Changes in Certain Aminoacyl Transfer Ribonucleic Acid Synthetase Activities in Developing Pea Roots

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ABSTRACT

Tyrosyl-, arginyl-, leucyl-, and phenylalanyl-tRNA synthetase activities were measured in extracts from three root sections of 3-day-old pea seedlings. The sections 0 to 2, 3 to 7, and 8 to 22 millimeters from the root tip were then chosen to represent the regions of cell division, elongation, and maturation, respectively. The specific activity for each aminoacyl-tRNA synthetase was highest in the 0- to 2-millimeter section and lowest in the 8- to 22-millimeter section. The changes in specific activity between the sections, however, varied with the particular synthetase. Tyrosyl-tRNA synthetase activity from each section was fractionated into two activity regions on a diethylaminoethyl cellulose column. Approximately 10, 22, and 44% of the total tyrosyl-tRNA synthetase activity in the 0 to 2, 3 to 7, and 8- to 22-millimeter sections, respectively, were associated with the first tyrosyl-tRNA synthetase region; the remaining activity was located in the second tyrosyl-tRNA synthetase region. Only one activity region for arginyl-tRNA synthetase was detected by diethylaminoethyl cellulose column fractionation.

Several studies (2, 6, 8-10) have shown that changes in the ratios of isoaccepting tRNAs for a particular amino acid are correlated with certain cellular or developmental changes. In plants, for example, Vold and Sypherd (13) reported that the ratio of isoaccepting seryl-, lysyl-, and prolyl-tRNAs in embryos and 2-day-old wheat seedlings were different. Vanderhoef and Key (12) have shown that the ratio of isoaccepting tyrosyl-tRNAs changed during the development of the pea root. Bick et al. (3) have also reported changes in the concentration of some isoaccepting leucyl- and tyrosyl-tRNAs during the development of soybean cotyledons.

Changes in aminoacyl-tRNA synthetase activities during plant development have also been reported. Anderson and Cherry (1) have shown that preparations from soybean hypocotyls and cotyledons differ in their ability to acylate isoaccepting leucyl-tRNAs. Hypocotyl extracts only acylated four isoaccepting leucyl-tRNAs, whereas extracts from cotyledons acylated all six tRNAs. More recently Kanabus and Cherry (6) reported the fractionation of leucyl-tRNA synthetase from soybean cotyledons into three activity regions on hydroxylapatite columns. The first fraction of synthetase activity eluting from the column acylated only two of six isoaccepting leucyl-tRNAs. The other two fractions did not effectively acylate the same two leucyl-tRNAs but were equally effective in acylating the four other leucyl-tRNAs. Synthetase preparations from soybean hypocotyls, however, were essentially deficient in the first synthetase and therefore only acylated four of the six leucyl-tRNAs. We have presented evidence for multiple tyrosyl-tRNA synthetases in extracts from pea roots (5).

The rate of protein synthesis decreases as the root develops from the meristematic region to the mature region; however, the mechanisms operative in the regulation of protein synthesis are not known. One essential step in protein synthesis which probably is involved in regulating the rate of protein synthesis is tRNA acylation. The experiments reported in this paper were designed to examine changes in the aminoacyl-tRNA synthetase activities for certain amino acids and quantitatively measure changes in tyrosyl-tRNA synthetase activity during the development of the pea root.

MATERIALS AND METHODS

Plant Growth and Tissue Extraction. Pea seeds (Pisum sativum, var Alaska) were germinated between layers of moist absorbent paper at room temperature (approximately 25 C). The roots from 3-day-old seedlings were cut into three sections, 0 to 2, 3 to 7, and 8 to 22 mm from the root tip and immediately frozen on Dry Ice. These sections represented cells primarily undergoing cell division, elongation, and maturation, respectively. The frozen root sections were transferred to a grinding solution (11) (1:1, w/v) containing 10 mm HEPES, pH 7.5; 1 mm MgCl2; 0.5 mm dithiothreitol; and 0.4 mm sucrose. After limited thawing, the root sections were homogenized with a Willems Polytron homogenizer at medium speed for approximately 2 min in the cold room. The homogenates were centrifuged 25 min at 27,000 g.

