Purification and Substrate Specificity of Arginyl-Ribonucleic Acid Synthetase from Rat Liver*

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It is generally accepted that in the first phase of protein biosynthesis, amino acid-specific enzymes, the aminoacyl-ribonucleic acid synthetases, carry out the activation of the various amino acids, and that the exacting specificity of these enzymes extends to the transfer of the amino acid to a particular soluble ribonucleic acid.

Several investigators (1-5) have achieved the purification of certain aminoacyl-RNA synthetases and have studied some of their properties.

The present work, as part of a program in which these enzymes are being studied with regard to structure and specificity, is chiefly a report on the partial purification of the arginyl-RNA synthetase and includes a study of the specificity of the enzyme with arginine analogues. Canavanine, an oxyguanidino analogue found in jack beans (6), inhibited the incorporation of arginine into sRNA, confirming an observation by Boman, Boman, and Maas (7) with Escherichia coli enzyme. In the rat liver system, the inhibition was shown to be of a competitive nature and the analogue was incorporated into both sRNA and protein.

EXPERIMENTAL PROCEDURE

Materials

ATP (crystalline, dipotassium), creatine phosphate, creatine kinase, and DEAE-cellulose were purchased from Sigma Chemical Company; GTP (sodium) from Pabst Laboratories; glutathione from Mann Research Laboratories; and yeast tRNA from General Biochemicals. E. coli (strain B) sRNA was prepared according to von Ehrenstein and Lipmann (8).

Uniformly labeled L-arginine-14C and L-leucine-14C were products of Schwarz BioResearch, Inc., and L-arginine-guanido-14C, L-homoarginine-14C, and L-tyrosine-14C as well as all the nonradioactive amino acids were purchased from California Corporation for Biochemical Research. Sephadex G-25 was purchased from Pharmacia, Uppsala.

Methods

Transamidinase was prepared as described by Rattner and Rochovansky (9). The method of Weinstein and Schechter (10) was employed for the preparation of rat liver 30,000 x g supernatant fraction. Rabbit liver pH 5 enzyme was prepared as described below for the rat liver enzyme except that acid precipitation was to pH 5.1 rather than pH 4.8. Crystalline bovine pancreatic ribonuclease was obtained from Nutritional Biochemicals Corporation.

Assay Procedures—The reaction mixture for the incorporation of amino acid into sRNA contained, in a total volume of 0.5 ml, 0.05 M Tris-HCl, pH 7.5; 0.005 M MgCl2; 0.0025 M ATP; 0.002 M glutathione; 0.01 M L-arginine-14C (specific activity, 5 mc per 

pmole) or other radioactive amino acid; 0.5 mg of yeast sRNA (except where otherwise noted), and arginyl-RNA synthetase. Dilutions of the enzyme were such that incorporating activity was proportional to enzyme concentration and sRNA was not limiting. The mixture was incubated for 10 minutes at 37°, and the reaction was terminated by the addition of 4 ml of 5% cold trichloroacetic acid containing approximately 0.001 m L-arginine-14C. The precipitate was washed twice with 4 ml of cold 5% trichloroacetic acid and with 4 ml of ethanol-ether, 1:1, and then dissolved in 0.2 ml of formic acid, plated, and counted in a windowless gas flow counter (efficiency, 42%). No corrections were made for self-absorption or geometry. Control incubations were those in which the enzyme was added after the trichloroacetic acid.

One unit of arginyl-RNA synthetase is defined as the amount of protein catalyzing the synthesis of 0.1 pmole of arginyl-sRNA in 10 minutes at 37°, and specific activity is defined as units per mg of protein. Protein was determined by light absorption or by the method of Lowry et al. (11). Specific activities of radioactive amino acids are expressed in micromoles per pmole. The concentration of sRNA was determined by absorbance at 260 mμ, assuming that 1 mg of RNA per ml has an optical density of 24.

Assays for protein synthesis were performed with the system described by Nirenberg and Matthaei (12), except that rat liver 30,000 x g supernatant fraction was employed. The final incubation volume was 0.5 ml, and incubation was for 1 hour at 37°. The reaction was stopped by the addition of 5% trichloroacetic acid, and the precipitated protein was heated for 15 minutes at 90°. The trichloroacetic acid was removed by filtration on a Millipore filter, the protein was washed on the filter several times with trichloroacetic acid containing the corresponding 14C-amino acid, and the Millipore filter was dried and counted as described for aminoacyl-sRNA.

