Phytochemistry, 1976, Vol. 15, pp. 1809–1812. Pergamon Press. Printed in England.

CHANGES IN LEUCYL-*t*RNAs AND AMINOACYL *t*RNA SYNTHETASES IN DEVELOPING AND AGING SOYBEAN COTYLEDONS*

VIJAYALAKSHMI SHRIDHAR and DATTA T. N. PILLAY
Department of Biology, University of Windsor, Windsor, Ontario, Canada

(*Revised received* 6 *July* 1976)

Key Word Index—*Glycine max*; soybean; Leguminosae; leucyl transfer RNAs; leucyl transfer RNA synthetase; hydroxylapatite column; reversed phase chromatography.

Abstract—Leucine specific *t*RNA of soybean cotyledons was fractionated into six peaks (1–6). The relative amounts of Leu-*t*RNA 5 and 6 are lower in developing cotyledons than in germinating cotyledons. Leu-*t*RNA synthetase from developing cotyledons is less active in aminoacylating Leu-*t*RNA 5 and 6 compared to enzyme from 5-day-old germinating cotyledons. Leu-*t*RNA synthetase from cotyledons of germinating seedlings and developing cotyledons can be fractionated into three peaks (1–3). Peak 1 in the developing cotyledon is about 36% less than peak 1 from 5-day-old germinating cotyledons. Peaks 2 and 3 from developing cotyledons are about 10 and 18% higher than from germinating cotyledons, respectively. Peak 1 from developing cotyledons acylates all six species of Leu-*t*RNA in contrast with peak 1 from germinating cotyledons, which essentially acylates only Leu-*t*RNA 5 and 6. The specificity of peaks 2 and 3 towards Leu-*t*RNA 1–4 is identical in both the organs.

INTRODUCTION

Alterations of specific *t*RNAs and appearance or disappearance of *t*RNA isoacceptors have been implicated as important steps in differentiation and regulation [21]. Differences in the elution profiles of *t*RNAs and aminoacyl-*t*RNA synthetases have been observed on phage infection of bacteria [14], in plant development [1, 10–13, 18], after administration of hormones [25] and during embryonic differentiation of various animals [9, 26]. Previous studies with soybean cotyledons revealed that the complement of Leu-*t*RNA isoaccepting species changes during cotyledon senescence [2, 20].

Protein synthesis in plants [3, 27] involves the same reactions and general mechanisms as described for bacterial and animal systems [24]. Usually in plants a quiescent or dormant state is associated with a low level of polyribosomes and certain enzymes in a repressed state [17, 22]. Increased metabolic activity, such as occurs on seed germination, is associated with increase in polysome content and activity of certain enzymes involved in storage compound hydrolysis [8]. Activation of the protein synthesizing machinery with germination may be attributed to one or more of the following components: increased availability of *m*RNA, changes in the amount of RNA species, aminoacyl-*t*RNA species, aminoacyl-*t*RNA synthetase and/or various factors (initiation, termination, etc.).

This report describes the changes in the relative proportions, of Leu-*t*RNA and synthetases, in developing and germinating soybean cotyledons.

*This work was supported by Grant A-1984 from the National Research Council of Canada.

RESULTS AND DISCUSSION

Changes in Leu-*t*RNAs and Leu-*t*RNA synthetases in aging soybean cotyledons following germination (5-, 15- and 20-days-old) and in developing cotyledons are compared, using synthetase preparations fractionated on hydroxylapatite (HA) columns (see Experimental).

