Induction by peroxisome proliferators and triiodothyronine of serine:pyruvate/alanine:glyoxylate aminotransferase of rat liver

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Abstract In rat liver, a single serine:pyruvate/alanine:glyoxylate aminotransferase (SPT or SPT/AGT) gene is transcribed from two transcription initiation sites. Transcription from the upstream site generates the mRNA encoding the precursor for mitochondrial SPT (pSPTm) and is markedly enhanced by the administration of glucagon or cAMP. In this report we show the increase in the downstream transcript, the peroxisomal SPT (SPTp) mRNA, caused by peroxisome proliferators and triiodothyronine (T_3). In the case of T_3, the pSPTm mRNA was also increased 72 h after a single administration of the hormone in addition to an earlier increase in SPTp mRNA.

Key words: Peroxisome proliferator; Triiodothyronine; Enzyme induction; Serine:pyruvate aminotransferase

1. Introduction

Serine:pyruvate aminotransferase (SPT; another name is alanine:glyoxylate aminotransferase, AGT) is an enzyme whose subcellular localization in rat liver is mitochondrial and peroxisomal [1,2]. Two mRNAs encoding SPTs with different organelle distributions are generated from a single gene through alternative transcription initiation from two sites, only 65 nucleotides apart, in the same exon 1 [3]. Transcription from the upstream site generates the mitochondrial SPT (SPTm) mRNA encoding the precursor for SPTm (pSPTm), which contains an N-terminal extension peptide of 22 amino acids as a mitochondrial targeting signal [3]. This upstream transcription is markedly and selectively enhanced by the administration of glucagon to rats [4] or the addition of cAMP to the culture medium of primary hepatocytes [5]. The transcription from the downstream site, on the other hand, generates the peroxisomal SPT (SPTp) mRNA which encodes the mature size of the enzyme, and this transcription is affected by neither glucagon nor cAMP. Thus the rat SPT gene has the unique characteristic that two promoters, which are quite different in their response to glucagon or cAMP stimuli, are located very closely. Several studies have revealed the induction of SPTp by peroxisome proliferators through enzymatic or histochemical analysis [6–10], but detailed analysis at the mRNA level remains to be performed. We analyzed in this study the induction of SPTp by peroxisome proliferators and triiodothyronine (T_3) at the mRNA and/or protein levels.

2. Materials and methods

2.1. Animals and drug administration

Male Wistar rats weighing 200–250 g were given daily intraperitoneal injections of clofibrate (ethyl *p*-chlorophenoxyisobutyrate) at doses of 20 or 40 mg/100 g body weight for 7 days, or a daily intragastric dose of di-(2-ethylhexyl)phthalate (DEHP) (0.25 g/100 g body weight) for 4 or 8 days. The rats were also given a single intraperitoneal injection of T_3 (50 μg/100 g body weight) 3, 6, 12, 24 or 72 h before they were killed, or a single intraperitoneal injection of glucagon (0.3 mg/100 g body weight) 3.5 h before they were killed.

2.2. Experimental procedures

Subcellular fractionation of a clofibrate-administered rat liver extract on a sucrose density gradient was performed as reported previously [2]. Dot blot analysis was carried out using 'probe E' [3] of SPT cDNA and the *Hin*fI (1278)-*Pvu*II (1702) fragment (424 bp) of rat serum albumin cDNA [11] as probes. For RNA blot analysis, 'probe A' [3] of SPT cDNA was also used as a specific probe for pSPTm mRNA. In vitro translation was performed as described previously [4], and the specific products were detected by use of rabbit anti-rat SPT serum and rabbit anti-rat serum albumin serum. Ribonuclease protection and primer extension analyses were carried out using 'probe a' and 'an oligonucleotide of 17 bases', respectively, as reported [3].

3. Results and discussion

Peroxisome proliferators such as clofibrate [12] and DEHP [13] are known to increase peroxisomes and also to induce peroxisomal enzymes. Enzymes of the β-oxidation system (acyl-CoA oxidase, etc.) are markedly induced by DEHP up to 30-fold, whereas the induction of catalase is only about 2-fold [14]. Fig. 1 shows the effects of clofibrate on the activities of SPT in mitochondrial and peroxisomal fractions which were separated by sucrose density gradient centrifugation. Fig. 1A shows that SPT was detected in two subcellular organelles, peroxisomes and mitochondria, and the soluble fraction as reported previously [1,2]. Clofibrate administration caused an increase in peroxisomes as shown by the catalase profile, and these peroxisomes also contained SPT (Fig. 1B,C). From the combined catalase activities in the particulate fractions 1–20, the induction ratios of catalase at doses of 20 and 40 mg/100 g body weight of clofibrate were calculated to be 1.66-fold and 1.43-fold of the control level, respectively. Because the activity profile of SPTp is similar to that of catalase, the induction ratio of SPTp was suggested to be 1.4–1.7-fold of the control level after clofibrate treatment. These values for SPT induction are in good agreement with those in other reports [6–8,10]. On the other hand, the induction ratio of glutamate dehydrogenase, a marker enzyme of mitochondria, was similarly calculated from the combined activities in

