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*t*RNA SPECIES IN THE DEVELOPING GRAIN OF *TRITICUM AESTIVUM*

R. D. NORRIS

Department of Biochemistry and Chemistry, The Medical College of St Bartholomew's Hospital, Charterhouse Square, London EC1

and

P. J. LEA and L. FOWDEN

Rothamsted Experimental Station, Harpenden, Hertfordshire, AL5 2JQ, England

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Abstract—The level of lysyl- and prolyl-*t*RNA in various stages of the maturing wheat grain was measured by the aminoacylation procedure. The levels of these *t*RNAs changed only slightly during the maturation period. Several species of lysyl- and prolyl-*t*RNA were obtained from different parts of the developing grain by fractionation on benzoylated-DEAE cellulose (BD-cellulose). The embryo contained three discrete species of prolyl and at least three species of lysyl isoacceptor *t*RNA throughout development, whilst the *t*RNA obtained from the endosperm gave more complicated elution profiles on chromatography on BD-cellulose. Small changes were noted in the levels of aminoacylation of individual isoacceptor *t*RNA species for lysine or proline during seed maturation. However, these were insufficient to account for the changing pattern of lysine and proline in the storage protein during the development of the endosperm.

INTRODUCTION

During the later stages in the maturation of cereal grains, storage protein rich in proline and glutamate [1] and low in lysine and tryptophan [2], is laid down in discrete protein bodies in the endosperm and/or aleurone layer [3–5]. However, the cytoplasmic protein of the endosperm is comparatively rich in lysine and tryptophan and has a lower content of proline and glutamate compared with storage protein [2].

Previous work from this laboratory describes changes in the levels of aminoacyl-*t*RNA synthetases during the maturation of the grain of *Triticum aestivum*. The decrease in the proportion of lysine and increase in proline and glutamate in the endosperm storage protein which occurs during seed maturation did not correlate with the changes of aminoacyl-*t*RNA synthetase activity in this tissue, although complications due to various factors such as multiplicity of synthetases and *t*RNAs could not be ruled out [6].

By the use of cell-free extracts from the pupae of the insect, *Tenebrio molitor*, the ratio of tyrosine–leucine incorporation into protein was shown to increase 10-fold during development, concomitant with the increase of tyrosine residues in cuticular protein [7]. This suggested that amino acid selection occurs at the level of either (i) aminoacylation or (ii) ribosomal–*t*RNA interaction with modified *t*RNA [8]. The emergence and/or disappearance of certain *t*RNA isoacceptors specific for phenylalanine has been observed during the seedling growth of barley [9]. However, no differences in the levels of cytoplasmic

isoaccepting *t*RNAs could be found during cotton seed embryogenesis [10], nor during the mitotic cycle of *Physarium polycephalum* [11].

Studies with mutant strains of cereals having high lysine contents compared with the wild type are of primary importance for understanding the factors responsible for the laying down of seed proteins [12]. High-lysine maize exhibits an increased ribonuclease activity compared with the normal strain [13,14]. A preferential breakage at the position of purines in the anticodon regions in *t*RNA by ribonuclease might affect the ratios of amino acids incorporated into different proteins [15], since some *t*RNA species split by ribonuclease in such a way cannot transfer amino acids to ribosomes, although they may retain aminoacylation activity. A greater incorporation of lysine in the presence of ribosomes from a lysine-rich genotype is observed compared with that using normal ribosomes [16]. Although this appears to be independent of the *t*RNA used, differences in aminoacyl–*t*RNA synthetase activity in normal and lysine-rich maize were observed using a particular *t*RNA preparation from the endosperm [17].

Since it is not clear whether the levels of individual *t*RNA molecules are modulated in accordance with the known changes in the amino acid composition of proteins from the endosperm during seed maturation, we decided to measure the changes in several *t*RNA species specific for amino acids whose proportions in storage protein vary markedly during the development of the wheat grain, e.g. those specific for proline and lysine.

RESULTS

tRNA and enzyme preparations. Since ribonucleases may cause *t*RNA to lose its ability to transfer amino acids into proteins in the presence of ribosomes [15], pilot experiments were performed with various inhibitors to ascertain whether ribonucleases in either the synthetase or *t*RNA preparations inactivated the *t*RNA [18]. Under the conditions used in the experiments reported in this paper, no significant loss of *t*RNA activity was attributed to ribonuclease action. Since prolyl- and lysyl-*t*RNAs are readily deacylated at neutral or alkaline pH's, care was taken to keep the acylated forms of these molecules at about pH6.