Transfer RNAs in aging cotyledons

Leu-*t*RNAs from cotyledons were fractionated into six discrete peaks on RPC-5 columns. An analysis of elution profiles of Leu-*t*RNA species in 5- and 20-day-old cotyledons acylated with leucine-[^{3}H], using 5-day-old cotyledon enzyme (crude preparation from DEAE column), confirms our previous observation [2, 20] that with aging, there is an increase in Leu-*t*RNA 5 and 6 (*ca* 12%) and a decrease in Leu-*t*RNA 2 (*ca* 7%) in 20-day-old cotyledons. However, when this 5-day-old cotyledon enzyme is used to acylate *t*RNA preparations from immature and mature developing cotyledons, we observe an 18% increase in acylation of Leu-*t*RNA 2 and a 12% decrease in Leu-*t*RNA 5 and 6. The inadequate or poor charging of Leu-*t*RNA 5 and 6 and the maximum charging of Leu-tRNA 2 indicates differences that could be based on one of the two rate limiting factors, i.e. synthetases or *t*RNA. No significant change in the acylation of Leu-*t*RNA 1, 3 and 4 was observed.

Transfer RNAs in developing cotyledons

In another system, synthetase preparations from mature developing cotyledons in the pods (pod enzyme) were used to acylate *t*RNAs from 5- and 20-day germinating cotyledons as well as *t*RNAs from immature and mature developing cotyledons. Pod enzyme is cap-

Table 1. Aminoacyl-*t*RNA synthetase activity of germinating and developing cotyledon

Source of enzyme	Source of *t*RNA	Relative amount of Leu-*t*RNA acylation of each peak (% of total) 1	2	3	4	5	6
Germinating cotyledon (5 days)	Cotyledon (5 days)	12.2	24.7	7.4	8.5	26.8	20.4
	Cotyledon (20 days)	7.1	16.7	3.8	5.4	39.2	27.8
	Seed pods (immature)	16.0	44.0	9.0	6.9	16.1	8.0
	Seed pods (mature)	11.6	51.6	8.4	7.4	14.2	6.8
Developing cotyledon (mature seed pods)	Cotyledon (5 days)	8.6	50.8	12.4	12.3	8.7	7.2
	Cotyledon (20 days)	10.5	56.2	13.8	11.0	5.5	3.0
	Seed pods (immature)	14.5	58.7	9.2	7.4	5.6	4.6
	Seed pods (mature)	9.3	60.6	11.2	10.0	5.9	3.0

*t*RNA was acylated in a 2 ml reaction mixture with leucine-[^{3}H] and fractionated on a Plaskon column as described in Experimental. The amount of radioactivity in each peak was summed and expressed as % of the sum total in the 6 peaks.

able of acylating all six species of Leu-*t*RNA obtained either from the germinating or developing cotyledons. In 5-day cotyledons Leu-*t*RNA 2 and Leu-*t*RNA 3 and 4 increase by 26 and 5%, respectively. In 20-day-old cotyledon Leu-*t*RNA 2, 3 and 4 show greater increases by 39, 10 and 15%, respectively. Regardless of the source of *t*RNA, acylation of Leu-*t*RNA 5 and 6 with pod enzyme decreases by 33 and 24%, respectively (Table 1). It should be noted that acylation of 20-day cotyledon *t*RNA with 5-day cotyledon enzyme shows an increase in Leu-*t*RNA 5 and 6. This clearly shows that the enzyme and not transfer RNAs is the limiting factor in developing cotyledons. Further acylation of 5- and 20-day cotyledon *t*RNA with 5-day cotyledon enzyme shows a much higher activity in Leu-*t*RNA 5 and 6 compared to the acylation by the pod enzyme. On the other hand, pod enzyme is more specific in giving higher activities for Leu-*t*RNA 2, 3 and 4 compared to cotyledon enzyme.

In the homologous system, acylation of pod *t*RNAs (immature and mature) with a pod enzyme results in about 16–20% less activity in Leu-*t*RNA 5 and 6, than one would expect with a homologous system of cotyledon *t*RNAs (5- and 20-day) acylated with a 5-day cotyledon enzyme. Activity of Leu-*t*RNA 2 increases up to 37% in the developing cotyledon system compared to the germinating cotyledons. Further, acylation of a cotyledon *t*RNA with a pod enzyme (heterologous system) also brings about this substantial increase in Leu-*t*RNA 2. Similarly, the pod enzyme acylation of either cotyledon *t*RNA or pod *t*RNA shows increases in Leu-*t*RNA 3 and 4 and decreases in Leu-*t*RNA 5 and 6. It is interesting to note here that pod enzyme is capable of higher acylation activities in Leu-*t*RNA 2, 3 and 4 but not in Leu-*t*RNA 5 and 6. Data summarized in Table 1 clearly shows the differential activities of these two synthetase preparations and *t*RNAs.

