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Transient induction of fatty acid synthase in rat liver after removal of a peroxisome proliferator

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Abstract Removal of a peroxisome proliferator from the diet triggered the degradation of peroxisomes and induced the transient expression of a 220 kDa soluble protein in rat liver. The 220 kDa protein was purified by conventional methods and analyzed by amino acid sequencing. A total of 99 amino acid residues in 4 lysylendopeptidase-digested peptides completely matched those in rat fatty acid synthase. The transient induction of fatty acid synthase mRNA during peroxisome degradation was confirmed by Northern blotting.

Key words: Peroxisome; Peroxisome proliferator; PPAR; Fatty acid synthase; Rat liver

1. Introduction

Peroxisomes are unique in their ability to proliferate in rodent liver cells in response to several disparate chemicals, which are designated 'peroxisome proliferators (PP)' (for a review, see [1]). The molecular mechanism of PP action and its physiological meaning have become clearer after the cDNA cloning of a PP-activated receptor (PPAR) (for a review, see [2]). In contrast, the process of reduction in the normal liver after removal of PPs is poorly understood. Upon removal of PPs, a dramatic decrease in the size and in number of peroxisomes occurs and hepatomegaly should be de-induced in a few days. Significant changes in metabolism should occur. Though an autophagic mechanism has been recently proposed for the degradation of the peroxisomes [3,4], the effects of the removal of PPs on other cellular processes have only been studied to a small extent [5]. Therefore we investigated the induced changes in the rat liver after removal of PPs and report here that the expression of fatty acid synthase is transiently induced.

2. Materials and methods

2.1. Materials

Clofibrate (2-(*p*-chlorophenoxy)isobutyric acid ethyl ester) was purchased from Tokyo-Kasei (Tokyo, Japan). DNA polymerase I, DNase I and T4 polynucleotide kinase were obtained from Toyobo (Osaka, Japan). [γ-^{32}P]ATP (~3000 Ci/mmol) and [α-^{32}P]dCTP were from ICN (Irvine, USA). Fatty acid synthase mRNA was detected using a 5'-end labeled synthetic 25mer oligonucleotide (Sawaday, Tokyo, Japan), (complementary to the published cDNA sequence, nucleotide number 555 to 579 [6]).

2.2. Feeding rats and preparation of light mitochondrial fraction

Male Fischer F344 rats (4 months old, 250–300 g) were given a diet containing 0.5% (w/w) clofibrate. At the indicated time, the rats were anesthetized with ether and decapitated. Post-mitochondrial fractions (PMS) were obtained from a portion of the livers as described [7].

2.3. Purification of a 220 kDa protein

The presence of a 220 kDa protein (P220) was assessed by SDS-PAGE and protein staining. PMS from the livers of the rats refed with a normal diet for 2–3 days after receiving that containing clofibrate, was centrifuged at 100,000 × *g* for 60 min at 0°C. The supernatant was fractionated by ammonium sulfate precipitation (10–40%), and purified by ionic exhange chromatography (DE-52, pH 8.5) followed by HPLC gel filtration (TSK G3000SW$_{XL}$).

2.4. Amino acid sequencing

The purified P220 was digested by lysylendopeptidase (20:1) and the peptides were separated by reverse phase HPLC as described [8]. Four peptides that were isolated from others on the chromatogram were recovered and their amino acid sequences were analyzed by automated Edman degradation using a ProSequencer (MilliGen) according to the manufacturer's instructions.

2.5. Isolation of RNA and Northern blotting

Total RNA was isolated and analyzed as described [9]. Hybridization with a 5'-end labeled synthetic oligonucleotide proceeded in hybridization buffer containing 10% (v/v) formaldehyde, and the membranes were washed finally with 2 × SSC, 0.1% SDS at 40 °C.

3. Results

3.1. Removal of a PP induced 220 kDa protein

SDS-PAGE analysis of the proteins in PMS fractions obtained from rats given clofibrate or a subsequent normal diet, showed the induction of peroxisome as assessed by the 77 kDa marker protein [10] at a level of over twenty times that of the control level [9], followed by a rapid decrease (Fig. 1). The decrease began 2–3 days after the removal of clofibrate from the diet and at almost the same time, a 220 kDa protein (P220) started to increase for a day or two. The time courses of the changes in the levels of the 77 kDa protein and P220 during the entire period are shown in Fig. 1B.

3.2. Purification and identification of P220

P220 was soluble in PMS because almost all of it was recovered in the supernatant after centrifugation at 100,000 × *g* for 60 min as assessed by SDS-PAGE (not shown). P220 was thus purified from the supernatant of PMS obtained from the livers of rats fed with clofibrate for 2 weeks followed by a normal diet for 2–3 days. The protein was purified almost to homogeneity by a combination of conventional methods (see section 2, and Fig. 2).

