EFFECTS OF THYROID HORMONES ON PHOSPHORYLATION OF LIVER RIBOSOMAL PROTEINS AND ON PROTEIN PHOSPHOKINASE ACTIVITY

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1. Introduction

Different reports have shown that the activity of liver polyribosomes decreases markedly after thyroidectomy [1–3]; this inhibition does not depend on the m-RNA's content but on a modification of the structure of the 80 S ribosomal particle [2, 3]. Simultaneously the activity of the liver pH 5 enzyme is also diminished [3].

Recently, it has been shown that the ribosomal proteins are phosphorylated [4, 7] and that glucagon increases their phosphorylation [8]. On the other hand the activity of soluble protein phosphokinases are stimulated by prolactin [9] and hydrocortisone [10].

These results suggested that some hormones could act, directly or not, on the activity of the translational machinery, through a series of reactions involving protein phosphorylation. The results reported in this work agree with this assumption as the phosphorus content of liver ribosomal proteins and the soluble protein phosphokinases activities are markedly decreased in the absence of thyroid hormones.

2. Materials and methods

2.1. *General*

Sprague-Dawley rats weighing 50–60 g were thyroidectomized; 15 days or later they were fasted overnight and sacrificed. In some experiments, 2 mCi of carrier-free ^{32}P-orthophosphate was injected intraperitoneally 30 min before the sacrifice. Ribosomes, post microsomal supernatant and pH 5 enzyme were prepared from livers as previously described [3].

Ribosomal proteins were purified by a modification of the Schneider technique [4]. The alkali labile protein phosphate was hydrolysed by 1 M NaOH treatment (18 hr at 37°). After precipitation of proteins by PCA (final conc. 20%) inorganic phosphate was isolated according to Delory [11]. Radioactivity was measured in a scintillation spectrometer (Intertechnique) and phosphorus was determined by the Delsal method [12]. Phosphoserine and phosphothreonine were isolated by paper electrophoresis after mild acid hydrolysis of purified ribosomal proteins [13].

The specific activity of the free orthophosphate and of the nucleotide pools was determined using the post microsomal supernatant, deproteinized by PCA treatment (final conc. 10%) and then filtered on a Norit column. The specific activity of P_i was measured on the filtrate. That of the nucleotides and ATP was measured after treatment of the washed charcoal with 5% PCA (15 min at 90°).

Proteins were determined by the Lowry technique.

2.2. *Protein phosphokinase assay*

The protein phosphokinase activity of the pH 5 enzyme was measured at pH 7.0 in the medium previously described [14] containing γ^{32}P-ATP (2×10^{-4} M or 2×10^{-5} M). 3',5'-c-AMP, when added, was at a concentration of 5×10^{-6} M. ^{32}P incorporated in the histones was measured according to Reimann et al. [15].

2.3. *Binding of ^{3}H-cyclic AMP*

The assays were carried out using the method described by Walton and Garren [16] in the presence of ^{3}H c-AMP 10^{-7} M (13 Ci/mmole).

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3. Results and discussion

3.1. *Phosphorus content and ^{32}P pulse labeling of ribosomal proteins*

Table 1 shows that the phosphorus content of liver ribosomal proteins is very significantly decreased (35%) after thyroidectomy. Table 2 shows the results of 3 experiments where the specific activities (cpm ^{32}P/mg of liver ribosomal protein) were measured 30 min after the *in vivo* injection of $^{32}P_i$. The ratio cpm/mg of ribosomal protein is decreased by 45–75% after thyroidectomy. However the specific activity of the P_i pool is also decreased (about 20%); the value for the nucleotide pool is also decreased (10 to 40%). The results (cpm/mg ribosomal protein) were therefore expressed as μg phosphorus/mg of ribosomal protein after correction for the nucleotide specific activity. These corrected values show that: i) during the ^{32}P pulse about 1/5 of the ribosomal protein phosphorus is replaced (0.110/0.534 for the controls and 0.76/0.358 for the thyroidectomized animals); the phosphorus turnover of the ribosomal protein is therefore approximately the same in the two physiological conditions; ii) the pulse labeling is markedly decreased (30 to 60%) after thyroidectomy. Injection, 24 hr before sacrifice, of 3,5,3′-triiodothyronine (25 μg) restores the values to the control.

The product of acidic hydrolysis of the purified ribosomal proteins was analysed by paper electrophoresis [13]; ^{32}P is present as phosphoserine and phosphothreonine.

Table 1
Phosphorus content of liver ribosomal proteins from normal and thyroidectomized rats.

Experiment no.	μg P/mg ribosomal protein	
	Normal rats	Thyroidectomized rats
1	0.500 (2)	0.397 (3)
	0.479 (2)	0.326 (3)
	0.487 (2)	0.290 (3)
2	0.506 (4)	0.300 (4)
	0.525 (4)	0.250 (4)
3	0.535 (1)	0.484 (1)
	0.621 (1)	0.440 (1)
	0.568 (1)	0.388 (1)
	0.552 (1)	0.228 (1)
Mean value	0.534 ± 0.017	0.348 ± 0.035

Phosphorus content (μg P/mg ribosomal protein) was measured as described under Methods. The numbers in parentheses represent the number of livers used in each assay. The mean value and the SEM are calculated in each case.

