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Non-participation of Opal Suppressor Phosphoseryl-Transfer Ribonucleic Acid (tRNA) in Phosphoserine Aminotransferase Catalysis

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The role of animal opal suppressor phosphoseryl-transfer ribonucleic acid (tRNA) as an intermediate in the metabolic pathway from 3-phosphoglycerate to glycine was studied. The labelled [^{32}P]phospho[^{3}H]seryl-tRNA was prepared and used as a substrate in the reaction of bovine brain phosphoserine aminotransferase in the presence of α-ketoglutarate. On analysis of the reaction product, no 3-phosphohydroxypyruvate was found even though phosphohydroxypyruvate was obtained in the control experiment by use of phosphoserine and α-ketoglutarate. The aminotransferase did not react with phosphoserine methylester. These results show that the carboxyl residue on phosphoserine is essential for recognition by the aminotransferase. A role of phosphoseryl-tRNA as an intermediary metabolite from serine to phosphohydroxypyruvate is excluded.

Keywords——opal suppressor; seryl-tRNA; phosphoserine aminotransferase; natural suppressor

It has been reported that one tRNA^{Ser} in higher eukaryotes accepts phosphate[1] and one corresponds to codon UGA as natural suppressor transfer ribonucleic acid (tRNA).[2] These two tRNAs^{Ser} are identical,[3] and the primary structure of this tRNA from bovine liver[4] and the tRNA gene structure of chicken[5] and human[6] were determined. As one role of suppressor tRNA, this tRNA suppresses the UGA termination codon in a rabbit reticulocyte system supplemented with exogenous globin messenger ribonucleic acid (mRNA), and the β-globin read-through product was found on a 15% gel as a new protein band radiolabeled with [^{35}S]methionine, not with serine on suppressor seryl-tRNA.[4] The present investigators have reported that a small amount of [^{32}P]phosphoserine (Ps) was incorporated into β-globin read-through protein *via* [^{32}P]Ps-tRNA, which was purified on Sephacryl S-200.[7] This result also showed that Ps-tRNA was able to bind to eEF-1α[3] and this complex had a normal function in the protein-synthesizing machinery of ribosomes. As another role of suppressor tRNA, it was suggested that Ps-tRNA was involved in the reverse metabolic pathway from serine to 3-phosphohydroxypyruvate (PHP).[1]

During the course of an investigation of the biological function of Ps-tRNA, the authors purified seryl-tRNA synthetase from bovine liver; its molecular mass is 170 kDa and it is of α_2 type.[8] This enzyme recognizes major tRNA^{Ser} and suppressor tRNA with the same K_m and V_{max} values. We have also purified from bovine liver an adenosine triphosphate (ATP): suppressor seryl-tRNA phosphotransferase,[9] which does not utilize major seryl-tRNA$^{Ser}_{IGA}$ but only suppressor ser-tRNA^{Ser}. This study suggested that the different regions between major tRNA^{Ser} and suppressor tRNA are similar to those between *E. coli* tRNA$_f^{Met}$ and tRNA$_i^{Met}$, because those regions might be recognized by bovine tRNA kinase or *E. coli* formyltransferase.[10,11] We wished to clarify the possible function of Ps-tRNA as an

intermediate from serine to PHP, and results demonstrating the non-participation of Ps-tRNA in the reaction of phosphoserine aminotransferase[12] are reported here.

Materials and Methods

Seryl-tRNA synthetase, tRNA kinase and opal suppressor serine tRNA were prepared from bovine liver according to the previous reports.[8,9,13] Ps aminotransferase was purified from bovine brain extracts by ammonium sulfate precipitation, chromatography on diethylaminoethyl (DEAE)-cellulose, and gel chromatography on Sephacryl S-200 according to the method of Hirsh and Greenberg.[12] The final preparation was concentrated by ammonium sulfate precipitation and used at a concentration of 20 mg/ml. The aminotransferase activity in the fraction of the final step was determined by coupling the Ps aminotransferase with glutamate dehydrogenase (a product of Boehringer Mannheim) and measuring the decrease in reduced nicotinamide adenine dinucleotide (NADH) absorption at 340 nm in the presence of glutamate. During the course of this investigation, the stability of the activity in the preparation of Ps aminotransferase on storage was confirmed. [^{32}P]ATP was prepared from carrier-free inorganic [^{32}P]phosphate according to the method of Walseth and Hornson.[14] [^{3}H]Serine was a product of Amersham. *O*-Phosphoserine and hydroxypyruvic acid phosphate dimethylketal were obtained from Sigma Chemicals. Phosphoserine methylester and PHP methylester were prepared by the general method with methyl alcohol and thionyl chloride.

