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Influence of thyroid hormone on ADP-ribosylation of nuclear proteins in cultured GH1 cells

Ana Aranda[1], Richard P. Copp[2], Angel Pascual[1] and Herbert H. Samuels[2]

[1]*Instituto de Investigaciones Biomedicas, CSIC, Madrid, Spain* and [2]*The Division of Molecular Endocrinology, The Rose F. Tishman Laboratories for Geriatric Endocrinology, Department of Medicine, New York University Medical Center, New York, USA*

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We present evidence that T3 can alter the ADP-ribosylation of chromatin associated proteins. Nuclei from GH1 cells were incubated with [adenylate-^{32}P]NAD and the radioactivity incorporated into histone and non-histone proteins was quantitated and analyzed by gel electrophoresis and autoradiography. Incubation of GH1 cells for 24 h with T3 lowered by 40–70% the [^{32}P]ADP-ribose incorporated into nuclear proteins. However, incubation for 3 h with T3 resulted in a stimulation instead of a decrease of in vitro [^{32}P]ADP-ribose incorporation. The major ADP-ribosylated component electrophoresed as a 120 000 molecular mass non-histone protein, and radiolabeled histones were also observed. The same protein species were observed for all the experimental groups and T3 affected the extent of ADP-ribosylation but did not alter the sedimentation of the [^{32}P]ADP-ribosylated components excised from chromatin after micrococcal nuclease digestion.

Thyroid hormone; Poly(ADP-ribosylation); Nuclear protein; GH1 cell

1. INTRODUCTION

Rat pituitary cell lines are effective systems to study thyroid hormone action. In these cells physiological concentrations of 3,5,3′-triiodo-L-thyronine (T3) rapidly stimulate growth hormone gene transcription [1,2] and after a lag period of 20-40 h stimulate cell growth [3,4]. Delayed effects of thyroid hormone may be related to the stimulation of intermediary factors or to an influence of hormone on chromatin structure which may modify gene expression. Hormonal effects on chromatin structure may be secondary to post-synthetic modifications of chromatin proteins [5]. ADP-ribosylation of chromatin proteins has been implicated in DNA replication and repair, gene expression, cell differentiation and transformation, or modification of chromatin structure (for review see [6]). We have recently shown that in pituitary GH1 cells the predominant ADP-ribose acceptor is a 120 000 molecular mass non-histone protein, presumably the poly(ADP-ribose) polymerase itself, and that histones are also modified [7]. The poly(ADP-ribose) polymerase activity of chromatin is preferentially associated with transcriptionally active, or at least extended forms of chromatin which is more sensitive to nuclease digestion [8]. Since thyroid hormone is known to regulate cellular events at the transcriptional level, we sought to determine whether T3 also influences post-synthetic modifications of chromatin proteins. In this study we report that T3 incubation influences the ADP-ribosylation of histones and non-histone chromatin proteins in GH1 cells.

Correspondence address: A. Aranda, Instituto de Investigaciones Biomedicas, CSIC, Arturo Duperier 4, 28029 Madrid, Spain

Abbreviations: T3, 3,5,3′-triiodo-L-thyronine; ADP-ribose, adenosine diphosphoribose; poly(ADP-ribose), polyadenosine diphosphoribose; HMG, high mobility group; SDS, sodium dodecyl sulfate