Purification of Arginyl-RNA Synthetase—The arginyl-RNA synthetase was prepared from an acetone powder of rat liver, partly according to procedures described by Schweet (13) for the isolation of aminoacyl-RNA synthetases from fresh guinea pig liver. Portions (45 g) of fresh rat liver were homogenized for 1 minute with 5 volumes of acetone at -15° in a Waring Blender.

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1 The abbreviation used is: sRNA, soluble or transfer ribonucleic acid.
The mixture was filtered on a large Buchner funnel, and the filter cake was rehomogenized with an additional 3 volumes of cold acetone and filtered. The filter cake was dried rapidly at room temperature and stored at -20°.

In the preparation, all operations were carried out at 0-4°, unless otherwise noted. All sedimentations were carried out in the Servall SS-1 centrifuge at 10,000 × g for 15 minutes. A typical preparation is described for 20 g of acetone powder in Table I.

The acetone powder was extracted with 10 volumes of 0.05 M Tris-HCl, pH 7.5, 0.001 M EDTA, and 0.005 M glutathione for 15 minutes and centrifuged. The supernatant fluid was adjusted to 0.02 M with NaCl and recentrifuged to remove clumped microsomal material. The supernatant solution was adjusted to pH 4.8 with 1 M acetic acid and centrifuged, and the precipitated enzyme was suspended in one-fourth the original extract volume of 0.1 M Tris buffer, pH 7.5, 0.001 M EDTA, and 0.005 M glutathione. If necessary the pH was adjusted to 7.5 with 1 M KOH. After 30 minutes of extraction, the insoluble material was removed by centrifugation. Aged calcium phosphate gel (14) (which had been centrifuged to minimize dilution) was added to the supernatant solution in an amount equal to total weight of protein (milligrams); the slurry was stirred gently for 30 to 45 minutes and centrifuged, and the supernatant was discarded. The gel was mixed with one-fourth the original volume of 0.2 M potassium phosphate buffer, pH 8.1, stirred for 30 minutes, and centrifuged. The 2 M extract, which contained about 20% of the enzyme activity, was discarded.

The extraction was repeated with one-half the previous volume of 0.2 M potassium phosphate buffer, pH 8.1. Glutathione was added to the 0.2 M extract to a final concentration of 0.01 M. The enzyme might be stored under these conditions at -20° without appreciable loss of activity for 2 weeks. For routine assay or to prepare for column chromatography, the solution was passed through a Sephadex G-25 column which had been equilibrated with 0.01 M Tris (pH 7.5), 0.001 M EDTA, and 0.005 M glutathione. Desalting was performed without loss of activity at room temperature on Sephadex columns 20 cm long, the column diameter being varied according to the volume of the protein solution.

DEAE-cellulose was equilibrated with the same buffer used in the Sephadex columns. The enzyme solution was applied to a column (for 10 to 20 mg of protein, a column 8 × 1.1 cm was used), which was then washed with 3 to 4 volumes of 0.01 M Tris buffer, pH 7.5. The enzyme was eluted with 0.05 M Tris buffer, pH 7.5; the tubes with the highest specific activities were combined, and glutathione was added to a final concentration of 0.01 M. The enzyme might be concentrated by readsorption on calcium phosphate gel or precipitation with ethanol (an equal volume at -10°). On continued elution of the DEAE-cellulose column with high salt concentrations, additional arginyl-RNA synthetase activity appeared (at 0.25 and 0.50 M), and contained as much as 12% nucleic acid. Other investigators (2, 13) have reported that aminocetyl-RNA synthetases appeared bound to nucleic acid during fractionations. The specific activities of these fractions were low, and they were not investigated further.

