ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. LIV, 6 SECTIO D 1999

Katedra i Zakład Chemii Ogólnej Akademii Medycznej w Lublinie
Kierownik: prof. dr hab. Kazimierz Pasternak

Katedra i Zakład Chemii Fizjologicznej Akademii Medycznej w Lublinie
Kierownik: prof. dr hab. Marta Stryjecka-Zimmer

KAZIMIERZ PASTERNAK, STANISŁAWA SZYMONIK-LESIUK,
HALINA BRZUSZKIEWICZ-ŻARNOWSKA, TOMASZ BORKOWSKI

Analysis of mitochondrial tRNAs in hyperthyroidism

Analiza mitochondrialnych tRNA w nadczynności tarczycy

Our previous experiments have shown that thyroxine potentiates the acceptor activity of both cytoplasmic and mitochondrial tRNA (15, 17). This is due to the ability of thyroxine to stimulate the nuclear and, mitochondrial genome (l, 5–6, 8, 10–13, 18–19). The mitochondrial genome of neoplasmal cells has been well known. The existence of specific tRNA for twenty amino acids has been found in cancer mitochondria (3, 9). In order to enrich our data concerning the role of thyroxine in the metabolism of mitochondrial tRNA we made an effort to perform a qualitative analysis of mitochondrial tRNA of brain and liver of thyroxinized rabbits.

METHODS

The experiments were carried out on 12 week–old rabbits of mixed breed. Hyperthyroidism was evoked by intramuscular administration of L–thyroxine (Sigma Chemical Co., St. Louis) in a dose of 200 mg/kg body weight, during four consecutive days (2). The level of triiodothyronine and thyroxine in serum was determined by fluorescence polarization immunoassay method (14) The livers and brains were taken from the thyroxinized and control rabbits.

Mitochondrial praparations were obtained from these tissues. The homogenized tissues were centrifuged at 2000 g for 15 min., and supernatant was centrifugated at 15000g for 30 min. The tRNA preparation was obtained from these mitochondrial preparations by phenol extraction according to Sein and Zubay (16, 20). Then, it was fractioned by DEAE 52 column chromatography, and deaminoacylated (7). tRNA concentration was determined spectrophotometrically and expressed in absorbance units.

The electrophoresis was carried on PAG in one and two dimensions. For first dimension 10% gel and for second dimension 20% gel was used (4). After electrophoresis detection of the tRNAs by coloured with stain–all.

RESULTS

One-dimensional electrophoresis (PAGE) has revealed significant differences in the amount of bands in mitochondrial tRNA of brain and liver (Fig. 1) in control and thyroxine–treated rabbits. Moreover, in hyperthyroidism some additional bands were detected.

tRNA isoacceptors map in human myometrial tissue (4) was used for comparison with our results 2D PAGE of tRNA (Fig. 2). Two-dimensional electrophoresis on PAG made possible the separation and comparison of spots of mitochondrial tRNAs of brain and liver of thyroxinized rabbits. Identical quantities of tRNA were used for each electrophoresis. When comparing the results of two–dimensional electrophoresis m PAG we used the numerous spots of tRNAs in the brain and the liver. The electrophoretogram of liver mitochondial tRNA of thyroxinized rabbit contains 35 distinct spots while there are only 19 in control tissues (Fig. 3). Similarly, brain mitochondrial tRNA in hyperthyroidism exhibits 25 spots in electrophoresis compared to 21 in control rabbits (Fig. 4). The spots numbered 1–4 refer to the acceptor activity of tRNA specific for serine.

DISCUSSION

In previous experiments we showed higher quantities of mitochondrial tRNA in rabbits with hyperthyroidism in comparison with rabbit control (17). The increased quantities of mitochondrial tRNA accompany increased quantities of individual, specific tRNA. In comparison with rabbit controls in which the quantity of specific tRNA was twenty, the quantity in rabbits with hyperthyroidism was distinctly greater. This fact may be explained by the appearance of tRNA isoacceptors. Experiments conducted at this time do not permit us to determine the increase in mitochondrial tRNA isoacceptors in animals with hyperthyroidism. Perhaps the expression of mitochondrial genome was increased under the influence of thyroxine. This suggestion requires further experimentation.

CONCLUSIONS

1. Two–dimensional electrophoresis (PAGE) has revealed the occurrence of various specific mitochondrial tRNA obtained from rabbit brain and liver.

2. In turn, the increased amount of spots of mitochondrial tRNAs in thyroxinized rabbits compared to control animals has been observed.

