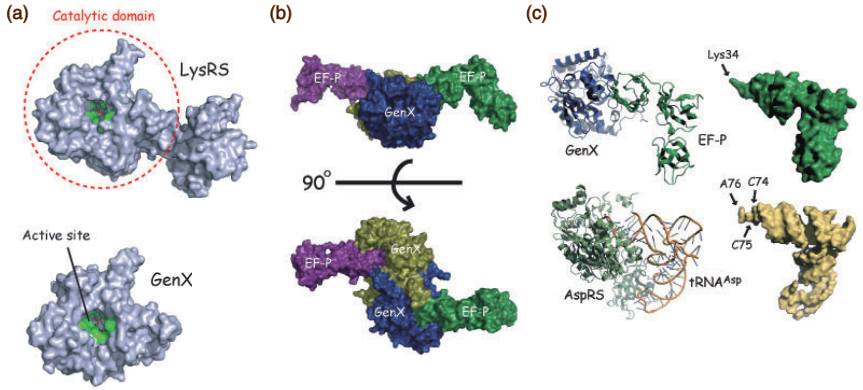

Figure 1
Structures of GenX and its complex with EF-P. (a) Comparison of the structures of LysRS and GenX. LysRS (left) and GenX (right) are represented as surface models. The GenX residues that are conserved with those of LysRS are colored green. The structure of the catalytic domain of LysRS is similar to that of GenX. (b) Structure of the GenX•EF-P complex. The GenX dimer (gold and blue) complexed with EF-Ps (violet and green) is represented as a surface model. The lower panel represents a view after a 90° rotation about the horizontal axis from the upper panel. (c) Structural comparison between the GenX•EF-P and aaRS•tRNA complexes. The complex of aspartyl–tRNA synthetase and aspartic acid tRNA (AspRS•$tRNA^{Asp}$) is shown, as an example of an aaRS•tRNA complex. The structures of the GenX•EF-P complex (upper left) and the aaRS•tRNA complex (lower left) are very similar. Lys34 of EF-P corresponds to the CCA terminus (A76) where an amino acid binds to the tRNA.

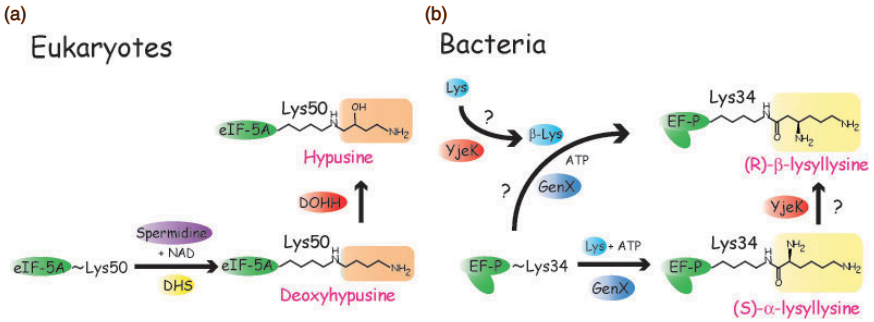

Figure 2
Post-translational modifications of eIF5A and EF-P. (a) Deoxyhypusine modification of eukaryotic eIF5A, and the following hydroxylation of deoxyhypusine-modified eIF5A. (b) Lysyl modification of bacterial EF-P. GenX produces (S)-α-lysyl–EF-P from (S)-α-lysine (Lys), ATP, and EF-P, and then YjeK may catalyze the isomerization of (S)-α-lysyl–EF-P to (R)-β-lysyl–EF-P *in vivo*. β-Lys represents (R)-β-lysine.

In bacteria, EF-P(Lys34) is post-translationally modified with lysine. Meanwhile, in eukaryotes, a conserved lysine residue (Lys50) of translation initiation factor 5A (eIF5A), a distant ortholog of EF-P, undergoes a unique post-translational hypusine modification (Fig. 2(a)) [5]. Hypusine is introduced in a two-step reaction, catalyzed by deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH). The conserved lysine residues at the tips of eIF5A and EF-P are both modified, and the modified residues (hypusine in eIF5A and lysyl–lysine in EF-P) are structurally similar. Our *in vivo* analyses revealed that YjeK (lysine 2, 3-aminomutase paralog), encoded next to EF-P in the *E. coli* genome, enhanced the lysyl modification of EF-P [1], and might convert (S)-α-lysyl–EF-P to (R)-β-lysyl–EF-P (Fig. 2(b)). Furthermore, the EF-P(Lys34) modification is essential for cell survival [1]. These results indicated that lysyl–EF-P is in the functional form, and the lysyl modification of EF-P(Lys34) is important *in vivo*.

In the present study, we crystallized the GenX•EF-P complex and determined its structure, which is very similar to that of an aaRS•tRNA. Based on this finding, we demonstrated that EF-P accepts the amino acid lysine from GenX, in a reaction similar to that of a tRNA. This is the first discovery of the striking similarities in both the structures and reactions between a nucleic acid and a protein, although they are completely different molecules. This phenomenon seems to be analogous to convergent evolution, in which different living organisms acquire similar shapes and living behaviors through evolution. GenX exists only in bacteria, and not in eukaryotes. Therefore, GenX is a promising target for new antimicrobial agents for pathogenic bacteria.

REFERENCES

[1] T. Yanagisawa, T. Sumida, R. Ishii, C. Takemoto and S. Yokoyama, *Nat. Struct. Mol. Biol.* **17** (2010) 1136.
[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. U.S.A.* **101** (2004) 9595.
[3] G. Blaha, R.E. Stanley and T.A. Steitz, *Science* **325** (2009) 966.
[4] M.C. Ganoza, M.C. Kiel and H. Aoki, *Microbiol. Mol. Biol. Rev.* **66** (2002) 460.
[5] E.C. Wolff, K.R. Kang, Y.S. Kim and M.H. Park, *Amino Acids.* **33** (2007) 341.

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