Preparation of L-Canavanine-14C—L-Canavanine-guanidino-14C was prepared enzymatically from l-arginine-14C and unlabeled L-canine with purified pig kidney transaminase as in the reaction shown in Fig. 1 (9). The incubation mixture contained, in a final volume of 2 ml, 0.001 M L-canaline; 0.05 M potassium phosphate buffer, pH 7.5; 60 units of transaminase (9) (specific activity, 8); and, in one experiment, 0.4 mM l-arginine-guanidino-14C (specific activity, 12.5), and in another experiment, 0.1 mM l-arginine-14C (uniformly labeled; specific activity, 140). The incubation time was 2 hours at 38°. The reaction was stopped by the addition of 5% trichloroacetic acid, and the protein precipitate was removed by centrifugation. The trichloroacetic acid was extracted three times with several volumes of ether. Carrrier arginine-14C (2 mg) was added, and the solution was applied to a Dowex 50-X8 (Na+) column for chromatography according to Moore and Stein (15, 16) as described for canavanine by Kruse et al. (17). In this system, control canavanine samples were eluted 20 to 30 ml after the start of the citrate buffer while arginine appeared at 70 to 90 ml. The amino acids were located by determination of radioactivity in aliquots of each fraction (Fig. 2).

A peak containing radioactivity was washed off the column at the solvent front; it consisted of urea formed by contaminant arginase and also possibly a degradation product of canavanine.
TABLE I
Purification of arginyl-RNA synthetase from rat liver

The purification is described for 20 g of acetone powder. Assays were performed as given in "Methods." Specific activity is defined as units per mg of protein.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein</th>
<th>Volume</th>
<th>Specific activity</th>
<th>Total units</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Acetone powder extract</td>
<td>94</td>
<td>20</td>
<td>0.44</td>
<td>4300</td>
<td>100</td>
</tr>
<tr>
<td>II. BaCl₂ supernatant extract</td>
<td>40</td>
<td>7</td>
<td>0.69</td>
<td>4210</td>
<td>90</td>
</tr>
<tr>
<td>III. pH 4.8 precipitate, extract</td>
<td>10</td>
<td>0.5</td>
<td>5.43</td>
<td>2710</td>
<td>59</td>
</tr>
<tr>
<td>IV. Ca₃(PO₄)₂ gel extract</td>
<td>2.3</td>
<td>0.3</td>
<td>19.6</td>
<td>1350</td>
<td>29</td>
</tr>
<tr>
<td>V. DEAE-cellulose, 0.05 M Tris eluate</td>
<td>0.04</td>
<td>0.03</td>
<td>125.0</td>
<td>150</td>
<td>3.3</td>
</tr>
</tbody>
</table>

**RESULTS**

The arginyl-RNA synthetase, although very labile at all stages of purity, was stabilized considerably by the addition of glutathione. The calcium phosphate gel eluate, stored in 0.01 M glutathione at –20°C, remained fully active for at least 2 weeks and then only gradually decreased in activity. The DEAE-cellulose chromatographed enzyme lost activity rapidly, however, even in the presence of glutathione or added protein, and after 24 hours was almost completely inactive. Eighty-five percent of the activity was lost on heating the enzyme for 1 minute at 50°C. No stabilization was observed during storage for 24 hours at –20°C with added thiglycerol, mercaptoethanol, 50% glycerol, arginine, RNA, or ATP. Magnesium appeared to have some stabilizing capacity but was not as effective as glutathione.

Because of the extreme lability and low yield of the DEAE-cellulose fractions, most of the studies described here were carried out with the gel eluate, Fraction IV, 40-fold purified.

As shown in Fig. 3A, the incorporation of ¹⁴C-arginine is proportional to enzyme concentration when sRNA is in excess. Incorporation is also dependent on the amount of sRNA present (Fig. 3B). Activity of this enzyme required added ATP and magnesium and was stimulated by glutathione. The pH optimum for the reaction indicated a broad range for maximal activity from pH 7.0 to 8.0 in Tris-HCl buffers. Potassium phosphate buffers, 0.005 M, were 50% inhibitory. The Kₘ for arginine was determined by a Lineweaver-Burk plot as 3 × 10⁻⁴ M (Fig. 4).