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Abbreviations: SPT, serine:pyruvate aminotransferase; AGT, alanine:glyoxylate aminotransferase; SPTm, mitochondrial SPT; SPTp, peroxisomal SPT; pSPTm, precursor for SPTm; T_3, triiodothyronine; DEHP, di-(2-ethylhexyl)phthalate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis

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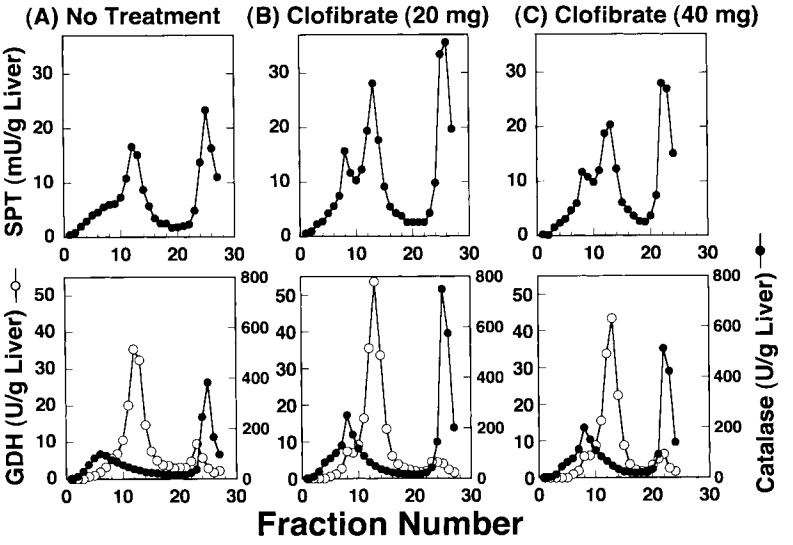

Fig. 1. Subcellular fractionation by sucrose density gradient centrifugation of liver extracts of rats treated with clofibrate. Rats were given daily intraperitoneal injections of clofibrate at doses of 20 (B) or 40 mg/100 g body weight (C) for 7 days. In control rats (A), only peanut oil, the solvent for clofibrate, was injected for 7 days. Livers were excised after 24 h fasting and then homogenized. Subcellular fractionation was carried out as described in Section 2, and the activities of SPT, glutamate dehydrogenase (GDH), and catalase in each fraction were determined as reported previously [2]. The left and right sides of the figures correspond to the bottom and top of the gradient, respectively.

fractions 1–20 to be 1.06-fold and 0.88-fold of the control level with 20 and 40 mg/100 g body weight of clofibrate administered, respectively. SPTm shows a similar activity profile, indicating that clofibrate has no effect on the glutamate dehydrogenase and SPTm activities. These results indicate that SPTp and catalase belong to the same group of peroxisomal enzymes, which are induced moderately by clofibrate.

In our preliminary experiment, the administration of DEHP, another peroxisome proliferator, also caused a small but significant increase in SPT activity. We then examined the effect of DEHP on the level of SPT mRNA. The effect of T_3 was also examined because several candidates for the half-site motif of the thyroid hormone-responsive element, AGGTCA, were observed in the 5′-flanking region of the SPT gene [15]. Fig. 2 shows the results of dot blot analysis. A cDNA fragment of rat serum albumin was used for normalization as to the change in the induction of SPT mRNA. Various amounts of poly(A) RNA were dotted in triplicate, and then hybridized with the SPT and serum albumin probes, autoradiographed and quantitated by densitometric analysis (Fig. 2A,B). By using the values within the linear range, the induction of SPT mRNAs at various time points after the DEHP or T_3 treatment was calculated (presented in Fig. 2C). The results show that both DEHP and T_3 induce SPT mRNA.

