Arginyl Transfer Ribonucleic Acid Protein Transferase and Endogenous Acceptor Proteins in Cultured Mammalian Cells*

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SUMMARY

The presence of arginyl-tRNA protein transferase has been shown in a variety of mammalian cells in culture. Analysis by sodium dodecyl sulfate acrylamide gel electrophoresis of the available endogenous acceptor proteins (proteins that can be arginylated at their amino terminus by this enzyme) reveals that there are at least two such proteins (designated Peaks I and II) with the same relative migration during electrophoresis in a variety of tissues. Analysis of baby hamster kidney cells (BHK), polyoma-transformed BHK, and herpes simplex-infected BHK showed Peak II, but in the general region of Peak I there were two peaks. Other peaks, which varied in migration and appearance, were also observed in the various cell preparations. The incorporation of labeled arginine was inhibited by ribonuclease, canavanine, hemoglobin, and hemin but not by puromycin nor cycloheximide. The specificity of the enzyme from different species for exogenous acceptor proteins of various species is reported.

In this report we have studied in various lines of tissue culture cells a range of cell types and animal species in which arginyl-tRNA protein transferase is found. The ability of the arginyl-tRNA protein transferase from these cells to add arginine to certain exogenous proteins as well as the capacity of various compounds to inhibit the enzymic activity are presented. These data show that the properties of the transferase in cultured cell extracts are similar to those already reported. In addition, we have characterized the endogenous acceptor proteins of these cell lines by sodium dodecyl sulfate polyacrylamide gel electrophoresis and report an identity in relative migration of some of the acceptor proteins amongst all the cell types.

EXPERIMENTAL PROCEDURE

Cells—BHK21/C13 (baby hamster kidney cells) and BHK21/C13/PyH3 (PyH3) a line cloned after polyoma virus transformation of BHK21/C13, were grown in Dulbecco’s Medium with 10% fetal calf serum. HeLa (human cervical carcinoma), HEP-2 (human carcinoma of the larynx), African green monkey kidney cell lines (BSC-1, Vero, and CV 1), and MDIHK cells (bovine kidney) were grown in Dulbecco’s Medium with 10% calf serum and L cells (mouse fibroblasts) in Minimum Essential Medium, Eagle (Hanks’ salts) with 10% fetal calf serum. L5178Y cells, a mouse leukemic cell line, were grown in suspension culture with Fischer’s medium containing 10% horse serum.

Preparation of Cell Extracts The cultured cells were drained of medium and washed with phosphate-buffered saline (137 mM NaCl, 8.1 mM Na2HPO4, 2.6 mM KCl, and 1.4 mM KH2PO4). More phosphate-buffered saline was added and the cells scraped off with a rubber policeman. The cells were collected by low speed centrifugation and the sediment resuspended in approximately 10 volumes of ice-cold suspension medium (250 mM sucrose, 100 mM mercaptoethanol, 150 mM Tris, pH 7.8, 30 mM KCl, 5 mM MgCl2, and 0.1 mM EDTA). The suspension was again centrifuged at low speed, the pellet collected and resuspended in a volume of suspension medium equal to the pellet volume. The suspension was frozen and thawed three times followed by three 10-s periods of sonicating with a Branson Biosonic Ultrasonicator. The disrupted cells were centrifuged for 2 hours at 5°C in a Sorvall 50

Arginyl-tRNA protein transferase which catalyzes the transfer of arginine from arginyl-tRNA to the amino terminal of specific proteins, has been found in rat (1-3) and rabbit liver (4, 5), rat hepatoma (6), and sheep thyroid (7, 8). Endogenous acceptor proteins occur in all these tissues, and in addition albumin, thyroglobulin (9), Bence-Jones proteins, and soybean trypsin inhibitor (10) serve as acceptor protein for arginine when a highly purified preparation of the transferase from rabbit liver is used. The endogenous acceptor protein (or proteins) have not been characterized except in a recent report by Soffer (10) wherein he offers evidence that a major endogenous acceptor protein in rabbit liver is an albumin-like protein. This finding concurs with the fact that (a) added bovine serum albumin acts as an acceptor, (b) albumin is a product of liver cells, (c) an acidic amino terminus is apparently a requirement for acceptor protein, and (d) rabbit serum albumin, an acceptor protein, does indeed have glutamate at the amino terminal.