Levels of unfractionated tRNAs in the wheat seed during maturation. The contents of *t*RNA species (assayed by acylation—see Experimental) specific for each individual amino acid was determined for different parts of the maturing grain. An approximately 2-fold increase in the level of *t*RNAs tested (those specific for phenylalanine, leucine, lysine, proline and asparagine) was observed in the developing endosperm between the 2nd and 4th week after anthesis. However, no significant changes occurred in the lysine–leucine acylation ratio. The proline–leucine and proline–phenylalanine ratios appeared to increase slightly (about 20%) between the 3rd and 6th week after anthesis. The level of *t*RNAs specific for leucine, phenylalanine, proline and lysine in the developing embryo increased 2–3-fold between the 2nd and 6th week after anthesis but, when expressed on a fr. wt basis, these levels appeared constant throughout this period. The levels of these four *t*RNAs decreased by less than 10% in the pericarp-testa fraction of the wheat grain over the first 4·5 weeks following anthesis but exhibited a marked decline after the 5th week.

Separation of isoaccepting species of lysyl- and prolyl-tRNA. Bulk *t*RNA extracted from grains and fully acylated by treatment with ^{14}C-amino acids was fractionated on BD-cellulose (see Experimental) in order to separate discrete species of *t*RNA specific for lysine and proline. The elution profiles of *t*RNA from endosperm tissue indicated the presence of at least three species of prolyl-*t*RNA and at least four species of lysyl-*t*RNA (Fig. 1). Elution profiles of embryo *t*RNA indicated the presence of three discrete species of prolyl-*t*RNA and three or possibly four species of

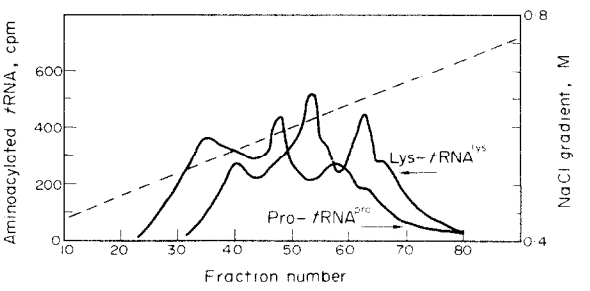

Fig. 1. Elution profile of Lys-*t*RNA^{Lys} and Pro-*t*RNA^{Pro} on a column of BD-cellulose: enzyme and *t*RNA obtained from endosperm 5 weeks after anthesis.

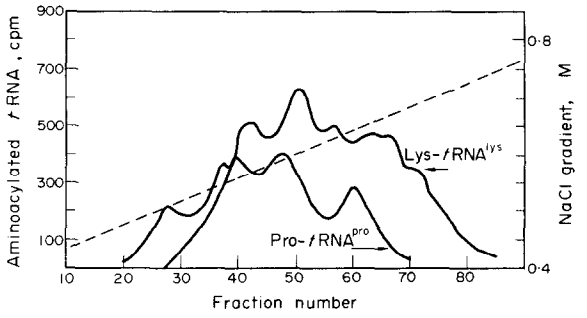

Fig. 2. Elution profile of Lys-tRNA^{Lys} and Pro-tRNA^{Pro} on a column of BD-cellulose: enzyme and tRNA obtained from embryo 5 weeks after anthesis.

lysyl-tRNA (Fig. 2). The elution profiles from the embryo were, in general, less complex than those obtained using material from the endosperm, but resolution was generally low.

No significant differences in the relative amounts of different isacceptor species of prolyl- or lysyl-tRNA were observed at different stages of the maturation of the wheat embryo (3–6 weeks after anthesis) or the whole grain. However, in the endosperm, slight variations in the lysyl-tRNA profile on BD-cellulose were noted between the 4th and 7th weeks after anthesis; slightly more (20%) of the earliest eluting species was observed at the 4th than at the 7th week.

DISCUSSION

The biochemical and physiological development of the wheat grain and laying down of the endosperm has been studied in some detail [19]. The experiments reported here were carried out under conditions where ribonuclease activity was minimal and extraction of tRNA maximal [6]. Care was taken to ensure that aminoacyl-tRNA synthetase was not limiting the reaction, a constant amount of synthetase activity from each stage of maturation being used for the acylation reaction, based upon the measurement of ATP-^{32}PPi exchange [20,21].

Results indicated that the level of tRNA as such did not regulate the changes in amino acid composition of the storage protein occurring during endosperm maturation, and no orderly emergence or disappearance of discrete species of prolyl- or lysyl-tRNAs was observed during this period. The constant lysine–leucine ratio observed during the maturation of the grain is further evidence that lysyl-tRNA is not modulated in accordance with the changes of the amino composition of the storage protein, since the proportion of leucine in these proteins remains fairly constant throughout the period when the proportion of lysine decreases. The slight increase in the proline–leucine ratio during maturation may reflect the increase in protein-bound proline which occurs in the endosperm, although confirmatory evidence is required. In general, these results agree with those previously reported for cotton seed embryogenesis [10] and support the idea that the aminoacylation system is not primarily responsible for the differences in the quality of storage protein of the wheat endosperm. It seems more likely that the change to the relatively lysine-poor protein during maturation of the grain results from modulation of the type of mRNA produced. Such modulation has been described in the lysine-rich variety of maize, where the O_2 gene is active only during the first 16 days after pollination [22]. The tRNA elution profiles obtained by the use of the BD-cellulose column were rather complex and poorly resolved. Similar profiles of lysyl- and prolyl-tRNAs from yeast have been reported [23]. The isoaccepting prolyl-tRNA species from the wheat embryo did not differ from those obtained from the endosperm except that the endosperm species eluting at the lowest salt concentration was absent from the embryo. The lysyl-tRNA isoacceptors appeared to differ slightly in elution position from those of the endosperm, although some of these differences may be due to differences in unfolding of tRNA or aminoacylation. The shape of the profiles of prolyl- and lysyl-tRNA was broader and slightly different from those observed with yeast tRNA, but eluted in approximately the same position, i.e. 0·5–0·7 M NaCl for prolyl-tRNA and 0·55–0·75 M NaCl for lysyl-tRNA. The multiplicity of tRNA species reported here accords well with the results of other workers [6,23,24].

