PRESENCE OF N-FORMYL-METHIONYL-TRANSFER RNA IN BEAN CHLOROPLASTS

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1. Introduction

The role played by N-formyl-methionyl-tRNA in the initiation of protein biosynthesis in bacteria is now well established [1–4]. In eukaryotes however, N-formyl-methionyl tRNA seems to be present only in mitochondria, as was shown by Smith and Marcker in the cases of yeast and of rat liver [5] and by Galper and Darnell in the case of HeLa cells [6]. That N-formyl-methionine is also involved in the initiation of protein synthesis in Euglena chloroplasts, was shown by the experiments of Schwartz et al. [7] who characterized N-formyl-methionine in the proteins synthetized in a cell-free system prepared from Euglena chloroplasts and stimulated by f_2 RNA, but these authors did not study the tRNA's from Euglena chloroplasts. We have isolated the tRNA's from bean chloroplasts and we describe here experiments which demonstrate the presence of N-formyl-methionyl-tRNA in these chloroplasts.

2. Methods

French bean plants (Phaseolus vulgaris) were grown from seeds for 10–12 days, placed in the dark for 2 days (to deplete the leaves of starch) and the young leaves were harvested. The leaves were washed, the midribs and lateral veins were removed and the leaves were freeze-dried at −25°C for 48 hr. The chloroplasts were then extracted by the non-aqueous technique of Charlton et al. [8]. Controls of our preparations of chloroplasts by electron microscopy showed that we had pure intact chloroplasts, without bacterial contamination.

Transfer RNA's were extracted from the chloroplasts by phenol extraction. Ribosomal RNA's were precipitated by NaCl M and DNA was hydrolysed by RNase free DNase. Transfer RNA's were further purified by DEAE-cellulose chromatography and were freed from esterified aminoacids by a treatment with 1.8 M Tris, pH 8 for 90 min.

An enzymatic preparation containing the aminoacyl-tRNA synthetases and the transformylase was obtained by sonication of the chloroplasts, centrifugation at 35,000 × *g* for 20 min, then at 150,000 × *g* for 2 hr and by Sephadex G 75 filtration.

Enzymes and tRNA's were also prepared from hypocotyls grown in the dark, using essentially the same techniques.

3. Results

Chloroplast tRNA's were charged with ^{35}S-methionine (850 mc/mM) using chloroplast enzymes. Cytoplasm (hypocotyl) tRNA's were also charged with ^{35}S-methionine, using either chloroplast or cytoplasm enzymes. The composition of the reaction mixture was similar to that described by von Ehrenstein and Lipmann [9]. After 15 min incubation at 37°C, formyltetrahydrofolate, prepared according to Jones et al. [10], was added and the mixture was allowed to incubate for another 15 min. The tRNA's were recovered by phenol extraction and alcohol precipitation. Redissolution was followed by overnight dialysis.

These tRNA's were hydrolysed by pancreatic RNase according to Marcker and Sanger [1] and the products were subjected to high voltage paper electrophoresis (3,000 V, 3 hr, pH 3.5). The paper was divided into 1 cm strips which were put in a scintillation mixture and counted.

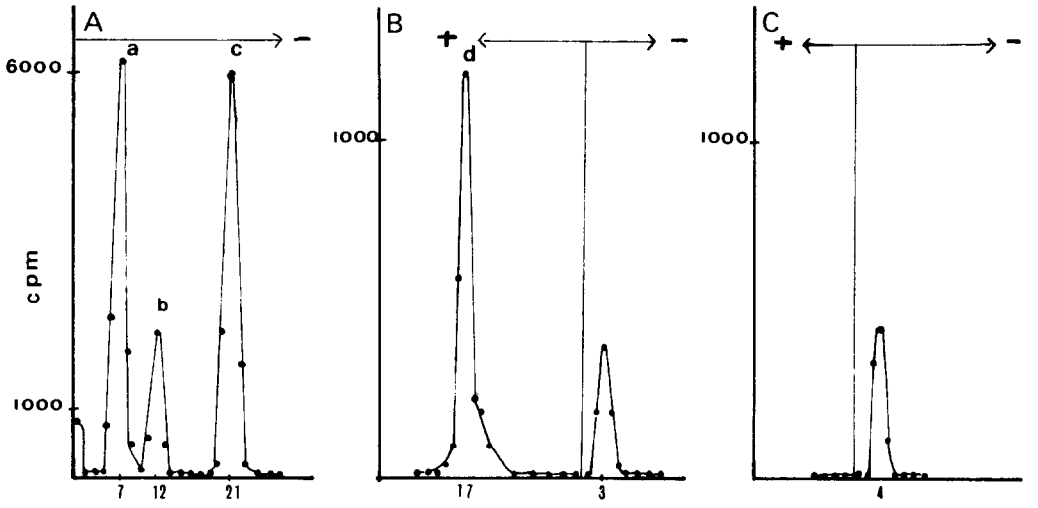

Fig. 1. Identification of the products of RNase digestion of chloroplast tRNA's after incubation with ^{35}S-methionine, formyl-tetrahydrofolate and chloroplast enzymes. A. RNase digest submitted to high voltage electrophoresis (3,000 V, pH 3.5, 2 hr); B. Material marked "b" (in A) was eluted with water, incubated with NH_4OH pH 10.5 for 1 hr at 37°C in N_2 atmosphere, neutralized and submitted to electrophoresis (3,000 V, pH 3.5, 90 min); C. Material marked "d" (in B) was eluted, treated with N HCl for 15 min at 100°C in N_2 atmosphere and submitted to electrophoresis (3,000 V, pH 3.5, 90 min).