**Activity with Arginine Analogues**—Structural analogues of arginine and other basic amino acids were tested as possible inhibitors of arginyl-RNA synthetase activity. For these (18). Approximately 20% of the ¹⁴C was recovered as canavanine, and the Rₑ corresponded to that of the control sample. All ¹⁴C applied could be accounted for in the eluted fractions. To eliminate the slightest possibility of contamination of the canavanine with ¹⁴C-arginine by overlap of peaks, the radioactive fractions corresponding to ¹⁴C-canavanine-¹⁴C were pooled, desalted on Dowex 50 (H⁺), and rechromatographed on Dowex 50-X8 (Na⁺) as before with 10 mg of arginine-¹⁴C added. Canavanine-¹⁴C was added to give a specific activity of 8.5 in one experiment and 12 in another. The canavanine-¹⁴C thus purified gave a single radioactive spot when subjected to electrophoresis at pH 9.1 as described by Richmond (19), and the Rₑ of the spot corresponded to that of a commercial sample. At pH 9.1 the Rₑ of canavanine was –1.5 (traveling to the anode) and for arginine, +9.2 (toward the cathode). Reisolation of ¹⁴C-amino acid material remaining after use in incorporation experiments showed that except for a constant small loss, presumably caused by cyclization to a nonamino acid form (18), all of the radioactivity could be recovered as canavanine.

![Fig. 3A](http://www.jbc.org/)

**Fig. 3A**, the effect of increasing enzyme concentration on ¹⁴C-arginyl-sRNA formation. The standard assay condition is described in "Methods." Enzyme Fraction IV and 0.5 mg of yeast sRNA were used. **Fig. 3B**, the effect of increasing yeast sRNA concentration on ¹⁴C-arginyl-sRNA formation. Standard assay conditions and 600 μg of enzyme Fraction IV were used.

![Fig. 4](http://www.jbc.org/)

**Fig. 4**, Lineweaver and Burk plot of L-arginine concentrations against rate of arginyl-sRNA formation. Standard assay conditions described in "Methods" were used, except that the incubation was for 5 minutes and the concentration of ¹⁴C-L-arginine was varied. Initial velocities, v, are given in counts of ¹⁴C-arginine incorporated per minute. ○—○ was assayed in the absence of inhibitor, and ●—● was assayed in the presence of 5 × 10⁻⁴ M canavanine.
Rabbit liver pH 5 enzyme fraction (specific activity, 8.5) replaced the arginine-14C; 1 mg of yeast sRNA and 100 µg of arginyl-RNA synthetase, Fraction IV, were used; and, where indicated, 25 µg of crystalline bovine pancreatic ribonuclease were added.

Table II

Incorporation of canavanine into sRNA

The assay system for amino acid incorporation into sRNA is described in "Methods." Arginine-14C was 0.01 mm; specific activity, 5. For incorporation of analogues, 0.02 mm L-canavanine-14C (specific activity, 8.5) replaced the arginine-14C; 1 mg of yeast sRNA and 100 µg of arginyl-RNA synthetase, Fraction IV, were used; and, where indicated, 25 µg of crystalline bovine pancreatic ribonuclease were added.

<table>
<thead>
<tr>
<th>[14C]-Amino acid</th>
<th>Incubation system</th>
<th>Activity (c.p.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Arginine</td>
<td>Complete</td>
<td>1880</td>
</tr>
<tr>
<td></td>
<td>-Enzyme</td>
<td>25</td>
</tr>
<tr>
<td>L-Canavanine</td>
<td>Complete</td>
<td>627</td>
</tr>
<tr>
<td></td>
<td>-Enzyme</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>-sRNA</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>+Ribonuclease</td>
<td>81</td>
</tr>
</tbody>
</table>

Table III

Incorporation of canavanine-14C into rat liver protein

The concentrations of components were, in a total volume of 0.5 ml: 0.10 M Tris-HCl, pH 7.5; 0.01 M MgCl2; 0.05 M KCl; 0.2 mM ATP; 0.05 mM each natural amino acid except arginine; and 2.3 mg of 30,000 X g supernatant fraction of rat liver homogenate. Puromycin was 0.2 mm, L-arginine-14C (specific activity, 5) was 0.01 mm, and L-canavanine-14C (specific activity, 12) was 0.007 mm.

