SPECIFICITY OF LEUCYL-tRNA AND SYNTHETASE IN PLANTS[a]

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SUMMARY

Transfer RNA and aminoacyl-tRNA synthetase were extracted from pea and soybean cotyledons. The amounts of leucylation by homologous and mixed tRNA and synthetase preparations were greatly different depending on the source of tRNA and enzyme. Generally the soybean homologous and soybean-pea mixed systems were superior to the pea homologous system. Charging of tRNA with radioactive leucine by homologous or heterologous enzyme and subsequent fractionation of the leucyl-tRNA on a Freon column reveal major differences in the leucyl-tRNA isoacceptor species. Pea cotyledons contain a much smaller proportion of leucyl-$tRNA_{1,4,5\&6}$ and virtually no leucyl-$tRNA_3$ in comparison to soybean cotyledons. Furthermore, synthetase preparation from pea cotyledons do not contain leucyl-$tRNA_{5\&6}$-synthetase activity. From these *in vitro* experiments it is concluded that the soybean homologous system charges six leucyl-tRNA species while the pea system only charges two (leucyl-$tRNA_{1\&2}$).

Several reports[1-6] in recent years have indicated the existence of a specificity in the synthesis of protein *in vitro*. This has been demonstrated by the incompatibility in the reaction between tRNA and aminoacyl-tRNA synthetase of different origin. Furthermore, fractionation of tRNAs charged with amino acids

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have shown major differences in a number of tRNA isoacceptor species.[7-12] Tissue-specific leucyl-tRNA and synthetase have been observed in animal[13-14] and plant systems.[15] While there is information on species specificity between tRNA and synthetase between bacteria, yeast and organs of animals and plants, little is known about the tRNA and synthetase specificity between homologous organs of different species. In this report we will show that the cotyledons of pea and soybean seedling vary greatly in their composition of leucyl-tRNAs and leucyl-tRNA synthetase activities.

MATERIALS AND METHODS

Plant material: Soybean seed (Glycine max L. var Hawkeye) were soaked in water overnight and then sown in moist Vermiculite. The seeds were allowed to germinate in darkness in a humid room with the temperature maintained at 27°. After seven days the cotyledons were removed, washed in distilled water and kept in the cold until use for tRNA extraction or synthetase preparation.

Pea seed (Pisum satioum L. var Alaska) were sown in moist sand and germinated in darkness at 27°. After eight days the cotyledons were removed, washed in distilled water and used for the extraction of tRNA and preparation of aminoacyl-tRNA synthetase.

Extraction of sRNA: Soluble RNA was isolated by the method of Anderson and Cherry[15], with the exception of an additional step. Following the solubilization of RNA in 1M NaCl, the insoluble pellet was re-extracted with 2M CH_3COOK. The 1M NaCl and 2M CH_3COOK extracts were combined and the sRNA precipitated in 2 volume of ethanol. Subsequent isolation procedures were the same as previously reported.[15]

Preparation of Synthetase: Preparations of aminoacyl synthetase was extracted from soybean and pea cotyledons following the method described for soybean tissue.[15]

Aminoacylation of tRNA and fractionation of RNA on the Freon Column: Leucine acceptor activity of tRNA and synthetase preparations were charged according to

a previously described method.[15] Details of the reaction mixture are given in each table or figure. Transfer RNA charged with radioactive leucine was fractionated on the Freon column (RPC-2) as described by Weiss and Kelmers.[16] The buffer employed by Anderson and Cherry[15] to elute RNA from the column was used in these experiments with the exception that EDTA was omitted.

RESULTS

The results presented in Table I indicate large differences in the amount of tRNA charged with leucine depending on the source of tRNA and synthetase. The soybean cotyledon homologous system is superior in the aminoacylation reaction. Other combinations of tRNA and synthetase gave activities much less than that for the soybean system. Furthermore, the pea homologous system gave the least activity of all combinations.

Table I

Species Differences in Leucyl-tRNA and Synthetase as Determined by the Aminoacylation Reaction

Source of sRNA	Source of Enzyme	p. moles leucine incorporated
soybean	soybean	53.2
soybean	pea	9.3
pea	soybean	17.2
pea	pea	5.9

Incorporation was measured over a period of 12 minutes. In each case, 60 μg of sRNA and 1 mg of synthetase protein was used in a 0.5 ml reaction. The concentration of ATP and other additives are the same as previously reported.[15]

Differences in the amount of tRNA charged with leucine by the various pea and soybean tRNA and synthetase preparations may be reflections of different amounts of leucyl-tRNA isoacceptor species and/or specific leucyl-tRNA synthetases. To test these possibilities, various combinations of tRNA and synthetases preparations from pea and soybean were acylated with either ^{3}H- or ^{14}C-leucine. Transfer RNA charged with ^{14}C-leucine from one reaction was mixed with tRNA charged with ^{3}H-leucine from another reaction and fractionated on the Freon column. Leucyl-tRNAs are fractionated into six major peaks (Figure 1); the first peak eluted from the column appears to contain degraded acylated tRNA and free radioactive leucine.

