Indentification of β-oxidation enzymes among peroxisomal polypeptides

Increase in Coomassie blue-stainable protein after clofibrate treatment

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Peroxisomes contain enzymes catalyzing the β-oxidation of fatty acids, which have been purified and partially characterized. Hypolipidemic drugs, including clofibrate, cause a marked proliferation of peroxisomes and a striking increase in the activity of their β-oxidation system. We have compared by sodium dodecyl sulfate–polyacrylamide gel electrophoresis the polypeptide patterns of normal and clofibrate-induced peroxisomes and the purified β-oxidation enzymes. The data allow a tentative identification of the β-oxidation enzymes among the peroxisomal polypeptides; these enzymes constitute only a small part of the protein of normal peroxisomes. A subset of peroxisomal polypeptides, including the β-oxidation enzymes, is preferentially increased by clofibrate.

Peroxisome composition *Hypolipidemic drugs* *β-Oxidation enzyme* *Organelle biogenesis* *SDS–PAGE*

1. INTRODUCTION

Peroxisomes catalyze the β-oxidation of fatty acids in rat liver [1,2]. The individual enzymes in this spiral reaction pathway have been purified and partially characterized [3–6]; their properties differ from the corresponding mitochondrial β-oxidation enzymes. In particular, the second and third enzymes (enoyl-CoA hydratase and β-hydroxyacyl-CoA dehydrogenase) form a bifunctional protein [3]. Highly purified peroxisomes have been analyzed by SDS–PAGE [7] and by two-dimensional mapping and found to contain at least 35 polypeptides (in preparation).

Clofibrate, a hypolipidemic drug, causes peroxisome proliferation [8,9] and a 10-fold increase in the activity of peroxisomal β-oxidation [1]. The bifunctional protein has been shown to be identical [10], as suggested [3], with a peroxisomal proliferation associated polypeptide [11]. The pattern of peroxisomal polypeptides is altered by clofibrate treatment: some, but not all, polypeptides are increased in abundance as shown by one and two-dimensional gel analyses (unpublished). Here, we compare by SDS–PAGE the purified β-oxidation enzymes and the total peroxisomal proteins of normal and clofibrate-treated rats. The results allow a tentative identification of the β-oxidation enzymes, and indicate that they are several of the peroxisomal polypeptides most noticeably increased by clofibrate.

2. EXPERIMENTAL

Peroxisome were purified from normal female Sprague-Dawley rats by differential and isopycnic centrifugation in sucrose according to [12]. Peroxisome were purified from clofibrate-treated male Fisher F-344 rats (0.5% clofibrate in the chow for 2 weeks) by differential centrifugation in sucrose

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[12] followed by isopycnic centrifugation in a metrizamide gradient [13]. Triton WR-1339 pretreatment was omitted because peroxisomes and lysosomes are well-separated in metrizamide gradients without it [13]. The peroxisomes from normal and clofibrate-treated rats were 85–95% pure according to marker enzyme analyses (see [7] for method). Control experiments have shown that neither sex nor strain differences are responsible for the changes to be reported below. Acyl-CoA oxidase [4], the bifunctional hydratase–dehydrogenase [3] and the thiolase [6] were purified as described from rats treated with di(2-ethylhexyl)phthalate to increase peroxisomal β-oxidation [14]. The bifunctional protein was also purified from clofibrate-fed rats by a modified procedure to be described elsewhere. SDS–PAGE was done as before using 7–15% acrylamide gradient gels [15].

3. RESULTS

Clofibrate treatment causes striking changes in the SDS–PAGE pattern of polypeptides of highly purified peroxisomes (cf. lanes 2,3 in fig. 1 which have equal amounts of protein). A number of Coomassie blue-stained bands increase in abundance while others decrease.

The bifunctional protein (enoyl-CoA hydratase and β-hydroxyacyl-CoA dehydrogenase, HD) (lanes 4,5) comigrates with a polypeptide that is well-stained in normal peroxisomes (lane 3) and which increases greatly after clofibrate treatment (lanes 2,6).

