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REGULATION OF RAT HEPATIC PEROXISOMAL ENOYL-CoA HYDRATASE-3-HYDROXYACYL-CoA DEHYDROGENASE BIFUNCTIONAL ENZYME BY THYROID HORMONE

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SUMMARY: Rat hepatic t protein that is negatively regulated by thyroid hormone in nuclear globulin extract was characterized by the antibodies. The following evidence indicated that t protein is a peroxisomal enoyl-CoA hydratase-3-hydroxyacyl-CoA dehydrogenase bifunctional enzyme (bifunctional enzyme). 1. Both proteins had an identical molecular size, and were immunologically indistinguishable from each other. 2. The t protein was abundant in mitochondrial fraction which contained abundant peroxisomes. 3. The amount of the t protein was increased by a peroxisomal proliferator. 4. The activity of the peroxisomal bifunctional enzyme corresponded to the t protein in CM-Sephadex column chromatography. The amount of peroxisomal bifunctional enzyme was increased by thyroidectomy and decreased by 3,5,3'-triiodo-L-thyronine treatment in the whole homogenate of rat liver. These results indicate that the levels of peroxisomal bifunctional enzyme were regulated by thyroid hormone in vivo.

It was shown in 1978 that two rat hepatic nuclear proteins were regulated by thyroid hormone(1). One, termed t protein, was increased by thyroidectomy and decreased by thyroid hormone, whereas the other, termed n protein, was decreased by thyroidectomy and increased by thyroid hormone. Previous studies showed that t protein levels in hepatic nuclei were more closely related to plasma thyroid hormone levels than n protein which was influenced by nutritional factors as well(2). In both proteins, nothing was known about their exact subcellular localization and function. In the present study, we analyzed t protein by the antibodies and provide the evidence that t protein is a peroxisomal enoyl-CoA hydratase-3-hydroxyacyl-CoA dehydrogenase

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bifunctional enzyme (simply referred to as the bifunctional enzyme). In addition, t protein levels were regulated by thyroid hormone in rat liver.

MATERIALS AND METHODS

Materials: Mono Q-Sepharose and CM-Sephadex were purchased from Pharmacia Fine Chemicals (Piskataway, NJ). Peroxidase conjugated anti-rabbit IgG (heavy chain and light chain specific) was from Cappel laboratories (Cochranville, PA). 3,5,3'-triiodo-L-thyronine (T_3) and Freund's complete and incomplete adjuvant were from Sigma Chemical Co.(St. Louis, MO).

Animals : Ninety-100 g male Wistar rats were thyroidectomized shortly after receipt and maintained on a low iodine diet and drinking water containing 0.9 % $CaCl_2$. After at least 4 weeks, rats were weighed and those which did not gain body weight and showed low thyroid hormone levels in the serum (Mean values for serum T_4 and T_3 were 0.3 μg/100ml and 35 ng/100 ml, respectively) were chosen for the experiment. These rats were referred to as thyroidectomized rats. When necessary, thyroidectomized rats were injected intraperitoneally with T_3 (5 μg/100 g body weight) or vehicle for 7 days. For di(2-ethylhexyl) phthalate (DEHP) treatment, rats were fed the diet containing 2 % (w/w) DEHP ad lib for more than 2 weeks(3,4).

Protein purification: Purification of t protein, peroxisomal bifunctional enzyme, and mitochondrial 3-hydroxyacyl-CoA dehydrogenase was performed as described previously(3,5).

Other methods : Preparation of antibodies and Western blotting analysis were performed as described previously(3,5). Subcellular fractionation of rat liver was performed according to the methods of de Duve et al.(6) with modifications(1,2,5). When necessary, the amount of t protein was measured by densitometry of the Western blot using Dual-wavelength Flying Scanner CS-9000 (Shimazu Co. Kyoto, Japan). For this purpose, we selected the condition where the signals on densitometry were proportional to the amount of t protein. Protein content was measured by the Lowry's procedures with modifications(7). Peroxisomal and mitochondrial 3-hydroxyacyl-CoA dehydrogenase activities were measured according to the methods of Osumi and Hashimoto(8).