3.2. *Protein phosphokinase and ^{3}H c-AMP binding activities of the liver soluble fractions*

Fig. 1 represents the kinetics of protein phosphokinase activities of the pH 5 enzymes. Two concentrations of ATP were used (fig. 1a: 2×10^{-4} M; fig. 1b: 2×10^{-5} M). In both cases the enzyme activities are markedly decreased after thyroidectomy. Similar results, but less precise, are obtained with the post microsomal supernatant. Fig. 2 represents a typical experiment where increasing amounts of the two types of pH 5 enzyme were used. The protein phos-

Table 2
^{32}P pulse labeling of liver ribosomal proteins from normal and thyroidectomized rats.

Experiment no.		Specific activity (cpm/μg P)		cpm/	μg P/
		P_i ($\times 10^{-4}$)	Nucleotides ($\times 10^{-3}$)	per mg ribosomal protein	
1	Control	4.3	10	1104	0.103
	Assay	3.7	8	615	0.076
2	Control	2.1	5.3	590	0.110
	Assay	1.6	4.9	196	0.040
3	Control	2.2	5.7	648	0.110
	Assay	1.8	3.4	269	0.079

^{32}P pulse labeling of ribosomal proteins was performed by injection of ^{32}P-orthophosphate (2 mCi) 30 min before sacrifice. The specific activity (cpm/mg of ribosomal protein) was measured and then corrected by the specific activity (^{32}P/mg ATP) of the nucleotide pool established as described under Methods.

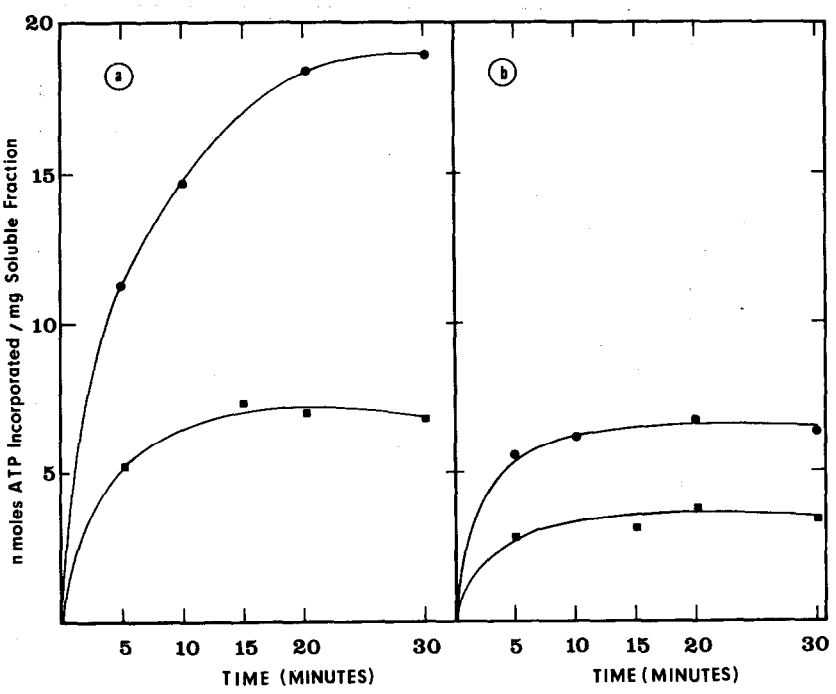


Fig. 1. Kinetics of protein kinase activity of the liver pH 5 enzyme from normal (●—●—●) and thyroidectomized rats (■—■—■). Protein kinase activity (nmoles ATP incorporated/mg of soluble fraction) was measured in the presence of 2×10^{-4} M (a) and 2×10^{-5} M (b) γ ^{32}P-ATP. The incubation mixture (0.5 ml) contained 50 mM β-glycerophosphate, 0.3 mM EGTA, 2 mM theophylline, 10 mM Mg acetate, 1 mM phosphate buffer pH 7.4 and 2 mg calf histones F_2A.

phokinase activity was measured in presence and absence of 3′5′-cyclic AMP. Again it is clear that thyroidectomy decreases markedly (about 50%) the level of enzyme activity. In the presence of saturating amounts of c-AMP (5×10^{-6} M) (fig. 2) both enzyme preparations are moderately activated (1.5–2-fold) but in the same proportion. These results suggest that the differences of activities observed do not depend on an increase in concentration of the regulatory inhibitory subunit which binds the c-AMP but rather on a decrease of both the catalytic and regulatory subunits. This conclusion was therefore controlled by a direct estimation of the binding of ^{3}H c-AMP to the regulatory subunit. Fig. 3 shows the results obtained for the two types of soluble fractions and pH 5 enzymes. A clear decrease of the ^{3}H c-AMP binding activity is observed in both cases.

