THE PEROXISOME PROLIFERATION-ASSOCIATED POLYPEPTIDE IN RAT LIVER

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Summary: A marked increase in the content of a polypeptide, with an apparent molecular weight of 80,000, was observed on sodium dodecyl sulfate-polyacrylamide gel electrophoresis, in the post-nuclear, large particle (crude mitochondrial) and microsomal pellets of liver of rats treated with the hepatic peroxisome proliferators, clofibrate, nafenopin and Wy-14,643. This protein band was also present when peroxisome proliferation was induced in the absence of catalase synthesis, by combined treatment of rats with Wy-14,643 and the inhibitor of catalase synthesis, allylisopropylacetamide; suggesting that it is not a catalase subunit. No increase in this polypeptide occurred in rats treated with phenobarbital or with allylisopropylacetamide, the compounds which cause a marked proliferation of smooth endoplasmic reticulum in liver cells. The nature and significance of the increase of this polypeptide in the liver of rats treated with hypolipidemic peroxisome proliferators and its specificity, if any, to peroxisome membranes remain to be elucidated.

A number of structurally unrelated compounds that lower serum lipid levels in man and animals, cause a striking increase in hepatic peroxisome (microbody) profiles in rats and mice (1-3). Morphometric analysis, of the ultrastructural changes in rat liver induced by the peroxisome proliferators, revealed a several-fold increase in the relative volume and surface density of peroxisomes and smooth endoplasmic reticulum (SER). These morphometric values for mitochondria, however, were not significantly altered (4,5). The increase in peroxisomes was accompanied by a significant elevation of hepatic catalase and carnitine acetyltransferase activities (6,7). The frequent association between hepatic peroxisome proliferation and hypolipidemia strongly suggests the possibility that these organelles may be involved in lipid metabolism (2). Evidence that hepatic catalase is not essential for the hypolipidemic action of peroxisome proliferators has been obtained from studies where the hypolipidemic drugs, clofibrate, methyl clofenapate, and nafenopin were administered to rats in combination with allylisopropylacetamide, an agent which inhibits catalase synthesis (8,9). From these various studies it is concluded

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that, peroxisome proliferators may induce the synthesis of yet unidentified peroxisomal proteins (8). The present study compares the polypeptide composition, using sodium dodecylsulfate-polyacrylamide gel electrophoresis, of subcellular fractions of liver, of control rats, with those treated with peroxisome proliferators.

MATERIALS AND METHODS

Animals and Treatment: Inbred male F-344 rats obtained from ARS Madison, Wisconsin, weighing 125-150 g, were fed 0.25% clofibrate (ethyl- α-p-chlorophenoxyisobutyrate); 0.1% nafenopin (2-methyl-2-(p-(1,2,3,4-tetrahydro-1-naphthyl) phenoxy) propionic acid); or 0.1% Wy-14,643 ((4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio) acetic acid) in the powdered Purina rat chow, for four weeks to induce hepatic peroxisome proliferation. Additional groups of rats were treated for one week, with phenobarbital (100 mg/kg b. wt. i.p. once daily) or with allyisopropylacetamide (AIA; 200 mg/kg b. wt., s.c. twice daily) to induce SER proliferation. To induce hepatic peroxisome proliferation in the absence of catalase synthesis, male rats were treated simultaneously with 0.1% Wy-14,643 in the diet and injected with AIA twice daily for seven days. The animals were sacrificed under light ether anesthesia. Small pieces of liver were fixed in O_sO_4 and processed for electron microscopy.

Subcellular Fractionation: This was done essentially as described by Kurup et al. (10). The livers were perfused with cold normal saline, weighed, and 10% homogenate in 0.25 M sucrose was prepared using Potter-Elvehjem homogenizer with 10 up and down strokes. After the removal of unbroken cells and nuclei by centrifugation at 700 g for 10 min, the supernatant was centrifuged, at 105,000g for 60 min in a Beckman L5-65 ultracentrifuge, to obtain post-nuclear pellet or at 8300 g for 10 min in RC-2 centrifuge to obtain large particle pellet consisting mostly of mitochondria, peroxisomes and lysbsomes. The supernatant, collected after sedimenting the large particle pellet at 8300 g, was centrifuged at 105,000 g for 60 min, in Beckman L5-65 ultracentrifuge, to obtain microsomal pellet. All pellets were washed in 0.25 M sucrose and resedimented using the appropriate centrifugal force. The pellets were solubilized with 3% sodium dodecyl sulfate and the total proteins estimated by the method of Lowry et al. (11).