To identify P220, the lysylendopeptidase-digested peptides

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Abbreviations: PP, peroxisome proliferator; PPAR, PP-activated receptor, PMS; post-mitochondrial supernatant, SDS-PAGE; SDS-polyacrylamide gel electrophoresis, FAS; fatty acid synthase.

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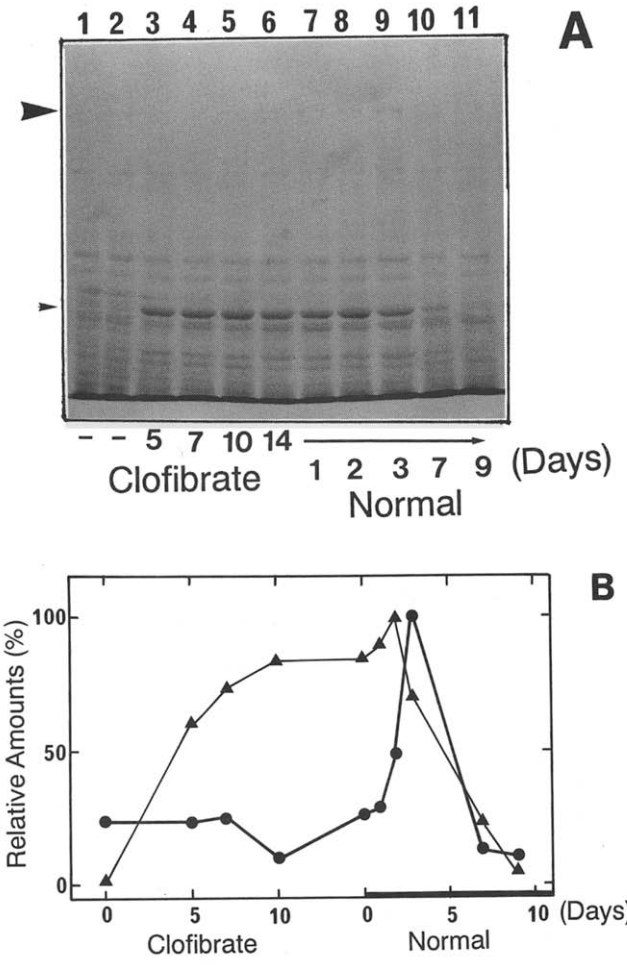

Fig. 1. Time course of changes in protein patterns of PMS from the livers of rats fed clofibrate. (A) PMS fractions (50 μg protein) were prepared from the livers of rats given a control diet (lanes 1 and 2, numbered from the left), clofibrate (0.5%) for 5 to 14 days (lanes 3–7), or clofibrate for 14 days and then the control diet for an additional 1 to 7 days (lanes 8–12), and analyzed by SDS-PAGE (7.5%). The arrowhead at the left shows the position of P220. (B) Time courses of the changes of P220 (●) and the 77 kDa marker protein (▲) quantified by densitometry of (A).

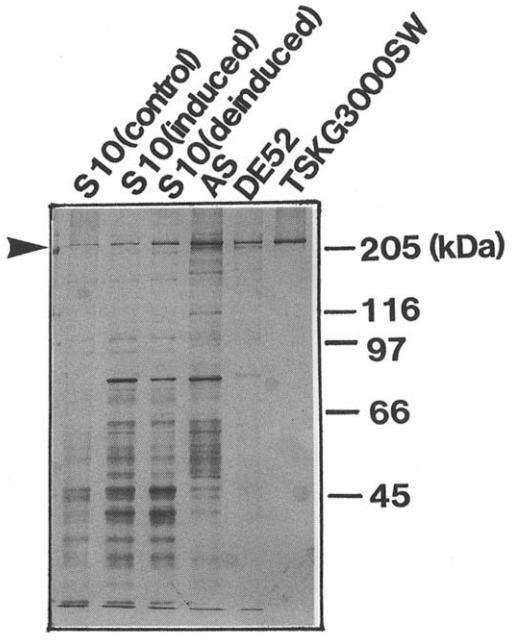

Fig. 2. Purification of P220. P220 was purified by centrifugation from the PMS fraction from the livers of the rats given the normal diet for 2–3 days after that containing clofibrate (S10), ammonium sulfate fractionation (AS), DE-52 column chromatography (DE52), and HPLC gel filtration (TSK G3000SW). A portion of each fraction (1–3 μg) was analyzed for purity by SDS-PAGE followed by silver staining. The arrowhead on the left indicates the position of P220 and the molecular weights are shown in kDa on the right.

were fractionated and the amino acids of 4 peptides (P220-1 to P220-4, with retention times at 76, 78, 81 and 83 min, respectively) were sequenced. The results are summarized in Fig. 3. The four peptide sequences were independently searched for similarity among data bases and all exactly corresponded to portions of the same protein, fatty acid synthase [6]. The molecular weight in SDS-PAGE and its soluble nature, as well as matching in amino acid sequences, all indicated that P220 is fatty acid synthase.

