Acta Biochimica Polonica

Vol. 42 No. 2/1995

227–232

QUARTERLY

Mitochondrial tRNA in hyperthyroidism

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Received: 2 October, 1994; accepted: 3 February, 1995

Key words: mitochondrial tRNA, thyroidism

The mitochondrial tRNA were prepared from liver and brain tissues of thyroxinized and control rabbits. The presence of tRNA for twenty amino acids both in liver and brain mitochondria was revealed. The quantity of radioactive amino acids bound to the mitochondrial tRNA was higher in hyperthyreosis than in control animals but considerable differences between the brain and liver tissues were observed.

Mammalian mitochondrial genome consists of circular DNA molecules containing a 16569 base-pairs that allow to encode mitochondrial mRNA and tRNA and several mitochondrial proteins [1, 2]. Heterogeneity of RNA in brain mitochondria as well as the aminoacyl-tRNA synthetase activity have been well documented [3–5].

Thyroid hormones that regulate gene expression, affect nuclear receptors for steroid and thyroid hormone as well as their respiratory activity [6–15]. It has been shown that triiodothyronine exerts some influence on the transcription of mitochondrial genes [1, 2].

Our previous experiments [16, 17] carried out on thyroxinized rabbits have shown a marked increase of acceptor activity of cell tRNA and increased activity of aminoacyl-tRNA synthetases. This effect can be explained by stimulation of nuclear, or may be even mitochondrial, genes. The aim of the present study was to evaluate the properties of mitochondrial tRNA isolated from thyroxinized rabbits.

MATERIAL AND METHODS

The experiments were carried out on 12-week-old rabbits of mixed breed. In the experimental group, hyperthyroidism was evoked by intramuscular administration of L-thyroxine (Sigma Chemical Co., St. Louis, U.S.A.) in a dose of 200 μg/kg body weight during four consecutive days [18]. The level of triiodothyronine and thyroxine in serum was determined by the fluorescence polarization immunoassay method [19]. The livers and brains were taken both from the thyroxinized and control rabbits.

Mitochondria were obtained from these tissues (isolated according to the method of Clark & Nicklas [20]). The homogenized tissues were centrifuged at 2000 × *g* for 15 min, and supernatants were centrifuged at 15000 × *g* for 30 min. The purity of mitochondrial preparations was checked by electron microscopy. tRNA was isolated from mitochondrial preparations by phenol extraction according to Sein *et al.* [21] as modified by Zubay [22]. Next, it was fractionated by DEAE 52 column chromatography, and deaminoacylated [23]. Concentration of tRNA was determined at 260 nm and expressed in absorbance units.

Aminoacyl-tRNA synthetases were obtained from liver and brain of control rabbits. The homogenized tissue was centrifuged at 5000 × *g* for 30 min, and microsomes were removed by ultracentrifugation at 105000 × *g* for 2 h. The

enzyme was precipitated at 0.4–0.7 $(NH_4)_2SO_4$ saturation [24]. The precipitated fraction was dialysed against two changes of 0.05 M standard Tris/HCl buffer, pH 7.5, containing 5 mM KCl, 1 mM $MgCl_2$, 0.5 mM phenylmethylsulphonyl fluoride (PMSF) and 5% glycerol.

Protein content in the preparations was determined by the method of Bradford [25].

The aminoacylation of tRNA was determined by measuring binding of ^{14}C-labelled amino acids to tRNA. The assay system consisted, in a total volume of 250 µl, of: 100 mM Tris/HCl buffer, pH 7.5, 10 mM $MgCl_2$, 10 mM ATP, 10 mM KCl, 0.4 mM dithiothreitol (DTT), 0.1 mM PMSF, 3.0 A_{260} units of tRNA, 50 µg of enzymic protein, and 18.5 kBq ^{14}C-labelled amino acids. The incubation was carried out at 37°C for 20 min. From the incubation mixture, 100 µl samples were withdrawn and transferred onto Whatman 3 MM discs, which were rinsed four times with cold trichloroacetic acid, and then by the Hokin solvent (0.8 ml 10 M KOH, 62.8 ml glacial acetic acid and 95% ethanol *ad* 1 litre) and finally with ethyl ether. Radioactivity was measured after drying the discs, in an Intertechnique-France scintillation counter. The results were statistically evaluated by the Cochran and Cox test *C*.