For control activity tests, the assay mixture contained 1 μmol of Ps, 5 μmol of α-ketoglutarate, and 0.4 mg of aminotransferase in 0.05 M Hepes buffer at pH 6.7 in a total volume of 0.2 ml. The mixture was incubated at 20 °C for 3 h. For sample activity tests, [^{32}P]phospho[^{3}H]seryl-tRNA (30000 cpm of ^{32}P and 5000 cpm of ^{3}H), which was prepared according to the previous report,[7] was used instead of Ps in the control activity tests. The optimum activity of aminotransferase was reported to be at pH 8.1,[12] but we employed pH 6.7, because Ps-tRNA was labile under alkaline conditions. After the incubation, the reaction mixture of the control activity tests was applied to an AG-1 column (10 × 0.3 cm). The mixture of the sample tests was treated with ethanol in order to remove free Ps liberated from Ps-tRNA during the incubation. The precipitate was dissolved in 0.2 M Tris buffer, pH 9, and incubated for 30 min at 20 °C to split Ps from Ps-tRNA (PHP from PHP-tRNA), then the mixture was applied to an AG-1 column. The columns were washed with water, then equilibrated with 2 mM HCl and eluted with a concave gradient formed from 2 mM HCl (16 ml) and 50 mM HCl (10 ml).[15] After the gradient, the column was flushed with 50 mM HCl. The elution positions of Ps and PHP were determined by the use of authentic samples. The amounts of cold Ps and cold PHP in the eluate from the AG-1 column were determined by estimation of the concentration of organic phosphate.[15] A part of the eluate from the column was counted with a liquid scintillation counter (Aloka LSC-1000). The residual part was used for the estimation of organic phosphate.

Results and Discussion

The reaction product of Ps and α-ketoglutarate formed catalytically by aminotransferase was analyzed on an AG-1 column, as was the product of Ps-tRNA and α-ketoglutarate. These results are shown in Fig. 1, in which arrows a and b indicate the elution positions of authentic Ps and PHP, respectively. The reaction product of Ps and α-ketoglutarate at 20 °C for 3 h at pH 6.7 contained PHP (9.6% of the estimated amount of the product). Under the above reaction conditions, the reaction proceeded to equilibrium, because the results of analysis of the reaction mixture at pH 8 for 20 h showed that the amount of PHP was 13% of the estimated amount from Ps. This reaction is reversible[12] and we confirmed the formation of phosphoserine from PHP and glutamate by analysis on an AG-1 column (data not shown). These results showed that PHP was produced from Ps and α-ketoglutarate by Ps aminotransferase.

Ps-tRNA and α-ketoglutarate were incubated with aminotransferase under conditions identical to those described above. The results of analyses showed that radioactivity of ^{32}P and ^{3}H was not found at the position of PHP but at the position of Ps, as shown in Fig. 1B. A portion of the radioactivity of ^{3}H was found in the passed-through fraction in Fig. 1B, and this may depend upon free [^{3}H]serine liberated from [^{3}H]seryl-tRNA which was not phosphorylated with tRNA kinase. The result in Fig. 1B shows that Ps-tRNA was not utilized by Ps aminotransferase. This result also suggests that Ps-tRNA does not play a role as an intermediary metabolite in the pathway between 3-phosphoglycerate and glycine through Ps.

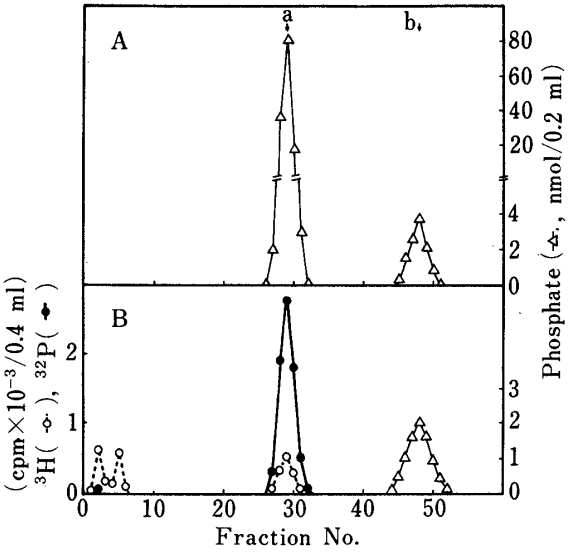

Fig. 1. Elution Patterns of the Reaction Products with Phosphoserine Aminotransferase on AG-1 Columns

