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The detection of natural opal suppressor seryl-tRNA in *Escherichia coli* by the dot blot hybridization and its phosphorylation by a tRNA kinase

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It was believed that there was no natural suppressor tRNA in *Escherichia coli*, however, it has been suggested that *selC*, relating to the synthesis of formate dehydrogenase of a selenoprotein [(1988) Nature 331, 723–725], codes for tRNA, even though the presence of tRNA has not yet been demonstrated. We detected the product of *selC* in the tRNA preparation of the *E. coli* MC 4100 strain by the dot blot hybridization method with a DNA probe (ACCGCTGGCGGC) corresponding to the extra arm of *selC* tRNA. Two hybridization peaks were found in the chromatographic pattern from Sephadex A50. The amount of tRNA was estimated to be about 0.03% of the total tRNA. The suppressor [^{3}H]seryl-tRNA was phosphorylated by a tRNA kinase in *E. coli* B. These results suggest that the opal suppressor seryl-tRNA in *E. coli* should be converted to selenocysteyl-tRNA through phosphoseryl-tRNA, and occurs in vertebrates as a general phenomenon.

Suppressor tRNA; Formate dehydrogenase; Selenocysteine; Opal termination codon; tRNA, phosphoseryl-

1. INTRODUCTION

Selenium is essential in prokaryotes and eukaryotes, as demonstrated by the fact that selenium is found as selenocysteine in active sites of formate dehydrogenase in *Escherichia coli* and glutathione peroxidase in vertebrates [1]. In these enzymes, selenocysteine corresponds to an in-frame UGA nonsense codon [2–4]. In the case of formate dehydrogenase, incorporation occurs co-translationally [5]. In mammals, it has been suggested that the natural opal suppressor seryl-tRNA should be converted to selenocysteyl-tRNA through phosphoseryl-tRNA [6].

In *E. coli*, it was believed that there was no natural suppressor tRNA, however it was then shown that one of the genes (*selC*) relating to the synthesis of formate dehydrogenase corresponded to the UGA nonsense suppressor tRNA [7]. This tRNA was used in the cotranslational incorporation of selenocysteine into formate dehydrogenase [5] in which selenocysteine was present at the active site [1]. It was suggested that this tRNA did not accept selenocysteine but serine [7]. However, the presence of suppressor tRNA in the cytosol of *E. coli* has not been shown. Meanwhile, a method for purification of tRNA was developed: a dot blot hybridization method is used with a DNA probe corresponding to various tRNA parts [8]. A major difference between suppressor serine tRNA and the major serine tRNAs in *E. coli* [9] was found in the extra arm. We prepared a 12-mer DNA fragment (ACCGCTGGCGGC) corresponding to the extra arm in the tRNA-type structure of *selC* [7] and used it as a probe in the dot blot hybridization method to detect the natural suppressor tRNA. We show in this paper the presence of tRNA in the *E. coli* tRNA preparation and the conversion of the

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seryl-tRNA to phosphoseryl-tRNA by a tRNA kinase in *E. coli*.

2. EXPERIMENTAL

Transfer RNA of *E. coli* W strain was purchased from Sigma. *E. coli* MC 4100 strain was obtained from Professor F. Zinoni. The preparation of tRNA from *E. coli* MC 4100 strain was carried out as described previously [10]. SerRS was partially purified from the 105 000 × *g* supernatant of the *E. coli* B extract by chromatography on DEAE-cellulose and Sephacryl S-300 [11]. The tRNA kinase used was an eluate from a DEAE-cellulose column of the 105 000 × *g* supernatant of the *E. coli* B extract. tRNA was chromatographed on columns (20 × 0.6 cm) with a linear gradient (0.3 to 0.6 M NaCl; total volume 20 ml and fraction volume 0.5 ml) in 0.02 M Tris-HCl, at pH 7.6. Acceptor activity was measured according to a previous report [12]. Dot blot hybridization was carried out with a DNA probe of 12-mer [^{32}P]d(ACCGCTGGCGGC) according to Kumazawa et al. [8]. Briefly, the tRNA (0.05 A_{260} unit) of each fraction was dotted on a membrane (Nihon Pall Ltd.) and cross-linked by UV irradiation for 30 min. Then the membrane was incubated in the DNA probe solution at 39°C overnight. After being washed with 3 × SSC, the membrane was autoradiographed. The hybridized activity of each dot was measured with a liquid scintillation counter.