<table>
<thead>
<tr>
<th>[14C]-Amino acid</th>
<th>Incubation system</th>
<th>Activity (c.p.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Canavanine</td>
<td>Complete</td>
<td>162</td>
</tr>
<tr>
<td></td>
<td>+Puromycin</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>-sRNA</td>
<td>3</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>Complete</td>
<td>169</td>
</tr>
<tr>
<td></td>
<td>-sRNA</td>
<td>12</td>
</tr>
</tbody>
</table>

* sRNA, 30,000 X g supernatant fraction.

Table IV

Mammalian aminoacyl-RNA synthetases with E. coli or yeast sRNA

The assay system for amino acid incorporation is described in "Methods." [14C]-L-Arginine (specific activity, 5) was 0.01 mm; [14C]-L-leucine (specific activity, 5) was 0.02 mm; and [14C]-L-tyrosine (specific activity, 10) was 0.01 mm. All reaction mixtures contained 1 mg of sRNA and 200 µg of pH 5 enzyme.

<table>
<thead>
<tr>
<th>Enzyme source</th>
<th>[14C]-Amino acid</th>
<th>E. coli sRNA (µmols/mg sRNA)</th>
<th>Yeast sRNA (µmols/mg sRNA)</th>
<th>Ratio of E. coli to yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat liver Fraction III</td>
<td>Arginine</td>
<td>0.69</td>
<td>0.21</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>Leucine</td>
<td>2.42</td>
<td>0.69</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>Tyrosine</td>
<td>0.08</td>
<td>0.54</td>
<td>0.15</td>
</tr>
<tr>
<td>Rabbit liver pH 5 enzyme</td>
<td>Arginine</td>
<td>0.98</td>
<td>0.27</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>Leucine</td>
<td>2.32</td>
<td>0.32</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>Tyrosine</td>
<td>0.10</td>
<td>0.18</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Discussion

Partially purified arginyl-RNA synthetase from rat liver showed requirements for its activity similar to those reported. Inasmuch as arginine and leucine are charged preferentially in E. coli sRNA rather than yeast sRNA by mammalian enzymes, tyrosine incorporation showed the reverse specificity. Absolute discrimination, however, was never observed. For example, while Benzer and Weisblum (20) found negligible incorporation of arginine with yeast sRNA and rabbit liver enzyme, we have detected 28% incorporation into yeast sRNA as compared to E. coli sRNA. In fact, yeast sRNA was used throughout this work with the rat liver enzyme. This discrepancy could be due to variations in the preparation of sRNA, activating enzymes, or differences in the strains of the organisms used. Partial charging of sRNA by experiments, the standard assay system was used, in which the concentration of arginine-14C was 0.01 mm. The analogues tested at 1 mm were L-ornithine, l-α-amino-γ-guanidinobutyric acid, L-citrulline, L-homocitrulline, L-lysine, and L-canavanine. Canavanine caused a significant inhibition (50%) of the incorporation of arginine into sRNA, as Boman et al. (7) observed with a 3-fold purified arginyl-RNA synthetase from E. coli. As seen in Fig. 4, the inhibition of canavanine appears to be competitive and the K<sub>i</sub> is 4.5 X 10<sup>-4</sup> M. The other analogues tested were not inhibitory (less than 10% variation from control values).

Incorporation of canavanine into sRNA and Protein—Because of the competitive nature of the inhibition of arginine activation by canavanine, it appeared possible that the analogue could actually be transferred to the sRNA in the place of arginine. As shown in Table II, the incorporation of canavanine-14C into trichloroacetic acid-precipitable material is dependent on sRNA and enzyme and is prevented by ribonuclease. Homoaarginine, another analogue, was not incorporated into sRNA when a 5-fold excess of the [14C] compound was present in place of [14C]-arginine under standard assay conditions. In addition it was found, as shown in Table III, that when canavanine-14C was present in a rat liver protein-synthesizing system which contained a complement of amino acids except arginine, the radioactive analogue was incorporated to a significant extent into protein. Homoaarginine, another analogue, was not incorporated into sRNA when a 5-fold excess of the [14C] compound was present in place of [14C]-arginine under standard assay conditions. In addition it was found, as shown in Table III, that when canavanine-14C was present in a rat liver protein-synthesizing system which contained a complement of amino acids except arginine, the radioactive analogue was incorporated to a significant extent into protein. This incorporation was inhibited by puromycin.