2. MATERIALS AND METHODS

2.1. *ADP-ribosylation assays*

The experiments were carried out in GH1 cells depleted of thyroid hormones [1,3]. GH1 cell nuclei were isolated [9], and the ADP-ribosylation assay was performed as described [7] by incubation for 15 min at 37°C in 0.5 ml of 0.1 M Tris-HCl, pH 7.9 at 25°C, 2 mM $MgCl_2$, and 1 mM dithiothreitol containing 5–50 μCi (0.3–3 μM) [adenylate-^{32}P]NAD. The nuclear material was extracted with 0.4 N sulfuric acid and the extracted histones precipitated as described [10]. The remaining nuclear material containing non-histone proteins was precipitated at −20°C with ethanol and dissolved in 10 mM sodium phosphate, pH 7.4, and 1% sodium dodecyl sulfate (SDS) [7]. An aliquot of the samples was utilized to analyze ^{32}P-incorporation by Cerenkov radiation and the sample was then used for protein determination by the method of Lowry et al. [11]. Another aliquot of the samples was saved for analysis by gel electrophoresis and autoradiography. Histones were electrophoresed in 15% polyacrylamide acid-urea slab gels in the presence of 2.5 M urea and 0.9% acetic acid [12], and electrophoresis of non-histone proteins was performed in SDS-polyacrylamide gradient (5–15%) slab gels [13]. After electrophoresis the gels were stained, destained, and autoradiographed.

2.2. *Micrococcal nuclease digestion of nuclei*

Nuclei were incubated with [adenylate-^{32}P]NAD (50 μCi/50 A_{260nm} of nuclei) for 15 min at 37°C and then digested [7] with 15 units of micrococcal nuclease/1 A_{260nm} unit of nuclear material at 0°C for 30

min. The samples were centrifuged at 3000 × g for 10 min and the supernatant (S1) was adjusted to 5 mM EDTA to stop the digestion. The pellet was extracted with 5 mM EDTA and centrifuged at 3000 × g for 10 min to yield a second supernatant fraction (S2). The S1 and S2 supernatants were centrifuged in isokinetic sucrose gradients, fractionated, precipitated with trichloroacetic acid and analyzed for ^{32}P-incorporation as described [7]. The fractions corresponding to different regions of the gradient were pooled and analyzed by electrophoresis in 5% to 15% exponential polyacrylamide-SDS slab gels.

2.3. *Determination of NAD levels*

0.5 N perchloric acid extracts of GH1 cells [7] were assayed for NAD using a spectrophotometric technique [14].

3. RESULTS

Table I illustrates the extent of [^{32}P]ADP-ribose incorporation into isolated nuclei after incubating GH1 cells for 24 h with 0.25 nM and 2.5 nM T3. These T3 concentrations result in approximately 35% and 80% occupancy of the thyroid hormone receptor [3,9]. As we have previously observed [7], approximately 10% of the radioactivity was present in the acid-soluble histone fraction and 90% was incorporated into non-histone proteins. T3 decreased [^{32}P]ADP-ribose incorporation into both fractions and the reduction was more pronounced at 2.5 nM T3. The effect of T3 was also examined with 350 μM [adenylate-2,8-^{3}H]NAD and the same decrease was found (results not shown), thus excluding ^{32}P-labeling by an alternative modification, such as protein phosphorylation. T3 incubation resulted in only a small decrease in NAD levels, the substrate for ADP-ribosylation over the 24 h period (from 2.3 ± 0.2 in control cells to 1.7 ± 0.1 nmol/mg protein in T3 treated cells).

The [^{32}P]ADP-ribose protein acceptors from control cells and cells incubated for 3 h and 24 h with 5 nM T3 were examined. In agreement with our previous results [7], two prominent bands electrophoresing to the same position as stained histones H1 and H3, as well as other radiolabeled bands having mobilities similar to the stained high mobility group proteins HMGs were observed (Fig. 1). The predominant acceptor among non-histones (Fig. 2) electrophoreses with an apparent molecular mass (M_r) of 120 000 and probably represents

Table I

[^{32}P]ADP-ribose incorporation (cpm/100 μg protein)

	Histones	Non-histones
Control	5879 ± 512	56 268 ± 4963
L-T3		
0.25 nM	3917 ± 215	38 957 ± 1867
2.50 nM	3280 ± 243	35 756 ± 1758

Effect of L-T3 on ADP-ribosylation of histone and non-histone proteins. Triplicate cultures of GH1 cells were incubated with T3 for 24 h. The nuclei were isolated and incubated with [adenylate-^{32}P]NAD, and the radioactivity associated with histones and non-histone proteins was determined. The data are mean ± SD of triplicate flasks.