Batch Fractionation. The following fractionations were carried out at 2 to 4 C. The supernatant from the above centrifugation was filtered through Miracloth and fractionated with 25 pl of 2% protamine sulfate (Sigma, grade 1) for each milligram of nucleic acid. After 10 min the solutions were centrifuged 10 min at 12,000 g. Saturated ammonium sulfate (Nutritional Biochemical Corp., enzyme grade) (pH 7.5) was added to the resulting supernatants to 40% saturation and allowed to stand 10 min. After centrifuging (10 min at 12,000 g) additional saturated ammonium sulfate was added to the supernatants to obtain 70% saturation. Again 10 min elapse prior to centrifuging 10 min at 12,000 g. The 70% ammonium sulfate pellets were rinsed with cold H2O and resuspended in 2 ml of 10 mm HEPES, pH 7.5, containing 2.5 mm dithiothreitol. A small portion of these extracts was used to assay for tyrosyl-, arginyl-, leucyl-, and phenylalanyl-tRNA synthetase activity. Glycerol was added to the remaining portions (30%, v/v) prior to storage at -70 C.

Fractionation on DEAE-cellulose Columns. The DEAE-
cellulose columns (2.0 × 15.0 cm) were equilibrated with 100 mm HEPES, pH 7.5, and then with 20 mm HEPES, pH 7.5, containing 0.5 mm diithioretilt (buffer A). The extracts containing glycerol were dialyzed for 4 hr (changed after 2 hr) against 2 liters of 5 mm HEPES, pH 7.5, containing 0.5 mm diithioretilt and then added to the DEAE-cellulose columns. The columns were eluted with 50 ml of buffer A and then with a linear KCl gradient; the mixing chamber contained 200 ml of buffer A, and the reservoir contained 200 ml of 0.15 M KCl in buffer A. Ten-milliliter fractions were collected and assayed directly for arginyl- and tyrosyl-tRNA synthetase activity. The data in Figures 1 and 2 are from one of three closely duplicating experiments.

**Aminoacyl-tRNA Synthetase Assay.** The reaction mixture for the assay of aminoacyl-tRNA synthetase activity in the ammonium sulfate-fractionated extract contained 80 µmoles of HEPES, pH 8.0; 4.8 µmoles of MgCl₂; 0.24 µmole of ATP; 80 µg of tRNA; 0.4 µC of ^14C-tyrosine, -arginine, -leucine, or -phenylalanine (50 µc/µmole); and 60 to 120 µg of protein in a final volume of 0.8 ml. Reaction mixtures for the assay of tyrosyl- and arginyl-tRNA synthetase activity in fractions from the DEAE-cellulose columns contained: 80 µmoles of HEPES, pH 8.0; 4.8 µmoles of MgCl₂; 0.24 µmole of ATP; 80 µg of tRNA; 0.4 µC of ^14C-tyrosine (200 or -arginine (200 µc/µmole); and 10 to 60 µg of synthetase protein in a final volume of 0.8 ml. The reaction mixtures were incubated at 37 C, and 0.2-ml aliquots were removed at 0-, 1-, 3-, and 10-min intervals and precipitated in 2 ml of ice-cold 10% trichloroacetic acid. The precipitates were collected on glass fiber discs (type GFA) and washed with 4 ml of 5% trichloroacetic acid, 4 ml of 0.5% trichloroacetic acid, and 3 ml of Hokin's reagent. The discs were dried, and the radioactivity was assayed in a liquid scintillation spectrometer. The counting solution contained 0.3 g of POPPOP and 5 g of PPO per liter of toluene.

**Other Methods.** The tRNA used in the assay mixtures was obtained by extracting the total RNA from the whole pea roots according to the method of Vanderheof et al. (11) with subsequent purification of the tRNA as described by Vanderheof and Key (12).

Protein concentrations were estimated from absorbance readings at 260 and 280 nm (14).

### RESULTS AND DISCUSSION

The fresh weight and protein content of the three root sections used in this investigation, 0 to 2, 3 to 7, and 8 to 22 mm, are reported in Table I. As expected, the fresh weight per cell increased as the cells enlarged and matured. The protein content per cell was about 3-fold greater in cells from the elongating region than from the dividing region. Cells in the mature region, on the other hand, contained 10 to 15% less protein than those in the elongating region. The decrease in protein per cell, even with the continual increase in cell mass, is in agreement with the data of Brown and Broadent (4).

