Glucose-6-phosphate dehydrogenase of rat liver peroxisomes

L. F. Panchenko and V. D. Antonenkov

Department of Biochemistry, All-Union Research Institute of Psychiatry, Kropotkinsky per. 23, Moscow 119034 (USSR),
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Summary. A study was made of the effect of chronic administration of clofibrate on the activity and intracellular localization of rat liver glucose-6-phosphate dehydrogenase. Clofibrate-activated glucose-6-phosphate dehydrogenase was found to be located in peroxisomes.

Peroxisomes of mammalian tissues play a role in lipid metabolism. These subcellular particles contain enzymes for β-oxidation of long chain fatty acids[1,2], aliphatic (branch) chain of cholesterol[3], as well as enzymes involved in the 'alternative' pathway of triglyceride syntheses[4]. However, there is little data concerning the participation of peroxisomes in the metabolism of biological molecules of other classes, though oxidation of purines, L-α-hydroxy acids and polyamines in these organelles has been reported[5].

The present paper describes the localization of glucose-6-phosphate dehydrogenase in rat liver peroxisomes and reports changes in the activity of this enzyme under the influence of the hypolipidemic drug clofibrate, which has been shown selectively to increase the amount and size of rat liver peroxisomes[6].

Materials and methods. Male Wistar rats (200–250 g) were used in the experiments. Clofibrate (400 mg/kg b. wt) was injected i.p. once daily in the course of 10 days. Liver perfusion, preparation of homogenate, differential centrifugation and enzymatic assay of the fractions were performed as described previously[7,8]. Purified peroxisomal fraction was obtained by centrifugation of the 'light' mitochondrial fraction ('L'-fraction) in a multistep sucrose density gradient as described[9], except that hypotonic treatment of the particles was not done.

The purity of the fractions obtained was estimated by determination of marker enzyme activities[7,8]. Catalase (EC 1.11.1.6) and urate oxidase (EC 1.7.3.3) were assayed as peroxisomal markers; glutamate dehydrogenase (EC 1.4.1.2) and malate dehydrogenase (EC 1.1.1.37) as mitochondrial; acid phosphatase (EC 3.1.3.2) as lysosomal, and antimycin-insensitive NADH: cytochrome c reductase (EC 1.6.2.2) and glucose-6-phosphatase (EC 3.1.3.9) as microsomal. Enzymes of the peroxisomal matrix were solubilized after 10-fold dilution of the peroxisomal suspension in 50% sucrose by 20 mM tris-HCL (pH = 7.6) with subsequent centrifugation at 15,000 × g for 40 min in the presence of 0.15 M KCl[9]. Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) was assayed spectrophotometrically (37 °C) by following $NADP^+$ reduction (340 nm) at pH 9.0[10]. Protein was determined as in Lowry et al.[11].

Results and discussion. Chronic administration of clofibrate did not alter the specific activity of glucose-6-phosphate dehydrogenase (table) in the homogenate, though elevation of the amount of enzyme in total particulate fration was observed. Differental centrifugation of liver homogenates from clofibrate-treated animals showed that the highest specific activity of glucose-6-phosphate dehydrogenase was in the soluble fraction (16.2 ± 3.7 nmoles/min/mg protein, recovery 65%), in the microsomes (table), and in the 'light' mitochondrial fraction enriched with peroxisomes and lysosomes (5.1 ± 2.0 nmoles/min/mg protein, recovery 8.4%, n = 4–5).