RESULTS

Identification of t protein as a peroxisomal bifunctional enzyme

After differential centrifugal fractionation of normal rat liver, t protein was present mainly in heavy mitochondrial, light mitochondrial and microsomal fractions and slightly in nuclear fraction (Fig.1). DEHP induced t protein in all subcellular fractions (Fig. 1). Since DEHP is a peroxisomal proliferator and induces peroxisomal beta oxidation enzymes, we tested the possibility that the t protein is a peroxisomal bifunctional enzyme. Electrophoretical mobility was identical in both proteins (Fig. 2 bottom panel). Recognition of the t protein and peroxisomal bifunctional enzyme by

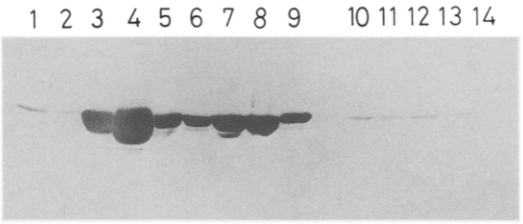

Fig. 1. Induction of t protein by di(2-ethylhexyl)phthalate in various subcellular fractions of rat liver.
Western blotting was performed using anti-t protein antibody. Samples are as follows: Nuclear globulin extract from thyroidectomized (lane 1) or T_3-treated rat liver (lane 2). Whole homogenate (lane 4), nuclear (lane 3), heavy mitochondrial (lane 5), light mitochondrial (lane 6), microsomal (lane 7,8), cytosolic (lane 9) fractions from 0.2 mg (wet weight) liver of rats treated with di(2-ethylhexyl)phthalate. Whole homogenate (lane 10), nuclear (lane 11), mitochondrial (lane 12), microsomal (lane 13), and cytosolic (lane 14) fractions from 0.2 mg (wet weight) liver of normal rats.

the respective antibodies was abolished when the antibodies were pretreated with pure peroxisomal bifunctional enzyme and t protein, respectively (Fig. 3). Since 3-hydroxyacyl-CoA dehydrogenase activity is present in mitochondria as well as peroxisomes, its activity was analyzed before and after treatment of the samples at 60°C for 5 min which destroys peroxisomal enzyme activity without altering mitochondrial enzyme activity(8). Peroxisomal enzyme activity, which was determined by subtracting the heat resistant activity from the activity before heating (Fig. 2 top panel), corresponded well to t protein levels quantified by Western blotting analysis of each fraction (Fig. 2 bottom panel), whereas the amount of mitochondrial enzyme determined by Western blotting using anti-mitochondrial 3-hydroxyacyl-CoA dehydrogenase antibody corresponded well to heat resistant mitochondrial enzyme activity (Fig. 2 middle panel).

Regulation of t protein/peroxisomal bifunctional enzyme by thyroid hormone

As is shown in Fig. 4, t protein levels were increased in whole hepatic homogenate after thyroidectomy. Administration of T_3 to thyroidectomized rats caused a reduction in t protein. Densitometry revealed that the t protein levels of T_3-treated rats were 1/5-1/7 of thyroidectomized rats.