The aminoacyl-tRNA synthetase activity for several amino acids was measured in cell extracts from the three different root sections. Relative rates were calculated from the linear portion of time course analyses (usually 1-3 min) made over a 10-min period. The synthetase activities per milligram of protein (specific activity) and per cell for tyrosine, arginine, leucine, and phenylalanine are reported in Table II. Cells from the 0 to 2 mm section contained the highest specific activity for these four aminoacyl-tRNA synthetases while cells from the 8 to 22 mm section had the lowest specific activity. Additionally, the four aminoacyl-tRNA synthetases had similar specific activities in the 0 to 2 mm section. The differences in specific activity of the aminoacyl-tRNA synthetases between the three sections varied with the particular aminoacyl-tRNA synthetase. For example, the specific activities of tyrosyl-tRNA synthetase in the 0 to 2 and 3 to 7 mm sections were very similar, whereas synthetase activity in the 8 to 22 mm section was less than 50% that in the other two sections. The specific activity of arginyl-tRNA synthetase in the 3 to 7 mm section, on the other hand, was approximately 4-fold less than in the 0 to 2 mm section but was only 30% greater than in the 8 to 22 mm section.

The aminoacyl-tRNA synthetase activity per cell for the four amino acids again was similar in the 0 to 2 mm section but varied considerably between sections. The difference in activity per cell in these sections also varied with the particular aminoacyl-tRNA synthetase. The aminoacyl-tRNA synthetase activity per cell (except for arginine) was greater in the 3 to 7 mm section, where protein accumulation per cell was greatest, than in the 0 to 2 mm section, while the 8 to 22 mm section contained 40 to 60% less synthetase activity per cell than the 3 to 7 mm section. The increase in tyrosyl- and leucyl-tRNA synthetase activities in the 3 to 7 mm section (about 2.5- and 1.6-fold, respectively) was greater than in the 0 to 2 mm sections (1.3- and 1.2-fold, respectively). The synthetase activity per cell for arginine was 3.0-fold greater in the 3 to 7 mm section than in the 0 to 2 mm section. The effect of protein interactions on synthetase activity per cell was complex. The magnitude of this effect was greater for the aminoacyl-tRNA synthetase activity per cell than for the tRNA synthetase activity per cell.

### Table I. Mass and Protein Content from Different Regions of the Developing Pea Root

<table>
<thead>
<tr>
<th>Section</th>
<th>Cells per section</th>
<th>Mass</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fresh wt/section</td>
<td>Fresh wt/cell</td>
</tr>
<tr>
<td>mm</td>
<td>X10^4</td>
<td>ug X 10^-3</td>
<td>ug X 10^-3</td>
</tr>
<tr>
<td>0 to 2</td>
<td>7.45</td>
<td>8.11</td>
<td>1.02</td>
</tr>
<tr>
<td>3 to 7</td>
<td>5.20</td>
<td>24.33</td>
<td>4.40</td>
</tr>
<tr>
<td>8 to 22</td>
<td>9.08</td>
<td>92.67</td>
<td>9.60</td>
</tr>
</tbody>
</table>

1 The cells per section were estimated from data reported by Brown and Broadent (4).

### Table II. Tyrosyl-, Arginyl-, Leucyl- and Phenylalanyl-tRNA Synthetase Activity from Different Regions of the Developing Pea Roots

The complete reaction mixture contained 80 µmoles HEPES, pH 8.0; 4.8 µmoles MgCl₂; 0.24 µmole ATP; 0.4 µC ^14C-tyrosine, -arginine, -leucine or -phenylalanine, (50 µc/µmole); 80 µg tRNA; and synthetase protein in a final vol of 0.8 ml. The reaction mixtures were incubated at 37 C and 200 µl aliquots of the mixtures were removed at 0, 1, 3, 5 and 10 min to determine the rate of tRNA acylation. The synthetase activity per cell for each section is based on the protein content and cell numbers reported in Table I.