RESULTS

Thyroxine injected intramuscularly caused the state of hyperthyroidism in rabbits as confirmed by determination of total plasma levels of thyroxine and triiodothyronine. The level of thyroxine was 0.24 µg/ml and of triiodothyronine 8.0 ng/ml. The levels of thyroxine and triiodothyronine in control animals was 0.15 µg/ml and 0.9 ng/ml, respectively. In each experiment the mitochondria were prepared from one liver and from one brain. Electron microscopy (Fig. 1) showed that the liver mitochondria obtained from thyroxinized animals were larger and more swollen than those from control animals, whereas the brain mitochondria of thyroxinized and control rabbits (Fig. 2), were similar. From mitochondrial preparations of thyroxinized liver we obtained 100–262.5 A_{260} of tRNA (average 210 A_{260}), while from mitochondria of control livers 22.5–112.5 A_{260} of tRNA (average 64.55 A_{260}). Similarly, from mitochondrial preparations of thyroxinized brain we obtained 28–112 A_{260} of tRNA (average 81.21 A_{260}), while from control preparations of brain mitochondria 13.5–60 A_{260} of tRNA (average 41.71 A_{260}). In both cases statistically significant differences were found by the Cochran and Cox test ($P < 0.008$).

Determination the aminoacylation extent of tRNA from liver and brain mitochondria evidenced that they were able to bind all the 20 amino acids tested. In thyroxinized animals the aminoacylation of tRNA obtained from brain and liver was higher than in controls with respect to most of the amino acids tested (Fig. 3, 4). The ratios of aminoacylation of mitochondrial tRNA in brain and liver in hyperthyroidism and euthyreosis are presented in Table 1. Both in brain and liver these ratios exceeded the value of 1 for all the amino acids tested. In the brain, the ratio was the highest for the phenylalanine tRNA, with gradually decreasing values for the proline, leucine, lysine, tyrosine, glutamic acid, aspartic acid, alanine, serine, cysteine and asparagine specific tRNAs. Actually, the aminoacylation of tRNA in mitochondria for the amino acids mentioned above was more than twice as high in thyroxinized rabbits than in control animals. In the liver, the

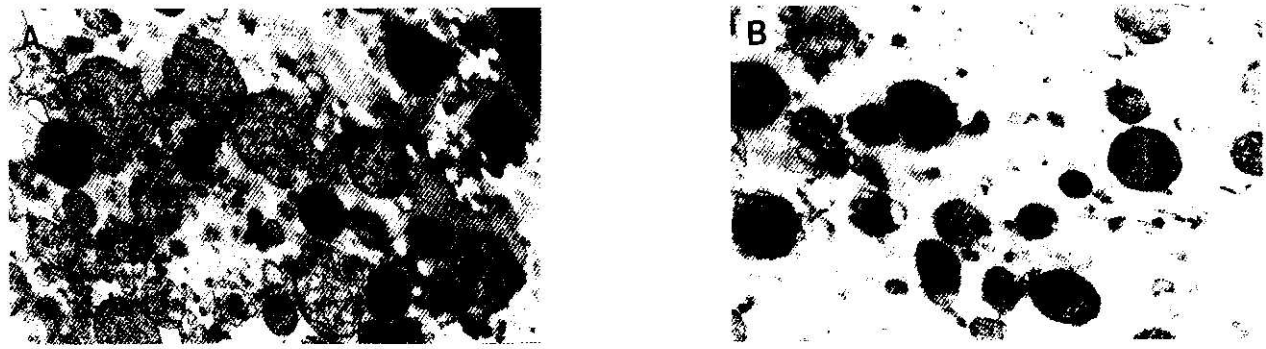

Fig. 1. The electron microscopy of mitochondria from liver of rabbit. A, thyroxinized; B, control.

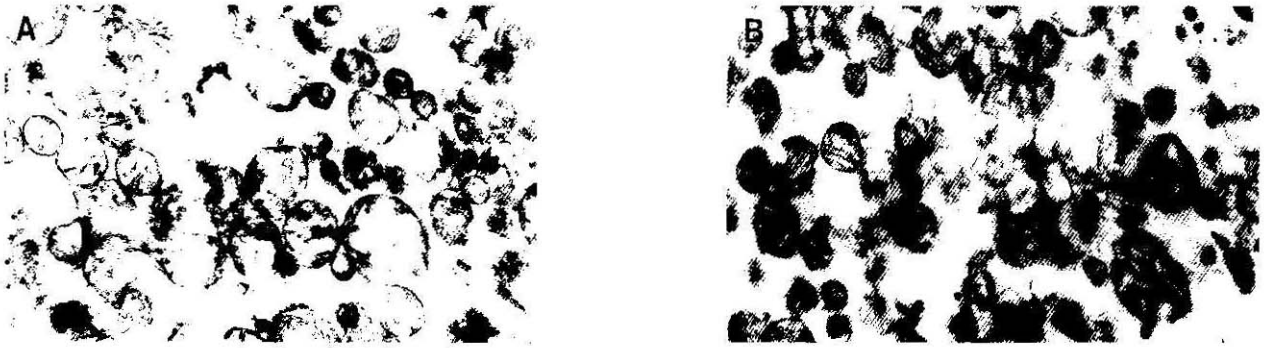

Fig. 2. The electron microscopy of mitochondria from brain of rabbit. A, thyroxinized; B, control.

