Phytochemistry, 1975, Vol. 14, pp. 85–87. Pergamon Press. Printed in England.

t-RNA-NUCLEOTIDYLTRANSFERASE ACTIVITY IN *LUPINUS LUTEUS* SEEDS

HENRYK CUDNY, MACIEJ PIETRZAK and SLAWOMIR BARTKOWIAK
Institute of Plant Genetics, Polish Academy of Sciences, Poznań, Strzeszyńska 2/4, Poland

(*Received* 21 *April* 1974)

Key Word Index—*Lupinus luteus*; Leguminosae; yellow lupin; seed; *t*-RNA-nucleotidyltransferase.

Abstract—*t*-RNA-nucleotidyltransferase activity was detected in *Lupinus luteus* seed. The enzyme was partly purified, and some of its properties are described.

INTRODUCTION

An intact CCA sequence at the 3′-hydroxyl terminus of *t*-RNA is an absolute requirement for biological activity. For a few years it has been known that the enzyme *t*-RNA-nucleotidyltransferase catalyzes the addition of AMP and CMP to *t*-RNA, which lack this intact CCA terminus. This enzyme has been found in *E. coli* [1,2], yeast [3], and animal tissues [4,5]. We have now found this enzyme in yellow lupin seed and we here describe its purification and properties.

RESULTS

Enzyme preparation

The crude protein of *Lupinus luteus* seed was found to catalyze the incorporation of some radioactive AMP and CMP into cold 5% trichloroacetic acid insoluble material. It was further purified by ammonium sulfate precipitation and chromatography on a Bio. Gel column. Activity for [^{14}C]AMP and [^{14}C]CMP incorporation was found to be in a single peak of the Bio. Gel column eluate. Addition of *t*-RNA to the reaction mixture stimulated radioactivity incorporation, but the enzyme was not fully *t*-RNA dependent (Table 1).

The fractions from Bio. Gel chromatography were pooled and further purified on DEAE-cellulose, when the enzyme was apparently *t*-RNA dependent (Table 1). The enzyme had an optimum activity at pH 8·5 and required magnesium for activity (optimum activity at 5 mM $MgCl_2$). Specific activity after DEAE chromatography was 6 nmol of AMP and 3 nmol of CMP incorporated in 10 min mg of protein (Table 1). GMP was not incorporated and incorporation of UMP was 10 fold lower than CMP (unpublished data).

CTP had an inhibitory effect on [^{14}C]AMP incorporation. During purification the ratio of [^{14}C]CMP to [^{14}C]AMP incorporation was changed, which suggests the existence of other activities than *t*-RNA-nucleotidyl transferase in the crude extracts [6]. The enzyme after Bio. Gel chromatography was free of ribonuclease activity [7].

Table 1. *t*-RNA Dependence of AMP and CMP incorporating activities during enzyme purification

Fraction	Incubation mixture	Specific activity* CMP	AMP
I. Homogenate	complete	5800	14770
	−*t*-RNA	4200	12700
II. 30–50% $(NH_4)_2SO_4$	complete	16100	41000
	−*t*-RNA	5100	21000
III. Bio. Gel	complete	16500	41800
	−*t*-RNA	1300	2500
IV. DEAE-cellulose	complete	33000	68000
	−*t*-RNA	1200	2400

Incubation mixture was as in experimental. Reaction was carried out at 30° for 10 min [^{14}C]-ATP and [^{14}C]-CTP were used.
* cpm Incorporated/mg protein/10 min.

Table 2. Analysis of [^{14}C]-CMP and [^{14}C]-AMP incorporation products into *t*-RNA

Radioactive nucleoside triphosphate in incubation mixture	Nucleotide incorporated total incorporation pmol/nmol *t*-RNA	Radioactivity recovered after hydrolysis	
		% Nucleoside	% Nucleotide
[^{14}C]-CTP	290	67	33
[^{14}C]-CTP add 2mM ATP	288	51	49
[^{14}C]-ATP	373	84	16

The enzyme incorporated AMP and CMP into the *t*-RNA from the yeast as well (unpublished date).

Analysis of [^{14}C]*CMP and* [^{14}C]*AMP incorporation products into* t-*RNA*

In order to prove that radioactive nucleotides are incorporated in the terminal positions in *t*-RNA, the *t*-RNA was isolated and hydrolyzed in 0·1 N NaOH after incubation. Products of hydrolysis were separated on AG1-X4 according to Deutscher [6]. In the case of [^{14}C]CTP, the ratio of nucleotide (CMP) to nucleoside (cytidine) was 1 : 2. This means that about 67% of [^{14}C]CMP incorporated into *t*-RNA was in the terminal position. When incubation was in the presence of nonradioactive ATP, only 50% of the cytidine was recovered in the *t*-RNA. This suggests that the terminal incorporation of AMP occurs fact that under the conditions used formation of the CCA terminus is not complete.

