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EFFECT OF SEED GERMINATION ON LEVELS OF *t*RNA AMINOACYLATION

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Abstract—In ungerminated seeds of *Lupinus luteus t*RNAs are aminoacylated 10% or less depending on species of *t*RNA. The levels of *t*RNA aminoacylation for specific *t*RNAs increase steadily during seed germination. Specific *t*RNAs in cotyledons and axes of 3-day-old seedlings are aminoacylated to a similar extent. No significant changes are observed in the *t*RNA population during germination.

INTRODUCTION

In seeds, protein synthesis probably takes place soon after the onset of germination. [1–4]. Some aspects of the synthesis such as the incorporation of radioactive amino acids into proteins [2,5] and the changes of isoaccepting *t*RNAs [6–9] during seed germination have been studied extensively. It was also found that the levels of cytosol *t*RNAs and aminoacyl-*t*RNA synthetases per cell are constant in germinated and ungerminated cotton cotyledons. By contrast the same constituents in chloroplasts were found to increase markedly in germinating cotyledons [10,11]. Although the above mentioned studies have been focussed on protein synthesis in germinating seeds, no information is available on the level of *t*RNA aminoacylation *in vivo*, neither in seeds, nor at other developmental stages of higher plants. In the work presented here we have examined the level of aminoacylation of lupin *t*RNA from dry and germinated seeds as well as from young seedlings.

RESULTS AND DISCUSSION

The procedure for *t*RNA isolation to determine the degree of *in vivo t*RNA aminoacylation in yeast cells [12] had to be greatly modified in order to give satisfactory results with our material due to the large amounts of polysaccharides present in *t*RNA isolated from seeds. These polysaccharides were removed together with highly polymerized RNA on DEAE-cellulose and Sephadex G-75 columns.

Because the determination of the level of *t*RNA aminoacylation is based on comparison of amino acid acceptor activities of periodate treated to control *t*RNAs, the acceptor activities of *t*RNAs must be determined very accurately. Thus incubation mixtures contained excess of aminoacyl-*t*RNA synthetase and several different concentrations of *t*RNA. Linear relationship of aminoacyl-*t*RNA formation under the conditions used indicated the correctness of acceptor activity determination. Using this method we have found that in seeds, valine, isoleucine and tyrosine specific *t*RNAs are aminoacylated 4,6 and 10%, respectively (Table 1). All other *t*RNAs investigated are either not aminoacylated, or the level of aminoacylation is lower than the error of estimation, depending on sp. act. of the radioactive amino acid used, acceptor activity of *t*RNA and blank sample.

Table 1. Level of aminoacylation of *t*RNA (%) during germination of lupin seeds. In parenthesis the acceptor activities are given in pmol of aminoacid/A_{260} unit of *t*RNA preparation

*t*RNA specific for	Time of germination (hr)				
	0	1	3	72	
				cotyledons	axes
Ala	0·2	0·3	10	41	48
	(48)	(29)	(30)	(29)	(31)
Ile	6	10	30	88	91
	(333)	(277)	(260)	(364)	(296)
Leu	0·1	4	20	58	68
	(139)	(142)	(132)	(135)	(128)
Lys	0·4	0·7	0·5	36	30
	(48)	(30)	(37)	(38)	(37)
Met	0·9	0·8	0·7	76	65
	(23)	(25)	(28)	(26)	(31)
Phe	0·2	3	12	48	50
	(48)	(62)	(50)	(61)	(58)
Trp	0·5	0·6	30	70	71
	(42)	(34)	(33)	(37)	(39)
Tyr	10	20	38	70	76
	(98)	(103)	(107)	(153)	(137)
Val	4	7	39	90	92
	(126)	(82)	(76)	(87)	(91)

Extraction, purification and periodate treatment of lupin seed *t*RNA does not cause significant deacylation of aminoacyl-*t*RNAs present *in vivo* in seeds. This has been proved for valyl-*t*RNA with valine-[^{14}C] added to the seed meal. Furthermore, *t*RNAs isolated in an identical way from germinated seeds and from seedlings are aminoacylated to a higher degree, depending on the time of germination. Thus we can conclude that the low level of *t*RNA aminoacylation determined in lupin seeds actually describes the status *in vivo*. Such a very low level of *t*RNA aminoacylation has also been detected so far in dormant spores of *Bacillus megaterium* [13]. It suggests that these deacylated forms of *t*RNAs are common for

resting stages of organisms, as in seeds, and spores. The low level of *t*RNA aminoacylation *in vivo* in seeds makes possible the partial degradation of the acceptor end of *t*RNAs isolated from seeds, which has been observed at this laboratory.

The level of *t*RNA aminoacylation increases slowly during early stages of seed germination, reducing the synthesis of protein not only because of the small amounts of aminoacyl-*t*RNAs, but also because deacylated *t*RNAs present in excess inhibit the formation of eukaryotic initiation complexes [14,15]. It is interesting to note that polysomes are absent in wheat embryos. They are formed from ribosomes and mRNA during water imbibition [16]. Elongation factors also undergo some changes in imbibed wheat embryos [17].