Figure A is the result of analysis of a product obtained with phosphoserine and α-ketoglutarate; B is that of a product obtained with [^{32}P]phospho[^{3}H]-seryl-tRNA and α-ketoglutarate. The products were analyzed on an AG-1 column by elution with a concave gradient formed from 2 mM HCl (16 ml) and 50 mM HCl (10 ml). Fraction volumes were 0.6 ml. Closed circles and open circles indicate the radioactivity of ^{32}P and ^{3}H, respectively. Open triangles indicate the amount of phosphate in eluates. Arrows a and b indicate the elution positions of authentic phosphoserine and phosphohydroxypyruvate, respectively.

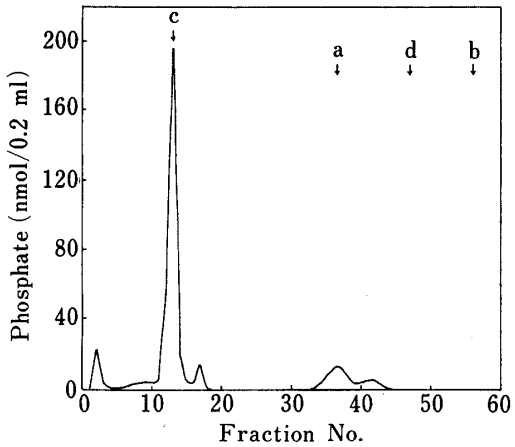

Fig. 2. Elution Pattern of a Reaction Mixture of Phosphoserine Methylester and α-Ketoglutarate with Phosphoserine Aminotransferase on an AG-1 Column

The conditions of chromatography were identical with those in Fig. 1. Arrows c and d indicate the elution positions of phosphoserine methylester and phosphohydroxypyruvate methylester.

It is possible that Ps-tRNA is present in a different compartment in the cytoplasm from the enzyme, such as aminotransferase, metabolizing free Ps. In order to confirm that Ps-tRNA does not participate in the reaction with aminotransferase, Ps methylester was prepared and used as a substrate for aminotransferase. Analysis of the reaction mixture showed no PHP methylester at the elution position of an authentic sample on an AG-1 column, as shown in Fig. 2. No Ps modified on the carboxyl residue was recognized by the aminotransferase, so the carboxyl residue on Ps is essential for recognition by Ps aminotransferase.

In this experiment, the pH of the reaction mixture was 6.7, because Ps-tRNA is unstable under neutral or alkaline conditions, as are other aminoacyl-tRNAs. Free Ps, liberated from Ps-tRNA, was found in the reaction mixture at pH 7.6 during the preparation of Ps-tRNA.[7] Meanwhile, the concentrations of free Ps in serum and liver are 5 and 100 μM, respectively. It is possible that Ps-tRNA is one source of this free Ps, although not a major one.

Finally, from these results, we considered that the main role of Ps-tRNA might be to serve in the protein-synthesizing system as one of the regulators or constituents. We have already reported that a small amount of ^{32}P radioactivity of suppressor Ps-tRNA was incorporated into β-globin read-through protein.[7] The suppressor tRNA content was about one-fiftieth of the total serine tRNA in liver. Therefore, the amount of suppressor tRNA was not negligible; this tRNA presumably has an important role in the normal state, though not in a specific state such as the oncogenic state. One role of suppressor tRNA in the protein-synthesizing system may be active regulation, such as a Trp-tRNA in attenuation mechanisms[16] or a Gln-tRNA in the first step of chlorophyll biosynthesis.[17] This UGA suppressor tRNA may have a role to

synthesize glutathione peroxidase whose mRNA contains the UGA codon for selenocysteine in the active site.[18] In this study, a role of Ps-tRNA as a metabolite from serine to PHP was completely excluded. A part of this study was presented at the 14th Nucleic Acids Chemistry Symposium held at Tokushima, Japan.[19]

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