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1 The abbreviations used are: BHK, baby hamster kidney cells; SDS, sodium dodecyl sulfate.
carbonate, pH 8.5, at tritill in 2 ml were dialyzed against 500 ml of 0.6 M ammonia alcohol were added to 1 was shaken for 18 hours at room temperature. The pH was then adjusted to 2 with concentrated HCl, and the unreacted trinitrofluorobenzene was removed by three 10-ml washings with ether. An equal volume of 10% trichloroacetic acid was added to the aqueous phase, and the mixture was spun at 3000 x g for 10 min to remove all glass homogenizer. The suspension of disrupted cells was centrifuged at 4° for 50 min at 10,000 x g. The resulting supernatant fluid was centrifuged at 4° for 2 hours at 39,000 rpm in a Spinco 50 Ti rotor. The supernatant fluid was collected.

**Assay of ArginyI-tRNA Protein Transferase and Formation of Arginyl-tRNA**—Arginyl-tRNA protein transferase activity in the 100,000 x g supernatant fluid was measured essentially as described by Dupras and de Laimirande (6). The contents of the incubation mixtures are indicated in the appropriate portions under “Results.” Radioactivity was measured in a scintillation mixture with Triton X-100 as described by Patterson and Greene (11).

**SDS-Acrylamide Gel Analysis of Endogenous Acceptor Protein**—One-milliliter samples of [14C]arginine-labeled proteins formed by incubation of the 100,000 x g supernatant fluid of a cell extract as described under “Results” were mixed with an equal volume of a 2% SDS solution containing sodium phosphate buffer (10 mM) at pH 7.0 and 2-mercaptoethanol (200 mM). The solutions were mixed, heated at 100° for 1 min, and then dialyzed against two 500-ml changes of electrode buffer described below. Sucrose and bromophenol blue dye were added to concentrations of 10 and 0.01%, respectively.

The treated [14C]arginine proteins (0.35 ml) were analyzed on SDS-acrylamide gel with a Buchler analytical polyacrylamide vertical-die gel electrophoresis apparatus. Neutral 7.5% gels were prepared by mixing one portion of Solution A (30 g of acrylamide and 1.0 g of bisacrylamide in 100 ml of water) and one portion of Solution B (3.24 g of NaH₂PO₄, 2.37 g of Na₂HPO₄·H₂O, 0.4 g of SDS, and 0.4 ml of N,N,N',N'–tetramethylethylenediamine diluted to 100 ml) with two portions of Solution C (0.4 g of ammonium persulfate diluted to 100 ml). The mixture was poured into glass cylinders (0.5 x 7.5 cm) immediately covered with a layer of water and allowed to polymerize. The composition of the electrode buffer was 0.8 g of NaH₂PO₄, 0.6 g of Na₂HPO₄·H₂O, and 1.0 g of SDS in 1 liter of H₂O. Electrophoresis was carried out at room temperature for approximately 2 hours at 200 volts. After completion of the electrophoresis the gels were frozen at −70°, cut into 1 mm sections with a Diversified Scientific Instruments, Inc., gel slicer. The slices were prepared for counting by the method of Dinsmore and Peacock (19), which gave a recovery of 80 to 90% of the counts per min on the gels.

**Amino-terminal Amino Acid Analysis**—The preparation of NH₂-terminal amino acids described here is similar to that carried out by Kaji (3). Ten milligrams of [14C]arginine-labeled protein in 2 ml were dialyzed against 500 ml of 0.6 M ammonium carbonate, pH 8.5, at room temperature for 4 to 6 hours. Two milliliters of 5% solution of 1-fluoro-2,4-dinitrobenzene in ethyl alcohol were added to 1 ml of the dialyzed sample, and the mixture was shaken for 18 hours at room temperature. The pH was then adjusted to 2 with concentrated HCl, and the unreacted dinitrofluorobenzene was removed by three 10-ml washings with ether. An equal volume of 10% trichloroacetic acid was added to the aqueous phase, and the mixture was spun at 3000 x g for 5 min. The yellow precipitate was washed with 10 ml of ethanol followed by an equivalent quantity of ether, and then dried at room temperature over paraffin shavings. The dried powder was suspended in 3 ml of 6 N HCl, sealed in a glass tube under nitrogen, and digested at 105° for 12 hours. The digestion mixture was centrifuged at 3000 x g for 10 min and the supernatant liquid was dried in vacuo in a small Petri dish. The powder was then dissolved in 1 ml of acetone which contained 1 mg of unlabeled dinitrophenyl-L-arginine (Schwarz BioResearch Inc.) as a marker. It was necessary to add a drop of concentrated HCl to dissolve the arginyl derivative. Descending chromatography of 0.1-ml samples of this solution containing 600 cpm was carried out on Whatman No. 1 paper using the butanol-1-acetic acid-water (4:1:5) solvent system for 15 hours. The yellow spot was cut from the paper, placed in 10 ml of toluene 1,4-bis[2-(6-phenylloxazolyl)]benzene (POPOP) and the radioactive content determined.

