BASE COMPOSITION OF MITOCHONDRIAL RNA SPECIES AND CHARACTERIZATION OF MITOCHONDRIAL 4 S RNA FROM *LOCUSTA MIGRATORIA*

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1. Introduction

Investigations in several organisms have shown that the mitochondrial rRNAs and at least a number of the mitochondrial tRNAs are coded for by the mitochondrial genome [1–12]. Recently, detailed studies demonstrated that yeast mitochondria contain a full complement of endogenously specified tRNAs [13–16]. With respect to higher animal cells, which possess only a small mitochondrial genome, the question remains open whether all of the nucleic acids taking part in mitochondrial protein biosynthesis are manufactured within the mitochondria or whether some of them have to be imported from the cytoplasm.

We wish to report here on a direct approach to study the characteristics of rRNA and tRNA from highly purified mitochondria of the insect Locusta migratoria [17]. The mitochondrial RNAs were found to be extremely low in (G+C) content and the tRNAs could be resolved into about 25 different species. From these findings we suggest that locust mitochondria have a full complement of endogenously specified rRNAs and tRNAs.

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2. Results

2.1. *Size and nucleotide composition of locust mitochondrial RNAs*

Intact RNA was extracted from purified mitochondria and each preparation was checked by electrophoresis on polyacrylamide gels as described [17]. Sucrose density gradient centrifugation served as a preparative means to separate the RNAs of the large and the small ribosomal subunits and a slowly sedimenting (4 S) RNA (fig.1). The latter was shown to possess amino acid acceptor activity. A 5 S RNA component was not detected in the gel electrophoreses.

The nucleotide composition of the RNAs was determined in three independent experiments by digestion with ribonuclease T_2, subsequent thin-layer chromatography, and spectrophotometric measurements of the separated components. The results are shown in table 1. It is obvious that both mitochondrial rRNAs differ in nucleotide composition and exhibit an unusually low (G+C) content compared to that of cellular ribosomal RNAs.

The mitochondrial tRNA has a content of only 30% guanosine plus cytidine, the two bases are found in nearly equal proportion. Also, adenosine and uridine are found in a nearly equimolar ratio (pseudouridine and thymidine were included in the

