THE ROLE OF THYROXINE IN THE REGULATION OF TRANSLATABLE MESSENGER RNA FOR α_{2u} GLOBULIN IN RAT LIVER

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1. Introduction

α_{2u} Globulin is an androgen-dependent rat urinary protein which is synthesized and secreted by the hepatic parenchymal cells [1,2]. The protein has been isolated and partially characterized. It has a relatively low mol. wt. ($\sim$ 23 800) and is rapidly filtered by the kidneys and therefore it comprises the major urinary protein in the male rat [1,2]. Hepatic synthesis of α_{2u} globulin in the normal male rat starts at the time of puberty and both α_{2u} globulin and its corresponding mRNA could be induced in the ovariectomized female rats with androgens [3–6]. Synthesis of α_{2u} globulin was also found to be dependent on the presence of pituitary gland [7]. Maximal induction of α_{2u} globulin in the hypophysectomized rat requires treatment with testosterone, growth hormone, corticosterone and thyroxine. Urinary levels of α_{2u} globulin in hypophysectomized rats treated with a combination of hormones containing testosterone, growth hormone and corticosterone was found to be only about 50% as much as those receiving testosterone, growth hormone, corticosterone plus thyroxine [8]. Removal of the thyroid gland from mature male rats results in a drastic reduction in the urinary output of α_{2u} globulin which could be reversed back to normal with thyroxine supplementation [8].

In order to explore the mechanism of thyroxine action on the synthesis of α_{2u} globulin we have investigated the levels of hepatic mRNA for α_{2u} globulin under conditions of deficiency and sufficiency of thyroid hormone. The results show a positive correlation between the hepatic levels of α_{2u} mRNA and the urinary levels of α_{2u} globulin under various thyroidal states indicating the role of thyroxine in the regulation of transcription of α_{2u} mRNA.

2. Materials and methods

Experiments were performed on Sprague-Dawley rats obtained from Zivic-Miller Laboratory, Allison Park, Pa. All of the thyroidectomized animals carried implantation of the parathyroid tissue. The animals were fed Purina rat chow and tap water ad libitum. Procedures for the collection of urine samples, purification and immunoassay of α_{2u} globulin have all been described earlier [8]. A 100 μg/ml solution of L-thyroxine (sodium salt) in 0.005 M NaOH containing 5% ethanol was administered intraperitoneally at a daily dose of 10 μg/100 g body weight of the animal.

Total hepatic RNA was extracted with phenol saturated with sodium dodecyl sulfate (SDS) buffer, pH 8.0 [9]. The total RNA mixture was chromatographed on cellulose to obtain the poly(A) containing RNA [10,11]. Poly(A) containing RNA fraction was translated in a heterologous cell-free system derived from wheat germ [12]. The translation was carried out in a 200 μl reaction mixture at 25°C for 2 h with 28 μCi of [^{3}H]leucine (59 Ci/mmole) as the tracer amino acid. The amount of [^{3}H]leucine incorporation into total proteins was determined in a 5 μl reaction mixture according to the procedure of Bollum [13]. Preliminary experiments revealed that maximum stimulation (9-fold) of leucine incorporation into

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total proteins was obtained with a RNA concentration of 40 μg per 200 μl reaction mixture. All subsequent experiments were therefore carried out with the above RNA concentration. The amount of the leucine incorporation into the released chains (150 000 *g* supernatant) was determined in a 5 μl aliquot of the supernatant. Aliquots of 80 to 120 μl supernatant containing 300 000 c.p.m. of the released peptide chains were used for the immunoprecipitation and quantitation of the newly synthesized α_{2u} globulin [6]. The washed immunoprecipitate was subjected to SDS-polyacrylamide gel electrophoresis (12% gel) according to Laemmli [14]. Macerated gel fractions obtained from 2 mm portions of the gel were counted in scintillation medium [15] containing 3% protosol (New England Nuclear Co., Boston, Mass.).

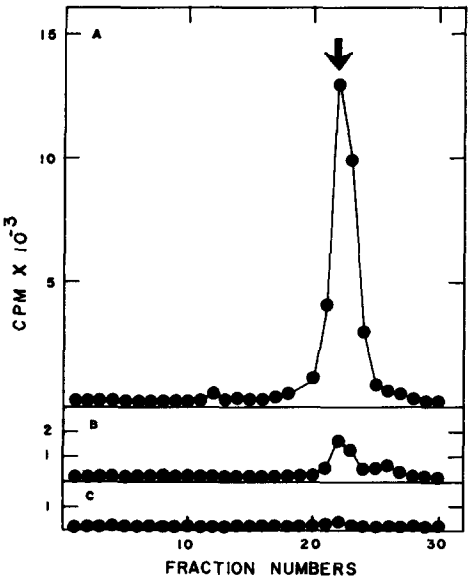

Fig.1. Distribution of [^{3}H]leucine radioactivity in the SDS-polyacrylamide gel after electrophoresis of the immunologically precipitated α_{2u} globulin synthesized in vitro directed by the hepatic mRNA from normal male (A), thyroidectomized male (B) and normal female (C) rats. Twenty-four hour urinary output of α_{2u} globulin of these animals before their sacrifice for hepatic RNA extraction were: normal male, 19.0; thyroidectomized male, 1.2; normal female, 0. The arrow shows the position of α_{2u} band in the gel stained with Coomassie blue.