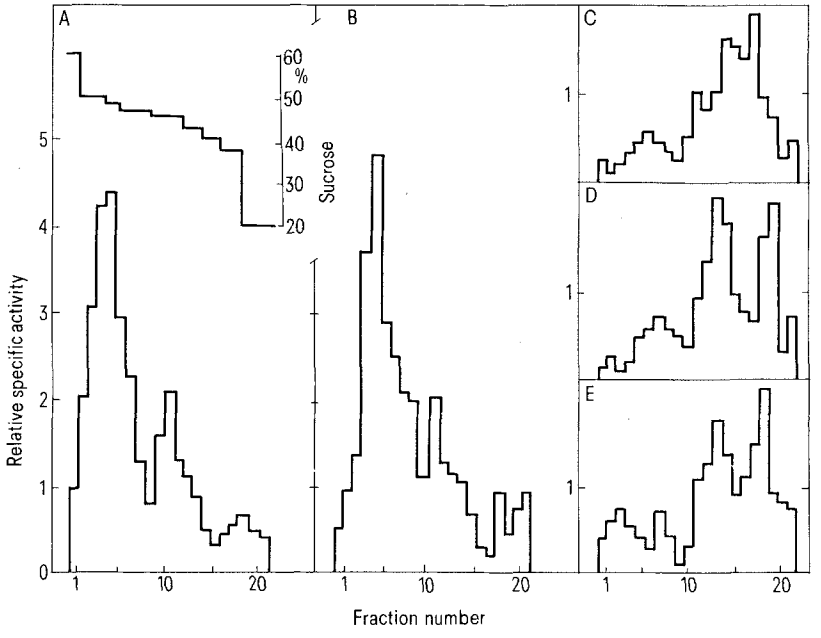
Centrifugation of 'L'-fraction from control or clofibrate-treated group in a multistep sucrose gradient revealed that the peroxisomal area, ranging in density from 1.22 to 1.26 g/cm^3, contained 65–70% of the total glucose-6-phosphate dehydrogenase activity of this fraction. As shown in the figure, the distribution of glucose-6-phosphate dehydrogenase and the peroxisomal marker catalase are similar, and the relative specific activity of glucose-6-phosphate dehydrogenase in peroxisomes significantly exceeds that of other gradient fractions. The specific activity of catalase and urate oxidase in control peroxisomes increased 25.9-fold and 22.7-fold respectively, in comparison with the activity in the homogenate (table). Determination of marker enzyme activities in the peroxisomal fraction indicated that contamination by mitochondria and microsomes was insignificant; peroxisomal protein constituted 70–75% of the fraction protein[12].

It is known that clofibrate administration not only increases the number of peroxisomes in liver cells but also affects their enzyme content. Consequently, the relative amount of catalase and urate oxidase molecules in peroxisomes is reduced under the action of clofibrate[1,5]. This can explain the observed decrease in specific activity of catalase and urate oxidase in the peroxisomal fraction from clofibrate-

Distribution of glucose-6-phosphate dehydrogenase and some marker enzymes in subcellular fractions of rat liver

Enzyme		Homogenate	Fractions		
			Peroxisomes	Mitochondria	Microsomes
Glucose-6-phosphate dehydrogenase	Control	11.4 ± 1.1	12.1 ± 1.5	3.4 ± 0.5	11.8 ± 1.8
	Clofibrate	11.8 ± 1.4	11.2 ± 2.0	3.6 ± 0.2	17.8 ± 1.7
Catalase	Control	0.13 ± 0.01	3.39 ± 0.41	0.89 ± 0.28	0.06 ± 0.01
	Clofibrate	0.20 ± 0.02	1.50 ± 0.04	0.23 ± 0.05	0.13 ± 0.02
Urate oxidase	Control	9.4 ± 0.7	214.5 ± 34.2	45.1 ± 11.7	11.3 ± 9.2
	Clofibrate	11.4 ± 1.3	90.9 ± 5.7	18.5 ± 3.4	7.0 ± 1.7
Glutamate dehydrogenase	Control	1016 ± 114	120 ± 82	1980 ± 337	52 ± 33
	Clofibrate	944 ± 73	72 ± 18	1688 ± 370	92 ± 24
Acid phosphatase	Control	56.8 ± 6.0	52.7 ± 7.7	123.8 ± 14.6	72.2 ± 12.6
	Clofibrate	52.7 ± 6.9	46.0 ± 5.3	119.5 ± 28.3	66.8 ± 6.6
Glucose-6-phosphatase	Control	43.7 ± 6.2	7.2 ± 2.4	35.9 ± 14.7	128.1 ± 8.0
	Clofibrate	48.0 ± 6.2	2.8 ± 1.8	34.9 ± 3.7	98.7 ± 13.2