In the case of [^{14}C]ATP, about 84% of [^{14}C]-AMP incorporation was in the terminal position of *t*-RNA; the presence of ^{14}C in other positions may be due to misincorporation. Abnormal incorporation has also been reported in the case of *E. coli* [8,9], in the rat liver [6].

Increase in amino acid acceptance of t-*RNA after AMP and CMP incorporation*

The enzyme after the DEAE step was used for AMP and CMP incorporation into *t*-RNA, which was further assayed for amino acid acceptance. When *t*-RNA was incubated with ATP alone and then assayed for leucine incorporation, there was about a 14% increase in its ability to accept leucine. The presence of CTP in addition to ATP in the incubation mixture resulted in a 26% increase in this. These results suggest that there is an intact CCA sequence at the *t*-RNA terminus, since the aminoacylation reaction may only take place with a *t*-RNA which has an intact CCA terminus. In our *t*-RNA from *Lupinus luteus*, adenosine was absent in about 30% of the molecules as assayed by the method of Vanderhoef [10] (Table 2).

DISCUSSION

In general, the properties of the enzyme isolated from lupin are similar to those obtained previously from *E. coli* [2], yeast [3], rat liver [4], and mouse brain [11]. There have been several reports of the existence of enzymes which are involved in the ribonucleic acid metabolism in dry seeds. Mazus

Table 3. Increase in amino acid acceptance of *t*-RNA for leucine after incorporation of AMP and CMP

Nonradioactive nucleoside-triphosphate present in incubation mixture	Amino acid acceptance (nmol leucine/nmol *t*-RNA	Relative activity (%)
1. None	0·048	100
2. 2·0 mM ATP	0·055	115
3. 2·0 mM ATP + 0·4 mM CTP	0·057	118
4. 2·0 mM ATP + 0·8 mM CTP	0·061	126

Incubation mixture for nucleotide incorporation was as described in Experimental with exception of radioactive triphosphates. After incorporation, mixture was heated at 60° for 5 min in order to destroy nucleotidyl-transferase activity and then assayed for [^{14}C]-leucine acceptance. Aminoacylation was as described in Experimental.

[12] have found DNA-dependent RNA polymerase in dry wheat, Wilson [13] has found ribonuclease in maize and Legocki *et al.* [14] has reported aminoacyl–t-RNA synthetases in *Lupinus luteus*. In 1965, Marcus *et al.* [15] found that in dry wheat seeds machinery for protein synthesis was ready to start immediately after imbibition. Since protein synthesis requires continued acylation of the terminal adenosine of t-RNA, there is also a requirement for a pool of t-RNA with an intact CCA terminus. By contrast, Dobrzańska *et al.* [16] report that newly synthesized t-RNA appears at a later period after protein synthesis has started. The enzyme which we have isolated seems to be responsible for the turnover of t-RNA molecules with uncompleted CCA terminus and provides molecules which are active in protein synthesis, until the new synthesis of t-RNA begins. We found that about 30% of t-RNA in dry *Lupinus* seeds are without the terminal adenosine. Therefore our data support the theory that dry seeds contain all the enzymes needed for protein synthesis to occur immediately after imbibition.

EXPERIMENTAL

Enzyme preparation. Lupinus luteus grains were ground and extracted with buffer A (0·05 M KH_2PO_4.KOH, pH 6·8; 10 mM $MgCl_2$; 10 mM 2-mercaptoethanol) 100 g/300 ml. After 30 min of extraction with stirring, the slurry was centrifuged at 25000 rpm and $(NH_4)_2SO_4$ was added to the supernatant to 20% saturation. The suspension was stirred for 2 hr and centrifuged at 15000 rpm for 30 min. The pellet was discarded and the supernatant brought to 60% saturation with $(NH_4)_2SO_4$. After a few hours of stirring, the suspension was centrifuged and the 20–60% $(NH_4)_2SO_4$ precipitate was redissolved in a small vol of buffer B (50 mM Tris–HCl, pH 8·0; 1 mM $MgCl_2$; 1 mM dithioerithritol; 10% glycerol). The protein solution in buffer B was placed in a Bio. Gel (A-1·5 m) column, previously washed with the same buffer. The protein was eluted with buffer B at the rate of 20 ml/hr. and the activity for t-RNA nucleotidyltransferase was assayed as described below. The fractions with t-RNA nucleotidyltransferase activity were pooled and brought to 60% $(NH_4)_2SO_4$ saturation. The suspension was centrifuged at 15000 rpm and the pellet was dissolved in buffer C (50 mM Tris–HCl, pH 7·9; 0·1 mM EDTA; 0·1 mM dithoerytritol; 10% glycerol) and dialyzed overnight against the same buffer. The protein was adsorbed on a DEAE column (2·5 × 1·5 cm) equilibrated with buffer C. The column was washed with buffer C until A_{280} was negligible and then an $(NH_4)_2SO_4$ gradient 0·01–0·3 M (800 ml total vol.) was used at the rate 20 ml/hr. Fractions with t-RNA nucleotidyltransferase activity were pooled and used for further study. All operations were conducted at 4°.