Although the wt of seeds soaked in H_2O at room temperature for 1 hr increases significantly (50%) *t*RNAs are still aminoacylated to a small extent. A two-fold increase in weight of seeds germinated for 3 hr is accompanied by a significant increase in amount of aminoacyl-*t*RNAs, with the exception of lysyl-*t*RNA. Tyrosine, valine and isoleucine *t*RNAs are aminoacylated 40, 38 and 30%, respectively, and remain the most aminoacylated species *in vivo* among *t*RNAs investigated. *t*RNAs isolated from 3-day-old seedlings are found mainly in acylated forms. No considerable differences in the degree of aminoacylation of specific *t*RNAs in cotyledons and in axes are observed. The increase in the degree of *t*RNA aminoacylation during germination varies significantly for specific *t*RNAs. This indicates different rates of synthesis of different aminoacyl-*t*RNAs and/or different rates of deacylation of aminoacyl-*t*RNAs on ribosomes.

The increase in the level of *t*RNA aminoacylation during seed germination is not accompanied by changes in the *t*RNA population (Table 1). In each of the *t*RNA preparations the highest acceptance of isoleucine has been observed, next of leucine, tyrosine and valine. Alanine and methionine *t*RNAs are present in amounts *ca* 10 times lower than the amount of isoleucine *t*RNA. The constancy in the *t*RNA population during seed germination was also found for cotton cytoplasmic *t*RNA [8].

EXPERIMENTAL

Lupin seeds (*Lupinus luteus* cv Express) were soaked in H_2O at 20° for 5 hr, then germinated on wet cellulose in the dark at 20° for 3 days.

Extraction of tRNA. This was carried out at 5° and the pH was kept at 5 to minimize deacylation of *in vivo* acylated *t*RNAs. Seeds, cotyledons or axes of lupin, frozen in dry ice, were ground. Meal (50 g) was stored at 0° for 30 min to remove dry ice, then mixed with 300 ml of PhOH and 300 ml of extraction buffer containing 100 mM NaOAc, pH 5, 100 mM NaCl and 0.5 mM EDTA. The mixture was shaken for 1 hr and centrifuged, then the aq. phase was shaken again with PhOH (200 ml) for 30 min. Following centrifugation, 0.1 vol. of 20% NaOAc pH 5 and 2.5 vol. of cold (−20°) EtOH were added to the aq. phase. RNA collected by centrifugation was dissolved in 80 ml of buffer pH 5 (containing 100 mM NaOAc and 10 mM $MgCl_2$) at room temp. for 30 min. Following centrifugation the supernatant was applied to a DEAE-cellulose column. The column was washed with above buffer. *t*RNA was eluted with M NaCl in this buffer, precipitated by cold EtOH and purified on a Sephadex G-75 column in buffer containing 100 mM NaOAc pH 5, 200 mM NaCl and 10 mM $MgCl_2$. *t*RNA was precipitated as usual, centrifuged, then washed with cold EtOH and dried in vacuum.

Periodate treatment and deacylation of tRNAs. The oxidation mixture contained in 2.5 ml: 200 mM NaOAc pH 5, 20 mM $NaIO_4$ and 5 mg *t*RNA. Following 30 min incubation in the dark at room temp. (the excess of periodate was checked by the decrease in A at 232 nm after addition of glycerol to a small vol. withdrawn from the oxidation mixture) *t*RNA was precipitated with cold (−20°) EtOH and 0.5 ml 5 M NaCl, collected by centrifugation and dissolved in 0.2 ml containing 200 mM NaOAc pH 5 and 20% glycerol. Then 1.8 ml 1.8 M Tris–HCl pH 8.2 was added. Following incubation at 30° for 90 min the soln was cooled and *t*RNA was precipitated with 6 ml EtOH and 0.2 ml 20% NaOAc pH 5. *t*RNA was collected by centrifugation, dissolved in 0.2 ml buffer pH 7.5 containing 30 mM Tris–HCl and 5 mM $MgCl_2$ and dialysed against two changes of above buffer for 18 hr. Acceptor activities of periodate treated *t*RNAs were compared to activities of control *t*RNA preparations subjected to all above procedure except $NaIO_4$ was omitted in the oxidation mixture.

Enzyme preparation. Lupin seed meal (30 g) was extracted with 100 mM BPK pH 6.8 containing 10% glycerol and 5 mM mercaptoethanol at 4° for 30 min. The extract was centrifuged at 15000 *g* for 30 min. The supernatant was fractionated with $(NH_4)_2SO_4$. The fraction precipitating between 35–50% of $(NH_4)_2SO_4$ satn contained the aminoacyl-*t*RNA synthetases, except seryl- and tyrosyl-*t*RNA synthetases which were present in the fraction precipitating between 50–70% of $(NH_4)_2SO_4$ satn. The ppt. was dissolved in a small vol. of 20 mM BPK pH 6.8 containing 10% glycerol and 5 mM mercaptoethanol and dialysed against two changes of above buffer for 18 hr. The enzyme preparation was stored in 50% glycerol at −20°.

Aminoacylation. Incubation mixture contained in 70 µl 100 mM Tris–HCl pH 7.5, 50 mM KCl, 10 mM $MgCl_2$, 2.5 mM ATP (diNa salt), 2 nmol of (^{14}C)-labelled amino acid, 0.3 mg enzyme prepn and different amounts of *t*RNA, periodate treated or control (0.05–0.50 A_{260} unit). Following 20 min incubation at 30° 50 µl of incubation mixture was withdrawn and applied to paper discs. The discs were washed ×3 in 5% TCA for 15 min then once in EtOH for 5 min, dried and counted for radioactivity. The activities of radioactive amino acids used were (in mCi/mmol): Ala-105, Ile-69, Leu-174, Lys-25, Met-59, Phe-125, Ser-53, Trp-46, Tyr-110, Val-105.

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