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Aminoacylation of tRNA in thyroid glands

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In pathologically changed tissues, especially in those less morphologically differentiated, the intensity of translating processes is significantly increased. This manifests itself in a higher content of tRNA and by the appearance of isoacceptor tRNA, characteristic of these tissues.

Table 1
Aminoacylation of tRNA in vitro

Amino acid	Thyroid adenoma (struma nodosa) pmole / A_{260}	Bovine thyroid pmole / A_{260}
Asparagine	1.01	0.79
Tyrosine	1.23	1.04
Proline	0.49	0.31
Glutamic acid	0.75	0.15
Methionine	1.95	1.56
Glutamine	5.39	0.98
Valine	0.42	0.41
Tryptophan	4.24	1.82
Cysteine	10.79	8.16
Phenylalanine	0.70	0.67
Aspartic acid	1.33	0.50
Histidine	0.65	0.84
Isoleucine	0.60	0.23
Lysine	1.03	0.59
Leucine	0.37	0.09
Arginine	1.30	0.70
Alanine	1.61	3.20
Glycine	2.89	0.96
Serine	1.25	0.43

Hormone-dependent tissues possess a particular kind of regulation of transcription and translation, which was demonstrated in our previous works [1, 2]. In the thyroid, which is a subject to neurohormonal regulation, effectiveness of synthesis of thyreoglobulin as well as of thyroxine and triiodothyronine is altered in different functional conditions (hyper- and hypothyroidism).

The results of our previous studies indicated that the extent of tRNA aminoacylation *in vivo* depends on the character of the tissue [3]. This led us to undertake comparative studies on the degree of RNA aminoacylation in the normal and adenomous thyroid gland. Human thyroid with a struma nodosa was taken during strumectomy. Bovine thyroid gland served as physiological material.

Preparations of tRNA obtained by phenol extraction from the tissues by the method of Sein *et al.* and Zubay [4, 5] were fractionated by chromatography on DEAE 52 column. Concentration of tRNA was determined spectrophotometrically. The preparation of aminoacyl-tRNA synthetase (ARS) was obtained according to Charęziński & Borkowski [6], and protein content determined by the method of Lowry *et al.* [7].

The degree of aminoacylation of specific tRNA *in vivo* was determined on the basis of differences in binding of radioactive amino acids by tRNAs before and after deaminoacylation *in vitro*. Deaminoacylation was conducted by splitting off amino acids at elevated pH [8]. Preparations of tRNA were incubated at 37°C for 10 min with homogeneous ARS in the presence of nineteen ^{14}C-amino acids. The incorporating system consisted of the follow-

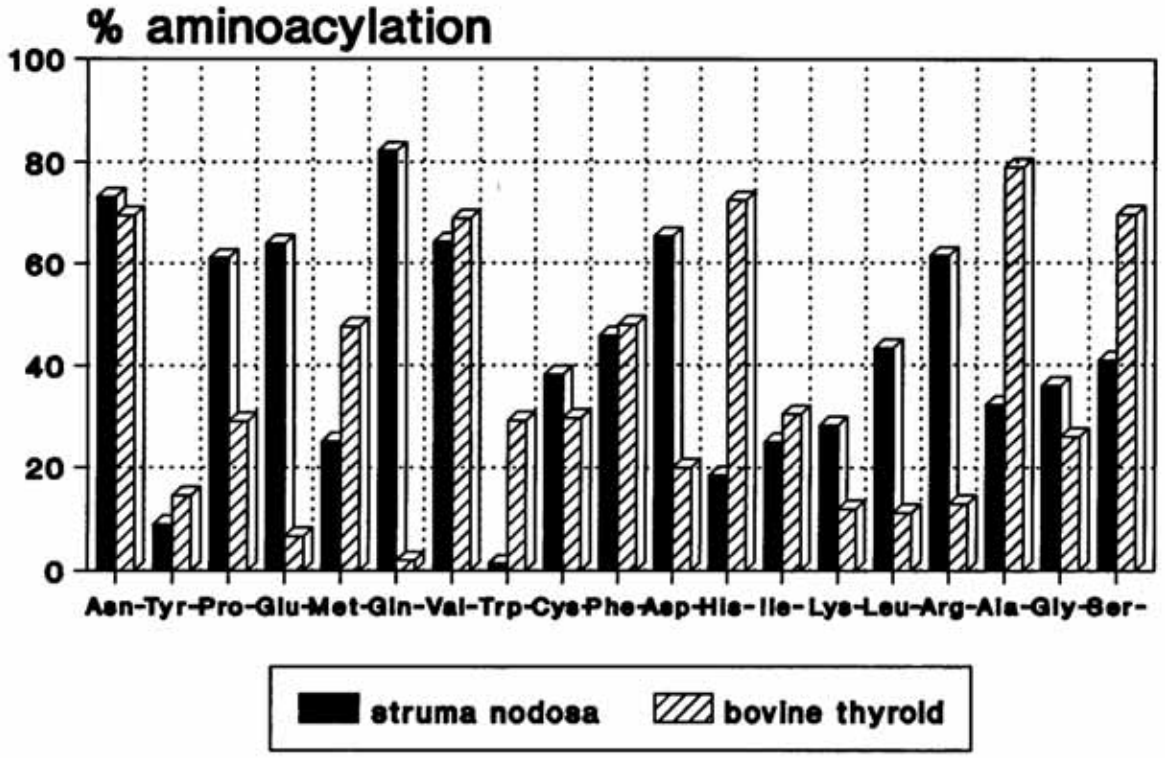

Fig. 1. Aminoacylation of tRNA in vivo.
The binding of radioactive amino acids by tRNA after deaminoacylation was taken as 100 per cent