3.3. Northern blots

To examine the step of regulation at which the level of fatty acid synthase is increased, the changes in the level of the synthase mRNA were measured by Northern blotting (Fig. 4). An mRNA larger than 28S rRNA was scarcely detected during the induction period, but it transiently increased 2–3 days after the removal of clofibrate, then decreased to the normal level. The timing of the increase in the mRNA well corresponded with that in the protein levels, confirming that the induced P220 is fatty acid synthase and suggesting that the increase was mediated by activation of the transcription rate and/or in stabilization of the mRNA.

4. Discussion

The induction and de-induction of peroxisomal β-oxidation and the microsomal ω-hydroxylation enzymes for fatty acids should be co-regulated with other enzymes in lipid metabolism to maintain lipid homeostasis. Fatty acid synthase, which plays

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                    1         10        20
P220-P1:            VLVQREEEPEAMLPGAQPTLISA
FAS:      (1845)K=======================(1869)

                    1         10        20
P220-P2:            GHALGXTLAXLPSXVQPGPSFLSQXXWESL
FAS:      (1354)K=====E===C===E==========EE====(1374)

                    1         10        20
P220-P3:            XLEXRVAAAVDLITRSXQSLDRRLS
FAS:      (2385)KS==D===========H========(2411)

                    1         10        20
P220-P4:            LSVPXYGLQCTQAAPLDSIXNLAAYYIXCI
FAS:      (2257)K====T==============P=======D==(2287)
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Fig. 3. Amino acid sequences of lysylendopeptidase-digested fragments in alignment with the published sequences of rat fatty acid synthase (FAS). The residues indicated by an 'X' could not be identified. Those substituted by '=' in the published sequences are the same as those which we identified.

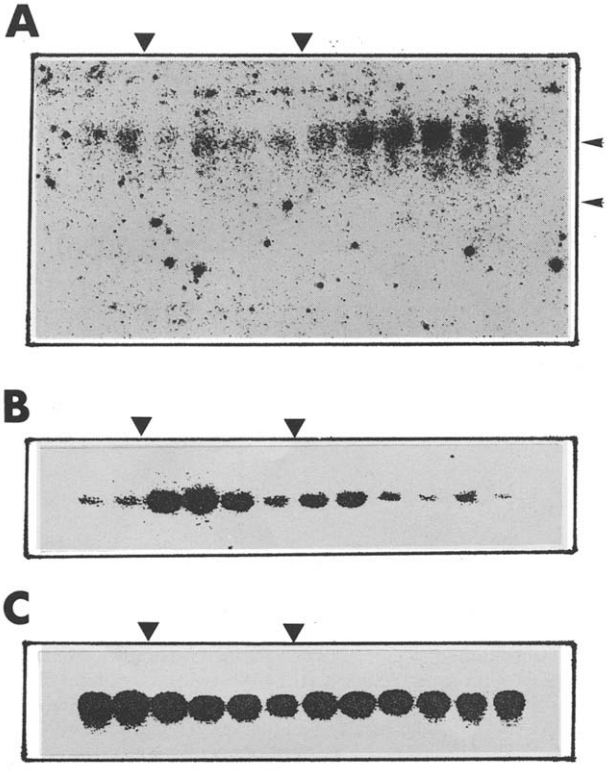

Fig. 4. Northern blots of changes in the levels of fatty acid synthase mRNA (A), peroxisomal thiolase mRNA (B), and control α_{2u} globulin mRNA (C). Arrow heads indicate the time of addition (left) and withdrawal (right) of clofibrate. See Fig. 1 for a detailed schedule.

a central role in de novo lipogenesis by catalyzing all of the reactions in the conversion of acetyl-CoA and malonyl-CoA to palmitate [11], may be one of these enzymes. The hormonal regulation of FAS gene transcription has been studied in detail [11,12], but there is no data at present about involvement of PPAR in its transcriptional regulation. The ability of PPARs to form a heterodimer with various transcription factors requires further study [2]. The sequence TGTCCAATGGTCT (located at nucleotides −509 to −496) is present in the promoter region of the rat FAS gene [13]. It is composed of a possible PPAR binding motif TGTCCA, a one-base spacer, and TGGTCT, which is homologous to the TGGACA motif found in the PPAR heterodimer binding sites in the promoters of the rat apolipoprotein CIII [14] and P450 IVA [15] genes. Changes in the levels of other enzymes as well as the cellular pool of fatty acids in relation to PP action should be studied to understand how complex lipid metabolism is regulated and the role of PPAR.

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