

Modulation of a major 30-kDa skeletal muscle protein by thyroid hormone

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Thyroidectomy results in the transformation of type II fibres to type I in rat soleus muscle. In vitro translations containing polyribosomes indicate that the template activity of mRNA coding for a 30-kDa protein is increased in hypothyroid (6 months) rats. The cellular content of this protein is also increased in hypothyroid rats. The in vitro synthesis of the 30-kDa protein is not observed in thyroidectomized (10 weeks) rats that have been treated with triiodothyronine. The synthesis and accumulation of this protein are directly related to the proportion of type I fibres in rat skeletal muscle and appear to be modulated by thyroid hormone.

Thyroidectomy Fiber type Soleus muscle Protein synthesis Soluble enzyme Triiodothyronine

1. INTRODUCTION

It is now well established that thyroid hormone influences the physiological properties of the rat soleus muscle. Hypothyroidism results in marked increases in the isometric contraction time and relaxation time in this muscle [1]. Thyroid hormone replacement reverses these effects [2,3]. Recently it has been reported that hypothyroidism influences the myosin isozyme transitions in developing rat muscle [4]. The synthesis of cardiac α - and β -myosin heavy chain mRNA also appears to be regulated by thyroid hormone [5].

Here we have investigated the synthesis and accumulation of a 30-kDa protein from soleus muscle of rats of different thyroid status. The results indicate that triiodothyronine (T_3) modulates the

template activity of the mRNA coding for a major 30-kDa polypeptide whose presence correlates with a high proportion of type I muscle fibres.

2. EXPERIMENTAL

Skeletal muscle fibre types were determined using the Ca,Mg-ATPase method [6] which distinguishes between type I, type IIA and type IIB in a single-step procedure.

Surgical thyroidectomy (TH-X) was performed on adult male Sprague-Dawley rats. Polyribosomes used in cell-free translation experiments were isolated from the soleus muscle of TH-X (6 months) rats and corresponding controls as well as from TH-X (10 weeks) rats or TH-X (10 weeks) rats that had been treated with T_3 (100 μ g/day) for 7 days.

Biologically active polyribosomes were isolated as in [7] except that they were resuspended (10 A_{260} units/ml) in 20 mM Hepes (pH 7.6), 0.1 M potassium acetate, 5 mM $MgCl_2$, 0.5 mM dithiothreitol.

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Abbreviations: IEF, isoelectric focusing; NEpHGE, nonequilibrium pH gradient electrophoresis; SDS-PAGE, SDS-polyacrylamide gel electrophoresis

Aliquots containing 0.01–0.05 A_{260} units were added to 15 μ l of a rabbit reticulocyte lysate [8] containing 40 μ Ci [35 S]methionine (1100 Ci/mmol). The potassium concentration is adjusted to 0.16 M using the acetate salt and the translation reaction is carried out for 60 min at 30°C. In vitro synthesized proteins and the low ionic strength soluble extract of the soleus muscle [9] were resolved by either IEF or NEpHGE followed by SDS-PAGE [10,11]. Proteins were visualized by fluorography [12] or by Coomassie blue staining. Quantitative analysis of the in vitro synthesized 30-kDa protein was performed by scintillation spectrometry or by densitometric scanning of stained gels [13]. The pH gradient of IEF gels was determined according to [10]. Gels that were prepared for fluorography were loaded with $\approx 400\,000$ cpm [35 S]methionine and Coomassie blue stained gels were loaded with ≈ 150 μ g pro-

tein. Acrylamide concentration in SDS gels was 12%.

