

Evidence for the Existence of Two Arginyl-Transfer Ribonucleic Acid Synthetase Activities in *Escherichia coli*

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Received for publication 16 October 1972

Two arginyl-transfer ribonucleic acid (tRNA) synthetase (EC 6.1.1.13, arginine: ribonucleic acid ligase adenosine monophosphate) activities were found in extracts of *Escherichia coli* strains AB1132 and NP2. The two arginyl-tRNA synthetase activities in extracts of strain AB1132 were found to be separable by diethylaminoethyl-cellulose column chromatography, Sephadex column fractionation, and by sucrose density gradient centrifugation. In addition, in the standard assay using extracts of strain AB1132 there were two pH optima for arginyl-tRNA synthetase activity. Furthermore, when arginyl-tRNA synthetase of strain NP2 was fractionated by hydroxylapatite column chromatography, two activities were observed which were similar to those of strain AB1132.

A number of aminoacyl-transfer ribonucleic acid (tRNA) synthetases have been extensively purified, and analyses of subunit structure have been performed. The evidence clearly indicates that several of these enzymes are composed of single polypeptide chains, whereas others have oligomeric structures composed of identical or nonidentical subunits (2, 6, 8, 10, 16, 18). The arginyl-tRNA synthetase has been purified to homogeneity as a single protein unit (7), and the genetic data suggest that there is only one structural gene for arginyl-tRNA synthetase (5). However, two active forms have been reported for leucyl- (17) and lysyl-tRNA synthetases (9).

In this communication, we present data for the existence of two arginyl-tRNA synthetase activities.

MATERIALS AND METHODS

The *Escherichia coli* strain AB1132, a threonine, leucine, methionine, proline, and histidine auxotroph, was obtained from E. A. Adelberg. Strain NP2 is a valine-sensitive K-12 prototroph maintained in our laboratory. The cells were grown in L broth (12), with aeration on a rotary-action shaker at 37 C. Growth was measured by optical density in a Hitachi Perkin-Elmer model 101 spectrophotometer at 420 nm (light path equals 1 cm).

Cells were harvested during the mid-exponential phase of growth, rapidly chilled, and collected by centrifugation. The resulting pellet was resuspended

in potassium phosphate buffer (4), and the cells were subjected to sonic treatment with a Branson sonifier as described by Chrispeels et al. (4). The protein content of the extracts was determined by the method of Lowry et al. (13).

Arginyl-tRNA synthetase activity was determined by the ¹⁴C-labeled amino acid attachment assay as described by Chrispeels et al. (4), with the exception that incubation was for 5 min. The RNA was from *E. coli* K-12 (General Biochemicals), and the radioactivity determinations were made with a Packard liquid scintillation spectrometer. For the pH optima study, the activity was determined as in the standard attachment assay, except that pH of the buffers used was as indicated in the experiment. For the activity determinations, with buffers with a pH above 7.0, potassium phosphate or tris(hydroxymethyl)amino-methane (Tris)-hydrochloride buffers were used; for the low pH value, Tris-acid maleate buffers were employed. Minus-adenosine triphosphate blanks were determined for each reaction mixture in each experiment.

The diethylaminoethyl (DEAE)-cellulose column (5 by 40 cm) was packed and equilibrated with 10 mM potassium phosphate buffer, pH 7.0, 1 mM magnesium chloride, 20% glycerol, 20 mM monovalent glycerol, 0.5% sucrose, and 60 μM L-arginine. The column was washed with 1 vol of the initial buffer (above), the extract was applied (40 to 90 mg), and the elution was run with a phosphate gradient (10 to 100 mM, pH 7.0). Twelve-milliliter fractions (flow rate of 48 ml/hr) were collected. The Sephadex G-50-80 column (5 by 40 cm) was packed and equilibrated with the initial phosphate buffer de-

scribed above. All other column procedures were as described for the DEAE-cellulose column. Hydroxylapatite (Bio-Rad) was packed in a column (2.4 by 18 cm) and all other column procedures were as described for DEAE-cellulose, except that gradient was 10 to 300 mM phosphate. For the sucrose-density gradient centrifugation, use was made of a 5 to 20% sucrose gradient containing 10 mM potassium phosphate buffer, pH 7.0, 1 mM magnesium chloride, 20 mM monothioglycerol, and 60 μ M L-arginine. The centrifugation was performed in a Spinco SW50.1 rotor using a Spinco-Beckman model L4 ultracentrifuge for 18 hr at 38,000 rpm. The fractions (equal volume) were collected with a ISCO density fractionator.