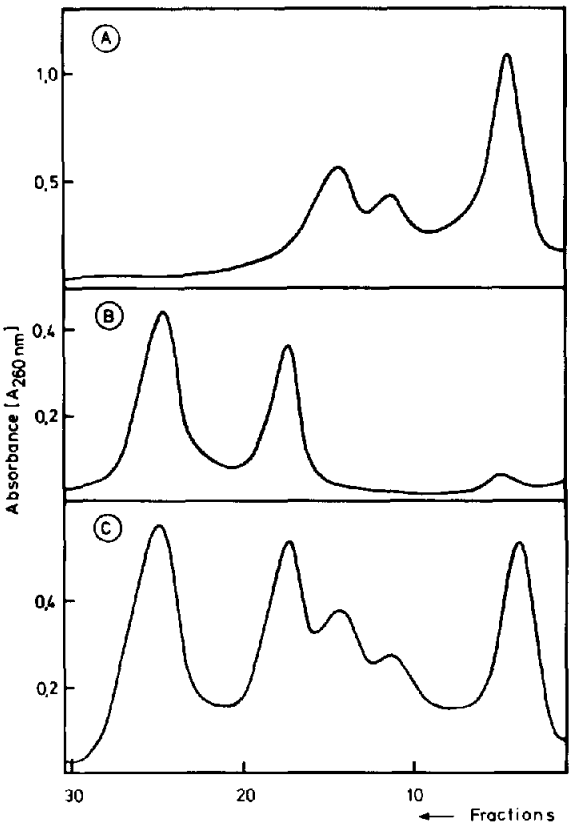

Fig.1. Separation of RNA from whole locust mitochondria by centrifugation on sucrose gradients. Mitochondria were purified and the RNA was extracted similarly as described [17]. Convex gradients were prepared similarly as reported [18] using 5% and 20% sucrose (RNAase free, Serva, Heidelberg) dissolved in a buffer containing 0.1 M NH_4Cl and 0.03 M Tris–HCl, pH 7.6. Centrifugation was performed in a rotor SW 41 Ti (Spinco, Beckman Instr.) for 14 h at 35 000 rpm. The content of each tube was pumped through a flow-cell (volume 50 µl, 0.5 cm light path), the absorbance monitored at 260 nm, and fractions of 0.4 ml were collected. A: 7.0 A_{260} units of mitochondrial RNA were loaded onto the gradient. (In some experiments, i.e. for preparing mitochondrial 4 S RNA, up to 70 A_{260}-units were used and the centrifugation time was extended to 21 h.) B: 1.84 A_{260} units of *E. coli* ribosomal RNA was used. C: A mixture of 3.2 A_{260} units of *E. coli* ribosomal RNA and 4.6 A_{260} units of mitochondrial RNA were loaded onto the gradient. The sedimentation values of the mitochondrial RNAs were calculated from these experiments to be 12.6 S, 9.6 S, and 4 S, respectively, taking the sedimentation coefficients for the *E. coli* RNAs (23 S, 16 S, and 4 S, respectively) as references.

calculations as uridine derivatives). By rechromatography of the material from the spots containing the major nucleotides in a different solvent system [21], some minor nucleosides were identified such as 1-methyl-adenosine, *N*(7)-methylguanosine, and *N*(2)-dimethylguanosine, but they could not be determined quantitatively. 4-thiouridine was absent in mitochondrial tRNA (10 A_{260} units tRNA were used) according to measurements of the ultra violet Spectra beyond 300 nm. The absence of Y-base was inferred from the evaluation of fluorescence excitation and emission spectra. Control experiments (data not shown) were performed with *E. coli* and yeast tRNAs.

In a comparison, using total cytosolic tRNA from locust muscle cells and the same methods (table 1), it was found that this tRNA had a base composition comprising about 56% (G+C) which is in a range found for most cellular tRNAs.

2.2. *Mapping of mitochondrial tRNA species on polyacrylamide gels*

Recently, we devised a method for mapping tRNAs by the use of two-dimensional electrophoresis on polyacrylamide gels [22]. This technique was applied to locust mitochondrial tRNA in order to get an estimate about the number of individual tRNA species and to prove the existence of isoacceptors. A comparison of the band patterns of locust mitochondrial tRNA, locust cytosolic tRNA and yeast tRNA on a 10% polyacrylamide gel is shown in fig.2a. It can be seen, that the patterns are definitely differing and that the one for mitochondrial tRNA is less complex than the one for cytosolic tRNA. The electrophoretic mobility of individual tRNAs in this type of gel is not strictly dependent on the length of the nucleotide sequence but influenced by its nucleotide composition. It is not possible, therefore, to get an estimate of the actual chain lengths of the mitochondrial tRNAs, which are rich in adenosine and uridine residues.

On two-dimensional gel electrophoresis, the mitochondrial tRNAs were separated into about 27 major spots. In addition, some minor spots were seen. It is unlikely that the fainter spots were due to a contamination of the mitochondrial tRNA preparation with

Table 1
Nucleotide composition of mitochondrial rRNAs and tRNAs from *Locusta migratoria*

Mol %	Mitochondria			Cytosol
	Large rRNA	Small rRNA	tRNA	tRNA
Gp	18.5	17.4	16.6	34.6
Ap	29.4	31.6	33.2	22.6
Cp	16.0	8.2	14.1	20.8
Up	36.1	42.8	30.6	17.2
ψp		–	4.5	3.8
Tp	–	–	approx. 1.0	approx. 1.0
(G+C)	34.5	25.6	30.7	55.4

1–3 A_{260}-units of tRNA were incubated in 10 μl of 0.05 M Na-acetate, 0.01 M $MgCl_2$, pH 4.5, with 0.05 units of ribonuclease T_2 (EC 3.1.4.23, Sankyo, Tokyo) in a sealed capillary tube for 4 h at 37°C. The sample was then charged onto a cellulose thin-layer plate (Merck, Darmstadt) as a 3 cm wide trace and chromatographed in the solvent system isopropanol-conc. HCl - water (114:29:24, by volume) together with reference markers [19]. The material containing the nucleotides was scraped off, extracted with 500 μl water twice, and the ultra violet spectra were measured in a Zeiss PMQ II spectrophotometer. Mol percent was calculated from the corresponding extinction coefficient [20]. The spots for Gp, Ap, Cp, and Up in this solvent system contained also the modified nucleotides. For detection of the minor nucleotides, the material from the bands mentioned above was rechromatographed in the solvent system described [21], together with reference markers.