[^{3}H]Seryl-tRNA, which was prepared with a tRNA fraction (10 A_{260} units) strongly hybridizing with the DNA probe, was purified on a Sephacryl S-200 column (40 × 1.1 cm) in 0.15 M NaCl/10 mM acetate buffer at pH 4.6. The peak of [^{3}H]seryl-tRNA was collected with ethanol precipitation and dried. The tRNA was dissolved in 0.1 M Hepes buffer at pH 6.5 and phosphorylated as in [13]. [^{32}P]Phospho[^{3}H]seryl-tRNA was purified on the Sephacryl S-200 column. One part of eluate was spotted on filter papers and treated with 10% cold or hot trichloroacetic acid in order to estimate the amount of phosphate on the tRNA. The peak of phosphoseryl-tRNA was collected by ethanol precipitation and hydrolyzed with 0.1 M NH_4OH. The hydrolyzate was mixed with authentic phosphoserine and then analyzed on an AG-1 column according to Mizutani et al. [14].

3. RESULTS AND DISCUSSION

Fig.1a shows the chromatographic pattern of the tRNA preparation from *E. coli* MC 4100 (cultured under anaerobic condition and in the presence of selenium). Fig.1b shows the results of the dot blot hybridization of each fraction from fig.1a. The amount of ^{32}P in each dot is plotted in fig.1a by closed circles and serine acceptor activity is shown by open circles. Strong hybridization (specific hybridization, cpm/A_{260} unit) is found in the last fraction of the tRNA peak (tubes 47–49) after the $tRNA_3^{Ser}$ peak of tube 44. Weak specific hybridization is found in tubes 42–43 but the amount of tRNA in these fractions is more than that in tubes

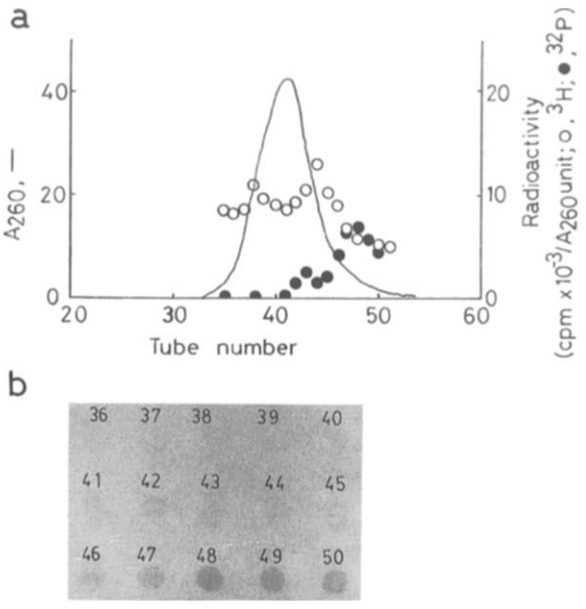

Fig.1. Chromatographic pattern of *Escherichia coli* tRNA on Sephadex A50 and hybridization pattern using a DNA probe. (a) Chromatographic pattern of tRNA obtained from *E. coli* MC 4100. (b) The results from dot blot hybridization of each fraction in a (number corresponds to tube number in the figure). Closed circles show the radioactivity of ^{32}P probe hybridized on tRNA. Open circles show the [^{3}H]serine acceptor activity.

47–49. Therefore, total amount of hybridization in tubes 42–43 is about twice as much as that in tubes 47–49. Thus, there are two tRNAs which hybridize with the DNA probe to *selC*. These two tRNAs are both active because the tRNAs are phosphorylated by a tRNA kinase described later. We think that these two tRNAs come from one gene (*selC*), one being the hypo-modified tRNA of mature tRNA.

Vertebrate opal suppressor seryl-tRNA was phosphorylated to become phosphoseryl-tRNA [15]. This phosphoseryl-tRNA should be converted to selenocysteyl-tRNA by a Se-transferase [6]. Thus in *E. coli*, the opal suppressor seryl-tRNA should also be converted to selenocysteyl-tRNA through phosphoseryl-tRNA. Therefore, as shown in fig.2, we investigated the phosphorylation of seryl-$tRNA^{su}$. [^{3}H]Seryl-tRNA (tubes 47–49 in fig.1a) was phosphorylated with a tRNA kinase in *E. coli* B. The product was chromatographed on Sephacryl S-200 as shown in fig.2a. A [^{32}P]phospho[^{3}H]seryl-tRNA peak can be seen in tubes 40–50 in fig.2a. The radioactivity (^{32}P and ^{3}H) of the peak disappeared following the hot TCA treat-