RESULTS AND DISCUSSION

The results in Fig. 1A show the fractionation of arginyl-tRNA synthetase activity from the DEAE-cellulose column. The fractions invariably rechromatographed at the same or similar positions as separate activities. Figure 1B shows the two activities obtained upon fractionation by Sephadex G50-80 chromatography. In both instances, it is quite clear that these fractionations resulted in two activities for arginyl-tRNA synthetase of strain AB1132. Since the slow migrating peak possessed the major activity in both studies, we then centrifuged the extract in a sucrose density gradient containing the same phosphate buffer at pH 7.0. The results of this experiment are shown in Fig. 2. Again, the major activity peak has the greatest apparent molecular weight. The possibility was considered that these results were somehow influenced by the strain (AB1132) from which the extracts were prepared. These considerations included the possibility that strain AB1132 harbored a phage carrying arginyl-tRNA synthetase, especially in view of the large number of mutations which had been introduced into this strain and the observation by Neidhardt and co-workers (15) that T-4 phage was capable of effecting a modification of valyl-tRNA synthetase. Therefore, we examined arginyl-tRNA synthetase activity of another *E. coli* strain (NP2), a valine-sensitive K-12 prototroph, which was fractionated using a hydroxylapatite column. The results of this experiment shown in Fig. 3 indicate the presence of two activities for this enzyme in extracts of NP2, as revealed by hydroxylapatite fractionation. For a given activity of both strains, there was similar inhibition of arginine acylation by canavanine, an arginine analog, which suggests that the activity obtained by different procedures was equivalent. Similar profiles have been obtained by this column procedure for arginyl-tRNA syn-

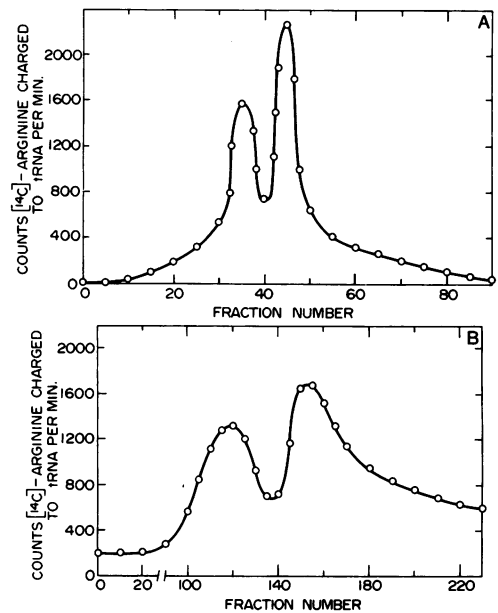


FIG. 1. (A) DEAE-cellulose column chromatography of a crude extract of strain AB1132. A crude extract of strain AB1132 was prepared as described in Materials and Methods. The crude extract was loaded onto the DEAE-cellulose column (5 by 40 cm), which was equilibrated with 10 mM potassium phosphate buffer (composition described in Materials and Methods). Elution was with a linear gradient using an equal volume of 10 and of 100 mM potassium phosphate buffers. Twelve fractions were collected, and arginyl-tRNA synthetase activity was determined as described in Materials and Methods. (B) Sephadex G-50-80 column fractionation of a crude extract of strain AB1132. A crude extract of strain AB1132 was prepared as described in Materials and Methods. The crude extract was loaded onto the Sephadex column (5 by 40 cm), which was equilibrated and eluted as described for A. The fractions were collected, and arginyl-tRNA synthetase activity was determined as described in Materials and Methods.

thetase from the two other *E. coli* strains (HP18 and HP 4; unpublished results). To approach the question of the physiological basis of these activities, we prepared an extract from AB1132 and determined arginyl-tRNA synthetase activity over a pH range of 5.5 to 9.0. Similar results were obtained using the separate fractions from the DEAE-cellulose column fractionation. These results (Fig. 4) are again consistent with the existence of two separate activities in strain AB1132.

In the present study, observations are reported suggesting the existence of two activities for arginyl-tRNA synthetase. The two activities are separable by DEAE-cellulose chromatography, Sephadex fractionation, sucrose den-

sity gradient centrifugation, and by hydroxylapatite chromatography. The results obtained with NP2 and other strains of *E. coli* suggest that this observation is not a unique property of strain AB1132. However, there was a shift in the major and minor activities of this strain as compared to strain AB1132. Interestingly, the activity reported in this report with a pH optimum of 8.0 appears identical to the activity purified by Hirschfield and Bloemers (7), in which they reported a loss of about 75% of the total activity units after ammonium