Fig. 1A shows the radioactive products of RNase digest after incubation of chloroplast tRNA's with chloroplast enzymes. Three products have migrated towards the cathode: peak "a" is free methionine (as shown by a parallel run of the standard aminoacid). Peak "b" is formyl-methionyl adenosine, as shown by the following control experiments: when the product of peak "b" is eluted, subjected to mild base hydrolysis (NH_4OH pH 10.5) for 1 hr at 37°C in N_2 atmosphere, neutralized and subjected to electrophoresis at 3,000 V for 90 min, two products are obtained: (i) formyl-methionine which migrates towards the anode (fig. 1B), as already described by Marcker and Sanger, and which was identified by the running of an authentic marker with it; the formyl [^{35}S] methionine marker was prepared according to Sheehan and Yang [11]; (ii) a compound which migrates towards the cathode and was not identified, but could perhaps be the amide of N-formyl-methionine (Dr. Marcker, personal communication). When the spot migrating towards the anode (just like formyl-methionine) was eluted in N HCl, incubated 15 min at 100°C in N_2 atmosphere and subjected to electrophoresis at 3,000 V for 90 min it gave a peak migrating towards the cathode and corresponding to free methionine (fig. 1C).

Peak "c" is the methionyl adenosine ester; if eluted and incubated with NH_4OH pH 10.5 for 1 hr at 37°C, in N_2 atmosphere it is converted to free methionine which can be characterized by its electrophoretic mobility and gives a peak identical to that of the standard aminoacid.

Fig. 2 shows the radioactive products of RNase digest after incubation of cytoplasmic tRNA's with chloroplast enzymes. Three areas can be distinguished peak "a" which is free methionine; peak "b", which represents only about 1/40 of peak (c_1+c_2) and is much less important than in fig. 1A where it represents 35% of peak "c". Peak "c" is here split into two peaks, c_1 and c_2, which were both converted into free methionine on treatment with NH_4OH at pH 10.5 and represent methionyl-adenosine; the presence of two peaks was also observed by Marcker and Sanger [1] who suggested that they were due to the methionine esterifying both the 2′ and 3′ OH groups of adenosine.

4. Discussion

Bean chloroplast tRNA's and cytoplasmic tRNA's were incubated in two identical incubation mixtures

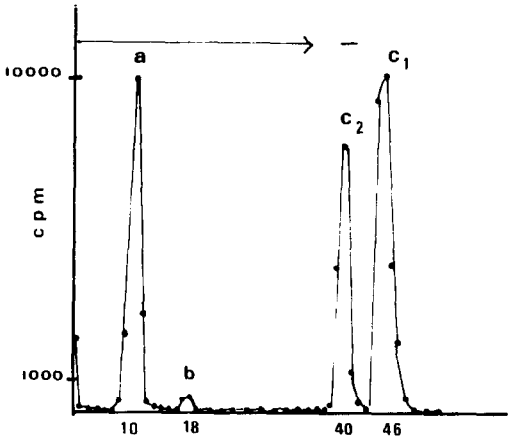

Fig. 2. Identification of the products of RNase digestion of cytoplasm (hypocotyl) tRNA's, after incubation with ^{35}S-methionine, formyl-tetrahydrofolate and chloroplast enzymes. The RNase digest was submitted to high voltage electrophoresis (3,000 V, pH 3.5, 3 hr).

containing all the factors necessary for methionine attachment and formylation. Upon re-extraction and RNase digestion, chloroplast tRNA's gave a product which could be identified as formyl-methionyl-adenosine and which represented about 35% of the methionyl adenosine ester yielded by that preparation. Cytoplasmic tRNA's gave only a very small peak in the position characteristic of formyl-methionyl-adenosine, which represented about 2.5% of the methionyl-adenosine ester yielded by the corresponding preparation. It is likely that this little amount of formyl-methionyl-adenosine obtained from the cytoplasmic tRNA's is due to small contaminations of our cytoplasm preparations by material either from proplasts or from mitochondria and we intend to study whether bean mitochondria contain N-formyl-methionyl-tRNA as do mitochondria from other organisms [5,6]. Our results suggest that initiation of protein biosynthesis in plant chloroplasts involves N-formyl-methionyl-tRNA as in bacteria and in mitochondria, so that the same initiation mechanisms seem to operate in bacteria and in cell organelles, all of which have 70S ribosomes.

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