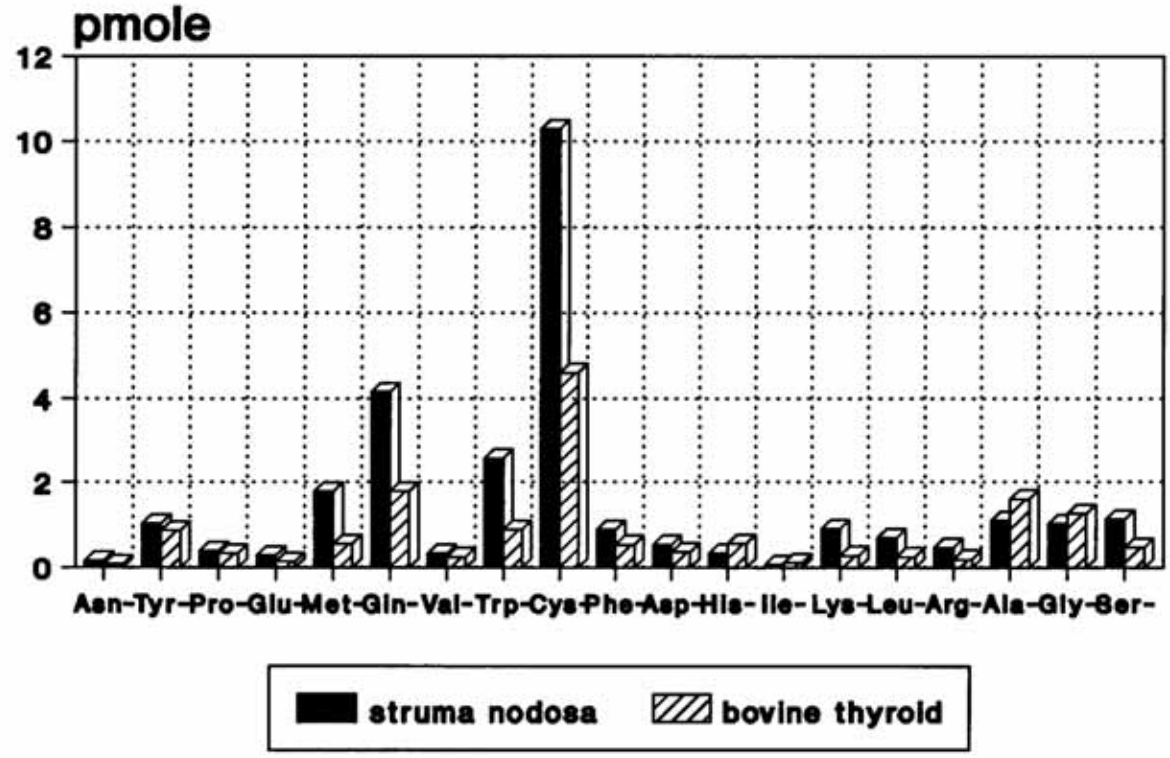

Fig. 2. Aminoacylation of tRNA in vitro

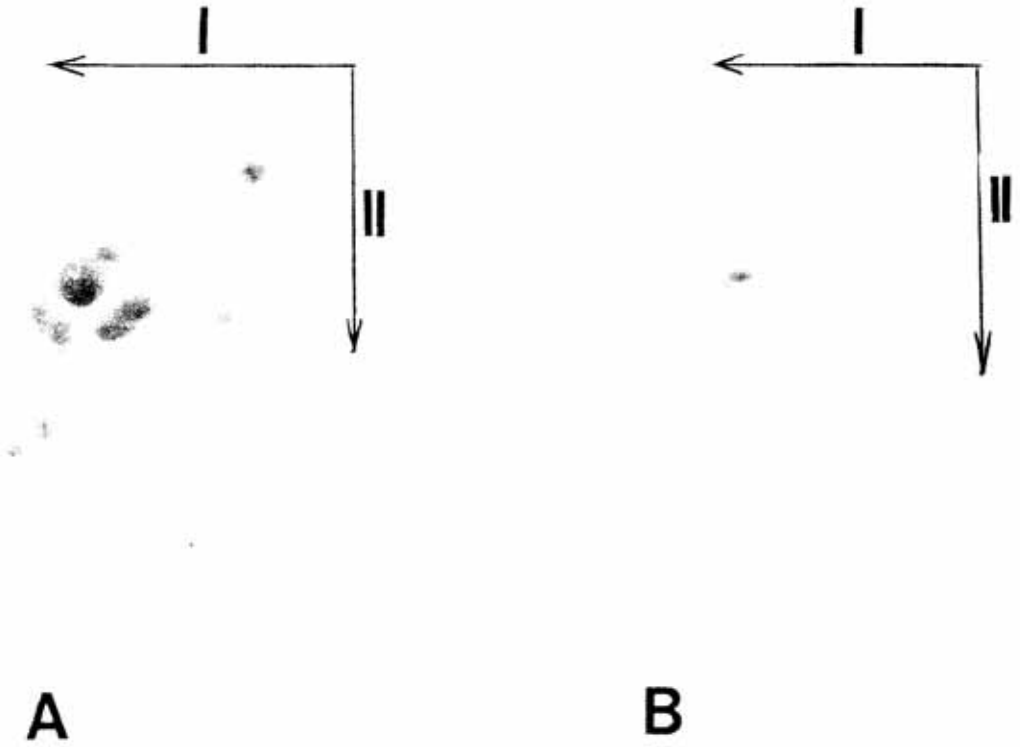

Fig. 3. Two-dimensional electrophoresis of thyroid tRNA in polyacrylamide gel.
A, bovine gland; B, struma nodosa. The electrophoresis was run in the first dimension in 10% gel (48 h, 300 V, 4°C) and 0.9 M Tris/borate buffer, pH 8.3, containing urea and EDTA [9], and in the second dimension in 20% gel, in the same buffer containing urea but not EDTA (72 h, 300 V, 4°C). The tRNA was detected with stains-all

ing components in a total volume of 300 µl : 100 mM Tris/HCl buffer, pH 7.5, 10 mM $MgCl_2$, 10 mM ATP, 10 mM KCl, 0.4 mM dithiothreitol, 0.1 mM phenylmethylsulfonylfluoride, 1.0 A_{260} units of tRNA, 50 µg of enzymes, and ^{14}C-amino acids (18.5 kBq). In blanks the enzymes were omitted.

Samples of 100 µl were applied onto Whatman 3 MM discs which were rinsed four times in cold trichloroacetic acid and then in Hokin fluid (0.8 ml 10 M NaOH + 62.4 ml glacial acetic acid + ethanol to 1 l) and ether, and dried. Radioactivity was measured in a scintillation counter. Activity was determined on the basis of binding of labelled ^{14}C-amino acids by tRNA.

Two-dimensional electrophoresis of tRNA was run in polyacrylamide gel according to Peacock & Dingman [9].

All tRNA preparations obtained from the bovine thyroid gland and the human thyroid with struma nodosa were subjected to deaminoacylation, and then used for aminoacylation investigations. Significant differences were found in the degree of tRNA aminoacylation *in vivo* between the two tissues for the nineteen amino acids tested: asparagine, tyrosine, proline, glutamine, glutamic acid, methionine, valine, tryptophan, cysteine, phenylalanine, aspartic acid, histidine, isoleucine, lysine, leucine, arginine, alanine, glycine and serine (Fig. 1). A higher degree of aminoacylation was found in the thyroid with the tumour for tRNA's specific for glutamic acid, glutamine, aspartic acid, asparagine, leucine, proline, lysine, glycine, arginine and cysteine. In the bovine thyroid tRNA's specific for tyrosine, glutamic acid, glutamine, lysine, leucine and arginine were characterized by exceptionally low aminoacylation *in vivo*.

