DISTRIBUTION OF MITOCHONDRIAL ENZYMES AFTER ISOPYCNIC CENTRIFUGATION OF A RAT LIVER MITOCHONDRIAL FRACTION IN A SUCROSE GRADIENT: INFLUENCE OF THE SPEED OF CENTRIFUGATION

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Summary

The distributions of mitochondrial enzymes after isopycnic centrifugation of a rat liver mitochondrial fraction in a sucrose gradient differ depending on whether centrifugation is performed at 39,000 rpm or 50,000 rpm in the Spinco SW50.1 rotor. This finding is best explained by postulating that mitochondria are damaged when centrifugation speed is too high.

In isopycnic centrifugation experiments, subcellular particles migrate in a density gradient until they reach a region with a density equal to their own. Normally, in this case, an increase in centrifugation speed should not affect particle distribution in a given gradient; it should merely shorten the time required for particles to reach their equilibrium position. Unexpectedly, we have observed that mitochondrial enzyme distributions differed after isopycnic centrifugation of a rat liver mitochondrial fraction in a sucrose gradient, depending on whether centrifugation was performed at 39,000 rpm or 50,000 rpm in the Spinco SW50.1 rotor.

Methods

Mitochondrial fractions were prepared from the livers of Wistar rats by the procedure of de Duve et al. (1965). Density gradient centrifugations were performed according to Beaufay et al. (1964). Cytochrome oxidase was assayed as described by Appelmans et al. (1955), monoamine oxidase by the procedure of Schnaitman et al. (1967). Glutamate dehydrogenase and alkaline deoxyribonuclease were assayed by the technique of Beaufay et al. (1959) and proteins by the method of Lowry et al. (1951).

Results and discussion

Figure 1 makes it possible to compare the distributions of enzymes that are characterized by different submitochondrial localizations: monoamine oxidase and cytochrome oxidase, which are markers respectively for outer and inner membranes (Schnaitman and Greenawalt, 1968), alkaline