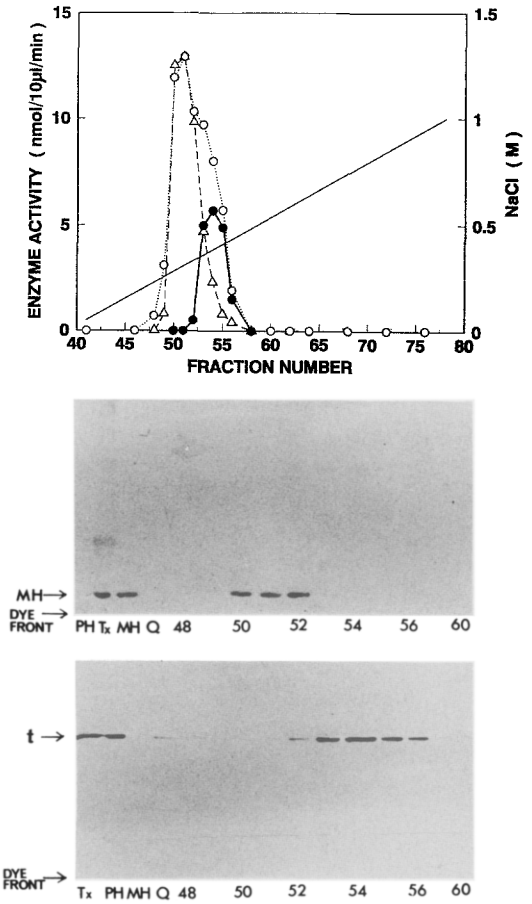

Fig. 2. CM-Sephadex column chromatography of t protein, peroxisomal bifunctional enzyme and mitochondrial 3-hydroxyacyl-CoA dehydrogenase. Thyroidectomized rat nuclear globulin extract was passed through a mono Q-Sepharose column and then applied to a CM-Sephadex column. Fractions after CM-Sephadex column chromatography were assayed for 3-hydroxyacyl-CoA dehydrogenase activity before (—○—) and after (—△—) treatment at 60°C for 5 min. Heat sensitive fraction (—●—), representing peroxisomal enzyme was also determined (top panel). Western blotting using anti-mitochondrial 3-hydroxyacyl-CoA dehydrogenase antibody (middle panel) or anti-t protein antibody (bottom panel) was performed. Samples are as follows: PH, pure peroxisomal bifunctional enzyme; Tx, nuclear globulin extract from thyroidectomized rat liver; MH, pure mitochondrial 3-hydroxyacyl-CoA dehydrogenase; Q,samples before CM-Sephadex column chromatography; 48-60, fraction numbers after column chromatography.

DISCUSSION

Present study shows that rat hepatic t protein is a peroxisomal bifunctional enzyme which catalyses the second and third step reaction of peroxisomal fatty acid beta oxidation. Although the enzyme is localized in peroxisome, it leaks out of the organelle during tissue homogenization and

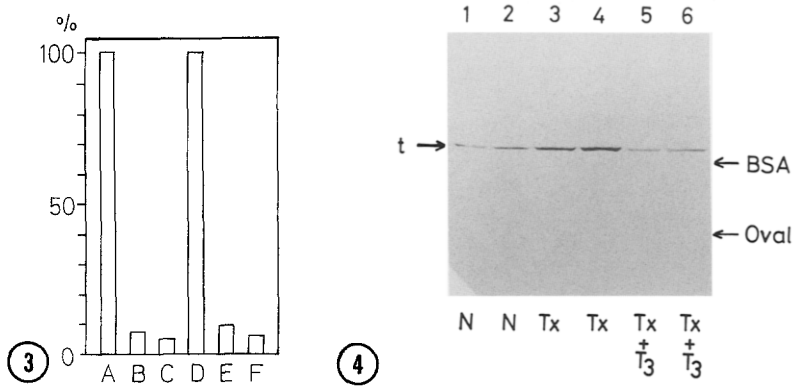

Fig. 3. Immunological cross-reactivity between t protein and peroxisomal bifunctional enzyme.
Thyroidectomized rat hepatic nuclear globulin extract was electrophoresed and Western blotting was performed by using anti-t protein antibody without (A) or with preadsorption by pure t protein (B) or by pure peroxisomal bifunctional enzyme (C). Anti-peroxisomal bifunctional enzyme antibody without (D) or with preadsorption by pure t protein (E) or by pure peroxisomal bifunctional enzyme (F) was also used. The amount of antibody binding was determined by densitometry and was expressed as % of that without preadsorption of the antibodies.

Fig. 4. Regulation of t protein/peroxisomal bifunctional enzyme levels by thyroid hormone.
Anti-t protein antibody was used for Western blotting of whole liver homogenate (50 μg protein, 0.3 mg liver wet weight) prepared from two normal rats (N)(lane 1 and 2), two thyroidectomized rats treated with vehicle (Tx)(lane 3 and 4), and two thyroidectomized rats treated with T_3 (Tx+T_3) (lane 5 and 6). Positions of bovine serum albumin (BSA) and ovalbumin (Oval) were indicated by arrows for molecular size marker.

contaminates the other subcellular fractions (4). Apparent nuclear localization of the t protein was thus a result of artificial contamination during subcellular fractionation. Therefore it is important to know whether total amount of t protein/peroxisomal bifunctional enzyme is regulated by thyroid hormone in whole hepatic tissue. Results in Fig.4 clearly show that hepatic levels of t protein/peroxisomal bifunctional enzyme is dependent on thyroid hormone.

Our previous studies indicated that rat hepatic peroxisomal enzymes, such as L-alpha-hydroxyacid oxidase and D-amino acid oxidase were altered by thyroid hormone(9). However, the activity of rat hepatic acyl-CoA oxidase, a key enzyme catalyzing the rate limiting step of peroxisomal beta oxidation was not affected by thyroid hormone. Therefore it is possible that changes in peroxisomal bifunctional enzyme caused by thyroid hormone do not affect the

overall beta oxidation of fatty acid by peroxisomes. It would be interesting to determine what metabolic consequences result from the alterations of this enzyme and to study further the mechanisms of the regulation of the enzyme by thyroid hormone.

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