ISOLATION OF PEROXISOMES FROM RAT LIVER BY HYPOTONIC TREATMENT

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A method of isolating peroxisomes from the rat liver by hypotonic treatment followed by centrifugation in a two-stage sucrose density gradient on a fixed-angle rotor is described. The resulting fraction consisted of peroxisomes to the extent of more than 90%.

KEY WORDS: liver peroxisomes; two-stage sucrose density gradient; hypotonic treatment.

Peroxisomes (microparticles) are the least studied organoids of animal cells. The main reason for this is the difficulty of obtaining a purified fraction of the intact organelles.

This paper describes a convenient method of obtaining a purified fraction of rat liver peroxisomes without preliminary injection of Triton WR-1339 into the animals. The method is based on the osmotic inertness of the peroxisomes of animal tissues.

Male rats weighing 170-200 g were used. The animals were decapitated, and the liver was perfused in situ and homogenized in a medium (1 : 10) containing 0.25 M sucrose, 5% ficoll, and 2 mM EDTA, pH 7.0. The residue obtained after centrifuging at 1700 g for 7 min was rehomogenized in the same medium (1 : 4), and the nuclear fraction was sedimented at 1700 g for 7 min. The supernatants were pooled and centrifuged for 15 min at 12,000 g. The fraction of light mitochondria, giving the highest yield of peroxisomal enzymes, was resuspended in medium (1 : 10) containing 5% ficoll and 2 mM EDTA, pH 7.0, and incubated for 20 min with periodic shaking at 0-4°C. The suspension was centrifuged (12,000 g, 15 min) and the residue again subjected to hypotonic treatment and centrifuged. The final residue was resuspended in 1.5 M sucrose containing 5% ficoll, at the rate of 1 ml sucrose to the amount of residue equivalent to 1 g weight of liver, and 3 ml of the suspension was applied to a two-stage gradient: 5 ml of 1.50 M sucrose (d = 1.20) and 10 ml of 1.58 M sucrose (d = 1.21), 5% ficoll, 2 mM EDTA, pH 7.0. Centrifugation was carried out on an 8 × 35 ml fixed-angle rotor (BAK-601) for 2 h at 160, 000 g. The residue (the fraction of peroxisomes) was washed by reprecipitation in the homogenization medium at 12, 000 g for 15 min.

For comparison the peroxisomes were isolated after preliminary injection of Triton WR-1339 into the rats by Baudhuin's method [3]. All procedures were carried out at 0-4°C. In another series of experiments fractionation was carried out in a continuous sucrose gradient (4.5 ml sucrose with density from 1.16 to 1.26) by centrifugation in a horizontal rotor (3 × 5 ml) for 3 h at 160,000 g. The activity of glycollate oxidase (EC 1.1.3.1) was determined by the method of Kornberg and Dixon [8], urate oxidase (EC 1.7.3.3) by the method of Schneider and Hogeboom [13], and glutamate dehydrogenase (EC 1.4.1.2) and acid deoxyribonuclease (EC 3.1.4.5) by Pokrovskii's method [1, 2]. Protein was determined by Lowry's method [10].

Studies of the properties of the peroxisomes of animal tissue have shown [3, 5] that these particles are osmotically inert and are not injured in a hypotonic medium. Under the same conditions mitrochondria and lysosomes undergo osmotic lysis. The density of the damaged particles falls sharply under these conditions in sucrose [11], and it is thus possible to separate them from peroxisomes in a sucrose density gradient.

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Adequate separation of the peroxisomes and mitochondria could not be obtained by the use of a continuous sucrose gradient, and the yield of the fraction of these particles was low. In the next experiments, the peroxisomes were therefore isolated by centrifugation in a stepwise sucrose gradient. Tests showed that a two-stage gradient is adequate for the isolation of a purified fraction. The density of the lower layer of this gradient must be [3, 5, 11] intermediate between the mean "buoyant" density of the peroxisomes in sucrose (ρ=1.240) and the buoyant density of injured mitochondria and lysosomes (ρ=1.150). During centrifugation the injured particles are distributed in the top layer of the gradient and the peroxisomes fall to the bottom of the tube. The use of the fixed-angle rotor shortened the time of centrifugation in the density gradient and enabled a sufficiently highly purified fraction of peroxisomes to be obtained in large quantities.

The specific activity of the marker enzymes of the peroxisomes was increased by 47 times for urate oxidase and by 43 times for glycollate oxidase compared with the activity in the homogenate. Taking the peroxisomes to account for about 2% of the total liver cell protein [6, 9], this degree of increase in specific activity of the peroxisomal enzymes means that the resulting fraction consisted of peroxisomes to the extent of more than 90%. The validity of this calculation, suggested by De Duve [6], is confirmed by the fact that the isolated fraction contained only 0.07% of the initial glutamate dehydrogenase activity and showed no acid deoxyribonuclease activity. Baudhuin et al. [3] isolated a fraction of particles by hypotonic treatment which had specific activity of the peroxisomal enzymes comparable with that obtained in the present experiments, but their isolation procedure was technically more complicated than that now described. Triton WR-1339 is known to cause considerable disturbance of lipid metabolism [4, 8, 13] and, in addition, as some workers [6, 10] consider, it increases the number of peroxisomes in the liver.

The evident advantage of the method described in this paper is that it is not necessary to inject Triton WR-1339 into the animals. It is thus a promising method for "artefact-free" isolation of peroxisomes from pathologically changed tissues.

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