**[14C]Arginyl-tRNA Preparation**—[14C]Arginyl-tRNA was prepared with stripped Escherichia coli B tRNA (General Biochemicals) and the supernatant fluid from an 80% ammonium sulfate-saturated solution of an E. coli B extract prepared as described by Voytek et al. (13).

### RESULTS

**Occurrence of Enzyme**—The supernatant fluids from 2-hour, 100,000 x g centrifugations of sonically disrupted cells of various types were tested for arginyl-tRNA protein transferase activity. Apparent transferase activity was found in the 100,000 x g supernatant fluids of L, BHK, PB13, Hela, PEP-2, L5178Y, MDBK,vero, BSC-1, and CV-1 cells. A typical experiment showing incorporation of [14C]arginine into a hot acid-insoluble product is shown in Fig. 1A. For BHK and L cells, the rate of incorporation diminishes after about 2½ hours. Similar time courses were obtained for the other cell lines tested. One explanation for the decrease in rate of incorporation could be that almost all of the available endogenous acceptor proteins have had arginine added. Such is the case, has been substantiated by the experiments depicted in Fig. 1B and C. First, as shown in Fig. 1B, after 120 min of incubation of an L cell preparation, when the rate of incorporation of [14C]arginine is markedly decreased, if additional [14C]arginine, ATP, and ATP-generating components (equivalent to the amount present initially) are added to the mixture, no increase in [14C]arginine incorporation ensues. This observation indicates that the decline in incorporation is not due to exhaustion of the supply of arginine or ATP. Second, Fig. 1C shows that after a similar 120-min incubation, the addition of bovine serum albumin, an exogenous acceptor protein (9), results in a prompt and marked stimulation of the incorporation of [14C]arginine into hot acid-insoluble product, thus indicating that the other components of the assay mixture are active. The most feasible interpretation of these experiments is that the incorporation of labeled arginine essentially stops due to exhaustion of available endogenous acceptor protein. Moreover, the plateau level observed should represent a reasonable estimate of the amount of available acceptor protein in any particular preparation, provided arginine and ATP are present in excess.

**Optimal Assay Conditions**—The optimal conditions for measuring arginyl-tRNA protein transferase activity were determined for BHK and L cells when an ATP-generating system, arginine, and uncharged tRNA were used with the 100,000 x g supernatant fluid. The optimum for such a crude preparation reflects,
Fig. 1. A, incorporation of [14C]arginine into hot acid-insoluble product in BHK and L cells. Extracts of BHK and L cells were prepared as described under “Experimental Procedure” and added to incubation mixtures containing: 100 mM Tris buffer (pH 7.8), 100 mM mercaptoethanol, KCl (35 mM for L cells, 65 mM for BHK), [14C]arginine (15 µM, 20 Ci per mole), 3 mM MgCl2, 3 mM ATP, 12 mM creatine phosphate, and approximately 10 units per ml of creatine phosphokinase. Incubation was at 37° and samples were removed as indicated for analysis as described under “Experimental Procedure.” BHK, O—O; L, O—O. B, effect of inclusion of additional reactants or exogenous acceptor protein on the incorporation of [14C]arginine into hot acid-insoluble product after the rate of [14C]arginine uptake had decreased in the L cell extract. After [14C]arginine incorporation has proceeded for 120 min (incubation constituents the same as in A), an additional quantity of reactants (except for the extract) equivalent to that present at time zero is added. C, conditions are as in B except that at 120 min sufficient bovine serum albumin is added for a final concentration of 5 mg per ml. D, specificity of Incorporation for arginine. BHK 100,000 X g supernatant fluid was incubated with the standard mixture (A) using either 15 µM [14C]arginine (20 Ci per mole) (O—O), or 15 µM [14C]leucine (20 Ci per mole) (O—O). E, BHK 100,000 X g supernatant fluid was incubated with the standard mixture (A) and a [14C]-amino acid mixture (arginine final concentration 0.4 µM, 255 Ci per mole; other amino acids at similar concentration and specific activity) (O—O) or the same [14C]-amino acid mixture plus added unlabelled arginine to a final concentration of 1.5 mM (0.17 Ci per mole) (O—O).