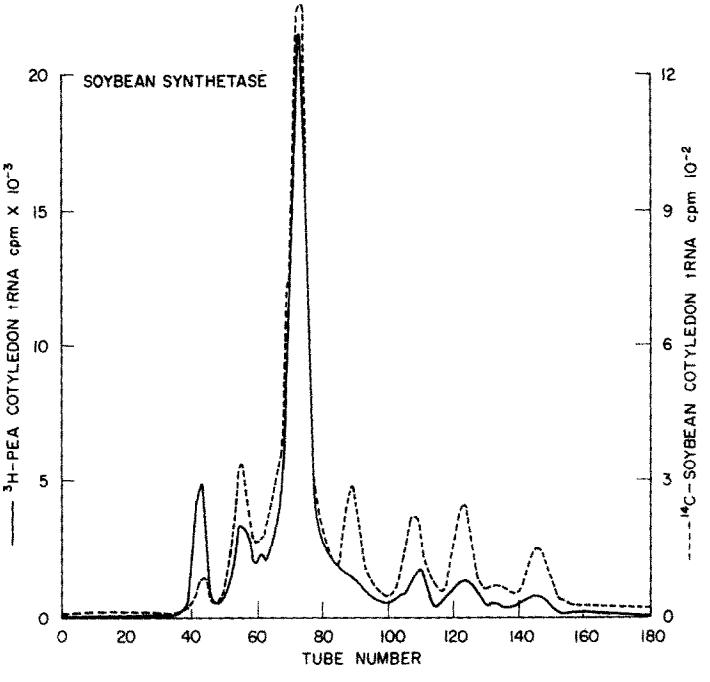

Figure 1. Comparison of the leucyl-tRNAs of pea and soybean cotyledons using a common soybean synthetase preparation. In each reaction mixture, 240 µg of pea or soybean sRNA was used. The reactions were carried out in the presence of 1 mg of soybean synthetase protein per ml for 30 min at 27°. The composition of the reaction mixture was the same as previously described.[15] At the termination of the reaction, the acylated tRNA was recovered using a DEAE cellulose column as previously described[15] and then loaded onto a Freon column. The tRNA was eluted with a 2-L linear gradient of NaCl from 0.425M to 0.725M. The flow rate of the column was 1.3 ml per min and approximately 11 ml per fraction were collected. After the fractions were collected, the tubes were chilled and 1.2 ml of 55% TCA was added to each. The radioactive leucyl-tRNA was collected on glass filter paper (GF/A) and determined in a Beckman scintillation counter.

The remaining peaks are numbered in order from 1 through 6 and are the same as those previously reported.[15] The data shown in Figure 1 indicate differences in leucyl-tRNA species of soybean and pea tRNA as soybean synthetase is used to charge both samples of tRNA. From these experiments using limiting quantities of RNA as substrate, it appears that pea cotyledon tRNA contains much less leucyl-$tRNA_{1,4,5\&6}$ and hardly any leucyl-$tRNA_3$ as compared to soybean tRNA.

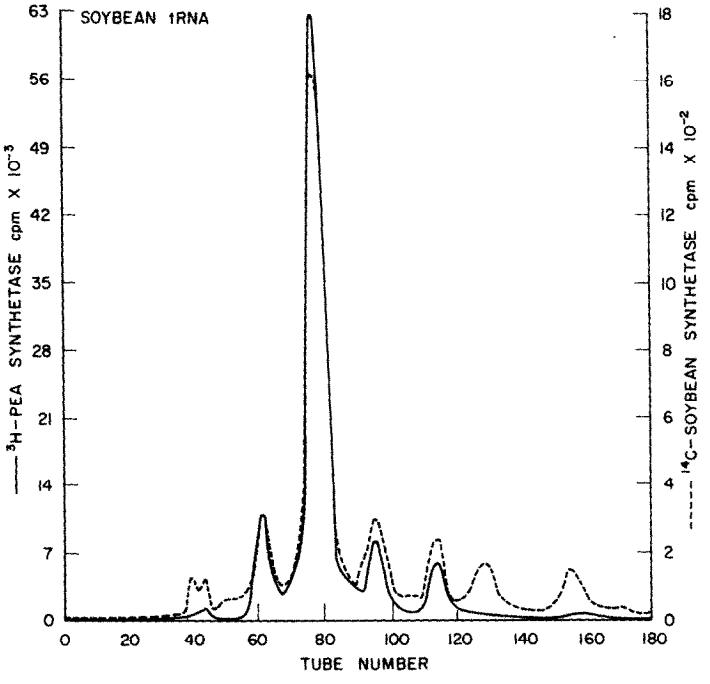

Figure 2. Comparison of the leucyl-tRNAs charged by pea and soybean synthetase preparations. In both cases 240 μg of soybean cotyledon sRNA was added to a 2 ml reaction mixture. The composition of the reaction mixture was the same as previously described.[15] Pea and soybean synthetase protein was added to give a final concentration of 1 mg per ml. The reaction was incubated for 30 min at 27°. Fractionation of tRNA on the Freon column was the same as described in Figure 1.