REFERENCES

1. Andersson M.L. et al.: Thyroid hormone alters the DNA binding properties of chicken thyroid hormone receptors α and β. Nucleic Acid Res., 20, 4803–4810, 1992.
2. Arai M. et al.: Effect of thyroid hormone on the expression of mRNA encoding sarcoplasmic reticulum proteins. Circ. Res., 69/2, 265–276, 1991.
3. Baggetto L.G.: Mitochondrie et cancer. Regard sur la Biochime, 4, 48–54, 1992.

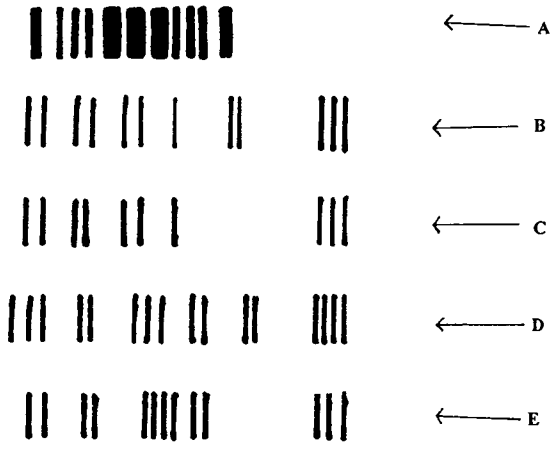


Fig. 1. Electrophoretogram of one dimensional tRNA spearation in PAGE; A – cytosolic liver tRNA, B – mitochondrial brain tRNA – hyperthyroidism, C – mitochondrial brain tRNA – control, D – mitochondrial liver tRNA – hyperthyroidism, E – mitochondrial liver tRNA – control

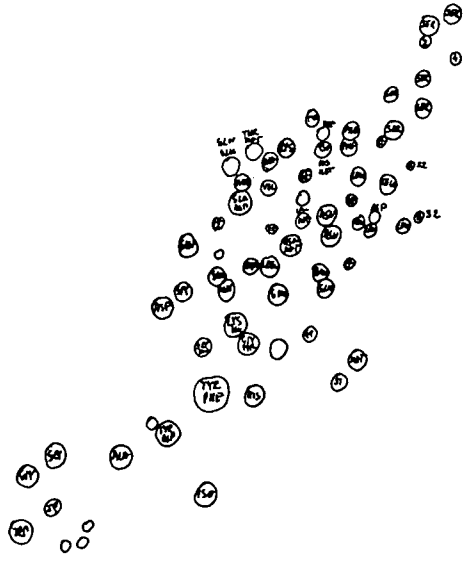
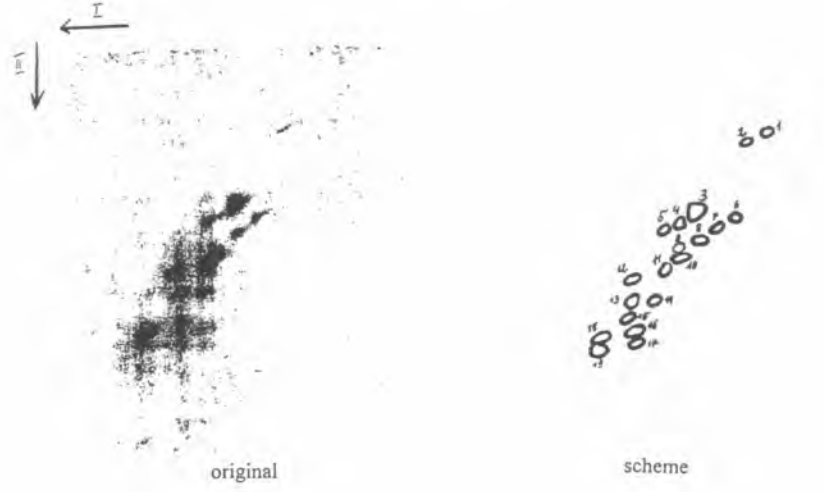


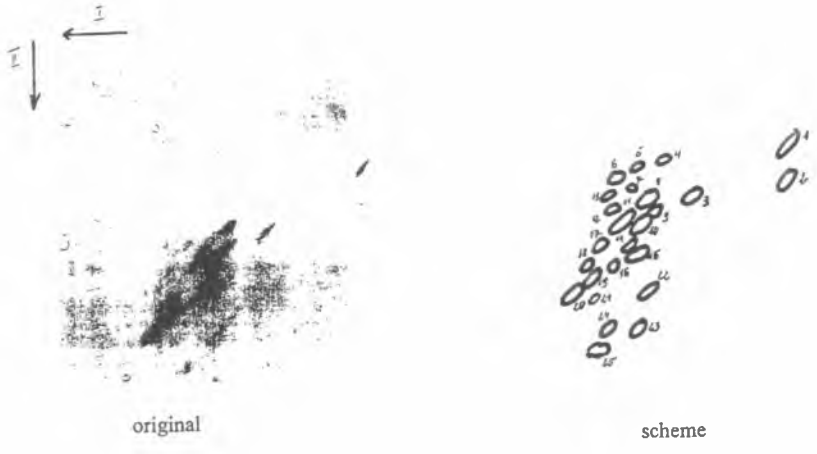
Fig. 2. tRNA isoacceptors map in human myometrial tissue