3. RESULTS

Table 1 summarizes the changes in body weights

Table 1

Effect of chronic hypothyroidism (6 months) on body weights and serum T_3 levels of male rats

	Control	Surgical thyroidectomy
Body weight (g)	477 $\pm$ 8 ^a (7)	302 $\pm$ 8 ^b (7)
Serum T_3 (ng/dl)	88 $\pm$ 3 (7)	13 $\pm$ 1 ^b (7)

^a $\bar{X} \pm SE$ (N)

^b Significantly different at $p < 0.001$ from control values

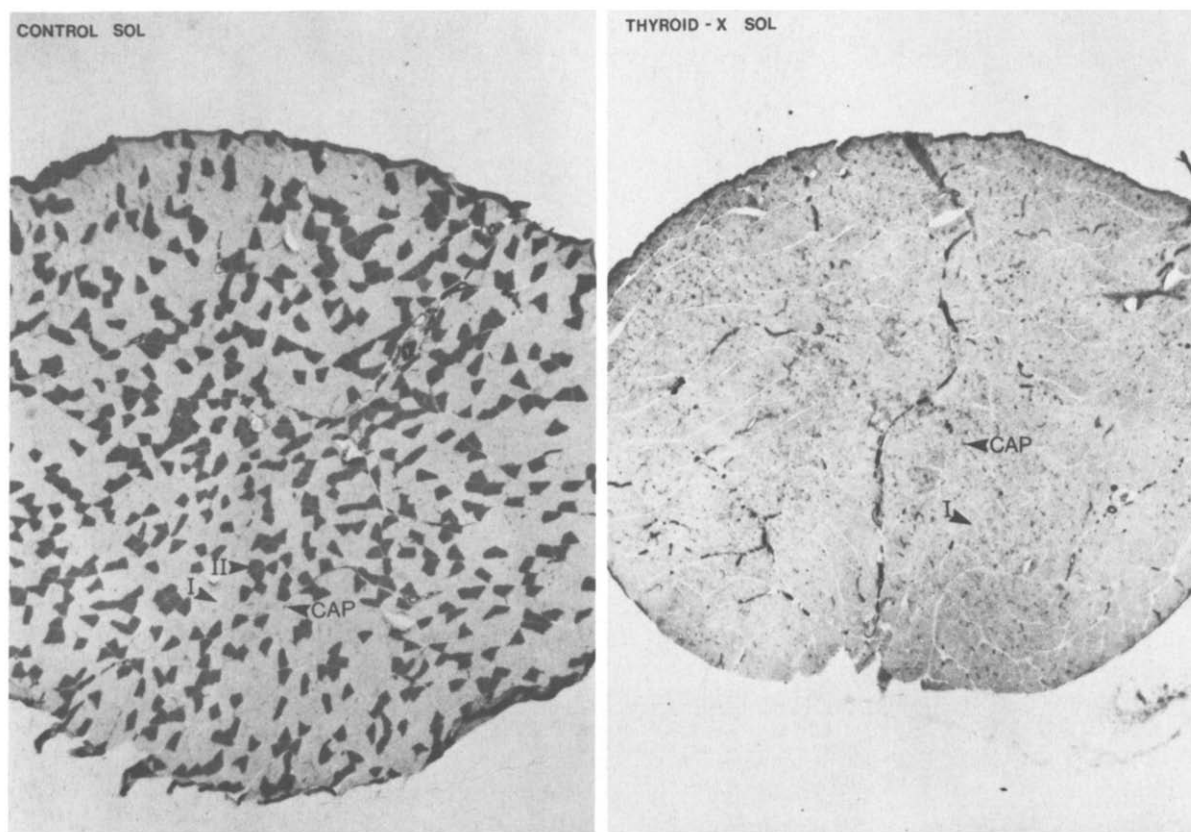


Fig.1. Comparison of sections of control and thyroidectomized (6 months) rat soleus muscles stained by Ca,Mg-ATPase [6]. The ATPase activity of the fibres follows the pattern light type I (slow) and dark type II (fast) fibres. CAP, capillary; Thyroid-X, thyroidectomy (6 months); SOL, soleus muscle.

and serum T₃ of rats following 6 months of chronic hypothyroidism. Thyroidectomized rats failed to grow and their mean body weight was 63% of control values at the time of sacrifice. Terminal serum T₃ level in TH-X rats was 15% of the corresponding controls.

