Specific Phosphorylation of Nuclear Non-Histone Proteins of Rat Liver After Thyreoidectomy and T_4 Treatment

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Abstract. The effect of thyroxine (T_4) on phosphorylation of non-histone proteins in rat liver nuclei was studied. Non-histone proteins obtained by extracting liver nuclei with 0.4 mol . l^{-1} KCl from normal (N), hypothyroid (T_x) and T_x rats treated with T_4 (100 μg kg^{-1} B.W.), respectively, were incubated with ^{32}P—ATP at 30 °C for 5 min followed by sodium dodecyl sulfate-gradient polyacrylamide gel electrophoresis and autoradiographic analysis. Endogenous phosphorylation in T_x rats was decreased. In T_4 treated T_x rats phosphorylation of some proteins was recovered, however the 84×10^3 mol wt protein was not completely recovered. Phosphorylation of nuclear nonhistone proteins was not altered by the addition cAMP into the incubation. These findings show that in vitro phosphorylation of non-histone proteins of the rat liver nuclei occurs in some specific fractions which may be thyroid hormone dependent.

Key words: Liver nuclei — Thyroid hormone — Phosphorylation

Introduction

The metabolic action of thyroid hormones comprises several intracellular reactions. Both 3,5,3′-triiodothyronine (T_3) and thyroxine (T_4) interact with specific binding sites in liver nuclei localized on non-histone proteins (NHP). This binding triggers responses which finally lead to mRNA and protein synthesis (Oppenheimer et al. 1976). Phosphorylation and dephosphorylation of NHP catalyzed by endogenous nuclear protein kinase or phosphatase, respectively, correlate with changes in the rate of RNA synthesis (Kleinsmith et al. 1966).

Recently it has been reported that nuclear fraction extracted from rat liver contains two globulins which may reflect the thyroid status. One of them was present in abundance in nuclei from normal rats: it diminished after thyroidectomy (Tx) and could be restored by T_3 treatment. The other globulin with a lower molecular weight prevailed in Tx rats and it was reduced by T_3 treatment of both normal and Tx rats (Barsano et al. 1980).

A marked endogenous phosphorylation of NHP was observed in both intact and T_3-treated Tx rats, however the identity of the phosphorylated proteins remained unknown (Taningher et al. 1977; Coleoni and DeGroot 1980).

The present studies were undertaken to examine whether some of the rat liver NHP are predominantly phosphorylated after T_x or T_4 treatment. Our results suggest that at least five distinct proteins have to be considered in this respect.

Materials and Methods

Adult male SPF Wistar rats weighing 200—250 g were used. One group of animals were thyreo-parathyreoidectomized (group T_x) and then were given drinking water containing 0.5% $CaCl_2$ and 0.1% 6n-propylthiouracil (PTU). During three weeks 100 μg T_4 per kg B.W. in 0.05—0.1 ml N NaOH — 0.9% NaCs was administrated to a subgroup of Tx animals intraperitoneally, 24 h after the T_4 administration animals were sacrificied by decapitation together with the remaining untreated Tx rats and intact controls. All the animals were fed a pelleted standard laboratory diet containing 60 μg iodine (100 g of food).

Isolation of nuclei

Livers were removed immediately after the death of the animals and perfused with ice-cold STM buffer containing (0.25 mol . l^{-1} sucrose; 1.1 mmol . l^{-1} $MgCl_2$; and 20 mmol . l^{-1} Tris-HCl) via the portal vein. The liver cells nuclei were prepared by sedimentation in a sucrose solution (Brtko et al. 1981).

Preparation of nuclear extract

A total of 1×10^8 nuclei was suspended in 2 ml of 0.4 mol . l^{-1} KCl with 5 mmol l^{-1} dithiothreitol. After 24 h extraction at 0—4 °C the suspension was centrifuged at 7000 xg for 10 min and the supernatant, as the main non-histone protein fraction, was dialysed against 10 mmol . l^{-1} Tris buffer (pH 7.8) and stored for analysis.

Assay of phosphorylated non-histone proteins

The phosphorylation reaction was carried out in small disposable tubes (70 × 9 mm) in a total volume of 200 μl medium (containing 50 mmol . l^{-1} Hepes; 10 mmol . l^{-1} $MgCl_2$;; 10 μmol . l^{-1} cAMP or H_2O; pH 7.8) with the nuclear extract in a final concentration of 2 mg ml^{-1} protein. The reaction was initiated by the addition of 20 μl of 1 pmol . l^{-1} γ-^{32}P-ATP (specific activity 134.5 TBq $mmol^{-1}$) at 30 °C for 5 min and it was terminated by the addition of 100 μl of a stop solution containing (9 % sodiumdodecylsulphate; 15 % glycerol; 0.05 % bromphenol blue; 30 mmol . l^{-1} Tris, pH 7.8). The tubes were then agitated and immediately transfered to a boiling water bath for a 5 min incubation, subsequently removed, 100 μl of an 8 % solution of β-mercaptoethanol was added to each tube. The samples were incubated overninght at room temperature and used for slab polyacrylamide gel electrophoresis; 40 μl of sample were loaded to stacking 6 % acrylamide gel. The lower separating gel was a 12.6—20% gradient. After electrophoresis, the gel was stained overnight with Coomassie blue R and then destained in 7% glacial acetic acid. Finally, it was dried and exposed to X-ray sensitive film (Foma, medix rapid, GDR) for 10 days.