All specific activities of enzymes (except catalase) are given in nmoles/min per mg protein. Catalase activity was expressed as described[12]. Peroxisomes and mitochondria were isolated by isopycnic subfractionation of the 'L'-fraction in a sucrose gradient[9]. Microsomes were obtained by differential centrifugation of homogenate. Protein content in the whole homogenate was: control group 178 ± 13 mg/g liver, clofibrate treated group 214 ± 11 mg/g. In the peroxisomal fraction the concentration of protein was 0.37 ± 0.04 mg/ml (control) and 1.08 ± 0.23 mg/ml (clofibrate). Each value represents on average 6–9 independent determinations ± SE.



Separation of peroxisomes and mitochondria from liver of a clofibrate-treated rat by centrifugation of the 'L'-fraction in a multistep sucrose density gradient. Successive fractions from the bottom of the tubes were collected (from left to right) and different enzyme activities and protein content are determined. Relative specific activity is defined as the percentage of total recovered activity present in the individual fraction divided by the percentage of total recovered protein present in that fraction. The recoveries of enzyme activities in all gradient fractions were: *A* Catalyse, 89%; *B* glucose-6-phosphate dehydrogenase, 112%; *C* malate dehydrogenase, 105%; *D* glucose-6-phosphate, 92%; *E* acid phosphatase, 110%.

treated rats, with a parallel increase in the recovery of peroxisomal protein (table). However, it should be noted that the total activity of these enzymes (recovery) in the peroxisomal fraction is unaffected by clofibrate.

The highest rate of $NADP^+$ reduction in the presence of glucose-6-phosphate was detected in the purified peroxisomal and microsomal fractions where it was consistent with the specific activity of glucose-6-phosphate dehydrogenase in the homogenate (table). In mitochondria the enzyme activity was significantly lower. Glucose-6-phosphate dehydrogenase of peroxisomes had a pH optimum at 9.0–9.2; K_m for glucose-6-phosphate is 2.0 mM. Solubilization of peroxisomal matrix proteins in the presence of 0.15 M KCl caused a leakage of more than 80% of the total glucose-6-phosphate dehydrogenase activity which was then recovered in the supernatant together with catalase, while the urate oxidase activity of the nucleoid, as well as the activity of the peroxisomal membrane marker NADH: cytochrome c reductase, remained in the pellet containing the peroxisomal 'ghosts'[9]. These results indicate that glucose-6-phosphate dehydrogenase is located in the peroxisomal matrix and differs in this respect from tightly membrane-bound microsomal hexose-6-phosphate dehydrogenase[13]. In peroxisomes obtained from clofibrate-treated animals the total glucose-6-phosphate dehydrogenase activity was more than twice as high as that of the control (mean values: control, 4.5 nmoles/min/ml fraction; clofibrate-treated, 12.1 nmoles/min/ml fraction). This result suggests that the enzyme content in the peroxisomal fraction was elevated under the influence of clofibrate. Clofibrate treatment also increased to some extent hexose-6-phosphate dehydrogenase activity in microsomes (table).

Glucose-6-phosphate dehydrogenase is the second $NADP^+$-dependent dehydrogenase of peroxisomes. Previously, isocitrate dehydrogenase was found in these particles[12]. The biological significance of both enzymes in peroxisomes may involve NADPH production which is then utilized in the 'alternative' pathway of triglyceride biosynthesis for reduction of acyl dihydroxyacetone phosphate to lysophosphatidic acid[4].

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