Fig. 1. Autoradiogram of the [^{32}P]ADP-ribosylated histones. GH1 cells were incubated with 5 nM T3 for 0, 3, or 24 h. After isolation of nuclei and incubation with [adenylate-^{32}P]NAD, the histone and non-histone fractions were prepared and analyzed by electrophoresis and autoradiography.

the enzyme poly(ADP-ribose) polymerase which is capable of self-modification [14,15]. In addition, several proteins with M_r values in the range of 60 000–80 000 were also observed. After a 24 h incubation with T3 the incorporation into the different protein

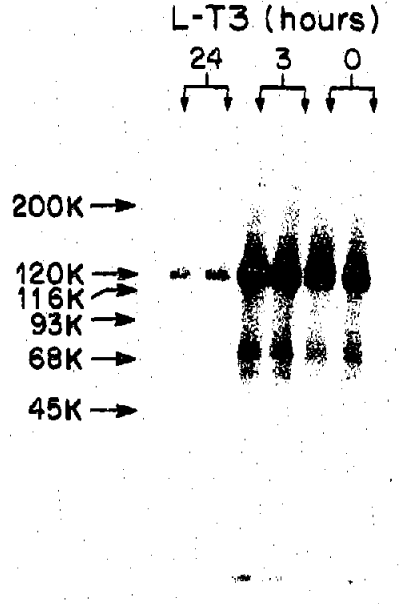


Fig. 2. Autoradiogram of the [^{32}P]ADP-ribosylated non-histone proteins. Non-histone proteins from the experiment shown in Fig. 1. Reference proteins (myosin, M_r = 200 000; β-galactosidase, M_r = 116 250; phosphorylase B, M_r = 92 500; bovine serum albumin, M_r = 68 000; and ovalbumin, M_r = 45 000) were electrophoresed in parallel lanes.

acceptors was lower than in control cells, in agreement with the data shown in Table I. However, nuclei from cells incubated for 3 h with T3 showed an increase rather than a decrease of labeling. The increase in [^{32}P]ADP-ribose incorporation into histones and non-histones ranged from 1- to 2.5-fold.

We assessed the sedimentation profile of the [^{32}P]ADP-ribosylated components released from chromatin after digestion with micrococcal nuclease. Fig. 3 shows the isokinetic sucrose gradients of the fraction released (S1) after nuclease digestion, and the fraction obtained by extraction of the residual nuclear pellet with 5 mM EDTA (S2) obtained from control cells and cells treated with 5 nM T3 for 3 h. Different regions of the gradient were pooled, as indicated by the arrows in the bottom of Fig. 3, and analyzed by electrophoresis. We have previously reported [7] that in the S1 fraction most of the radioactivity sediments to the same position as the bulk mononucleosome peak (11.5 S, pool 1) and that a less abundant fraction sediments at about 4 S (pool 2). The sedimentation profile in the S2 fraction was similar to S1 except that [^{32}P]ADP-ribose also migrated to the same position as the mono (11.5 S, pool 5), di (16 S, pool 4) and tri (19 S, pool 3) nucleosomal particles, and proportionally less sediments than 4 S (pool 6). Incubation with 5 nM T3 for 24 h inhibited the extent of [^{32}P]ADP-ribosylation in both the S1 and S2 fractions but did not alter the sedimentation profiles (not illustrated). The autoradiograms obtained from the different regions of the gradient are shown in Fig. 4. Only [^{32}P]ADP-ribosylated products of very low molecular weight corresponding to histone proteins