Sodium Dodecyl Sulfate (SDS)-Polyacrylamide Gel Electrophoresis: The SDS-polyacrylamide gel electrophoresis was performed according to the technique of Laemmli (12) with minor modifications. Gels consisting of 7.5%, 8.5% and 10% acrylamide were prepared from a stock solution of 30% acrylamide and 0.8% N,N'-bis methylene acrylamide. The separation gel of 9 to 10 cm length had the final concentration of the following: 0.375 M Tris-HCl (pH 8.8) and 0.1% SDS. Polymerization of the gels was accomplished by the addition of 0.024% tetramethylethylenediamine (TEMED) and 0.1% ammonium persulfate. The stacking gels of 3% acrylamide contained 0.125 M Tris HCl (pH 6.8) and 0.1% SDS. The electrode buffer, Tris-glycine (pH 8.3) contained 0.1% SDS. The samples were solubilized with final sample buffer, consisting of 0.0625 M Tris HCl (pH 6.8), 2% SDS, 20% glycerol, 5% mercaptoethanol and 0.001% bromophenol blue. After proteins were completely dissociated by immersing the final samples in boiling water bath for 3 min, 100 µg of protein was loaded on the stacking gel. Electrophoresis was carried out at 4 mA/gel until the marker dye reached the bottom of the gel (about 5 hr). The proteins were then fixed in the gel with 20% TCA for 30 min and stained overnight with 0.1% Coomassie brilliant blue in 20% TCA. Destaining was done by repeated washing in 40% ethanol and the gels were

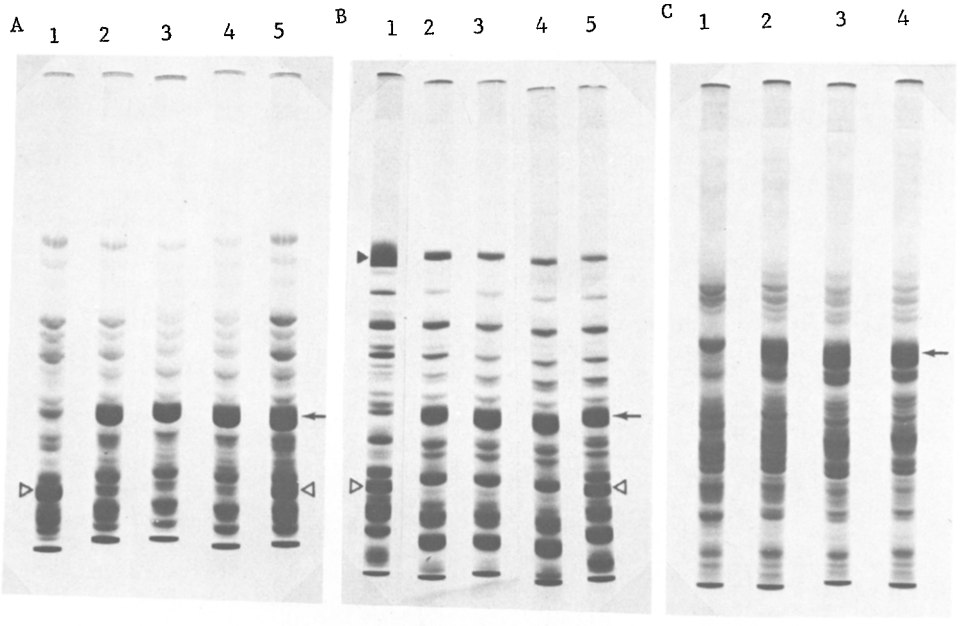

Figure 1. SDS-polyacrylamide gel electrophoresis of (A) post-nuclear; (B) large particle; and (C) microsomal pellets of liver, of normal and peroxisome proliferator-treated rats. Approximately 100 µg samples were analyzed from each subcellular fraction; A and B represent 7.5% and C, 8.5% SDS-polyacrylamide gels. The sample order is: 1, normal; 2, clofibrate; 3, nafenopin; and 4, Wy-14,643 treated rats. The sample 5, in A and B represents Wy-14,643, to which 5 µg of catalase was added. Catalase was also added to sample 1 in A and B. The position of added catalase (60,000 MW) in the gel, is indicated (◁). Arrows indicate the position of 80,000 MW polypeptide in rats treated with peroxisome proliferators. The polypeptide was not increased in post-microsomal supernatant of treated rats (not illustrated). In the large particle pellets of treated rats (B) the 145,000 MW band appears reduced (▶).

stored in 7% acetic acid. Relative mobility of the following standard proteins were obtained: chymotrypsinogen A (25,000); aldolase (35,000); ovalbumin (45,000); catalase (60,000), bovine serum albumin (68,000), phosphorylase (94,000), β-galactosidase (130,000) and γ-globulins (160,000).

RESULTS AND DISCUSSION

The treatment of rats with the hypolipidemic agents, clofibrate, nafenopin and Wy-14,643 caused as expected, a marked increase in liver weight and a striking proliferation of hepatic peroxisomes. In addition, these drugs also increased the total protein content of post-nuclear and large particle pellets of the liver by about 40%. This increase is attributable to increases

in the relative volume and surface density of peroxisomes and SER induced by these drugs (4,5).