To test whether differences in the charging of leucyl-tRNAs exists between pea and soybean synthetases, soybean tRNA was charged with leucine by pea and soybean synthetase preparations and the two samples of tRNA (double labelled) were fractionated on the Freon column. The most striking difference between the leucyl-tRNA of these two systems is that pea synthetase does not charge soybean

leucyl-$tRNA_{5\&6}$ (Figure 2). Both synthetase preparations charged soybean leucyl-$tRNA_{1-4}$ equally well.

When pea tRNA is charged with leucine by pea synthetase only two major peaks (leucyl-$tRNA_{1\&2}$) are noted (Figure 3). Thus, it is quite clear that soybean and pea cotyledons differ markedly in their respective leucyl-tRNAs and leucyl synthetases.

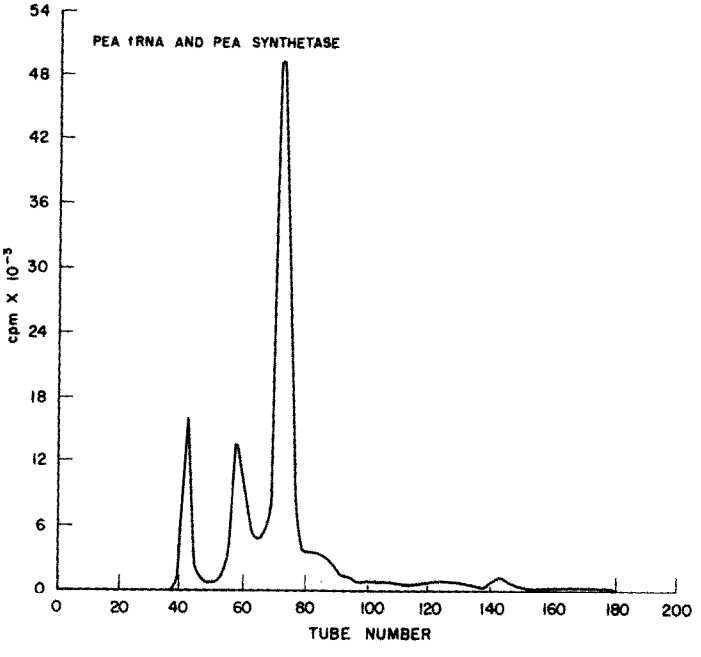

Figure 3. Fractionation of leucyl-tRNA of the pea homologous system on the Freon column. Pea cotyledon sRNA (240 µg) was acylated with ^{3}H-leucine by a pea cotyledon synthetase preparation (1 mg per ml) in a total volume of 2 ml. The details of the reaction mixture and the Freon column fractionation procedure are presented in Figure 1.

DISCUSSION

Similar organs, whose function in seed germination appear to be identical, of two related plant species have different quantities of leucyl-tRNAs and leucyl-tRNA synthetase activities. Soybean cotyledons contain tRNA and synthetase to acylate six major leucyl-tRNA species. On the other hand, pea cotyledons appear to have much less leucyl-$tRNA_{1,4,5\&6}$ and virtually no leucyl-$tRNA_{3}$ as compared to soybean cotyledons. Synthetase preparations from pea cotyledons do not contain

leucyl-tRNA synthetase activity to charge leucyl-$tRNA_{5\&6}$. Preliminary results from one of these laboratories[17] indicate that the leucyl-tRNA synthetase activity can be separated into three protein fractions. One of these fractions acylates only leucyl-$tRNA_{5\&6}$.

Even though the soybean system appears to contain all the leucyl-tRNA and synthetases, it cannot be assumed that the same is true for other amino acids. Other data[c] from these laboratories show that the soybean synthetase preparation apparently contains a nuclease which degrades tyrosyl-tRNA but not leucyl-tRNA. Therefore, the main message to be reached from our results is that extreme caution must be exercised when dealing with any amino acid incorporation system. In the past, tRNA and synthetase preparations have been used indiscriminately from a number of sources to support mixed protein synthesis mainly because of convenience. However, it is clear from our data on pea and soybean cotyledon tRNA and synthetase that the presence of specific tRNAs, activities of specific synthetases and compatibilities of mixed systems should be tested before routine use.

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[c] Unpublished results of Joe H. Cherry and Daphne J. Osborne.