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SHORT COMMUNICATION

CHARACTERIZATION OF *N*-FORMYL-METHIONYL-*t*RNA IN BEAN MITOCHONDRIA AND ETIOPLASTS

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Key Word Index—*Phaseolus vulgaris*; Leguminosae; *N*-formyl-methionyl-*t*RNA; mitochondria; etioplasts.

Abstract—*t*RNAs isolated from bean mitochondria and etioplasts have been charged with ^{35}S-methionine using a homologous enzyme preparation and formylated using either a homologous or an *E. coli* transformylase. On the other hand, mitochondria have been incubated in the presence of ^{35}S-methionine and formyltetrahydrofolate. In both cases, after hydrolysis of *t*RNAs by pancreatic RNase, methionyl-adenosine and formyl-methionyl-adenosine have been characterized, demonstrating the presence of formylatable methionyl-*t*RNA and of an active transformylase in mitochondria and etioplasts.

INTRODUCTION

N-FORMYL-METHIONYL-*t*RNA is known to be responsible for the initiation of protein biosynthesis in bacteria (for a review see Ref. 1). It is also present in the mitochondria of yeast,[2] fungi[3] and mammalian cells,[2,4] but although it could be inferred that the same initiator *t*RNA should also function in plant mitochondria, its presence in these organelles has not as yet been reported, probably because of the difficulties encountered in purifying plant mitochondria and in extracting measurable amounts of mitochondrial *t*RNAs. Using a method described in this article, we have been able to obtain pure and intact bean mitochondria, to extract their *t*RNAs and to demonstrate the presence of formylatable methionyl-*t*RNA and of a transformylase in these organelles.

After *N*-formyl-methionine was shown to be the first aminoacid in polypeptides synthesized in a cell-free system prepared from *Euglena* chloroplasts and programmed by phage f_2 RNA as a messenger,[5] *N*-formyl-methionyl-*t*RNA has been characterized in purified bean chloroplasts,[6] and then also shown to be present in the chloroplasts of *Acetabularia*,[7] maize,[8] cotton[9] and wheat germ.[10] In addition, the tissues of *Vicia faba*[11] have been shown to contain a formylatable $t\text{RNA}^{met}$ species, to which has been ascribed the role of initiator *t*RNA in the organelles of this plant.

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It has been reported that some plastid-specific *t*RNA species are either absent, or present only in relatively low amounts in the plastids of dark-grown or bleached *Euglena* cells,[12,13] in etiolated bean leaves,[14] and in the purified etioplasts of dark-grown bean leaves,[15] suggesting that the synthesis of these *t*RNAs can be stimulated upon illumination. It was therefore of interest to determine whether *N*-formyl-methionyl-*t*RNA, first characterized in mature chloroplasts,[6] is already present in the etioplasts of dark-grown bean plants. The results which we are reporting here show that in the etioplasts both a formylatable $tRNA^{met}$ and an active transformylase are present.

RESULTS AND DISCUSSION

When isolated mitochondrial *t*RNAs are charged with ^{35}S-methionine using a mitochondrial enzyme preparation, re-isolated, formylated in the presence of a mitochondrial enzyme, isolated again and subjected to RNase digestion, both methionyladenosine (met-A) and formyl-methionyl-adenosine (*f*-met-A) are found. In this case the formylation ratio (*f*-met-A $\times$ 100/*f*-met-A + met-A) is 28%, which represents a maximal value (plateau obtained in the presence of an excess of mitochondrial transformylase). However, if, after charging, the mitochondrial ^{35}S-methionyl-*t*RNAs are isolated and incubated in the presence of an *E. coli* enzyme preparation, the formylation ratio reaches 56%.

When isolated etioplast *t*RNAs are charged with ^{35}S-methionine in the presence of an etioplast enzyme preparation, re-isolated and formylated, the formylation ratio is 33% if an etioplast transformylase is used and 60% if an *E. coli* transformylase is used. The value of 33%, obtained in the case of the etioplast *t*RNAs using a homologous etioplast transformylase should be compared with the value of 26%[6] obtained with chloroplast *t*RNAs using a homologous chloroplast preparation (in our previous studies the formylation ratio was expressed as *f*-met-A $\times$ 100/met A and was 35%).