Fractionation of the leucyl-tRNA synthetase from germinating and developing soybean cotyledons

It was of interest to determine, if the variations in charging capacities of these two synthetase preparations could be due to specificities of the enzyme during different stages of development or aging of the cotyledons. To check this, synthetase preparations from 5-, 15- and 20-day germinating (aging) cotyledons and developing cotyledons were routinely prepared and their multiple forms separated on an HA column. Aliquots taken from every second fraction were assayed for leucine acceptor activity in 0.2 ml reaction mixture (see Experimental). The activity profiles corresponding to each of the three enzyme peaks are present in both 5-day germinating and developing cotyledon enzyme. (Data not presented here.) Although the relative amounts of the three peaks are somewhat different in the two systems. The three peaks from the HA column are designated as peaks 1, 2 and 3 in order of elution. Isolation of comparable forms of synthetases from developing and germinating cotyledons indicates no qualitative deficiencies in these tissues.

On a quantitative basis, peak 1 from the developing cotyledon is about 30% less in activity than cotyledon peak 1. The amounts of peaks 2 and 3 in the developing cotyledons are much higher in quantity (19% and 17% more than the cotyledon peak 2 and 3). Because of the variations in Leu-*t*RNA 5 and 6 reported earlier, it was necessary to show if there existed any differences in the two enzyme systems. Leu-*t*RNA synthetase from three stages of germinating cotyledons (5-, 15- and 20-day old) were extracted, purified and separated on HA columns, and charged with *t*RNAs prepared from germinating and developing cotyledons. Regardless of the age of germinating cotyledons or developing cotyledons, Leu-*t*RNA synthetases from these two systems have all three enzyme peaks (Table 2). However, there is a quantitative change from one stage to the next. In aging cotyledons there is an increase in cotyledon peak 1, while peaks 2 and 3 do not change greatly. Thus, the observed differences in the relative amounts of three enzyme species in developing cotyledons and different stages of germinating cotyledons could explain the variations in the relative acylation patterns of their isoaccepting Leu-*t*RNA 2, 5 and 6.

Transfer RNA specificity of individual enzyme fractions

A previous report [16] has shown that cotyledon peak 1 was specific in acylating Leu-*t*RNA 5 and 6 and enzymes 2 and 3 were equally effective in acylating Leu-*t*RNA 1 to 4. To determine whether or not Leu-*t*RNA synthetases 1, 2 and 3 in the developing and germinating cotyledon differ in their specificities, *t*RNA samples from 5-day-old cotyledons and mature developing cotyledons were charged and cross charged and fractionated on

Table 2. Aminoacyl-tRNA synthetase activity of germinating cotyledons and developing cotyledons (seed pods)—multiple forms of enzyme separated on hydroxylapatite (HA) column

Source of enzyme	Source of tRNA	Relative amount of Leu-tRNA acylation of each peak (% of total) 1	2	3
Developing cotyledons (mature seed pods)	Cotyledon (5 days)	17.6	45.8	36.6
	Seed pods	15.3	41.6	43.1
Germinating cotyledons (5 days)	Cotyledon (5 days)	41.8	32.5	25.7
	Seeds pods	39.3	33.0	27.7
Cotyledon (15 days)	Cotyledon (15 days)	48.1	28.2	23.7
	Seed pods	47.4	26.6	26.0
Cotyledon (20 days)	Cotyledon (20 days)	48.7	26.4	24.9

About 50 mg protein was loaded onto a HA column (2.5 × 10 cm) in 50 ml of 50 mM KPi (pH 6.5) and eluted with a linear gradient of KPi from 0.05–0.4 M. Fractions were assayed for Leu-tRNA synthetase activity as described in Experimental.