Table 1

The ratios of acceptor activity of mitochondrial tRNA in hyperthyroidism and euthyreosis (control animals).

The values were calculated from eight experiments.

Amino acids	Brain	Liver
Alanine	2.47	1.39
Glutamic acid	2.63	2.14
Phenylalanine	6.71	2.04
Valine	1.42	2.14
Arginine	1.41	5.39
Serine	2.31	1.61
Isoleucine	1.03	1.89
Aspartic acid	2.56	2.95
Tyrosine	2.77	1.33
Lysine	3.00	1.08
Methionine	1.19	3.42
Leucine	5.57	2.53
Histidine	1.49	1.65
Glycine	1.11	1.05
Aspargine	2.15	2.15
Glutamine	1.98	3.09
Proline	5.91	4.18
Cysteine	2.21	1.23
Tryptophan	1.67	1.26
Threonine	1.66	3.60

ratio was particularly high for arginine, proline, threonine, methionine, glutamine, aspartic acid and leucine, and somewhat less so for asparagine, glutamic acid, valine and phenylalanine.

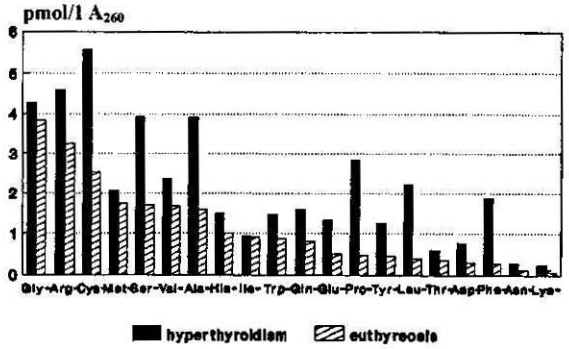

Fig. 3. The aminoacylation of tRNA in liver mitochondria.

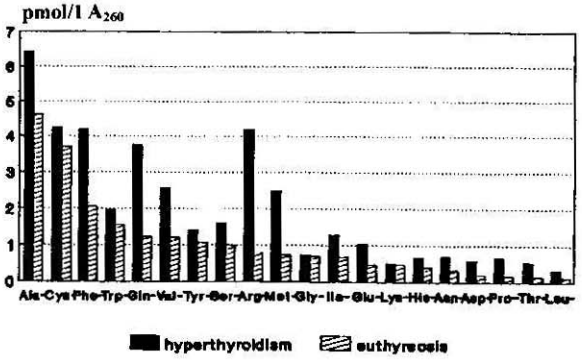

Fig. 4. The aminoacylation of tRNA in brain mitochondria.

DISCUSSION

Previous investigation have shown that in thyroxinized rabbits the aminoacylation of cellular tRNA is increased [16, 17]. Obviously, the mitochondrial genome contains informations that encode all specific tRNAs [1, 2]. It could be assumed that the increase of the total cellular tRNA level was partly due to stimulation of its own biosynthesis also in mitochondria. This was confirmed by our current results. We ob-

served a distinct increase of aminoacylation of tRNA in brain and liver mitochondria of thyroxinized rabbits and the ratios of aminoacylation of all mitochondrial aminoacyl-tRNAs in hyperthyroidism and euthyreosis were greater than 1. Coordination of nuclear and mitochondrial genome expression is poorly understood; however, it has been observed during proliferation, differentiation and cell transformation [1, 3, 12–15, 26, 27]. Our experiments suggest simultaneous stimulation of nuclear and mitochondrial genes following thyroxine treatment. As a matter of fact, this refers only to genes connected with tRNA synthesis.

CONCLUSIONS

–1. Significant increase of aminoacylation of mitochondrial tRNA for 20 amino acids was shown in thyroxinized rabbits.
–2. The analysis of aminoacylation of different mitochondrial tRNA indicate for considerable differences between the brain and liver tissues.

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