3.3. *Conclusions*

The results reported suggest that thyroid hormones regulate: 1) the phosphorylation of the ribosomal proteins; the phosphorus content is markedly decreased after thyroidectomy. 2) The protein phosphokinase activity of the liver soluble fraction which is diminished by 50% in the absence of hormones. The ^{3}H c-AMP binding activity is simultaneously decreased in the same proportion. It is therefore likely that the concentration of both subunits of the kinase is reduced.

Different effects of thyroid hormones could be secondary to the regulation of this key regulatory step. It is too early to know whether these results could explain the well known physiological effect of thyroid hormones on growth and on the properties of the soluble and ribosomal components of the liver machinery for protein synthesis. However the observed

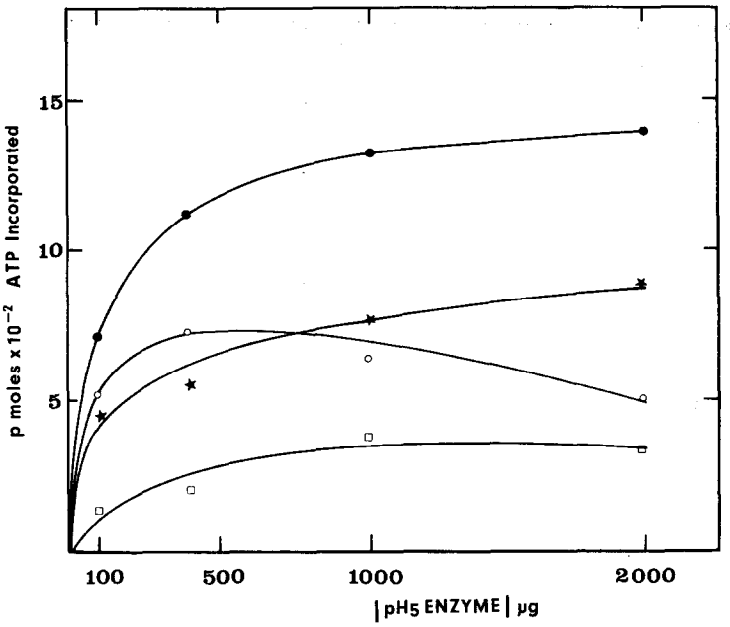
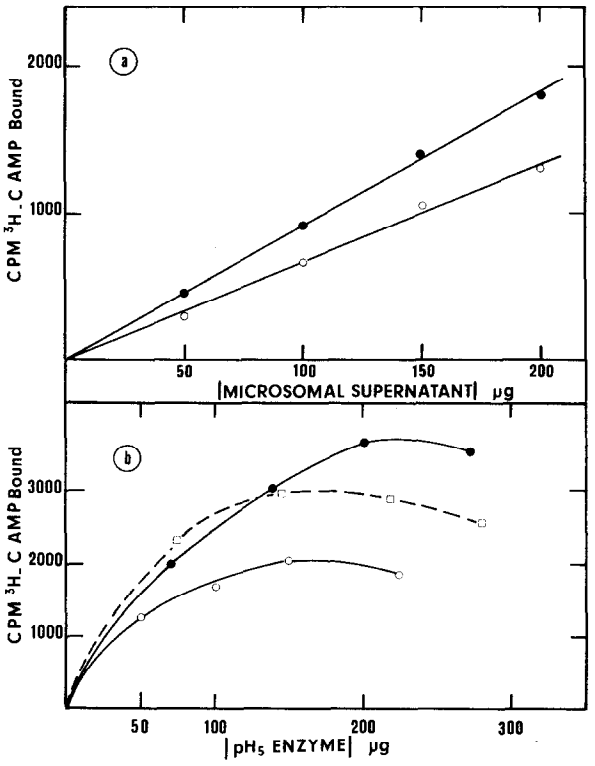


Fig. 2. Protein kinase activity of liver pH 5 enzyme from normal rats in the absence (○—○—○) or presence of c-AMP (●—●—●) and from thyroidectomized rats without (□—□—□) or with c-AMP (★—★—★). Increasing amounts of pH 5 enzyme were incubated 20 min at 30° as described in fig. 1.



decrease in the phosphorus content of ribosomal proteins is the first precise data reported showing a structural difference between the two types of polyribosomes. Correlatively it seems also significant that the protein phosphokinase activity could be regulated by thyroid hormones.

Fig. 3. ^{3}H c–AMP binding activity of the liver post microsomal supernatant or the pH 5 enzyme. Increasing amounts of post microsomal supernatant (a) and pH 5 enzyme (b) were incubated 30 min at 30° in a medium (final vol 0.2 ml) containing 67 mM Tris-HCl buffer, pH 7.4, 10 mM $MgCl_2$, 8 mM theophylline, 10^{-7} M ^{3}H c–AMP (13 Ci/mmole). The liver soluble fraction used was prepared from normal rats (●—●—●), thyroidectomized rats (○—○—○) and thyroidectomized rats injected 24 hr before sacrifice with 25 µg 3,5,3'-triiodothyronine (□—□—□).

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