Thiolase (lane 7) comigrates with a band of moderate intensity in clofibrate-induced peroxisomes (lane 6). No band can be seen at this position in normal peroxisomes in this experiment (lane 8). The band in lane 8 marked with a dot migrated significantly faster than did thiolase. In another, more heavily stained gel (fig. 2), a faint band with the mobility of thiolase was observed in normal peroxisomes (→). Fig. 2 also reveals that a minor component of the purified thiolase preparation likewise comigrates with a clofibrate-induced peroxisomal polypeptide (▷).

The purified acyl-CoA oxidase preparation (fig. 1, lane 9) contains the components A,B,C described in [4] as well as some larger polypeptides presumed to be contaminants. The middle oxidase component B comigrates with a Coomassie blue-stained polypeptide in normal peroxisomes (lane 8) and in clofibrate-treated peroxisomes (lane 10). When equal amounts of protein are loaded (lanes 2,3), there is clearly much more of this oxidase band after clofibrate treatment.

The large oxidase component A comigrates with a polypeptide present in peroxisomes of clofibrate-treated rats (lane 10) and with what appears to be the lower band in a not fully resolved doublet in

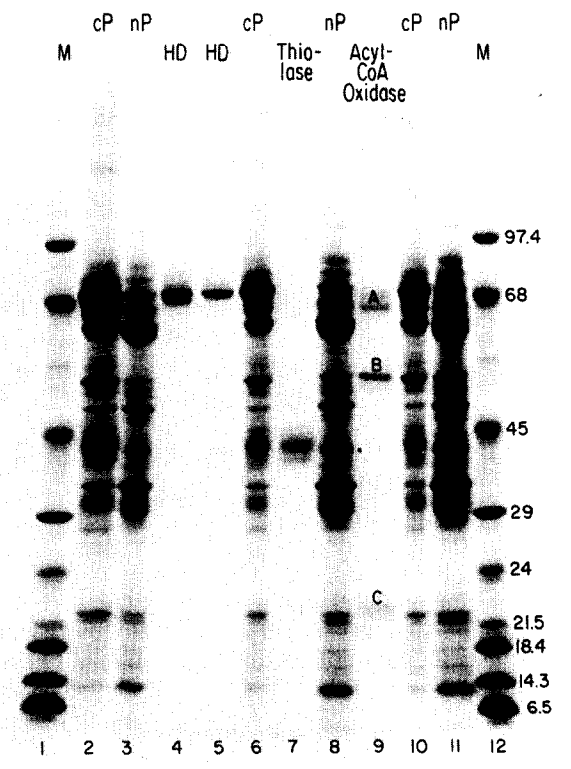

Fig. 1. SDS–PAGE analyses: Normal peroxisomes (nP, lane 3 (30 μg) and lanes 8 and 11 (50 μg)). Peroxisomes from clofibrate-treated rats (cP, lane 2 (30 μg) and lanes 6 and 10 (15 μg)). Purified hydratase–dehydrogenase bifunctional protein (HD, 2 μg, lane 4 di(2-ethylhexyl)phthalate-induced; lane 5 clofibrate-induced). Purified thiolase (lane 7, 2 μg). Purified acyl-CoA oxidase (lane 9, 4 μg). Molecular mass standards (lanes 1,12): rabbit muscle phosphorylase *b* (97400), bovine serum albumin (68000), ovalbumin (45000), bovine erythrocyte carbonic anhydrase (29000), bovine pancreas trypsinogen (24000), soybean trypsin inhibitor (21500), bovine β-lactoglobulin (18400), egg white lysozyme (14300), and bovine lung trypsin inhibitor (aprotinin) (6500).