RPC-5 columns. Results obtained confirm the above observation [16] for the cotyledon system, and further show that the same specificity does not reside in developing cotyledon peak 1. (Data not presented here.) It is clear that this peak 1 acylates all six leucyl tRNA regardless of the source of tRNA. This is contrary to expected results that cotyledon peak 1 exclusively acylates Leu-tRNA 5 and 6 with traces of activity in regions of Leu-tRNA 1 to 4 possibly due to contamination from cotyledon peak 2. The range of specificity for developing cotyledon peaks 2 and 3 in acylating Leu-tRNA 1 to 4 is identical to cotyledon peaks 2 and 3. No attempt was made to study the kinetics of acylation reactions, as these have already been reported earlier [1].

Our results show differences in the relative amount of isoaccepting Leu-tRNAs and multiple forms of synthetases between developing and germinating soybean cotyledons. Further we also observe a gradual increase in the capacity of cotyledon peak 1 in acylating Leu-tRNA 5 and 6 during cotyledon senescence. In contrast, peak 1 in developing cotyledons is limiting; yet capable of acylating all six Leu-tRNA species. It is tempting to speculate that the differences in the enzyme patterns between the green developing cotyledon and the germinating etiolated cotyledons [9] possibly involve chloroplasts or proplastids. Such differences in enzyme activity have been observed in cotton [10] and beans [12]. Since the cotyledons serve as first leaves, until true leaves emerge, an increase in enzyme 1 during germination could indicate an increase in the chloroplasts.

EXPERIMENTAL

Soybean seeds (*Glycine max* var-Harosoy 63) were surface sterilized in 10% chlorox, soaked in H_2O for 18 hr and sown in moist vermiculite. Cotyledons were harvested after 5, 15 and 20 days of germination in the dark at 27–29°. Samples of two different stages of developing cotyledons (immature and mature) were collected after 7 weeks from the day of planting and stored after freezing in dry ice.

Transfer RNA was prepared from total RNA of 5-, 15- or 20-day-old (freshly harvested) germinating cotyledons or developing cotyledons stored in a freezer for several days; tRNA was extracted by the phenol technique of ref. [4] with minor modifications [20].

Leucyl-tRNA synthetase. Freshly harvested germinating cotyledons, or frozen developing cotyledons were used. Extraction, purification, and fractionation of the enzyme was performed at 0–4° as described in ref. [18].

Hydroxylapatite (HA) column chromatography. Enzyme fractions eluted from DEAE columns were diluted 1 : 1 with H_2O and the pH was adjusted to 6.5 with 50 mM KH_2PO_4. The soln was applied to a HA column and fractionated as described in ref. [18].

Transfer RNA aminoacylation assay. The aminoacylation reaction was carried out at 5 min intervals starting from 0 to 20 min, when the reaction reached a plateau. Therefore, routinely tRNA was aminoacylated for 20 min and then used for RPC column chromatography. The reaction was carried out at 30°. Unless otherwise stated, 1 ml of the reaction mixture contained: 10 μmol Tris–HCl, pH 7.8; 5 μmol $MgCl_2$; 0.5 μmol ATP: 0.2% soluble PVP–0.2 mg/ml of tRNA: 0.2 mg/ml of enzyme and 20 μl leucine-[^{3}H], unneutralized soln (60 Ci/mmol).

Reversed-phase column chromatography (RPC-5). A mixture of 8 ml of Adogen 464 in 400 ml of $CHCl_3$ was coated on to 200 g of polychlorotrifluoroethylene (Plaskon) support, as described in ref. [19]. The coated Plaskon was suspended in 0.5 M NaCl in buffer A (NaOAc buffer pH 4.5) for packing of the columns. For RPC-5 column fractionation, tRNA was aminoacylated as described above in a 2 ml reaction mixture containing larger quantities of tRNA and protein. Charged Leu-tRNA was recovered using a small DEAE-cellulose column as described in ref [20] and applied to a RPC-5 column (2.5 × 30 cm) in 0.5 M NaCl in buffer A. Elution was at room temp with a linear gradient of NaCl from 0.5–0.9 M in buffer A. Fractions (10 ml) were collected at 1 ml/min, made 5% with respect to TCA, filtered through glass fibre filters, and counted.