Figure 1, illustrates the appearance of typical SDS-polyacrylamide gels of post-nuclear, large particle and microsomal pellets, from the livers of normal and hypolipidemic drug-treated rats. In rats treated with these hypolipidemic peroxisome proliferators, approximately the same number of polypeptide bands were seen as in the corresponding fractions of normal rats; however, a band of approximately 80,000 MW consistently increased in all three subcellular fractions (Fig. 1). Calibration of the gels was carried out by adding 5 µg of catalase, to some of the solubilized samples, just before electrophoresis (Fig. 1A and B) in addition to, determining the relative mobility of proteins of known molecular weights. The increase in the 80,000 MW polypeptide was also observed in the livers of rats treated concomitantly with the peroxisome proliferator Wy-14,643, and allylisopropylacetamide, an inhibitor of catalase synthesis. Our earlier observations on rats treated with allylisopropylacetamide and with clofibrate (8) or with other peroxisome proliferators (9), demonstrated that peroxisome proliferation also occurs in the absence of catalase synthesis, suggesting that these drugs may induce the synthesis of not yet identified peroxisomal proteins. The increase of 80,000 MW polypeptide in rats treated with Wy-14,643 and allylisopylacetamide, suggests that this polypeptide is not a catalase subunit and further supports the contention that peroxisome proliferators may induce the synthesis of yet unidentified peroxisomal proteins (8). This polypeptide band did not increase in the post-microsomal supernatants of treated rats, and though not shown here, treatment of pellets with 30 mM KCl and 20 mM EDTA solution, does not result in the extraction of this polypeptide. These observations suggest that this polypeptide is possibly membrane bound (13).

The presence of this 80,000 MW polypeptide in all three subcellular fractions of liver of rats treated with the peroxisome proliferators, may be due, either to its induction in membranes of all subcellular organelles or

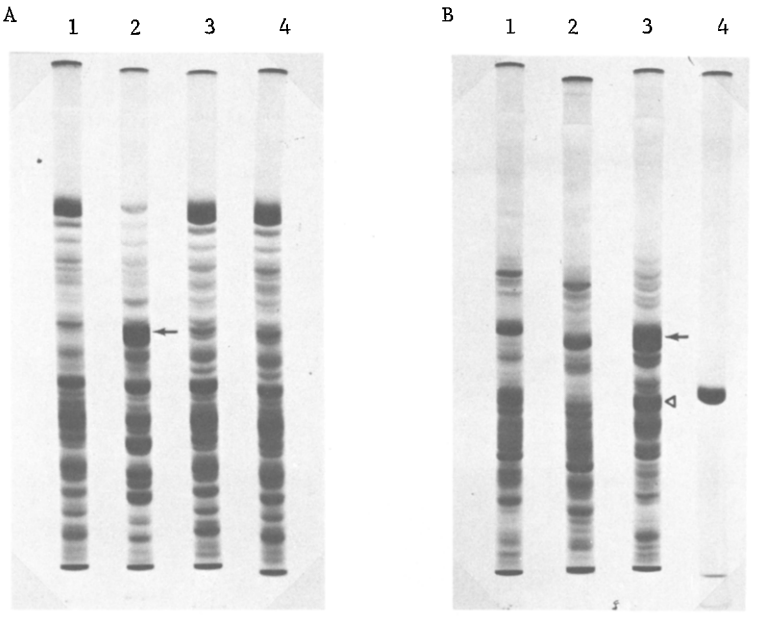

Figure 2. Electrophoretic profile of (A) large particle; and (B) microsomal pellets, analyzed on 8.5% SDS-polyacrylamide gels. The sample order in A: 1, normal rat; 2, Wy-14,643 for 1 week; 3, phenobarbital; and 4, allylisopropylacetamide. The sample order in B: 1, phenobarbital, 2, allylisopropylacetamide; 3, Wy-14,643 with 5 μg of catalase added; and 4, catalase standard. The position of 80,000 MW polypeptide is indicated by arrows. The position of internal catalase standard is indicated by ◁ .

that it may be specific for proliferated peroxisomes and/or SER. Several, morphologic and biochemical, studies have clearly established that peroxisomes do not exist as individual entities but represent accumulation of peroxisomal proteins in dilated channels of SER (14-16); implying that the peroxisomal and SER membranes are similar in their composition. Accordingly, the increase of this polypeptide band in SER, as well as in post-nuclear and large particle pellets, may be due to the induction of this protein specifically in peroxisomes and the peroxisome-associated SER membranes. The presence of this polypeptide band in microsomal pellets excludes the possibility of its being exclusively mitochondrial in origin. Electron microscopy of microsomal pellets failed to reveal any mitochondrial profiles.

Evidence, that agents which cause predominantly the proliferation of SER do not induce this polypeptide, is derived from the livers of rats treated with

phenobarbital or allylisoproplyacetamide. Figure 2, illustrates a comparison of the SDS-polyacryl amide gels of large particle and microsomal pellets of liver obtained from normal rats and rats treated with phenobarbital, allylisopropylacetamide and Wy-14,643. The increase in 80,000 MW polypeptide is seen in the gels of Wy-14,643 treated rats. In contrast, this band showed no increase in the livers of rats treated with compounds which cause a significant increase in liver weight and proliferation of SER (17,18).

In conclusion, these studies demonstrate that hepatic peroxisome proliferators cause a striking increase in a 80,000 MW polypeptide in subcellular fractions of the liver, whereas agents that induce proliferation of SER do not. Additional studies are needed to characterize the nature of this polypeptide, its specificity to peroxisome membranes, and its role, if any,in lipid metabolism.

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