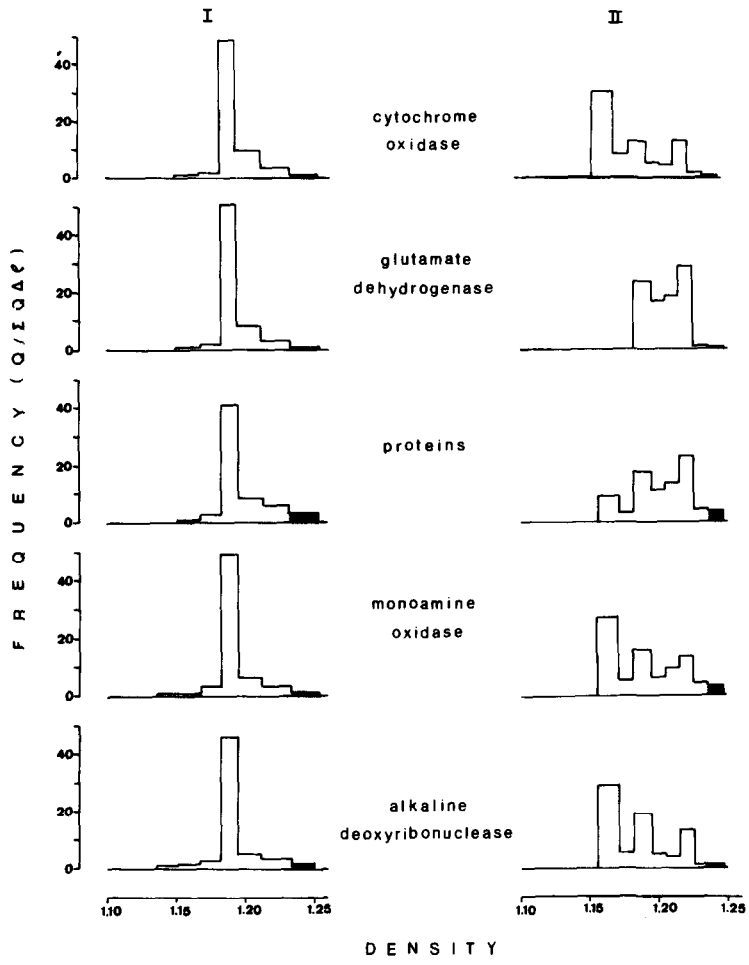

Fig.1

Distribution of mitochondrial enzymes after isopycnic centrifugation of a rat liver mitochondrial fraction in a sucrose gradient. Time integral of the square angular velocity $w = 14.4 \times 10^{10}$ rad^2 sec^{-1}; (I) centrifugation at 39,000 rpm; (II) centrifugation at 50,000 rpm in the SW50.1 rotor. For plotting the curves, the average frequency of the component was calculated for each fraction $Q/\Sigma Q.\Delta\rho$ where Q represents the activity found in the fraction, ΣQ represents the total recovered activity and $\Delta\rho$ represents the increment of density from top to bottom of the fraction. These values were plotted against density in histogram form. Recoveries were 105.1% and 119.8% for cytochrome oxidase, 104.6% and 76% for glutamate dehydrogenase, 117.4% and 118.1% for proteins, 120.8% and 130.5% for monoamine oxidase, and 114.5% and 119.8% for alkaline deoxyribonuclease.

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deoxyribonuclease, which according to Baudhuin et al. (1969) is situated in the intermembrane space, and glutamate dehydrogenase, an enzyme associated with the granule matrix (Schnaitman and Greenawalt, 1968).

The distributions have been established after isopycnic centrifugation of a rat liver mitochondrial fraction in a sucrose gradient. The time integral of the square angular velocity ($w=\int_0^t \omega^2 dt$) was 14.4×10^{10} rad^2 sec^{-1} and was obtained by centrifuging the particles at 39,000 rpm (I) or 50,000 rpm (II) in the SW50.1 rotor of the Spinco centrifuge. When the rotor is spun at 39,000 rpm, the four enzymes and the proteins show a very similar distribution; they are mostly recovered in a narrow region centered around a density of 1.19; this distribution pattern is similar to that described for cytochrome oxidase by Beaufay et al. (1964) under comparable experimental conditions. When an equal w is achieved by centrifuging at 50,000 rpm, the results are quite different. Monoamine oxidase, alkaline deoxyribonuclease and cytochrome oxidase exhibit a trimodal distribution; the three fractions where the activity peaks are observed have mean densities respectively of 1.165, 1.181 and 1.220. Glutamate dehydrogenase is relatively widely distributed over a region extending from density 1.18 to 1.23, but shows a significant bimodal distribution; one activity peak is found in the fraction of mean density 1.189 and a second in the fraction of mean density 1.220.

The distribution curves of mitochondrial enzymes after centrifugation under isopycnic conditions at 50,000 rpm differ in two respects from those observed after centrifugation at 39,000 rpm. First, for a given enzyme, the distribution pattern is considerably more heterogeneous. Secondly, different mitochondrial enzymes do not exhibit the same general distribution pattern. These results are most plausibly explained by postulating that mitochondria are damaged during centrifugation at 50,000 rpm. A marked dissociation is indeed observed between monoamine oxidase, cytochrome oxidase and alkaline deoxyribonuclease on the one hand, and glutamate dehydrogenase and to some extent, proteins on the other. This strongly suggests that disruption of the mitochondria occurs during centrifugation, leading to partial separation of the outer and inner membranes from the mitochondrial matrix. Preliminary experiments indicate that hydrostatic pressure is probably the main causal agent involved. The immediate relevance of these findings is that they indicate that care must be taken before attributing to mitochondria heterogeneity a multimodal distribution of some of their enzymes after isopycnic centrifugation.

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