A - hyperthyroidism



B – control

Fig. 3. 2D PAGE mitochondrial tRNA of rabbit liver



A - hyperthyroidism

B – control

Fig. 4. 2D PAGE mitochondrial tRNA of rabbit brain

4. Baranowski W.: Badania cytoplazmatycznych transportujących kwasów rybonukleinowych (tRNA) w tkankach nowotworów złośliwych jajnika u kobiet. Praca habilitacyjna, AM Lublin, 1994.
5. Brand M.D. et al.: The mechanism of the increase in mitochondrial proton permeability induced by thyroid hormones. Eur. J. Biochem., 296, 775–781, 1992.
6. Datta S. et al.: Thyroid hormone receptor mediates transcriptional activation and repression of different promotors *in vivo*. Molecular Endocrinology. 6, 81 S–825, 1992.
7. Denney R.M.: Detection and partial purification of rapidly sedimenting forms of aminoacyl-transfer ribonucleic acid synthetases from human placenta. Arch. Biochem. Biophys., 183, 156–167, 1977.
8. Glass Ch.K., Holloway J.M.: Regulation of gene expression by the thyroid hormone receptor. Biochim.Biophys. Acta, 1032, 157–176, 1990.
9. Goglia F. et al.: Mitochondrial DNA, RNA and protein synthesis in normal and mildly hypothyroid rat liver during cold exposure. Mol. Cell. Endocrinol., 55, 141–147, 1988.
10. Jannini E.A. et al.: Developmental regulation of the thyroid hormone receptor alpha 1 mRNA expression in the rat testis. Mol. Endocrinol., 8, 89–96, 1994.
11. Karin M. et al.: Various modes of gene regulation by nuclear receptors for steroid and thyroid hormones. Eur. J. Clin. Pharm., 45, 9–15, 1994.
12. Mano H. et al.: Thyroid hormone affects the gene expression of retionid X receptors in the adult rat. J. Biol. Chem., 269, 1591–1594, 1994.
13. Nagaya T. et al.: Thyroid hormone receptor mutants that cause resistance to thyroid hormone. J. Biol. Chem., 267, 13014–13019, 1992.
14. Oppenheimer J.H.: Role of plasma proteins in the binding, distribution and metabolism of the thyroid hormones. N. Engl. J. Med., 278, 1153–1161, 1968.
15. Pasternak K. et al.: Activity of aminoacyl-tRNA synthetases in experimental hyperthyroidism in muscle tissues of the rabbit. Acta Biochimica Polonica 41, 35–38, 1994.
16. Sein K.T. et al.: A simple modified method for the extraction of rat liver sRNA. Anal. Biochem., 28, 65–69, 1969.
17. Szymonik-Lesiuk S. et al.: Influence of hyperthyroidism on acceptor activity of tRNA in some rabbit tissues. Appl. Biol. Commun., 3/1–2, 1–8, 1993.
18. Tallini G. et al.: Analysis of nuclear and mitochondrial DNA alterations in thyroid and renal oncocytic tumors. Cytogenet. Cell Genet., 66, 253–259, 1994.
19. Yen P.M., Chin W.W.: New advances in understanding the molecular mechanisms of thyroid hormone action. Trends Endocrinol. Metab., 5, 65–72, 1994.
20. Zubay G.: The isolation and fractionation of soluble ribonucleic acid. J. Mol. Biol., 65, 375–378, 1972.

Otrz.: 1999.02.12

STRESZCZENIE

Celem pracy było przeprowadzenie analizy tRNA otrzymywanych z wątroby i mózgu królików, u których sztucznie wywołano hipertyreozę i kontrolnych. Do rozdziału uzyskiwanych tRNA zastosowano metodę dwukierunkowego rozdziału w żelu poliakrylamidowym. Wykazano, że większa ilość plam izoakceptorowych tRNA występowała w przypadku tRNA mózgu i wątroby królików z hipertyreozą. Przeprowadzono częściową identyfikację plam izoakceptorowych tRNA. Plamy izoakceptorowych tRNA 1–4 wykazywały aktywność akceptorową specyficzną dla seryny.