3. Results and discussion

Since earlier studies with α_{2u} mRNA were performed in ascites cell-free system [6], it was imperative to establish the authenticity of α_{2u} globulin synthesized in vitro by the wheat germ translational system under the direction of the hepatic mRNA and the applicability of the system for quantitation of the messenger activity for α_{2u} globulin. Fig.1 shows that poly(A) containing RNA fraction from the liver of normal male rat directs the synthesis of a peptide product which possesses both the same immunoreactivity and same electrophoretic mobility in the sodium dodecyl sulfate acrylamide gel as those of α_{2u} globulin (fig.1A). Poly(A) containing RNA preparation from female rats which do not synthesize α_{2u} globulin, although almost equally effective in stimulating the incorporation of [^{3}H] leucine into total proteins, did not significantly promote the incorporation of the labelled amino acid into the peptide which could be identified as α_{2u} globulin (fig.1C). The product synthesized under the direction of poly(A) containing RNA from the liver of thyroidectomized male rats showed a relatively small peak of radioactivity within the α_{2u} band in the gel (fig.1B) which is also in accordance with the low level of α_{2u} globulin produced by these animals.

The quantitative relationship between the hepatic level of α_{2u} mRNA activity and the 24 h urinary output of α_{2u} globulin at different thyroidal states is presented in table 1. Daily treatment of the hypothyroid male rat with thyroxine for 4 days resulted only in an insignificant increase in the hepatic α_{2u} mRNA activity and the urinary output of α_{2u} globulin. The observation of weak initial stimulation of α_{2u} synthesis by thyroxine in the thyroidectomized rat is consistent with our earlier observations [8]. However, after 8 daily injections of thyroxine there was a sharp rise in both the urinary output of α_{2u} globulin (22-fold) and the hepatic mRNA activity for α_{2u} globulin (7-fold) as compared to thyroidectomized animals without any thyroxine treatment. Withdrawal of thyroxine treatment for 4 days after 8 days of treatment resulted in an abrupt drop in the level of hepatic α_{2u} mRNA activity and the urinary level of α_{2u} globulin. The coordinated rise and fall of the hepatic mRNA activity for α_{2u}

Table 1
Quantitative relationship between the hepatic α_{2u} mRNA activity and the urinary level of α_{2u} globulin under different thyroidal states

Thyroid status and treatment	Hepatic mRNA activity for α_{2u} globulin		Urinary level of α_{2u} globulin	
	α_{2u} mRNA activity (c.p.m.)	% Control	mg α_{2u} Globulin in 24-h urine	% Control
Intact thyroid + no treatment (unoperated control)	39 727	100	25.0	100
Thyroidectomized + 8 days of testosterone, 250 μg/100 g, b.w. (testosterone-treated control)	4049	10.2	0.61	2.44
Thyroidectomized + no treatment	5386	13.5	0.75	3.0
Thyroidectomized + 4 days of thyroxine	5673	14.2	1.1	4.4
Thyroidectomized + 8 days of thyroxine	37 593	94.6	16.8	67.0
Thyroidectomized + 8 days of thyroxine followed by 4 days of withdrawal	16 781	42.2	6.0	24.0

All animals (300–350 g male rats) received two weeks of post-operative rest before any further experimental treatment. Twenty-four hour urine samples for the determination of the urinary output of α_{2u} globulin were collected just before the sacrifice of the animal for removal of the liver. Post-ribosomal supernatant from the wheat germ translational system containing 300 000 c.p.m. of [^{3}H] leucine incorporated into the peptide chains were used for immunological and electrophoretic separation of the newly synthesized α_{2u} globulin (due to the differences in counting efficiency 300 000 c.p.m. on filter paper was found to be equivalent to 1 605 000 c.p.m. in macerated gel; the counts were not multiplied with any conversion factor and are given as original counts per minute). Messenger RNA activity for α_{2u} globulin is expressed as the c.p.m. of [^{3}H]leucine within the α_{2u} band after SDS-polyacrylamide gel electrophoresis of the α_{2u}–anti-α_{2u} immunoprecipitate of the in vitro product of translation. Each value is an average of three animals.

globulin and the urinary output of this protein in the various thyroidal states may indicate a temporal relationship between the hepatic level of α_{2u} mRNA and the rate of synthesis of α_{2u} globulin. The low level of α_{2u} output in the hypothyroid rats could be explained as primarily resulting from the decreased level of α_{2u} mRNA in the hepatic tissue. Similarly the thyroxine mediated rise in the urinary output of α_{2u} globulin is also found to be associated with increased level of the hepatic mRNA for α_{2u} globulin. The above results suggest that thyroxine exerts its regulatory influence on α_{2u} synthesis by controlling the α_{2u} mRNA concentration. It is possible that the presence of both thyroxine and testosterone are concurrently required for the derepression of α_{2u} gene. On the other hand, thyroxine-mediated increase in the androgen sensitivity of the hepatic tissue can also explain these results.

Although the rise and fall in the level of the hepatic mRNA activity for α_{2u} globulin in different experimental groups followed the same pattern of changes as in the urinary output of α_{2u} globulin, the urinary level of α_{2u} globulin was always found to be lower than the predictable level based on the hepatic mRNA

activity. The above observation suggests that the level of urinary output of α_{2u} globulin may be regulated by additional factors other than the level of the hepatic mRNA for α_{2u} globulin. A positive translational stimulation of the α_{2u} mRNA superimposed over the transcriptional control may explain these results. However, other possible explanations such as increased degradation of α_{2u} globulin in the hypothyroid animals could also be equally compatible with the observation. Precise clarification of this point would require further experimentation.

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