The rat soleus muscle consists of two fibre types of differing stain intensity that are identified as type I (light stain) and type II (dark stain) found in the proportion of 80% and 20%, respectively. The soleus muscle of TH-X rats is found to be homogeneously type I (fig.1).

Long-term thyroidectomy exerted a selective effect on the *in vitro* translational activity of a limited number mRNAs of the soleus muscle. Of particular interest is the 4.3-fold increase in the incorporation of [³⁵S]methionine into a major 30-kDa translation product with a *pI* of 6.9 (fig.2).

Extraction of the soleus muscle in a low ionic strength buffer [9] revealed the presence of a major 30-kDa protein that comigrates on two-dimensional gels with the 30-kDa protein synthesized *in vitro*. Densitometric scanning of the

stained 30-kDa protein resolved by two-dimensional gel electrophoresis (see fig.3) indicates that thyroidectomy resulted in a 4.0-fold increase in the cellular content of this protein. Treatment of hypothyroid (10 weeks) rats with T₃ (100 µg/day) for 7 days resulted in the cessation of synthesis of the 30-kDa protein in cell-free translation system (fig.4). Translation of polyribosomes from rat extensor digitorum longus (EDL), a fast-twitch muscle consisting of primarily type II fibres, revealed that little if any of 30-kDa protein is synthesized when compared to the soleus muscle (fig.5).

4. DISCUSSION

ATPase activity [1,14], myosin phenotype [14,15], contractile properties [1,14] and calcium uptake by isolated sarcoplasmic reticulum [16] have all been shown to change in the direction of fast to slow in the skeletal muscle of hypothyroid rats. Moreover, slow to fast fibre conversion has been reported in hyperthyroid rats [1,2,15]. Slow-twitch muscle is considerably more responsive to

