The Effect of Hairpin DNA Fragments on *Escherichia coli* Poly(U)-Dependent Poly(Phe) Synthesis

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In the poly(U)-dependent poly(Phe) synthesis, we found that small hairpin DNA fragments overcome the decline in the translation efficiency caused by an excess amount of *E. coli* $tRNA^{Phe}$. Of these fragments, hairpin DNAs with similar sequences to the unmodified D-arm or TΨC-arm of $tRNA^{Phe}$ were most effective.

Escherichia coli poly(U)-dependent poly(Phe) synthesis system is a useful model system for studying the elongation *in vitro*, since the use of poly(U) as mRNA can bypass the physiological initiation events.[1-3] The *in vitro* translation system is prepared from *E. coli* cell extracts and comprises ribosomes, tRNAs, and S-100 fraction that contains other proteinous factors. In the *E. coli* system, some antibiotics[2] and oligoribonucleotides[4,5] are known to inhibit aminoacylation of tRNAs as well as protein synthesis.

We found that some DNA fragments enhanced the efficiency of the poly(U)-dependent poly(Phe) synthesis in the presence of an excess amount of $tRNA^{Phe}$. The addition of a small synthetic DNA fragment (0.27 mM) containing a hairpin structure, CGGCAAAAGCCGCGCCGCG (Fragment **1**), increased the translation efficiency in the presence of 14 μM $tRNA^{Phe}$ (Figure 1a). This effect depended on the concentration of $tRNA^{Phe}$; it was hardly observed in a lower concentration of $tRNA^{Phe}$ (2.8 μM)(Figure 1b).

Although some poly- or oligo-ribonucleotides inhibit the aminoacylation of tRNA in translation,[4-5] Fragment **1** scarcely affected the aminoacylation (Figure 2), suggesting that the small hairpin DNA is affecting some elongation steps in translation. It is known that an excess amount of unaminoacylated (uncharged) tRNA inhibits the protein synthesis due to the binding of the uncharged-tRNA to the P-site of ribosomes.[6-8] In our system, the translation efficiency also decreased with the increase of the $tRNA^{Phe}$ concentration. Thus, the hairpin DNA may compensate for the translation inhibition by the uncharged $tRNA^{Phe}$.

We then examined the effect of several DNA variants with hairpin or linear structures on the poly(Phe) synthesis. The effect of each DNA fragment in the presence of 14 μM $tRNA^{Phe}$ was compared with that of Fragment **1**. The relative values of enhancement shown in Table 1 were derived from the translation efficiency in the presence of 5 nmol (0.25 mM) each of DNA variants divided by the efficiency using 5 nmol of Fragment **1**. The DNA fragments with hairpin structures enhanced the translation efficiency much more than single-stranded DNA fragments. Interestingly, Fragments **7** and **8** corresponding to the respective D-arm and TΨC-arm of *E. coli*

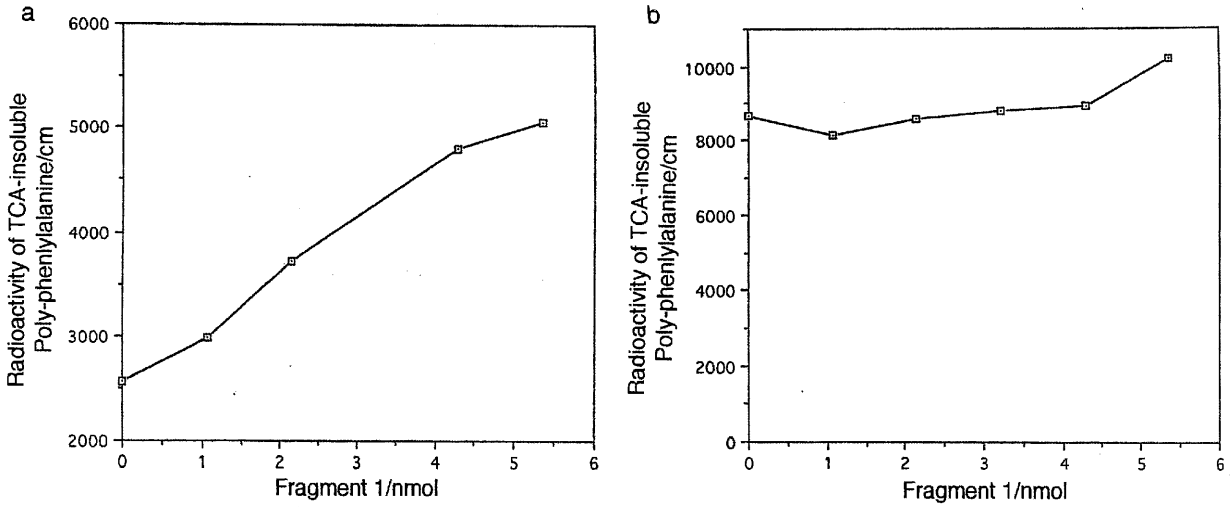

Figure 1. The effect of a hairpin DNA (Fragment **1**) on the poly(U)-dependent poly(Phe) synthesis in the presence of 14 μM (a) and 2.8 μM (b) $tRNA^{Phe}$. The reaction mixure (20 μl) contained 6.6 μg of S-100 fraction, 1.4 pmol of 70S ribosomes, 50 pmol of poly(U), 5.5 μM of [^{14}C]-phenylalanine (16.6 GBq/mmol), and 0 - 5 nmol of Fragment **1**. After incubation at 37 °C for 30 min, a portion of the mixture was put onto a filter (Whatmann 3MM). The filter was boiled with 10% trichloroacetic acid, then washed by usual procedures.[3] The radioactivity remaining on the filter was measured with a liquid scintillation counter (Wallac 1216 RACKBETA, LKB).