EXPERIMENTAL

Plant material. Developing grains were harvested from winter wheat plants (*Triticum aestivum* L. var. Cappelle) grown in the field in 1973 under a controlled nitrogen regime at Rothamsted Experimental Station. Seeds were surface sterilized and tested for bacterial or fungal infection, as previously described [6]. Developing grains were dissected into their component tissues for extraction of tRNA and synthetases.

Development stages selected for harvesting the grains were as previously described [6].

Radioisotopic chemicals. ^{32}P-orthophosphate (Radiochemical Centre) was converted to ^{32}P-pyrophosphate by pyrolysis [25]. Radioactive amino acids (^{14}C or ^{3}H) were obtained from the Radiochemical Centre, and dil. where necessary with unlabelled amino acid (Analar grade).

Other chemicals. Benzoylated-DEAE-cellulose was purchased from Cambrian Chemicals Ltd; DEAE-cellulose was obtained from Whatman Ltd.

Determination of protein and nucleic acids. Protein was determined by the method of Lowry *et al.* [26] with bovine serum albumin as a standard, or from extinction values measured at 260 and 280 nm [27]. The amount of RNA in the synthetase preparations was determined by the method of Key and Shannon [28].

Enzyme fractionation. The various tissues were ground manually for 3 min in a buffer, pH 7·8, containing Tris–HCl 0·1 M, 0·3% polyvinylpyrrolidone (soluble, MW 44000), 40 mM mercaptoethanol, 10 mM $MgCl_2$ and 15% (v/v) glycerol. After centrifugation at 24000 *g* for 30 min, the supernatant was passed through a column of Sephadex G-25 (equilibrated with extraction buffer). Prolyl- and lysyl-*t*RNA synthetases were partially purified by $(NH_4)_2SO_4$ pptn and Sephadex G-75 or DEAE-cellulose chromatography as previously described [29,30]. Synthetase preparations were only used if substantially free from ribonuclease activity.

Extraction of tRNA. The modification of the general phenol method as described by Vanderhoef *et al.* [31] was used to isolate *t*RNA from the various tissues of the developing wheat grain. Carbohydrates were removed by DEAE-cellulose chromatography and the *t*RNA deacylated by incubation at pH 9 for 30 min; the *t*RNA preparation was separated on Sephadex G-25 with an elution buffer of 0·1 M NaOAc, pH 6. The absence of ribonuclease activity in the *t*RNA preparations was established by incubating the unfractionated *t*RNA in Tris–HCl buffer pH 8 for 1 hr: *t*RNA remaining was assayed by aminoacylation.

Fractionation of tRNA on BD-cellulose. Prolyl- or lysyl-*t*RNA were completely aminoacylated by incubating a mixture containing (per ml) 10 μmol $MgCl_2$, 1·5 μmol ATP, 50 μmol triethanolamine buffer pH 7·2, *t*RNA preparation, 0·05–0·125 mg, radioactive amino acid (5 μCi) and synthetase preparation (50–200 μg protein) at 35° for 45 min. The course of aminoacylation was followed by the assay described below. Protein was removed from the aminoacyl-*t*RNA preparation by extraction with phenol and the washed EtOH-precipitated aminoacyl-*t*RNA was applied to a column (80 × 1 cm) of benzoylated DEAE-cellulose, prepared in pH 5 buffer and eluted using a linear gradient of 0·4–1·2 M NaCl, as described in [23]. Fractions were collected and 0·5 ml aliquots added to 10 ml samples of Bray's fluid [32], contained in scintillation vials and radioactivity determined with a Packard scintillation counter.

Assay procedures. (a) ATP-^{32}PPi exchange. Incubation mixtures contained: Tris–HCl buffer pH 8 (100 μmol), $MgCl_2$ (14 μmol), ATP (2 μmol), ^{32}PPi (2 μmol), amino acid (20 μmol) and enzyme preparation (about 100 μmol); total vol. 1 ml. The mixture was incubated for 15 min at 35° and assayed as previously described [20,21]. (b) Aminoacylation reaction: this was performed at 25° as previously described [6,20,21]. The initial rate of charging was calculated after aliquots of the reaction mixture were withdrawn at 1, 2 or 5 min to minimize the spontaneous deacylation which occurs with prolyl- and lysyl-*t*RNAs.

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