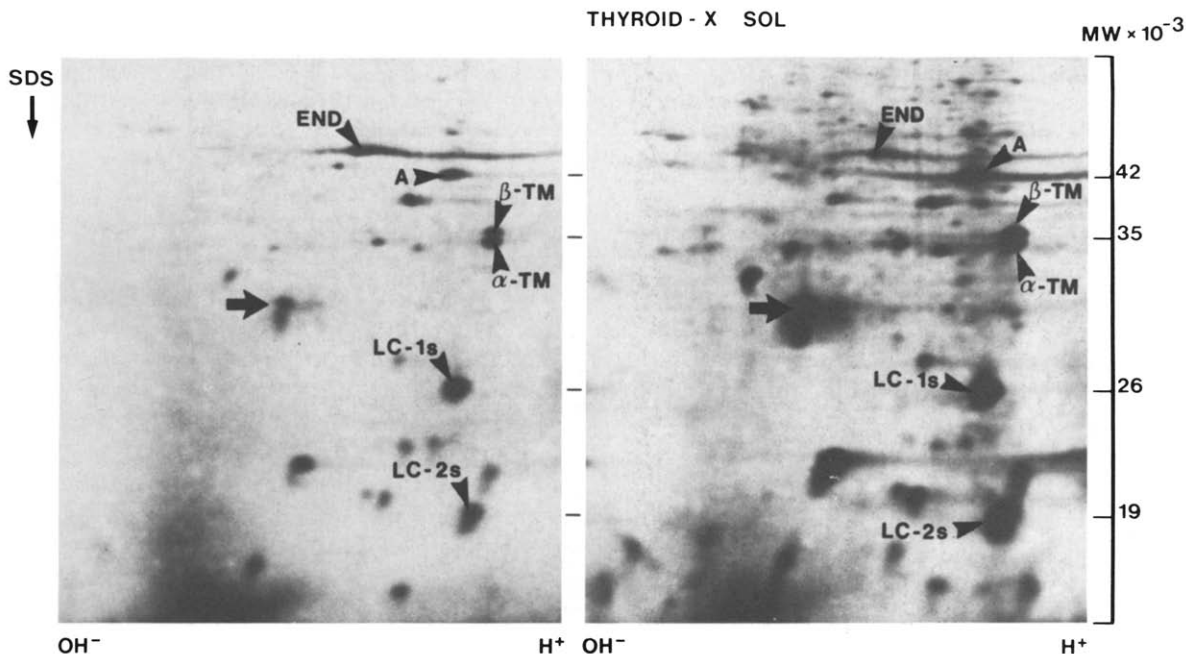


Fig.2. Fluorograms of electrophoretically resolved *in vitro* synthesized polypeptides from control and thyroidectomized (6 months) rat soleus muscles on 2-D gels (NEpHGE, SDS-PAGE). Identification of protein spots was ascertained by apparent *M_r* and isoelectric point. END, endogenous protein synthesized by the reticulocyte lysate; A, actin; α-TM, α-subunit of tropomyosin; β-TM, β-subunit of tropomyosin; LC-1s, myosin light chain 1 (slow); LC-2s, myosin light chain 2 (slow); arrow, 30-kDa protein; refer to fig.1 for others.

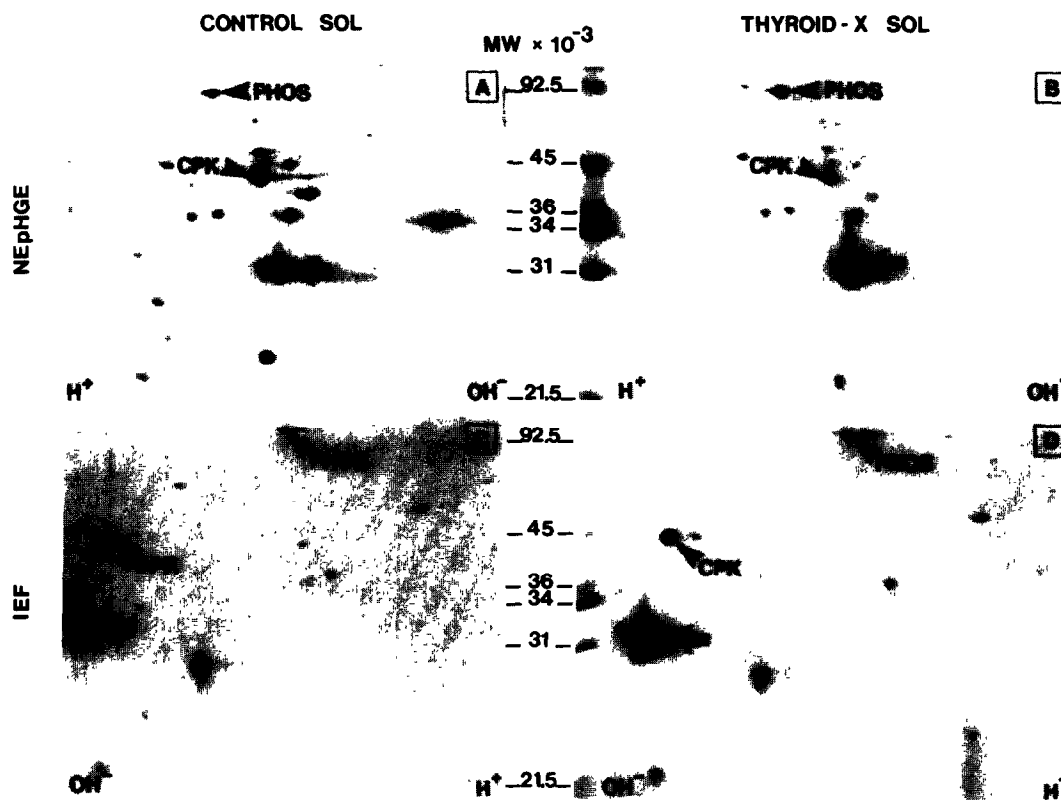


Fig.3. Two-dimensional electrophoretic separation of a low ionic strength extract containing 150 μ g protein prepared from rat soleus muscle of control and thyroidectomized (thyroid-X, 6 months) rats. The soluble protein extracts were resolved by NEpHGE (A and B) or by IEF (C and D) followed by SDS-PAGE. PHOS, phosphorylase; CPK, creatine kinase; arrow, 30-kDa protein.

thyroid hormone changes than fast-twitch muscle and thyroid-induced changes in muscle properties can be elicited in the absence of motor innervation which suggests a direct action of thyroid hormone over a neural mechanism in muscle [17].