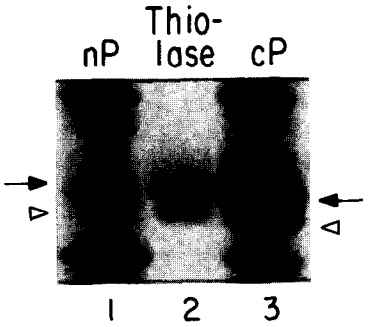

Fig. 2. Thiolase region of SDS gel: (1) normal peroxisomes; (2) purified thiolase; (3) peroxisomes from a clofibrate-treated rat.

normal peroxisomes (lane 8). This polypeptide's abundance is elevated in peroxisomes after clofibrate treatment (cf. lanes 2,3). The small oxidase component C has no visible counterpart in either peroxisome preparation and is noticeably fuzzy in appearance.

Because mobilities in SDS–PAGE vary slightly, depending on protein load and salt concentrations, we prepared mixtures of the purified enzymes and the purified peroxisomes, and subjected these mixtures to SDS–PAGE analysis. The results (fig. 3) confirm the conclusions drawn from fig. 1,2.

4. DISCUSSION

Based on these results, we tentatively identify the β-oxidation enzymes with those peroxisomal polypeptides with which they comigrate in SDS–PAGE. The identification is strengthened by the fact that the Coomassie blue staining intensity of each of these polypeptides increases strikingly after clofibrate treatment, which is known to cause large increases in the peroxisomal β-oxidation enzyme activities [1,2]. Other hypolipidemic drugs also increase peroxisomal β-oxidation enzyme activities [16–20], as does the plasticizer, di(2-ethylhexyl)phthalate [14].

Some uncertainty remains concerning the acyl-CoA oxidase, whose small component C is not detected in peroxisomes in these experiments. Component B is found in normal peroxisomes and is induced by clofibrate. The significance of the large component A has been questioned [5], where a purified enzyme of high specific activity was prepared that contained little of the large component. However, in [4] the amino acid composition of A was close to the sum of B + C, and suggested that they are related by proteolysis in vivo. Further work is required to resolve these questions.

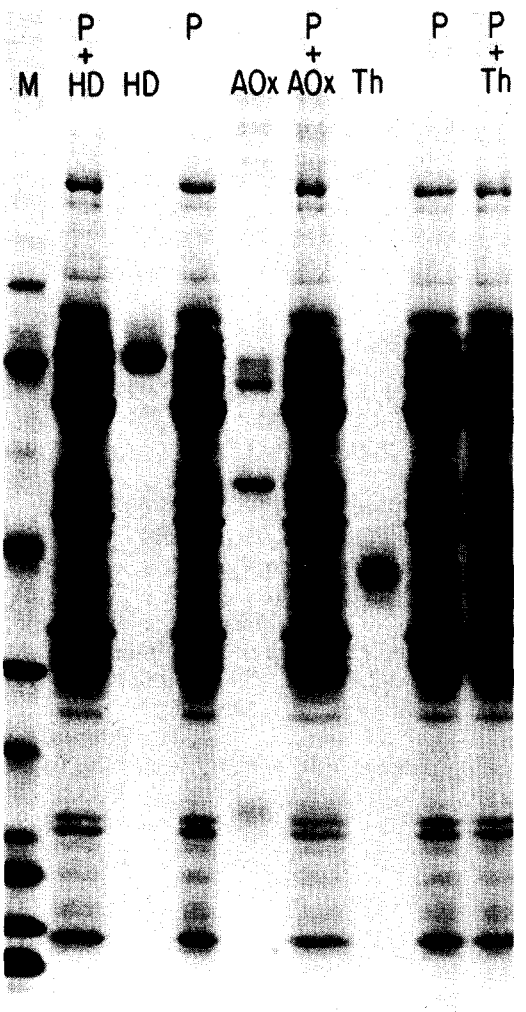

Fig. 3. SDS–PAGE analyses of mixtures of normal peroxisomes and purified β-oxidation enzymes. *Abbreviations*: *p*, peroxisomes (50 μg); HD, hydratase–dehydrogenase (2 μg); AOx, acyl-CoA oxidase (2 μg); Th, thiolase (2 μg); M, molecular mass standards as in fig. 1.

These results indicate that a subset of peroxisomal proteins, including the β-oxidation enzymes, is selectively elevated by clofibrate. Other peroxi-

somal polypeptides decrease in abundance or are unaffected.

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