To determine whether the effects of these reagents are specific for SPTp, RNA blot analysis and SDS-PAGE analysis of the immunoprecipitated in vitro translation product were carried out (Fig. 3A,B). Both DEHP and T_3 caused an increase in SPTp mRNA of 1700 nucleotides (Fig. 3A, SPT probe E), and the induction of SPTp of 43 kDa (Fig. 3B). However, pSPTm mRNA of 1900 nucleotides (Fig. 3A, lane 9) and pSPTm of 45 kDa (Fig. 3B, lane 9) were detected only when poly(A) RNA prepared 72 h after T_3 treatment was used. This was confirmed by detecting the pSPTm mRNA signal of 1900 nucleotides using 'SPT probe A', which is specific for pSPTm mRNA (lower panel in Fig. 3A, lane 9). There were no significant changes in the amount of in vitro translated serum albumin. As the pSPTm mRNA and its translation product, pSPTm, seemed to be induced 72 h after T_3 administration, we tried to confirm this by means of a ribonuclease protection assay (Fig. 3C) and primer extension analysis (Fig. 3D). Lane 9 in Fig. 3C shows that 72 h after T_3 treatment, transcription from the upstream site, generating pSPTm mRNA, was significantly enhanced. This was also confirmed by primer extension analysis, as shown in Fig. 3D (lane 4).

Two modes of action of T_3 on SPT mRNA induction are suggested by the above results. One is an early effect, inducing SPTp mRNA, and the other is a delayed effect, inducing pSPTm mRNA. The early effect of T_3 may be explained in part by the peroxisome proliferator-like action of T_3, as it was shown in a previous report [16] that hyperthyroidism caused by T_3 results in an increase in the peroxisome number in rat liver. It has been reported, concerning the delayed effect of T_3, that thyroid hormones induce hyperinsulinemia in the rat [17], and that insulin, as well as glucagon, causes the induction of pSPTm mRNA [18].

The dual subcellular localization of SPT in rat liver is probably related to its specific physiological role in each organelle. SPTm may contribute to the gluconeogenesis from or catabolism of serine in mitochondria [19] while the role of SPTp is to reduce oxalate formation by the conversion of glyoxylate, an immediate precursor of oxalate, into glycine as manifested by the SPTp-deficient human disease, primary hyperoxaluria

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Fig. 2. Dot blot analysis of SPT mRNA after DEHP or T_3 administration. Various amounts of poly(A) RNAs prepared from the livers of normal (N), DEHP-fed (DEHP), or T_3-administered (T3) rats were spotted in triplicate onto nylon membranes, and then hybridized with ^{3}P-labeled SPT probe E (A) or albumin probe (B). A part of the results of several experiments is shown in (A) and (B). C: An autoradiogram of each dot was quantitated by densitometric analysis using an ATTO Densitograph AE-6900M. The extent of induction of SPT mRNA after DEHP or T_3 treatment was determined by normalizing the SPT mRNA signals with albumin signals, using values in the linear range of the densitometric data.

Fig. 3. RNA blot (A), in vitro translation (B), ribonuclease protection (C) and primer extension (D) analyses of SPT mRNA after DEHP or T_3 treatment. A: Two types of SPT probes, E and A, were used for RNA blot analysis. Probe E is common to both pSPTm (1900 nt) and SPTp (1700 nt) mRNAs, whereas probe A is specific to pSPTm mRNA, as reported previously [3]. Poly(A) RNA prepared from the livers of rats injected with glucagon 3.5 h before they were killed (G3.5) was used as the glucagon-induced control, the major species of mRNA being pSPTm mRNA of 1900 nt, as reported [18]. B: For in vitro translation analysis, an anti-SPT serum and an anti-serum albumin serum were used. C: The antisense probe used in this experiment was 'probe a', which was used for the detection of the difference in the 5′-end of SPT mRNA in the previous study [3]. 2 μg of poly(A) RNA was used in each lane, except in lane 1, where 0.2 μg of the RNA was used because of the strong signal. D: Primer extension analysis was carried out as described Section 2 using 10 μg of poly(A) RNA prepared from the livers of normal rats (N), rats administered T_3 for 72 h (T72), or rats injected with glucagon 3.5 h before they were killed (G3.5). The 5′-end-labeled *Hin*fI digest of pBR322 was applied in lane 1 as size markers: a (504 bp), b (396 bp), c (344 bp), d (298 bp), e (221/220 bp), f (154 bp) and g (75 bp).