The results of the experiments in this study indicate that chronic hypothyroidism leads to an increase in the cellular content of a 30-kDa protein in rat soleus muscle and that the *in vitro* template activity of polyribosomal mRNA coding for the 30-kDa protein is also increased. It is possible that this increase in cellular content is a direct result of an increase in the availability of the corresponding mRNA [13].

When hypothyroid (10 weeks) rats are treated for 7 days with T_3 the *in vitro* synthesis of the 30-kDa protein is clearly diminished. Although T_3 has been shown to stimulate gene transcription, there are increasing numbers of reports of proteins

whose concentration decreases as a result of this treatment ([18,19] and references therein).

The *in vitro* synthesized 30-kDa protein co-migrates on two-dimensional gels with a 30-kDa protein which constitutes a major fraction of the low ionic strength extract of slow-twitch skeletal muscle. Indeed, *in vitro* translation of polyribosomes from TH-X rat soleus muscle suggest that the 30-kDa protein is synthesized in quantities comparable to slow myosin light chain 1.

The synthesis of the 30-kDa protein appears to be related to fibre type. *In vitro* synthesis of this protein by polyribosomes derived from rat EDL muscle ($\approx 85\%$ type II fibres) is significantly less than the synthesis by polyribosomes from the soleus muscle ($\approx 80\%$ type I fibres). It is most interesting therefore to note that the increased *in vitro* synthesis of the 30-kDa protein is accompanied by type II to type I fibre transformation in

