THE LOCALISATION OF THE PURINE-PHOSPHORIBOSYLTANSFERASE IN RAT LIVER ORGANELLS

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The liver is one of the most important organs where purine bases will be accumulated during catabolic processes and re-utilized. There exists a lot of information on preparing the different enzymes of the purine-phosphoribosyltransferase of cow and pigeon livers (1) or microorganism but there is less information about the localisation of these enzymes within cell organelles. We therefore investigated rat liver organelles with regard to their purine-phosphoribosyltransferase activities.

MATERIALS AND METHODS

The preparation of rat liver organelles followed a combined method of Widnell (2), Sottocasa (3) and Chandra (4). 12 female Sprague Dawley rats starved for 12 hours were decapitated, the livers prepared and perfused with ice cold isotone sodium chloride. 150 grams of liver were pressed through a sieve of steel and suspended in 1000 ml 0.24 M sucrose complemented by $3 \cdot 10^{-4}$ M CaCl$_2$ and homogenized (5). The homogenate was centrifuged at 600 g for 15 minutes. From this sediment containing cell wall fragments, nuclei and whole cells the nuclei were prepared according to the method of Widnell (2). The supernatant of the 600 g centrifugation was centrifuged at 6500 g for 20 minutes. The sediment contained the mitochondria fraction. To separate the light mitochondria from the heavy part the sediment was suspended in 0.45 M saccharose gently homogenized and the suspension overlayed on 1.18 M sucrose (1:1/V/V). The cell material was centrifuged in a Beckman SW 40 rotor at 24,000 rpm for 3 hours. The mitochondria fraction divided into four parts. On the top there was a light yellow soluble
fraction, then a yellow one, representing the light mitochondria, then a colourless one, only gradient and on the bottom a dark brown sediment, the heavy mitochondria.

The supernatant of the 6.500 g centrifugation was centrifuged at 27.000 g for 15 minutes. The sediment was discharged. The supernatant was run at 105.000 g for 60 minutes resulting in a sediment containing the microsomal fraction. The ribosomes were purified according to Chandra (4) from the microsomes.

The resting supernatant was the cytoplasmic part. The cytoplasm the fraction of the soluble mitochondria and the ribosomes were dialysed against TRIS-HCl buffer (0.1 M, pH 7.4 supplemented by 5·10^{-3} M MgCl2) overnight at 40 °C. The sediments of separated organelles were washed twice in the same buffer and homogenized. After centrifugation the cell free extracts were used for the enzyme determination.

A part of the original squeezed liver material was homogenized in buffer and the enzyme activity of the whole liver estimated. Protein was measured according to Lowry (6). The determination of the purine-PRT activities followed a radio isotope method described earlier (7). To get information about the contamination of microsomal particles in the different fractions the specific glucose-6-phosphatase was measured in each portion (8).

RESULTS AND DISCUSSION

As summarized in Table 1 the purine-phosphoribosyltransferases are ubiquoquaer in rat liver organelles. As expected the main activity is located in the cytoplasm. The A-PRT activity showed the highest rate followed by the G-PRT and the H-PRT activity. This result is divergent to the values obtained from the cell free extract of the whole liver. The possible reason for this and also in other samples is the different protein content of the preparations.

A very high enzyme activity could be determined in the 105.000 g sediment. This is a very heterogenous fraction. The so-called microsomal part of the preparation contains parts of the endoplasmatic reticulum, ergastoplasm, but also parts of the lysosomes and microbodies. The distribution of the enzyme activities in the whole microsomal fraction is completely different to the cytoplasm. The A-PRT shows the highest activity followed by H-PRT and G-PRT. The high enzyme activities lead to the conclusion that the microsomal fraction is contaminated by parts of the cytoplasm. We believe that due to the performed preparation this result is not a false one but reflects the natural enzyme activity.