Fig. 2. SDS-acrylamide gel patterns. The 100,000 X g supernatant fluid of extracts of the indicated cells were incubated with the standard BHK mixture described in Fig. 1A (for L cells the KC1 concentration was 35 mM) for 6 hours at 37° and analyzed on gels as described under “Experimental Procedure.” The specific activity of the 15 µM [14C]arginine was increased to 255 Ci per mole. The ordinate is percentage of total counts per min recovered from the gels which was 80 to 90% of the amount put on.

of course, the optimum for the dual process of charging tRNA and transferring the arginine to the acceptor protein.

The optimal KCl concentration for L cell extracts was 35 mM and for BHK, 65 mM. For both cell types, NaCl was equally effective and at the same concentrations as KCl. At 100 and 150 mM mercaptoethanol there was maximal transferase activity in L and BHK cell extracts, respectively. The pH curve transferase activity relationship for both BHK and L cells in the range tested (pH 7.4 to 9.6, 100 mM Tris-HCl, 30-min incubation) showed a broad peak from about 7.6 to 8.6. Above pH 8.6, activity decreased gradually so that at pH 9.6 the activity was 50% that of the maximum. Little can be made of the effect of pH on transferase activity since the change in activity may reflect an effect on arginyl-tRNA synthetase activity as well as increasing lability of arginyl-tRNA at the higher pH (6).

Specificity of Arginyl-tRNA Protein Transferase for Arginine—
The specificity of the enzyme for transferring only arginine was tested to determine whether it was the same as reported for the transferase from excised tissue. First, as may be seen in Fig. 1D in a preparation of transferase from BHK cells in which there is marked incorporation of labeled arginine a similar incubation with [14C]leucine shows no incorporation of radioactivity into the hot acid-insoluble fraction. Second, a mixture of several [14C]-labeled amino acids (New England Nuclear, [14C]-labeled, tRNA amino acid mixture) incubated with the BHK preparation also results in incorporation of radioactivity (Fig. 1E). Addition of excess unlabeled arginine alone to this incubation almost completely prevents measurable incorporation of radioactivity (Fig. 1E), thus showing that of all the labeled amino acids in the mixture only arginine is incorporated in significant amounts. The small residual incorporation may reflect a small incorporation of amino acid other than arginine as reported by Kuji (3). Addition of either unlabeled leucine or phenylalanine did not similarly diminish incorporation of radioactivity.

SDS-Acrylamide Gel Patterns of Endogenous Acceptor Protein—The SDS-acrylamide gel patterns of [14C]arginine-labeled acceptor protein incorporated via the usual incubation are shown for several of the cell lines (Fig. 2). Of note, is the great similarity of pattern in the extracts from the various species represented by the different cell lines. In all the cell extracts analyzed, a major peak is evident between Slices 15 to 20 (17) of the electrophoretograms. The protein in this peak has a molecular weight of approximately 65,000 based upon the migration of bovine serum albumin under the same conditions. In
the gels of the extracts from the various cells examined, one or two lesser peaks are also observable in gel Slices 9 to 13 and are presumably of greater than 65,000 molecular weight. Two peaks in Slices 9 to 13 (Ia and Ib) were always seen with gels of incubations of extracts from BHK, herpes-infected, and polyoma-transformed (PyH3) BHK. In extracts from other cells only one peak (I) was always clearly discernible. The number and position of the peaks of radioactivity beyond the major peak are somewhat variable from preparation to preparation from even the same cells although a peak was almost always seen in the Slice 30 to 40 range. Treatment of the incubation mixture after incubation and prior to gel analysis with 50 μg per ml of pancreatic ribonuclease for 30 min did not noticeably change the gel patterns thus indicating that the observed peaks are not arginyl-tRNA. 2,4-Dinitrophenol analysis indicated that after correcting for 2,4-dinitrophenol quenching 80 to 90% of the [14C]arginine associated with the endogenous acceptor proteins is at the amino terminal.