Differences in the degree of aminoacylation of specific tRNA's could be related either to differences in the type of glands or could result from the hypertrophic character of the human thyroid with struma nodosa. A higher degree of tRNA aminoacylation found *in vivo* in struma nodosa could testify to a more intense translation in this tissue.

Similar results were obtained on comparing the degree of tRNA aminoacylation *in vivo* in other hormone-dependent tissues [1, 2, 10].

In tRNA preparations obtained from the bovine thyroid and from the human one with the tumour, acceptor activity for nineteen ^{14}C-labelled amino acids was examined as well (Fig. 2).

On the whole, the acceptor activity of tRNA preparations from the two tissues studied was lower than values obtained by other authors [11, 12] which may be explained by differences in the character of the tissues studied.

Generally, greater acceptor activity towards the majority of the 19 amino acids tested was manifested by tRNA's obtained from struma nodosa. Particularly, the high acceptor activity of tRNA for cysteine, glutamine, tryptophan and methionine is to be noted. These observations may suggest accumulation of increased amounts of the tRNA in struma nodosa.

The tRNA preparations from both tissues were subjected to two dimensional electrophoresis on polyacrylamide gel (Fig. 3). The electrophoretic patterns obtained are consistent with the qualitative and quantitative differences between isoacceptor tRNA's obtained from different sources. These suggestions could be confirmed by further research on evaluation and activity of specific isoacceptor tRNA's in the tissues examined.

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