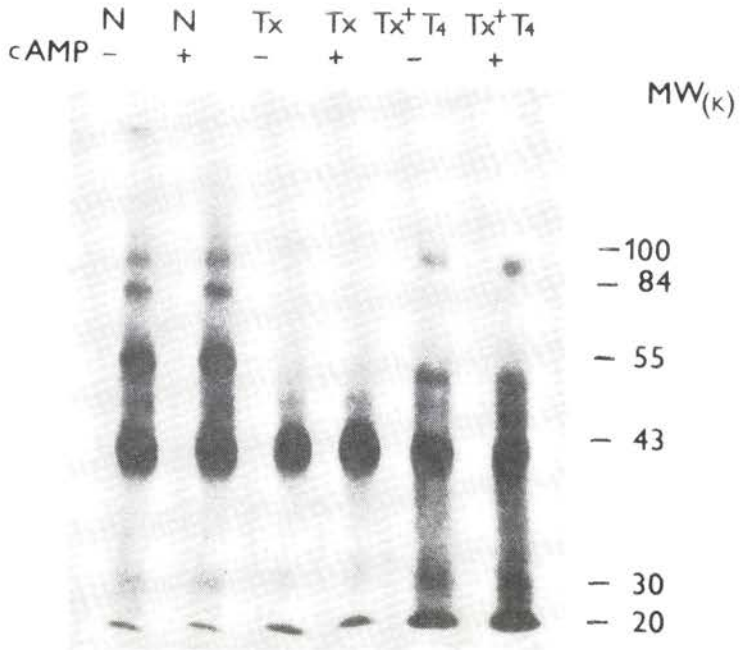

Fig. 1. Autoradiograph of ^{32}P-labeled rat liver nuclear non-histone proteins (NHP) separated by SDS (Sodiumdodecylsulphate) polyacrylamide gel electrophoresis. NHP obtained from normal (N), tyreoidectomized (Tx) and Tx rats injected with 100 μg T_4 kg^{-1} B.W. were incubated with ^{32}P-ATP in the absence (−) or presence (+) of 10 μmol . l^{-1} cAMP. Numbers indicate the mobilities of standard molecular weight markers.

Results

As shown on the autoradiogram (Fig. 1), in normal (N) rats seven phosphoproteins could be distinguished. In Tx rats two proteins with mol wt of 100 and 84×10^3, respectively were completely and one additional protein with a mol wt of 55×10^3 partially dephosphorylated, while in the protein with a mol wt of 20×10^3 the phosphorylation was even more prononced then in control rats. The administration of T_4 (100 g B.W.) to Tx rats resulted in an almost total recovery of phosphorylation in the protein band with a mol wt of 100×10^3, and to a partial recovery in bands with mol wt of 84 and 55×10^3, respectively. Moreover, T_4 administration to T_x rats marked phosphorylation was noted in the bands with mol wt of 30 and 20×10^3 respectively. Finally, the addition of 10 μmol . l^{-1} cAMP did not influence phosphorylation in either group.

Discussion

The present data provide evidence that both thyreoidectomy and T_4 administration alter endogenous phosphorylation of only some specific nonhistone proteins isolated from rat liver nuclei. Of the phosphoproteins preferentially stimulated by T_4, the 84×10^3 mol wt protein was not completely recovered following the hormone administration to T_x rats. Thyroidectomy resulted in a decrease in the protein phosphorylation; after T_4 treatment however, the phosphorylation was almost completely recovered in the protein bands 100 and 55×10^3, respectively. Although both the identity and function of the majority of these phosphorylated proteins are unknown the protein with a mol wt of about 50×10^3 has already been identified as nuclear receptor for T_3 (DeGroot et al. 1974; Latham et al. 1976; Latham et al. 1981).

Our data are in agreement with results of Barsano et al. (1980) who reported decreased or increased incorporation of ^{32}P-orthophosphate into rat liver proteins in vivo after thyreoidectomy and T_3 replacement, respectively. However, from our results a T_4-dependent specific phosphorylation of several distinct non-histone proteins is evident. In T_4-treated Tx rats, phosphorylation of the protein with a mol wt of 43×10^3 is reduced. Also, a protein component of the rat cytosol with a similar mol wt undergoes dephosphorylation after T_3 treatment (Nakamura and DeGroot 1983). It is likely that this protein is identical with the nuclear 43×10^3 mol wt protein, which is transported from the cytosol into the nucleus. We suppose that the effect of T_4 on phosphorylation is specific, as the conversion of T_4 to T_3 could be blocked, in vivo at least, by PTU treatment. This is a further evidence in support of the existence of an intrinsic T_4 activity, operating probably via specific nuclear binding sites for T_4 (Knopp and Brtko 1981).

Two types of cAMP dependent protein kinase have been found in soluble proteins of rat liver nuclei (Sikorska and MacManus 1980). However, in our experiments no substantial effect of cAMP on phosphorylation of nuclear non-histone proteins was observed. Although one of the nuclear globulin fractions contains protein kinase activity (Nakamura and DeGroot 1983) the presence of this enzyme in NHP is uncertain (Kuo et al. 1971; Nakazawa and Sano 1975). Another possibility would be inhibition of the protein-kinase by high salt concentrations (Mednieks et al. 1979) as used in our experiments for extractions.

These findings allow the conclusion that in vitro phosphorylation of NHP of the rat liver nuclei occurs predominantly in some specific fractions and is thyroid hormone-dependent.

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