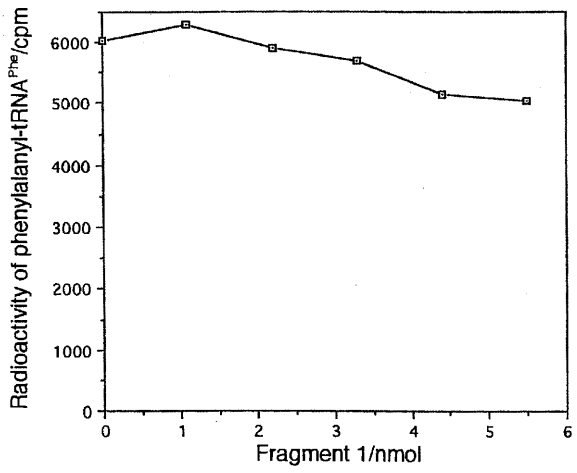

Figure 2. Aminoacylation of tRNA in the presence of Fragment **1**. The reaction mixture (20 μl) contained 280 pmol of $tRNA^{Phe}$, 6.6μg of S100 fraction, 5.5 μM (1.85 kBq) of [^{14}C]-phenylalanine, and various amounts of Fragment **1** as indicated.

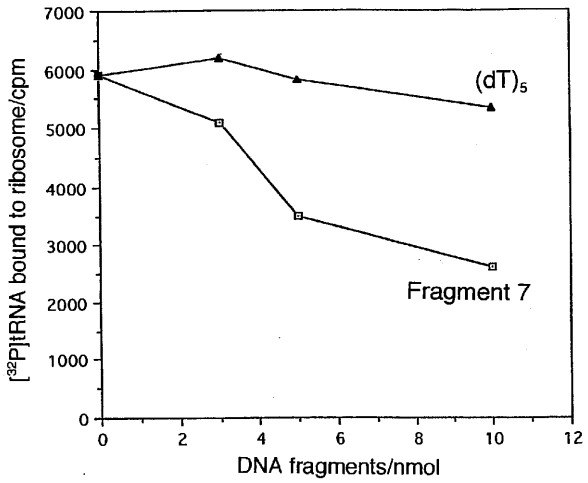

Figure 3. Inhibition of $tRNA^{Phe}$ binding to 70S ribosome by Fragment **7** and pentathymidylic acid [$(dT)_5$]. The reaction mixture (20 μl) contained 3 fmol of ^{32}P-labeled $tRNA^{Phe}$, 10 mg of poly(U), 4.6 pmol of 70S ribosome, and various amounts of Fragment **7** or pentathymidylic acid as indicated.

$tRNA^{Phe}$ showed larger effects than the other DNA fragments.

The sequence similarity between the hairpin DNAs and the tRNA stem-loops suggests that the hairpin DNAs inhibit binding the uncharged $tRNA^{Phe}$ to the ribosomal P-site. Thus, the inhibition of $tRNA^{Phe}$ binding to ribosomes by the DNA hairpin was examined using the 5'-labeled $tRNA^{Phe}$. While pentathymidylic acid [$(dT)_5$] as the control did not affect tRNA binding, an excess amount of Fragment **7** efficiently inhibited the tRNA binding (Figure 3). This inhibition may result in increasing the efficiency of poly(Phe) synthesis even in the presence of an excess amount of $tRNA^{Phe}$.

Here, we show the positive effect of hairpin DNAs on the poly(U)-dependent poly(Phe) synthesis, which has not been reported so far. The sequence similarity of the effective hairpin DNAs to the tRNA stem-loops is of interest in connection with the interaction between the tRNA loops and ribosomes.[9,10] Further studies concerning the mechanism are in progress.

Table 1. Relative effects of small DNA fragments on the poly(U)-dependent poly (Phe) synthesis[a]

CGGC A A GCGCCGCGCCG A A **Fragment 1**	**1**[b]
CGGC T T GCGCCGCGCCG T T **Fragment 2**	**1.3**
CGGC A A GCCG A A **Fragment 3**	**0.5**
CGCCGCG **Fragment 4**	**0.1**
TACCGAC **Fragment 5**	**-0.1**
CGGC A A TACCGACGCCG A A **Fragment 6**	**1.4**
GCTC A G T C GGGACGAG A T G G **Fragment 7** ($tDNA^{Phe}$ D-arm)	**2.4**
CTTGG T T C CCTGAGCC G T T A **Fragment 8** ($tDNA^{Phe}$ TΨC-arm)	**2.4**
GGGGA T T G A TGTGCCCCT A A A **Fragment 9** ($tDNA^{Phe}$ anticodon-arm)	**1.2**
GCTC A G T C CGAG A T G G **Fragment 10** ($tDNA^{Phe}$ D-arm)	**1.8**

[a]Relative effect of each DNA variant was estimated on the basis of the addition of 0.25mM of Fragment **1** in the presence of 14μM $tRNA^{Phe}$.
[b]The average experimental error of the relative effects is ±0.2.

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