t-RNA Nucleotidyltransferase assay. The standard reaction mixture contained 40 mM tricin KOH, pH 8·5; 15 mM $MgCl_2$; 0·2 mM [^{14}C]-ATP (5 mCi/mmol) or [^{14}C]-CTP (5 mCi/mmol): 50 μg/ml of enzyme and 100 μg/ml t-RNA. The reaction was carried out at 30° for 10 min, 50 μl aliquots were placed on Whatman 3MM paper discs and washed with 5% trichloroacetic acid (× 3), with acetone (× 2) and with Et_2O (× 1). The discs were dried, placed in a 10 ml toluene scintilator and the radioactivity was counted in a Packard liquid scintillation spectrometer (model 3375). The assay measured the incorporation of radioactive [^{14}C]-ATP or [^{14}C]-CTP into an insoluble product. Repairing of t-RNA which was further used for aminoacylation reaction, was carried out under the same conditions, but with unlabeled nucleotide triphosphate.

Preparation of aminoacyl-t-RNA synthetases and aminoacid acceptance assay. The aminoacyl-t-RNA synthetases pool was from the same origin as t-RNA nucleotidyltransferase, and was purified in the same manner with the exception of the Bio. Gel A-1·5 m step. Amino acid acceptance assay was according to Jakubowski *et al.* [17].

Preparation of t-RNA. t-RNA was isolated from *Lupinus luteus* grains according to Vanderhoef *et al.* [10].

Other procedures. The analysis of the radioactive product of nucleotide incorporation into t-RNA was carried out according Deutscher [6]. Protein determination was carried out according to Lowry *et al.* [18] and ribonuclease activity according to the procedure of Tuve *et al.* [7].

Acknowledgement—This work was supported by The Polish Academy of Sciences within the project 09.3.1.

REFERENCES

1. Miller, J. P. and Philipps, G. R. (1970) *Biochem. Biophys. Res. Commun.* **38,** 1174.
2. Miller, J. P. and Philipps, G. R. (1971) *J. Biol. Chem.* **246,** 1274.
3. Morris, R. W. and Herbert, E. (1970) *Biochemistry* **9,** 4819.
4. Deutscher, M. P. (1972) *J. Biol. Chem.* **247,** 459.
5. Anthony, D. D., Starr, J. L., Kerr, D. S. and Goldthwait, D. A. (1963) *J. Biol. Chem.* **238,** 690.
6. Deutscher, M. P. (1972) *J. Biol. Chem.* **247,** 469.
7. Tuve, T. W. and Anfinsen, C. B. (1960) *J. Biol. Chem.* **235,** 3437.
8. Gross, H. J., Wuernick, F. R. and Fiers, W. C. (1970) *European J. Biochem.* **17,** 116.
9. Best, A. N. and Novelli, G. W. (1971) *Arch. Biochem. Biophys.* **142,** 539.
10. Vanderhoef, L. N., Bohannon, R. F. and Key, J. (1970) *Phytochemistry* **9,** 2291.
11. Chou, L. and Johnson, T. C. (1972) *Brain Res.* **45,** 217.
12. Mazus, B. and Buchowicz, J. (1972) *Phytochemistry* **11,** 2443.
13. Wilson, C. M., (1968) *Plant Physiol.* **43,** 1332.
14. Legocki, A. B. and Pawelkiewicz, J. (1967) *Acta Biochim. Polon.* **14,** 313.
15. Marcus, A. and Feeley, J. (1965) *J. Biol. Chem.* **240,** 1675.
16. Dodrzanska, M. Tomaszewski, M. Crzelczak, Z. Rejman, E. and Buchowicz, J. (1973) *Nature* **244,** 507.
17. Jakubowski, H. and Pawelkiewicz, J. (1973) *FEBS Letters* **34,** 150.
18. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265.