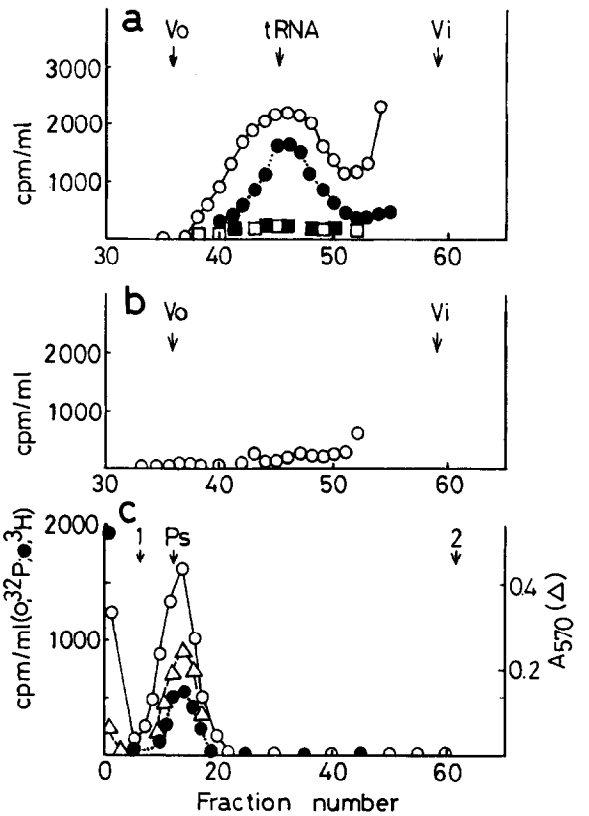

Fig.2. Chromatographic pattern and analyses of phosphoseryl-tRNA. (a) Chromatographic pattern of [^{32}P]phospho[^{3}H]seryl-tRNA on Sephacryl S-200 in 0.15 M NaCl/0.01 M acetate buffer at pH 4.6. Open and closed circles are ^{32}P and ^{3}H bound on tRNA after treatment in 10% cold TCA, respectively. Open and closed squares are ^{32}P and ^{3}H on tRNA after treatment in 10% hot TCA for 5 min. The peak of tubes 40–50 corresponds to the position of tRNA. (b) Chromatographic pattern of a mixture of nonacylated tRNA, tRNA kinase and [^{32}P]ATP (control experiment of a). (c) Analyses of [^{32}P]phospho[^{3}H]serine liberated from phosphoseryl-tRNA (the peak in a), on an AG-1 column. Arrow Ps shows the position of phosphoserine eluted from the column and phosphoserine was detected by the color reaction with ninhydrin (as shown by A_{570}). Arrows 1 and 2 are the starting and end points of gradient elution composed of 2 mM HCl (16 ml) and 50 mM HCl (10 ml).

ment, as indicated by the squares in fig.2a. These results show that the phosphate became bound to the serine on the tRNA. When non-acylated tRNA (serine free tRNA) was used as a phosphorylation substrate, no radioactivity of ^{32}P was found on tRNA, as shown in fig.2b. This result supports the fact that phosphate on the tRNA in fig.2a binds the OH residue of serine (seryl-tRNA). The presence of phosphoserine on phosphoseryl-tRNA was confirmed by analyses on an AG-1 column (fig.2c). Radioactivity of ^{32}P was found at the position of the authentic phosphoserine (color reaction of ninhydrin) with ^{3}H radioactivity in fig.2c. These results showed that seryl-tRNA was phosphorylated by a tRNA kinase of *E. coli*.

Thus, this report is the first to show the presence of the tRNA kinase activity in *E. coli* and the phosphorylation of UGA nonsense suppressor seryl-tRNA. This tRNA kinase is an unexpected enzyme in *E. coli*. Other results suggest that this tRNA kinase is a product of the *selB* gene [16] and acts as dimer. Phosphoseryl-tRNA in *E. coli* should be converted to selenocysteyl-tRNA by Se-transferase (a product of *selD*), in the same manner as in vertebrates [6]. It has been confirmed that phosphoseryl-tRNA is present in vertebrates [13]. These results suggest that all biological systems have a similar system to synthesize phosphoseryl-tRNA, which acts as an intermediate in the conversion of seryl-tRNA to selenocysteyl-tRNA and corresponds to the UGA nonsense codon as a natural opal suppressor tRNA. The DNA probe for *E. coli* suppressor tRNA did not become hybridized with the total tRNA preparation of bovine and yeast. Also, the DNA probe for the bovine suppressor tRNA did not become hybridized with the tRNA preparation of *E. coli* and yeast (Hitaka and Mizutani, unpublished). In yeast, it is assumed that natural opal suppressor tRNA differs in structure from the suppressor tRNA of bovine or *E. coli*. In *E. coli*, the UGA nonsense codon was recognized by releasing factor 2 [17]. However, the mechanisms used to discriminate the in-frame UGA codon for selenocysteine from the natural termination UGA codon have not yet been resolved.

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