REFERENCES

1. Anderson, M. B., and Cherry, J. H. (1969) *Proc. Natl Acad. Sci.* **62**, 202–209.

2. Bick, M. D., Liebke, H., Cherry, J. H. and Strehler, B. L. (1970) *Biochim. Biophys. Acta* **204,** 175–182.
3. Boulter, D., Ellis, R. J. and Yarwood, A. (1972) *Biol. Rev.* **47,** 113–175.
4. Brantner, J. H. and Dure III, L. S. (1975) *Biochim. Biophys. Acta* **414,** 99–114.
5. Breidenback, R. W. and Beevers, H. (1967) *Biochim. Biophys. Res.* **27,** 462.
6. Cherry, J. H., Chroboczek, H., Carpenter, W. J. G. and Richmond, A. (1965) *Plant Physiol.* **40,** 582.
7. Cherry, J. H. (1967) in (Woodhouse, H. ed.) *S.E.B. Symposium* XXI. pp. 231–247. Cambridge University Press, London.
8. Chrispeels, M. J. and Boulter, D. (1975) *Plant Physiol.* **55,** 1031.
9. DeWitt, W. (1971) *Biochim. Biophys. Res. Commun.* **42,** 266.
10. Dure III, L. S. and Merrick, W. C. (1971) *Autonomy and Biogenesis of Mitochondria and Chloroplasts*, pp. 413–422. North Holland, London.
11. Guillemaut, P., Steinmetz, A., Burkard, G. and Weil, J. H. (1975) *Biochim. Biophys. Acta* **378,** 64.
12. Guillemaut, P. and Weil, J. H. (1975) *Biochim. Biophys. Acta* **407,** 240.
13. Ihle, J. N. and Dure III, L. S. (1972) *J. Biol. Chem.* **247,** 5048.
14. Kan, J., Kano-Sueoka, T. and Sueoka, N. (1968) *J. Biol. Chem.* **243,** 5589.
15. Kanabus, J. (1971) Ph.D. Thesis. Purdue University, Indiana.
16. Kanabus, J. and Cherry, J. H. (1971) *Proc. Natl Acad. Sci. U.S.* **68,** 873.
17. Marre, E., Cocucci, S. and Sturani, S. (1965) *Plant Physiol.* **40,** 1162.
18. Patel, H. V. and Pillay, D. T. N. (1976) *Phytochemistry* **15,** 401.
19. Pearson, R. L., Weiss, J. F. and Kelmers, A. D. (1971) *Biochim. Biophys. Acta* **228,** 770.
20. Pillay, D. T. N. and Cherry, J. H. (1974) *Can. J. Botany* **52,** 2499.
21. Sueoka, N. and Kano-Sueoka, T. (1970) *Progress* in *Nucleic Acid Research and Molecular Biol.* vol. 10, pp. 23–55. Academic Press, New York.
22. Varner, J. E. and Ramchandra (1964) *Proc. Natl. Acad. Sci. U.S.* **52,** 100.
23. Vigil, E. L. (1970) *J. Cell Biol.* **46,** 435.
24. Wilkinson, B. R. and Kirby, K. S. (1966) *Biochem. J.* **19,** 786.
25. Wright, R. D., Pillay, D. T. N. and Cherry, J. H. (1972/73) *Mech. Aging Dev.* **1,** 403.
26. Yang, S. S. and Comb, D. G. (1968) *J. Mol. Biol.* **31,** 139.
27. Zalik, S. and Jones, B. L. (1973) *Ann. Rev. Plant Physiol.* **24,** 47.