Fig. 2. Electrophoresis of locust tRNAs on polyacrylamide gels. (a) 10% gel, technical details were as described [22]. A: mitochondrial tRNA. B: cytoplasmic tRNA. C: yeast tRNA (Boehringer, Mannheim) for comparison. Mitochondrial tRNA was prepared from the 4 S fraction of sucrose gradient centrifugations (fig.1a). Cytoplasmic tRNA was prepared from locust muscle cells. These were homogenized in SMT-buffer (0.25 M sucrose, 0.01 M $MgCl_2$, 0.01 M triethanolamine–HCl, pH 7.2). The homogenate was centrifuged for 10 min at 10 000 × *g* and the RNA was extracted from the supernatant as described [17]. Under these conditions all of the cytosolic rRNA remained associated with the pellet, and only the 4 S RNA was recovered from the supernatant. Mitochondrial S 100 supernatant was prepared from purified mitochondria [13]. The pellet was resuspended (35 mg protein/ml) in a standard buffer (0.01 M Tris–HCl, pH 7.8, 0.06 M NH_4Cl, 0.01 M Mg-acetate, 0.02 M mercaptoethanol) containing 10% glycerol [2,11] and sonified (6 × for 15 s, step II, microtip, Branson sonifier). After centrifugation (2 h, 50 000 rpm in a rotor Ti 50, Spinco, Beckman), the upper half of the supernatant was aspirated and dialyzed against the standard buffer. For storage at −20°C this preparation was made 50% in glycerol [11]. Cytosolic S 100 supernatant was prepared from locust muscle: 1 g of wet weight cells per ml was homogenized in the standard buffer in a Dual homogenizer with Teflon pestle (10 strokes). The homogenate was centrifuged for 15 min at 12 000 × *g* and then recentrifuged for 2 h at 226 000 × *g*. The upper half of the supernatant was treated as described above. The conditions for aminoacylation of the tRNAs were those described [22]. ^3H- or ^{14}C-labeled amino acids with high specific activities (The Radiochemical Centre, Amersham) were used. The bands indicated in Lane A and B corresponded to the tRNAs charged with leucine or methionine, respectively, by the use of the homologous tRNA synthetase preparations. (b) Two-dimensional gel electrophoresis (10 and 20% polyacrylamide) of mitochondrial tRNA. The designation of the specific tRNAs was reached in several runs under identical conditions, in which the mitochondrial tRNA had been charged with labeled amino acids. Technical details were as described [22].