### Table III. Aminoacyl-tRNA Synthetase Activity

<table>
<thead>
<tr>
<th>Aminoacyl-tRNA Synthetase</th>
<th>0 to 2 mm section</th>
<th>3 to 7 mm section</th>
<th>8 to 22 mm section</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosine</td>
<td>8450</td>
<td>8170</td>
<td>26.50</td>
</tr>
<tr>
<td>Arginine</td>
<td>8110</td>
<td>2110</td>
<td>6.85</td>
</tr>
<tr>
<td>Leucine</td>
<td>7580</td>
<td>6660</td>
<td>21.60</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>8220</td>
<td>3950</td>
<td>12.79</td>
</tr>
</tbody>
</table>

1 One unit = 1 cpm/min incubation; 8000 cpm are equivalent to approximately 180 pmoles
3.0-fold, respectively) corresponded with the increase in protein content (3.0-fold) in this section. Arginyl-tRNA synthetase activity, on the other hand, decreased, and phenylalanyl-tRNA synthetase activity only increased 30% in this section.

Preliminary data on 12 other aminoacyl-tRNA synthetases support the following general conclusions: the 0 to 2 mm section has the highest specific aminoacyl-tRNA synthetase activity, the 3 to 7 mm section contains the highest synthetase activity per cell, and the difference in synthetase activity in the three sections varies with the particular aminoacyl-tRNA synthetase. The assay conditions for certain of these 12 aminoacyl-tRNA synthetases probably were not optimum, since several of the synthetase activities were low or had abnormal kinetics.

The results in Table II support the view that the decreased rate of protein synthesis during cell maturity could be, in part, a result of decreased aminoacyl-tRNA synthetase activity. It is still possible that the decrease in aminoacyl-tRNA synthetase activity is a result of decreased protein synthesis per se and not related directly to the reduced rate of protein synthesis. However, as noted for arginyl- and phenylalanyl-tRNA synthetases, the decrease in synthetase activity does not occur in concert with the change in protein synthesis.

The fractionation of partially purified extracts of the 0 to 2, 3 to 7, and 8 to 22 mm sections on DEAE-cellulose columns resulted in two tyrosyl-tRNA synthetase regions from each section (Fig. 1). Similar results have been reported for whole pea root extracts (5). The relative proportion of the two synthetases were different in each section; approximately 10% of the total tyrosyl-tRNA synthetase activity in the 0 to 2 mm sections was associated with the first tyrosyl-tRNA synthetase region (T, synthetase) whereas in the 3 to 7 and 8 to 22 mm sections the T, synthetase activity represented approximately 22 and 44%, respectively, of the total tyrosyl-tRNA synthetase activity. These values are an average of closely replicating results from three experiments. The relative percentage of T, synthetase in vivo is probably higher than the data show since T, synthetase is considerably less stable than T, synthetase in vitro (5).

Arginyl-tRNA synthetase activity from each of the three sections fractionated on the DEAE-cellulose columns in a

![Fig. 2. Fractionation of arginyl-tRNA synthetase on DEAE-cellulose columns.](image-url)
single activity region (Fig. 2). The DEAE-cellulose column was an effective means of purifying arginyl-tRNA synthetase. The arginyl-tRNA synthetase activity in extracts from the 0 to 2 mm section, for example, increased from approximately 32,000 cpm per min incubation per mg protein (specific activity 200 µc/µmole) in the ammonium sulfate fraction to approximately 300,000 cpm per min incubation per mg protein in the peak fraction of the column.

Not only does tyrosyl-tRNA synthetase activity per mg protein and per cell change during pea root development, but the relative activities of the two tyrosyl-tRNA synthetases also change. The physiological advantage for two tyrosyl-tRNA synthetases in pea root cells is not apparent, especially since the two synthetases appear to be equally effective in acylating the two major isoaccepting tyrosyl-tRNA species (5). However, the acylation of tyrosine possibly is involved in the regulation of protein synthesis in developing plant roots. Evidence to support this view includes (a) the difference in tyrosyl-tRNA synthetase activity in each root section representing a different physiological state, (b) the difference in the relative ratios of the two tyrosyl-tRNA synthetases in each section, and (c) the study of Vanderhoeft and Key (12) which showed changes in the relative levels of the isoaccepting tyrosyl-tRNAs in developing pea roots.

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LITERATURE CITED