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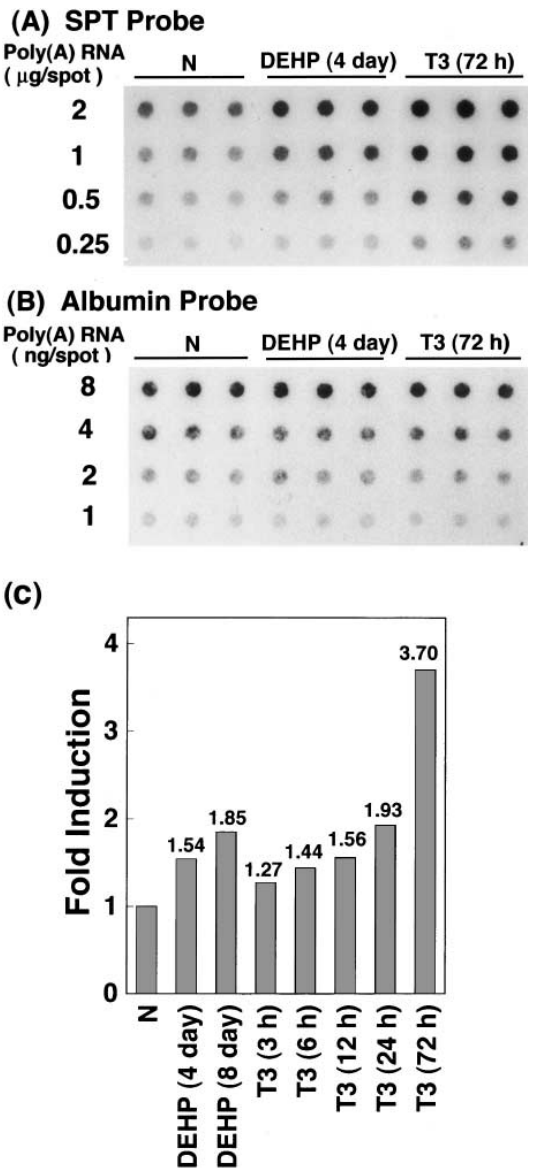

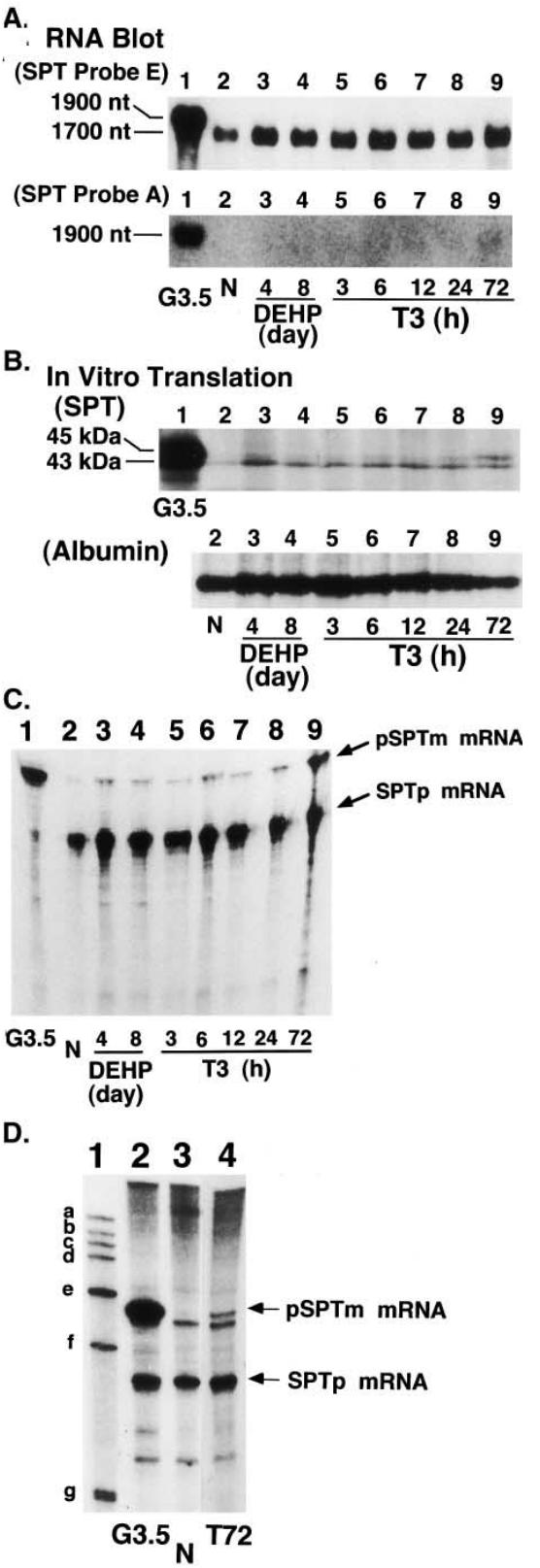

type 1 [20]. In rat liver, the level of mitochondrial and peroxisomal SPT appears to be primarily determined by the transcription from the upstream and downstream initiation sites, respectively, although the turnover rate of SPTm and SPTp may also contribute to the steady-state concentration of this

enzyme in each organelle. It is, therefore, necessary to analyze the transcriptional regulation of the SPT gene to understand the proper distribution of SPT to mitochondria and peroxisomes under various metabolic conditions.

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