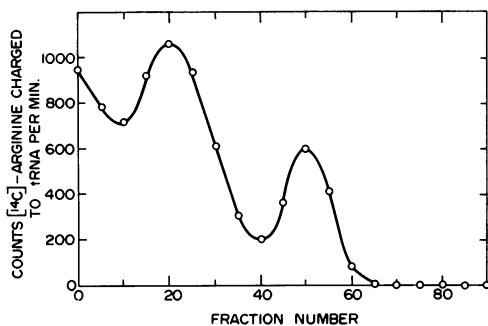


FIG. 2. Sucrose density gradient centrifugation of a crude extract of strain AB1132. The crude extract of AB1132 was applied onto a gradient of a 5 to 20% sucrose, which also contained 10 mM potassium phosphate buffer, pH 7.0, 1 mM magnesium chloride, 20 mM thioglycerol, and 6×10^{-7} M L-arginine. The tubes were centrifuged for 18 hr at 38,000 rpm in a Beckman ultracentrifuge using a SW50.1 rotor. Fractions of 4 drops each were collected, and arginyl-tRNA synthetase activity was determined as described in Materials and Methods. The fractionation was from the bottom of the tube.

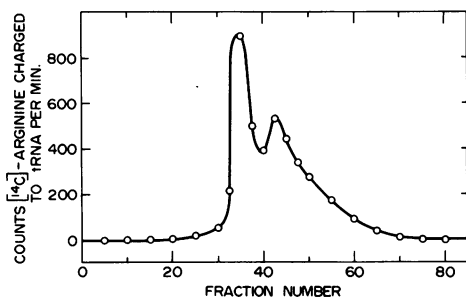


FIG. 3. Hydroxylapatite column chromatography of a crude extract of strain NP2. A crude extract of strain NP2 was prepared as described in Materials and Methods. The crude extract was loaded onto the hydroxylapatite column which was equilibrated with 10 mM potassium phosphate buffer. Elution was with a linear gradient using equal volumes of 10 and of 300 mM potassium phosphate buffers. Twelve-milliliter fractions were collected, and arginyl-tRNA synthetase activity was determined as described in Materials and Methods.

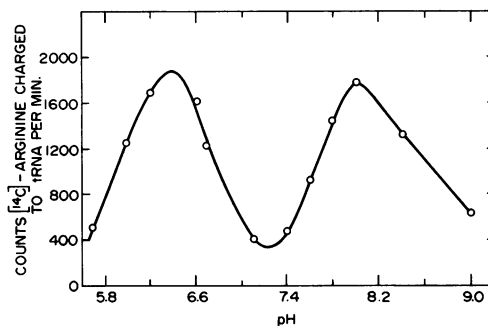


FIG. 4. Arginyl-tRNA synthetase activities of a crude extract of strain AB1132 assayed at different pH values. Arginyl-tRNA synthetase activity was determined as described in Materials and Methods. The assays were performed using phosphate or Tris-hydrochloride buffers, or both, for the high pH value and Tris-acid maleate buffer for the low pH value. All other procedures were as in the standard attachment assay.

sulfate fractionation. The present genetic evidence does not support the existence of two entirely different enzymes, in that only one position for the arginyl-tRNA synthetase structural gene on the *E. coli* chromosome has been reported (5).

However, there is only one known structural gene for the leucyl- (1) and lysyl-tRNA synthetases, yet Rouget and Chapeville (17) have reported two forms of the leucyl-tRNA synthetase and Kisselev and Baturina (9) have reported two enzymatically active forms of lysyl-tRNA synthetase. Furthermore, the active prolyl-tRNA synthetase is a dimer (11), phenylalanyl-tRNA synthetase is composed of subunits (3), and glycyl-tRNA synthetase is a tetramer (16). For the arginyl-tRNA synthetase activities described in this report, it seems premature to make a prediction of the possibility of an active subunit and multi-subunit enzyme. In addition, the physiological significance of these results is not immediately apparent. At present two main considerations are being made. (i) One of the activities may represent arginyl-tRNA synthetase complexed with arginyl-tRNA, or ribosomal binding components, thereby accounting for the column fractionation and sucrose density gradient results. (ii) Arginyl-tRNA synthetase is activated by tRNA^{Arg}, a process obligatory for the activation step (14); thus, the enzyme could exist in two states in vivo, and these states could be related to its ability to perform a strictly catalytic as compared to regulatory role (i.e., control of arginine biosynthesis by generating the effector for the repression signal). The latter consideration is the hypothesis for more detailed studies

of the existence and possible significance of these activities.

ACKNOWLEDGMENTS

This investigation was supported by a Public Health Service research grant GM 18905-02 from the National Institute of General Medical Sciences and an American Cancer Society grant, P574. Some of the studies reported here are from a thesis submitted by (D.Y.) to the Graduate School, Purdue University, in partial fulfillment of the requirements for the Master of Science degree. L. S. Williams is a Public Health Service Career Development awardee (K4-GM-32-981-02) from the National Institute of General Medical Sciences.

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