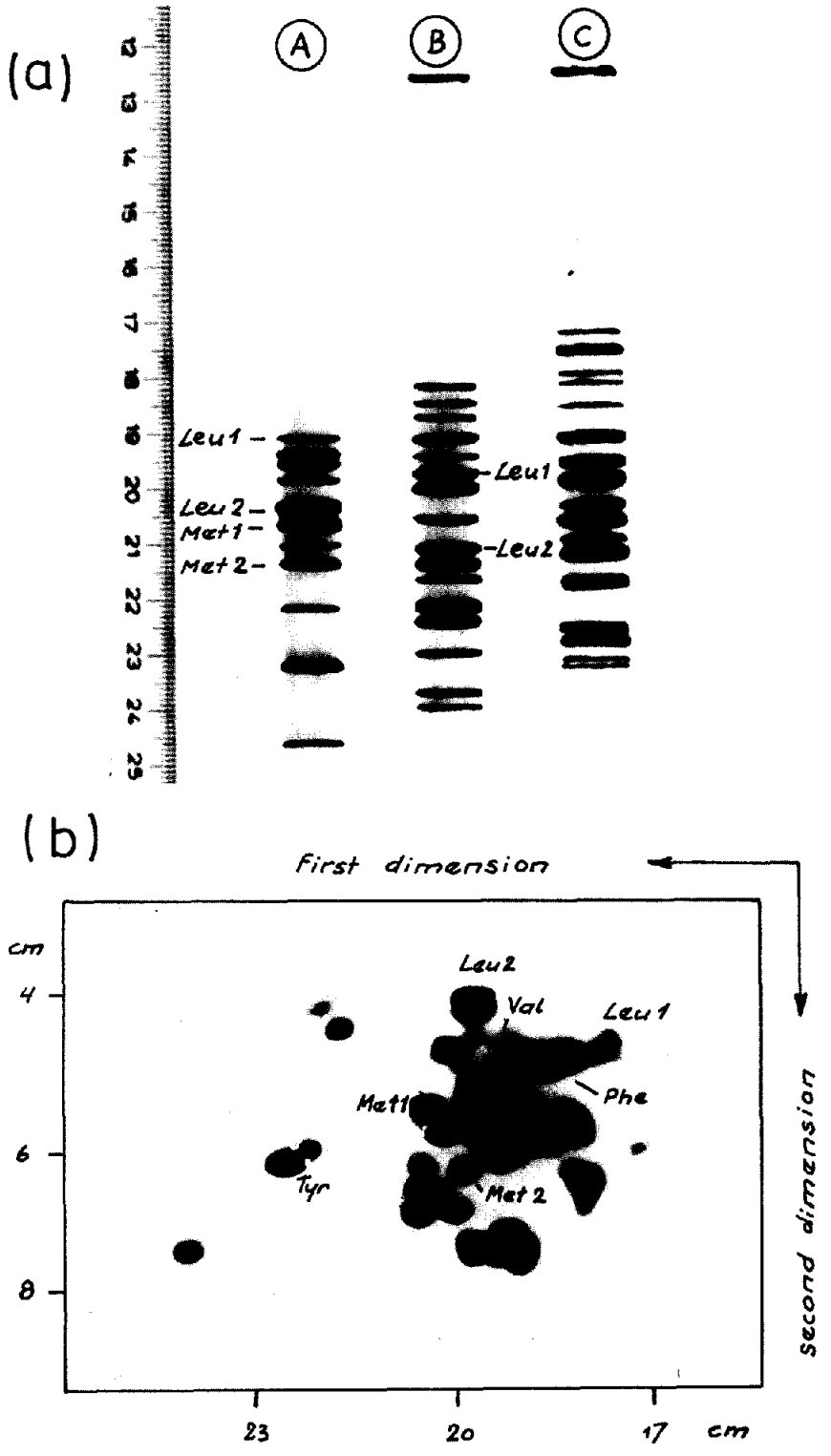
(a)
A
B
C
Leu 1 –
Leu 2 –
Met 1 –
Met 2 –
Leu 1
Leu 2
(b)
First dimension
Second dimension
cm
4
6
8
Leu 2
Val
Leu 1
Met 1
Phe
Tyr
Met 2
23
20
17
cm

Fig.2

cytosolic tRNA, as the mitochondria were highly purified. A contamination exceeding 4% should have been detected in electrophoreses on 2.5% gels (W. Kleinow, to be published) since the mitochondrial RNAs (including the 4 S RNA) migrated to different positions as compared to the cytosolic counterparts. Thus, we prefer the interpretation that locust mitochondria contain a full complement of tRNA species, possibly some minor tRNA species.

Furthermore, we were able to demonstrate that several of the spots resolved on two-dimensional electrophoresis corresponded to specific tRNAs (fig.2b). The existence of isoaccepting tRNA species was shown for leucine and methionine. For the other amino acids, which were tested, no isoacceptors were found. It may be noted that the isoacceptors for leucine in the cytoplasm and in the mitochondria differed in their electrophoretic mobility (fig.2a).

3. Discussion

From the experiments reported here, we suggest that locust mitochondria possess their own, endogenously specified population of tRNAs and ribosomal RNAs. This is supported by our finding that the (G+C) content of locust mitochondrial RNA is extremely low (the values reported here are the lowest percentages of (G+C) so far found for mitochondrial RNAs). A low (G+C) content is expected for these RNAs, if they are transcription products of the mitochondrial genome which is a DNA rich in (A+T) residues. Because of the small quantity of mitochondrial tRNA available, we have not specified acceptor activity for all amino acids. However, the existence of at least 27 different tRNA species indicates that locust mitochondria contain a full complement of tRNAs, with some isoaccepting tRNA species. On the other hand, the number of mitochondrial tRNAs is by far lower than that of a cytosolic tRNA population. The coding capacity even of a small mitochondrial genome would be sufficient for the two ribosomal RNAs, which are shorter than the cytosolic counterparts, and about 20–30 tRNA species.

In some respects, our results are similar to those reported recently for the tRNAs from yeast mitochondria [14–16]. Further work is intended to characterize locust mitochondrial DNA and its hybridizing properties for locust mitochondrial tRNA. A preliminary note on the data reported here has been published [23].

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