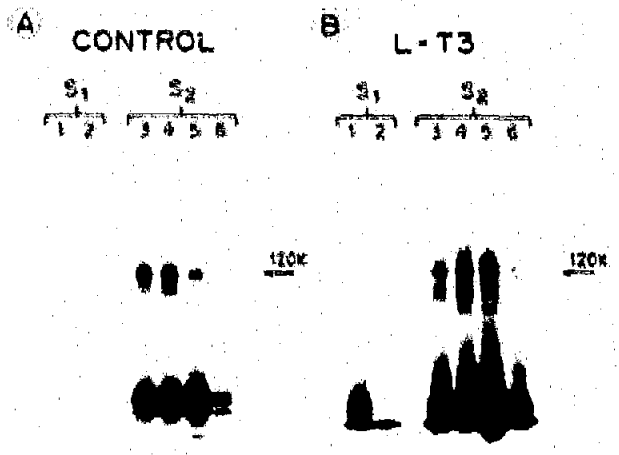


Fig. 4. Autoradiogram of ADP-ribosylated proteins of the pooled S1 and S2 chromatin fractions. (A) Control cells; (B) T3 incubated cells. The number of each lane corresponds to the number indicated above the arrows in Fig. 3.

were identified in lanes 1 and 2 correspond to the 11.5 S and 4 S region of the S1 fraction. In the S2 fraction the abundant 120 000 M_r ADP-ribosylated component was also present in lanes 3-5 which correspond to the region of the gradient enriched in oligonucleosome and mononucleosome particles. In addition, several other radiolabeled bands between 30 000 and 120 000 M_r were also observed. In the 4 S region of the S2 fraction (lane 6) the 120 000 M_r radiolabeled component was also present. The abundant radiolabeled material at the bottom of the autoradiogram represents histones and HMG

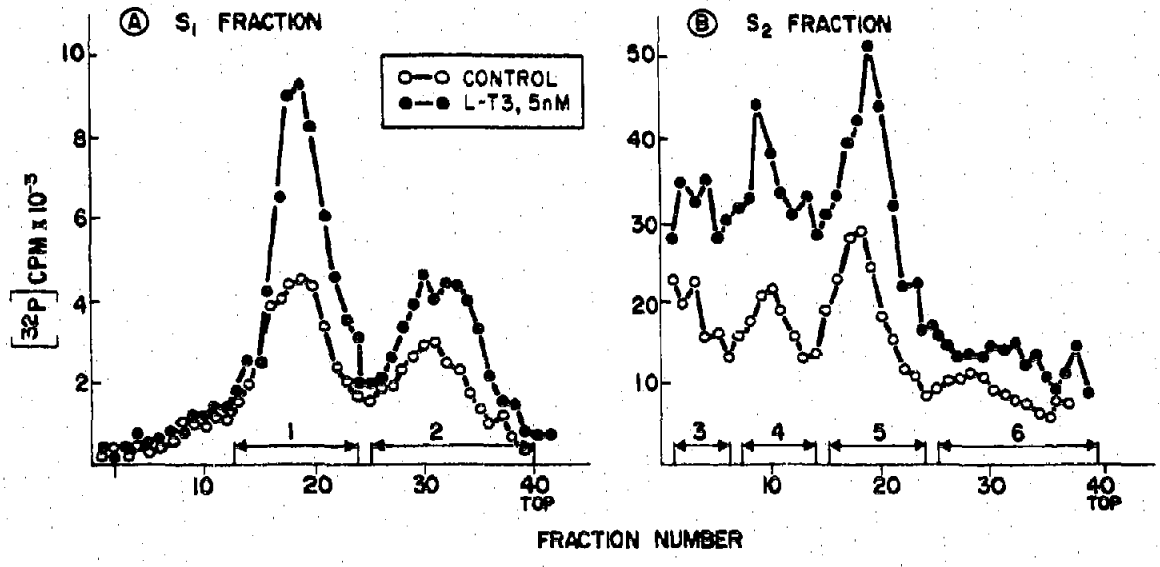


Fig. 3. Gradient centrifugation of ADP-ribosylated chromatin after micrococcal nuclease digestion. Nuclei were incubated with [adenylate-^{32}P]NAD and then digested with micrococcal nuclease. The S1 (A) and S2 (B) fractions were centrifuged in isokinetic sucrose gradients and fractionated. Control cells (○), cells incubated with 5 nM T3 for 3 h (●). The fractions corresponding to different regions of the gradients were pooled and electrophoresed in 5-15% polyacrylamide-SDS gels. The arrows at the bottom of the figure indicate the fractions which were pooled prior to electrophoresis.

proteins. These were not present in the experiment shown in Fig. 2 since the nuclei were extracted with 0.4 N H_2SO_4 before the non-histone proteins were electrophoresed.

