Influence of thyroid hormones on the human ATP synthase \( \beta \)-subunit gene promoter

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Abstract

The action of thyroid hormones on the expression of the mitochondrial ATP synthase \( \beta \)-subunit gene (ATPsyn\( \beta \)) is controversial. We detected a binding site for the thyroid hormone receptor between –366 and –380 in the human ATPsyn\( \beta \) gene by DNase I footprint analysis and band-shift assays. However, expression vectors in which the chloramphenicol acetyl transferase (CAT) reporter gene is driven by the 5' upstream region of ATPsyn\( \beta \) gene were unresponsive to \( T_3 \) when transiently transfected to HepG2 or GH4C1 cells. CAT constructs driven by the rat phosphoenolpyruvate carboxykinase (PEPCK) or the growth hormone (GH) promoters were stimulated several fold by \( T_3 \) in parallel experiments. It is proposed that the biological effects of thyroid hormones on the ATPsyn\( \beta \) expression occur through indirect mechanisms. (Mol Cell Biochem 154:107–111, 1996)

Key words: ATP synthase \( \beta \)-subunit gene, mitochondria, thyroid hormone, (human)

Introduction

One of the physiological roles of thyroid hormones is to increase the respiratory capacity of mammalian tissues [1]. Part of this effect is due to the positive action of thyroid hormones on mitochondrial biogenesis [2]. However, the molecular bases of this phenomenon are unknown. Thyroid hormones exert their biological function by modulating the transcription rate of specific genes and/or affecting post-transcriptional events of gene expression. The action of thyroid hormones on gene transcription is due to the interaction of thyroid hormone receptors on specific regions present in target genes (thyroid hormone response elements, TREs) [3]. The long-term effects of thyroid hormones promoting tissue respiratory activity and mitochondrial biogenesis are expected to be due to a complex array of effects of thyroid hormones on the transcription of the mitochondrial genome and of nuclear genes for mitochondrial proteins, especially those involved in the respiratory activity/oxidative phosphorylation system.

The ATP synthase \( \beta \)-subunit (ATPsyn\( \beta \)) is a major component of the catalytic site of the mitochondrial FoF1 ATP synthase complex and it is encoded by the nuclear genome [4]. The effects of thyroid hormones on the expression of the ATPsyn\( \beta \) gene are unclear. Thyroid hormones have been reported to increase the ATPsyn\( \beta \) mRNA levels in perinatal rat liver due to increased gene transcription [5, 6]. DNA sequences in the human ATPsyn\( \beta \) gene which resemble TREs have been also described [5]. Other laboratories have found that ATPsyn\( \beta \) mRNA levels are not specifically affected by thyroid hormones in the adult rat liver [7]. However, studies on the effect of thyroid hormones on the activity of the ATPsyn\( \beta \) gene promoter are lacking. The aim of the present study was to explore the possible presence of thyroid hormone response elements in the ATPsyn\( \beta \) promoter.

Materials and methods

Materials

DNA-modifying enzymes and poly(dI-dC) were purchased from Boehringer Mannheim or Promega. [\( \alpha ^{32} \text{P} \)] dCTP was
purchased from Amersham and D-threo-[1,2-14C]chloramphenicol was from ICN. Tissue culture media and fetal calf serum were obtained from Whittaker. Synthetic oligonucleotides were obtained from Oligos Inc (USA).

**DNase I footprint and electrophoretic gel mobility shift assays**

The DNA probe for DNaseI footprint assays was prepared by end-labeling the XbaI-digested [3381-CAT plasmid DNA (see below) using [α-32P]dCTP and Klenow enzyme. The DNase footprinting assays were performed as described [8]. Binding reactions contained from 5–25 μg of protein from rat liver nuclear extracts. Maxam and Gilbert G and G+A reactions of the labeled fragments were used to identify the position of protected sites. For electrophoretic gel mobility shift assays, double stranded synthetic oligonucleotides containing 5’ extruding ends were end-labeled using [α-32P]dCTP and the Klenow enzyme. 20–30,000 cpm of labeled probe were incubated in each single assay. Probes were incubated with different amounts of highly purified recombinant T3 receptor α1 (see below). In the competition experiments different fold molar excess of the unlabeled oligonucleotide TREpal (see Fig. 1B for sequence) were included in binding reactions. Reactions were carried out at room temperature for 30 min and contained 25 mM Tris-HCl (pH 7.8), 0.5 mM EDTA, 100 ng aprotinin, 10 mM 2-mercaptoethanol, 0.05% Triton X-100, 75 mM KCl and 10% glycerol. Samples were analyzed by electrophoresis in 5% polyacrylamide gels using buffer composed of 10 mM Tris base, 7.5 mM acetic acid and 0.04 mM EDTA, pH 7.8. Electrophoresis was performed at 4°C at a voltage gradient of 15V/cm with rapid buffer recircularization. Gels were analyzed by autoradiography.

**Preparation of nuclear protein extracts and recombinant purified T3 receptor α1**

Nuclear proteins were isolated from rat liver according to previous reports [9]. Purified, bacterially expressed T3 receptor α1 was a kind gift from H.H. Samuels. T3 receptor purity was checked by silver staining of an SDS-polyacrylamide gel and quantitated by ligand binding assays [10].

**Plasmids**

The β786-CAT plasmid is a previously reported expression vector in which CAT is driven by the −786/+89 fragment of the human ATPsynβ gene [8]. The β381-CAT is a deletion mutant obtained using a polymerase chain reaction strategy described previously [8]. Expression vectors for chicken thyroid hormone receptor α and human thyroid hormone receptor β have been described previously [10, 11] and were kindly provided by Drs. H.H. Samuels and M. Rosenfeld, respectively. The expression plasmids where CAT is driven by the rat PEPCK promoter (PEPCK−490/+73-CAT) [12] or the rat GH promoter (GH−540/+7) [13] were provided by Dr. R.W. Hanson and Dr. H.H. Samuels, respectively.

**Cell culture, DNA transfection and determination of CAT activity**

HepG2 cells were grown in Dulbecco’s modified Eagle’s essential medium containing 10% fetal calf serum. GH4C1 were grown in Dulbecco’s minimal essential medium containing 15 mM HEPES, 0.1 mg/ml pyruvate and 5% fetal calf serum. Transfections were carried out by the calcium phosphate precipitation procedure [14]. Each transfection contained 10 μg of CAT vector and, where appropriate, 5 μg of the expression vectors for hormone receptors. After transfection cells were grown for 36 h in the corresponding culture medium supplemented with 10% charcoal-treated calf serum with or without the appropriate concentration of T3. CAT activity was analyzed as described [15]. The amount of cell extract used was adjusted to maintain a percentage conversion of chloramphenicol in the linear range of the assay (< 30%).

**Statistical analysis**

Where appropriate, statistical analysis was performed by the Student’s t test and significance is indicated in the text.

**Results and discussion**

DNase I footprinting assays of the ATPsynβ gene promoter using nuclear extracts from rat liver were performed (see Fig. 1A) and they revealed the binding of nuclear proteins to the CCAAT element at −248/−230 (not shown in the figure) as well as an ETS-domain binding site at −306/−276 (E site), as previously reported [8]. Together with these two sites another protected domain was observed at −380/−366. DNA sequence analysis of this site (named T site from now on) indicated a significant homology with a palindromic alignment of two of the half sites known to bind thyroid hormone receptors (see Fig. 1B) [16]. The homology included a full conservation of two GG pairs placed in an opposite alignment and known to be essential for thyroid hormone receptor binding [14, 15]. In order to assess the actual ability of thyroid hormone