As a control, cytoplasmic *t*RNAs were charged with ^{35}S-methionine in the presence of a cytoplasmic enzyme preparation, re-isolated and formylated in the presence of an *E. coli* enzyme. In this case the formylation ratio was only 3·8%, a low value which can be attributed to small contaminations of cytoplasmic *t*RNAs by *t*RNAs from broken organelles. If *E. coli* *t*RNAs are charged and formylated under the same experimental conditions using *E. coli* enzymes, the value obtained for the formylation ratio (66%) is comparable to the value of 60% obtained by Marcker.[16]

If intact bean mitochondria are incubated in the presence of ^{35}S-methionine and formyltetrahydrofolate and if their *t*RNAs are then isolated and analyzed, the formylation ratio is 36%. If *E. coli* cells are incubated under the same conditions, the formylation ratio is 67%.

The actual values of the formylation ratio obtained in our experiments may not reflect the true relative proportions of $tRNA^{met}$ (M) and $tRNA^{met}$ (F) in plant organelles. Among possible causes of error, the fact that the ester linkage between methionine and *t*RNA is less stable than the bond between formyl-methionine and *t*RNA, as already reported,[16] could be responsible for a more rapid hydrolysis of methionyl-$tRNA^{met}$ (M) during the *in vitro* incubation and this would result in a higher formylation ratio. But our results clearly

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demonstrate the existence of formylatable methionyl-*t*RNA and of an active transformylase in bean mitochondria and etioplasts.

EXPERIMENTAL

Purification of organelles. Seeds of *Phaseolus vulgaris* were placed on moist vermiculite (sterilized at 180°) in the dark at 30° and the hypocotyls were harvested after 4–5 days. 200 g of hypocotyls were homogenized at 4° in a Turmix mixer in 200 ml of a medium containing 0·7 M mannitol, 10^{-3} M EDTA, 4×10^{-4} M ATP, and 1 mg/ml bovine serum albumin, and adjusted to pH 7·2 with triethanolamine. The homogenate was filtered through fine nylon cloth and centrifuged at 1000 *g* for 5 min. The supernatant was placed in new centrifuge tubes (50 ml in each tube) and 20 ml of a 27% sucrose solution, EDTA 10^{-4} M, containing 2 mg/ml bovine serum albumin, pH 7·2 (adjusted with triethanolamine) were gently introduced below the supernatant. After 10 min centrifugation at 8000 *g*, the mitochondria were found as a pellet which was resuspended in an appropriate buffer (either for *t*RNA extraction or for enzyme preparation) and filtered through a fine nylon cloth (pore size = 50 μ). All these operations were carried out at 4° within 20–25 min. Etioplasts were obtained from lyophilized etiolated bean leaves by a non-aqueous technique as previously decribed.[15]

tRNAs and enzyme preparations. *t*RNAs and enzymes were obtained from hypocotyls, from mitochondria (after a treatment with 0·2% Triton X 100 which lyses the organelles but not the contaminating bacteria, as checked on a suspension of these contaminating bacteria), and from etioplasts.[17]

Aminoacylation and formylation of isolated tRNAs. *t*RNAs were charged with ^{35}S-methionine,[18] re-isolated by phenol treatment and alcoholic precipitation from the aqueous phase, and dialysed at pH 5. After formylation[9] the *t*RNAs were re-isolated.

Incubation of intact mitochondria. Mitochondria (20–30 mg protein) were incubated in 2 ml of a medium containing 6 μmol ATP, 10 μmol potassium acetate, 40 μmol phosphoenolpyruvate, 0·08 mg pyruvate kinase, 25 μCi ^{35}S-methionine (6–20 Ci/mmol), 0·4 mg reduced formyltetrahydrofolate[19] and 1 mmol mannitol, for 30 min at 37°. After addition of 0·2 ml potassium acetate (20%, pH 5) the mitochondria were lysed by Triton X 100 (final concentration 0·2%) and the debris removed by centrifugation at 20 000 *g* for 10 min. The *t*RNAs were then isolated from the supernatant.[17]

Determination of the formylation ratio. The *t*RNAs aminoacylated and formylated *invitro* and the *t*RNAs extracted from incubated mitochondria were hydrolysed by pancreatic RNase. The resulting methionyl-adenosine (met-A) and formyl-methionyl-adenosine (*f*-met-A) were separated by high-voltage electrophoresis, identified and their amounts were determined as previously described.[6]

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