4. DISCUSSION

Nuclei from GH1 cells which had been incubated for 24 h with T3 showed decreased incorporation of [^{32}P]ADP-ribose into both the histone and non-histone protein fractions. The relative inhibition produced by 0.25 nM and 2.5 nM T3 suggests that this decrease is mediated through the interaction of T3 with its nuclear receptor. In contrast with the 24 h study, shorter T3 incubation times (3 h) resulted in an increase rather than a decrease of [^{32}P]ADP-ribose incorporation into both the histone and non-histone protein fractions. The decrease in in vitro [^{32}P]ADP-ribose incorporation observed after 24 h of T3 incubation could result from a reduction of poly(ADP-ribose) polymerase activity secondary to self-modification of the enzyme [16].

Administration of T3 by 4 days has been reported to decrease [^{32}P]ADP-ribosylation in isolated cardiac nuclei by about 40% [17]. This occurred without any change in the level of poly(ADP-ribose) glycohydrolase activity which can cleave poly(ADP-ribose) chains. This in vivo study is in keeping with our observations 24 h after T3 incubation in GH1 cells. Our studies in cultured GH1 cells indicate that the effect of thyroid hormone on ADP-ribosylation in rat heart nuclei probably reflects a cellular action of T3 and is not secondary to an altered metabolic state which could increase the secretion of other factors and/or hormones (e.g. glucocorticoid hormones or growth hormone) which are also known to modulate nuclear poly(ADP) ribosylation [18].

The influence of T3 on ADP-ribosylation showed a similar alteration in both histone and non-histone protein fractions, and the hormone produced a generalized effect in the different protein acceptors which were the same in all experimental conditions studied. To explore whether selective changes in the extent of [^{32}P]ADP-ribosylation might be restricted to specific chromatin domains we examined different chromatin fractions excised by micrococcal nuclease. We have found that in the S2 fraction ADP-ribosylated proteins are associated with the regions of the gradient corresponding to oligonucleosomes, mononucleosomes, and subnucleosomes, with the 120 000 M_r radiolabeled component present in all regions of the gradient. In contrast, the 120 000 M_r component band was not detected in the S1 fraction and only ADP-ribosylated proteins of very low molecular weight were identified. Again, T3 altered the extent of labeling but not the sedimentation profile or composition of the ADP-ribosylated chromatin particles.

ADP-ribosylation or other post-synthetic modifications of nuclear proteins may play a role in gene expression [6]. We find detectable changes in [^{32}P]ADP-ribose incorporation several hours after growth hormone gene transcription is maximally stimulated (1 h) by thyroid hormone [1]. This suggests that alterations in ADP-ribosylation reflect a secondary rather than a primary action of the thyroid hormone/receptor complex on gene expression and may influence delayed responses to thyroid hormone.

We do not find evidence that ADP-ribosylation of a protein or group of proteins is preferentially affected by T3, which suggests that the changes observed reflect an alteration in the activity of the poly(ADP-ribose) polymerase. Our studies, however, do not exclude the possibility that thyroid hormone rapidly alters the ADP-ribosylation of a minor subset of nuclear regulatory proteins which are essential for control of gene expression by thyroid hormone. Tanuma et al. [19] have reported that dexamethasone causes a selective and rapid reduction of ADP-ribose from HMG 14 and 17 over the same time frame at which glucocorticoid stimulated mouse mammary tumor viral RNA synthesis. In addition, Kimura et al. [20] reported that *N'*-methylnicotinamide, which lowers cellular NAD levels but does not inhibit poly(ADP-ribose) polymerase, increases the rate of growth hormone production in GH3 cells about 2-fold and enhances the effect of T3. Therefore, some influence of ADP-ribosylation on the control of gene expression by T3 is suggested and the role of this post-synthetic modification event in certain actions of the thyroid hormones in cultured cells [20] and rat tissues in vivo [17] warrants further investigation.

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