Mammalian Activating Enzymes with Yeast or E. coli sRNA—In view of the report that yeast sRNA was unable to accept arginine activated by extracts of rabbit liver (20), and since throughout this work yeast sRNA was used successfully with a rat liver system, a comparison is presented here between the yeast and E. coli sRNA amino acid acceptor capacities, with the use of both rat and rabbit pH 5 enzymes. As shown in Table IV, both rat and rabbit pH 5 enzymes catalyzed the incorporation of the three amino acids tested into both types of sRNA. The E. coli sRNA, however, was more effective for the incorporation of arginine and leucine, and less effective than the yeast sRNA for tyrosine. With respect to the arginine incorporation, similar results were observed with three different yeast sRNA preparations, two commercial and one prepared by the method of Holley et al. (21).
enzymes from different species might be related to the phenomenon described by Bennett, Goldstein, and Lipmann (23), who found that yeast enzyme charged only one of two separate leucine-specific sRNAs from E. coli. Aggar, Holley, and Merrill (24) and Sueoka and Yamane (25) have presented evidence that suggests widespread heterogeneity of various sRNAs.

The narrow limits of substrate specificity of arginyl-RNA synthetase are demonstrated by the failure of the enzyme to be inhibited by analogues identical with arginine except for the number of carbons in the side chain or those varying in the guanidino moiety. Canavanine, a naturally occurring amino acid, competitively inhibits the incorporation of arginine into sRNA, showing an affinity for the enzyme of the same order as that of arginine, and is itself incorporated into sRNA and protein.

There has been considerable speculation as to the mode of inhibition of growth by canavanine in various organisms (26-28) and viruses (29). This amino acid is a substrate for many of the enzymatic reactions that involve arginine (30). As early as 1948, Volcani and Snell (28) proposed that canavanine inhibited cell growth in bacteria by interfering in the synthesis of proteins. Schwartz and Maas (31) have since reported not only that canavanine interfered with cell growth of E. coli but also that the formation of β-galactosidase and ornithine transcarbamylase was prevented under certain conditions. Earlier, Kruse et al. (17) had isolated canavanine from a protein fraction of Walker carcinosarcoma 256 cells grown in tissue culture in the presence of the analogue.

The observation that arginyl-RNA synthetase from rat liver, as shown in the present work, as well as from E. coli (7), was inhibited by canavanine gives further support to the conclusion that canavanine curtails the utilization of arginine in the synthesis of proteins. The finding that canavanine could be incorporated into sRNA demonstrates that arginyl-RNA synthetase, like other arginine-metabolizing enzymes, cannot fully distinguish this analogue from the normal substrate.

The incorporation of canavanine into protein is in agreement with the evidence that after the formation of the aminoacyl-sRNA, the latter stages of protein synthesis show no specificity for the amino acid moiety (32).

The proteins synthesized in the presence of the analogue would contain a certain proportion of canavanine in the peptide chain positions which are normally occupied by arginine. In view of the large difference in the ionization of the guanidino and the oxyguanidino groups (33), the proteins containing canavanine would very likely have some altered properties.

The extent of replacement of arginine by canavanine in various systems and the properties of the proteins formed, for example behavior of proteolytic enzymes toward canavanyl peptide bonds, remain to be investigated.

SUMMARY

Arginyl-ribonucleic acid synthetase was purified 280-fold from rat liver acetone powder, and some of its properties were studied. Of several amino acid analogues tested, only canavanine inhibited arginine incorporation. The inhibition was competitive, and canavanine-14C could be incorporated into both soluble ribonucleic acid and protein.

Substrate specificities were also studied with soluble ribonucleic acid from yeast and Escherichia coli, as well as enzyme from rat or rabbit liver. Differences were found in the capacity of soluble ribonucleic acid from the two sources to accept the particular amino acids tested.

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REFERENCES

Purification and Substrate Specificity of Arginyl-Ribonucleic Acid Synthetase from Rat Liver
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