The SDS-acrylamide gel pattern of incubations of extracts from polyoma-transformed (PyH3 cells) and herpes simplex-infected BHK cells were examined for differences (Fig. 3, top and bottom left). No striking difference was found in gel pattern in incubated extracts from these cells.

To show that the gel patterns observed are not an artifact of, or peculiar to, tissue culture-grown cells, freshly excised mouse kidney, processed as described under “Experimental Procedure,” was also analyzed by SDS-acrylamide gel. Incubation conditions were the same as for the L cells. Essentially the same pattern obtains with the mouse kidney preparation (Fig. 3, bottom right).

The results are partly summarized in Table I where the RF values of the I, In, Ib, and II peaks are compared in electrophotograms of the different cell preparations. Note the almost perfect agreement for the RF values of Peaks I and II from the various cell extracts and the close agreement for the Peaks Ia and Ib in the gels of incubations of extracts from the BHK and related cells.

Exogenous Acceptor Proteins—As mentioned above, bovine serum albumin exhibits acceptor activity. Such activity is shown for BHK cells in Fig. 4. This is in agreement with Soffer’s work with rabbit liver (8) as is the apparent acceptor activity of bovine thyroglobulin (Fig. 4).

The fact that the activity has been shown in a variety of cells from different species allowed the investigation of species specificity of the enzyme activity. No such specificity was found. Preparations from cells of various species were tested with proteins (all albumins except for immunoglobulin G (IgG)) for acceptor activity. However, as may be seen (Fig. 5), there are differences amongst the various combinations of enzymes and acceptor protein. For example, mouse albumin acted as a much better acceptor protein for the transferase from human HeLa cells than mouse L cells, and bovine albumin-stimulated arginine incorporation by human HeLa cell extracts much better than

### Table I

<table>
<thead>
<tr>
<th>Cell</th>
<th>Peak RF Values</th>
<th>Endogenous Acceptor Protein</th>
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<tr>
<td>HeLa</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>L5178Y</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>L cell</td>
<td>0.30 0.17</td>
<td></td>
</tr>
<tr>
<td>Mouse kidney</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>BHK</td>
<td>0.17 0.19 0.23</td>
<td></td>
</tr>
<tr>
<td>PyH3</td>
<td>0.14 0.25 0.35</td>
<td></td>
</tr>
<tr>
<td>Herpes-infected BHK</td>
<td>0.18 0.24 0.36</td>
<td></td>
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</tbody>
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Values for RF were calculated by dividing the distance migrated by the [14C]arginine-labeled protein band by the distance migrated by bromphenol blue.

![Fig. 3. Comparison of SDS-acrylamide gel patterns of extracts of BHK cells, of polyoma virus transformed BHK cells (PyH3), herpes simplex (Rolly II), infected BHK cells, and freshly excised mouse kidney cells. The 100,000 x g supernatant fluid of extracts of the indicated cells were incubated for 6 hours, 37° with the standard BHK mixture (Fig. 1A) but using 15 μM [14C]arginine with a specific activity of 255 Ci per mole. For the herpes simplex infection the cells were infected at a multiplicity of 10 plaque-forming units per cell and adsorbed for 1 hour. Infection was allowed to proceed for 12 hours before harvesting the cells for analysis. The ordinate is as in Fig. 2.](http://www.jbc.org/)

![Fig. 4. Effect of exogenous proteins on the incorporation of [14C]arginine. BHK 100,000 x g supernatant fluid was incubated with the standard mixture (Fig. 1A) with no further additions (●—●), plus 80 μM bovine thyroglobulin (△—△), or plus 80 μM bovine serum albumin (○—○).](http://www.jbc.org/)
The properties of the enzyme in cultured mammalian cells reported here are similar to those described by other investigators.

**DISCUSSION**

The arginine analog canavanine inhibits arginine incorporation (Table II). The relatively high concentration of canavanine required for such inhibition can be accounted for solely or in part by the reports that high concentrations of canavanine are needed to inhibit binding of arginine to tRNA by both bacterial (16) and rat liver (17) preparations of purified arginyl-tRNA synthetase. Allen and Allende (17) reported that with rat liver arginyl-tRNA synthetase 100-fold more canavanine than arginine (1 mM versus 0.015 mM) caused 50% inhibition of arginine binding. This is in excellent agreement with the present finding that 100-fold more canavanine than arginine (1.5 mM versus 0.015 mM) caused about 50% inhibition of incorporation into hot acid-insoluble product (Table II).