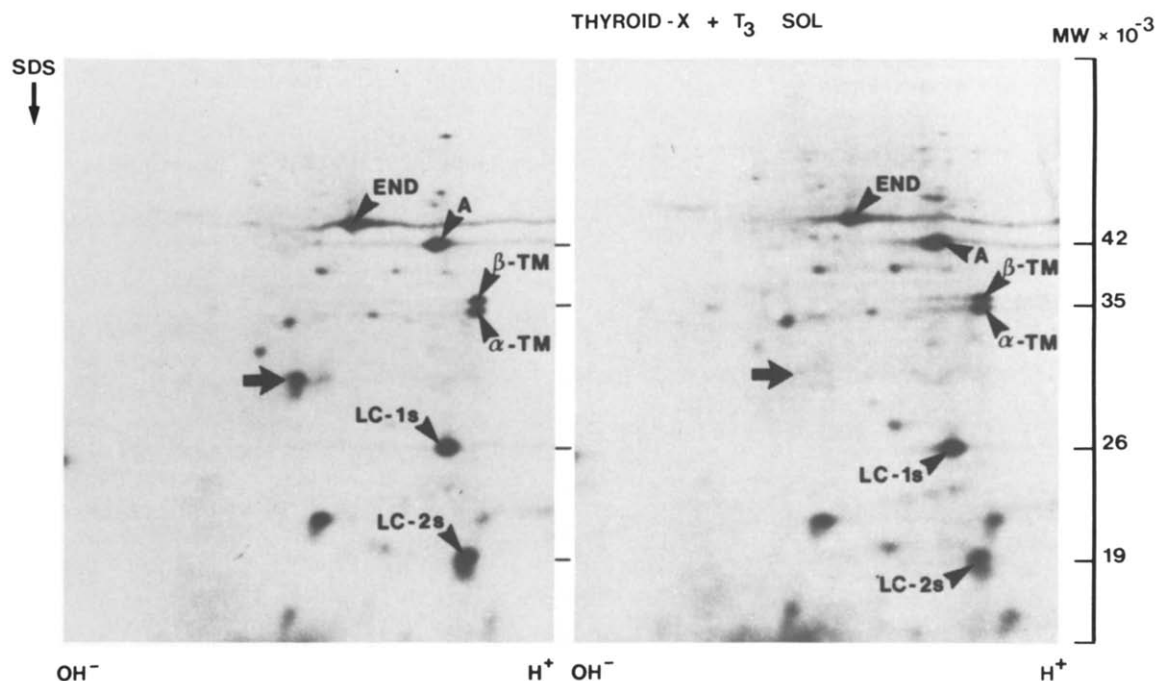


Fig.4. Fluorograms of electrophoretically resolved in vitro synthesized polypeptides of the soleus muscle from thyroidectomized rats (10 weeks) and thyroidectomized rats (10 weeks) that were treated with T₃ (100 μ g/day) for 7 days. Refer to fig.2 for abbreviations.

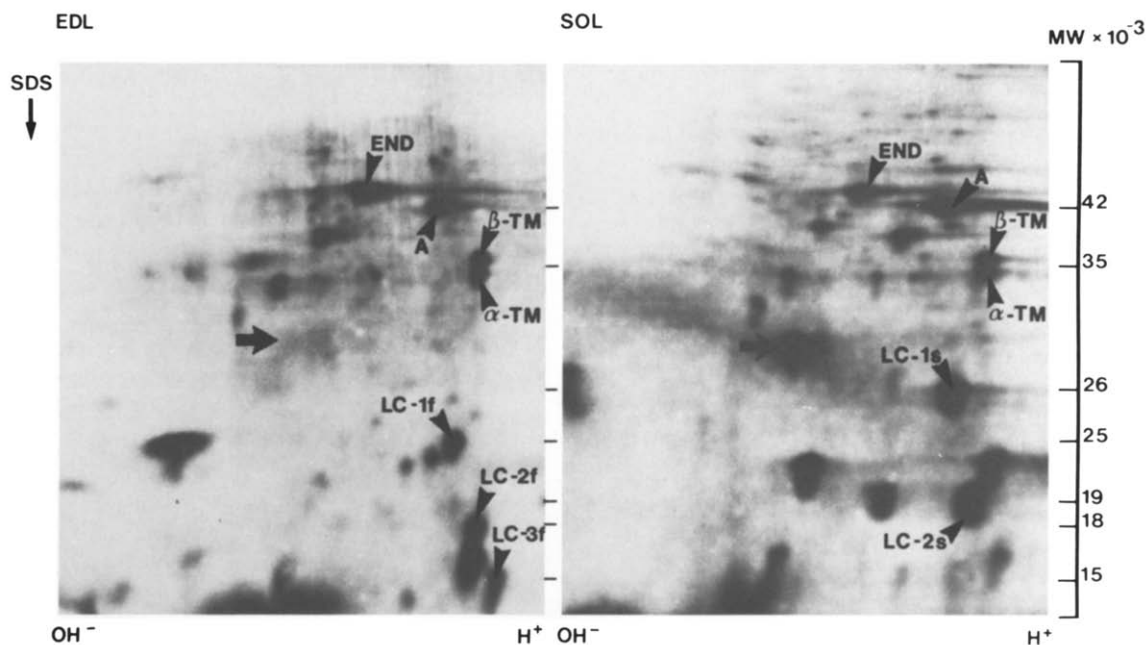


Fig.5. Fluorograms of electrophoretically resolved in vitro synthesized polypeptides from the extensor digitorum longus (EDL) and soleus (SOL) of euthyroid rats. END, endogenous band; A, actin; α -TM and β -TM, α - and β -subunits of tropomyosin; LC1-2-3 (s-f), myosin light chains 1-2-3 (slow and fast); arrow, 30-kDa protein.

the soleus muscle. Although the 30-kDa protein remains unidentified we have noted that a 30-kDa sarcoplasmic reticulum protein, which is characteristic of slow-twitch muscle, increases in experimentally induced type II to type I fibre transformation [20].

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