Soffer and Mendelsohn (7) reported that the enzyme from sheep thyroid is not inhibited by puromycin. Similarly, the arginyl-tRNA protein transferase from BHK is inhibited neither by 2 x 10^-4 M puromycin nor by 30 μg per ml of cycloheximide (Table II).

Soffer (9) has reported that hemoglobin does not have acceptor activity. We also find this to be the case with the cell extracts examined in this paper and, moreover, find that bovine hemoglobin appears to inhibit the transfer of arginine to other proteins which do have acceptor activity (Fig. 6A). Hemoglobin is a similar effect (Fig. 6A). Increasing the arginine concentration or adding additional acceptor protein (bovine serum albumin) does not overcome the inhibition by hemoglobin (Table III). In a concentration-inhibition curve for human hemoglobin (Fig. 6B) run with the added exogenous acceptor protein, bovine serum albumin, and 250 μM [14C]arginine, 50% inhibition occurs with about 15 μM hemoglobin. As the usual incubation system has three enzymes, creatine kinase, arginyl-tRNA synthetase, and arginyl-tRNA protein transferase, it was necessary to test whether the primary site of hemoglobin inhibition is specifically at the transferase step. Fig. 7 shows that similar inhibition by hemoglobin occurs when the need for the kinase and synthetase is obviated by addition of previously prepared [14C]arginyl-tRNA. Thus, hemoglobin appears to be specifically inhibiting the transferase reaction.

**Inhibition of Arginine Incorporation—Pancreatic ribonuclease**

Pancreatic ribonuclease (1 μg per ml final concentration) virtually prevents any incorporation of [14C]arginine into protein (Table II).
The 100,000 × g supernatant fraction of an extract of BHK cells was incubated for 20 min with the standard BHK mixture (Fig. 1.4) varying the arginine concentration, with or without bovine serum albumin and in the presence or absence of human hemoglobin. The amount of incorporation of [14C]arginine into hot acid-insoluble product in the absence of human hemoglobin is the control level (90% inhibition).

Table III

<table>
<thead>
<tr>
<th>Arginine</th>
<th>Albumin</th>
<th>Inhibition with hemoglobin</th>
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<tbody>
<tr>
<td>2 µM</td>
<td>µM</td>
<td>%</td>
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<td>15</td>
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<td>74</td>
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<tr>
<td>500</td>
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<td>85</td>
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Table III: Effect of increased arginine concentration and additional acceptor protein on inhibition of arginine incorporation by human hemoglobin

For arginyl-tRNA protein transferase from freshly excised tissues. The specificity for arginine, amino-terminal addition of arginine, acceptor protein activity of bovine serum albumin and thyroglobulin, sensitivity to ribonuclease, and lack of inhibition by puromycin have all been reported as properties of arginyl-tRNA protein transferase.

The most significant findings are those relating to the endogenous acceptor proteins. In Soffer's recent report (10) on isolation of an acceptor protein from rabbit liver, he finds a protein which appears to be albumin based on its electrophoretic mobility, molecular weight, and amino acid composition. As stated in the introduction, it is not surprising to find an albumin acceptor protein in the site of albumin synthesis. The major available endogenous acceptor protein (Peak II) that we find in Peak II which accounts for approximately 25% of the available endogenous acceptor protein is therefore also present in significant proportion (0.13% total cell protein). The fact that available endogenous acceptor protein appears to be present in this amount argues that the form of these proteins without arginine at the amino terminal is the predominant species as it is not likely that the total acceptor protein with or without arginine represents 1% or more of total cellular protein. Unfortunately, there is at present no means for measuring endogenous acceptor protein with arginine already added at the amino terminus. In this regard, it should be mentioned that terminal analysis of soluble proteins in HeLa cells (19) shows no arginine and 8 and 9% aspartate-asparagine, glutamate-glutamine, respectively, at the amino terminus.

At present, no cellular role has been attributed to the enzyme arginyl-tRNA protein transferase. Hopefully, the search for the role of this enzyme will be facilitated by the use now of tissue culture grown cells in which conditions may be more readily and precisely monitored and controlled than in the intact animal.

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