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Intracellular Localization of Catalase in Rat Liver

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Catalase is associated with cytoplasmic particles to the extent of about 70 % in homogenates of rat liver tissue. Fractionations performed under a variety of conditions indicate that the particles which contain this enzyme are different from mitochondria and from lysosomes, and are closely similar to, or identical with, those bearing urate oxidase. Catalase is partly latent in intact particles; it can be simultaneously unmasked and solubilized by several treatments, though not by exposure to distilled water.

Catalase is one of the numerous haemoproteins to the study of which Hugo Theorell has devoted some of his most inspiring research activities. The intracellular localization of this enzyme has been investigated by a number of authors and with a variety of techniques[1–11]. In general, it has been recovered partly in cytoplasmic particles and partly in the final supernatant, the ratio of soluble to particulate activity depending on the species and sex of the animal and on the fractionation method employed. The particles which contain the enzyme sediment largely with the mitochondrial fraction and are believed by most workers to be mitochondria. However, this view has been questioned by Thomson and Klipfel[8], who have found that the sedimentation pattern of particulate mouse liver catalase is very similar to that of urate oxidase. Feinstein[12] has quoted this finding, together with the observation by Adams and Burgess[9] that the catalase activity of the particles is enhanced by small amounts of Triton X-100, in support of the hypothesis that the enzyme may be associated with the lysosomes. Since it has now been found that urate oxidase is associated with a special group of cytoplasmic particles, different both from the mitochondria and from the lysosomes[13–15], it seemed of interest to investigate further the localization of catalase in liver tissue. The results of these investigations are described in the present paper. Some of them have already been mentioned briefly in preliminary reports[14,15] and at a recent symposium[16].

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EXPERIMENTAL

Rat liver was homogenized in 0.25 M sucrose and fractionated as described by de Duve *et al.*[17]. The scheme followed differs from the classical one in that two separate mitochondrial fractions are isolated: a heavy one (M), containing the larger part of the mitochondrial nitrogen and cytochrome oxidase activity and relatively poor in lysosomal enzymes and in urate oxidase; and a light one (L), containing the residual mitochondria and some microsomes, together with a particularly high proportion of lysosomes and of bodies containing urate oxidase. When density gradient experiments were performed, these two fractions were first isolated together, washed and then centrifuged in suitable density gradients until they had reached a position in the gradient corresponding to their own density. The techniques used in these experiments were those described by de Duve *et al.*[18] and Beaufay *et al.*[13].

Catalase was assayed at 0°C according to a modification of the method described by Chantrenne[19]. For measurements of total activity, the preparations were pretreated with 1 % Triton X-100 to release the enzyme. The reaction was found to be of first order with respect to hydrogen peroxide concentration under the conditions of the assay and the observed activities were expressed in units, one unit being the amount of enzyme causing the disappearance of 90 % of the substrate in one min in a volume of 50 ml at 0°C. Nitrogen, protein and reference enzymes were measured as described before[13,17].

RESULTS

Intracellular distribution pattern

In Table 1 are summarized the results obtained in 7 fractionation experiments. Only the data for catalase are shown, but each experiment included measurements of cytochrome oxidase, glucose-6-phosphatase, acid phosphatase and urate oxidase. The distributions observed for the latter enzymes were similar to those reported by de Duve *et al.*[17]. As indicated in Table 1, catalase exhibits a distinct peak of specific activity in fraction L, thus resembling urate oxidase and the lysosomal hydrolases, rather than the mitochondrial cytochrome oxidase which has its highest specific activity in fraction M. Approximately 30 % of the total catalase activity was recovered in the final supernatant.

Subfractionation in density gradients

In Figs. 1 and 2 are shown the enzymatic distributions found after isopycnic centrifugation of mitochondrial fractions in linear density gradients made up respectively with sucrose in D_2O and with glycogen in 0.5 M sucrose. In the

Table 1. Intracellular distribution of catalase (total activity of liver = 60.5 ± 6.0 U/g). Means of 7 experiments ± S.E.M.

Fraction	Total activity % of total	Specific activity % activity/% nitrogen
Nuclear (N)	7.4 ± 2.1	0.37 ± 0.08
Heavy mitochondrial (M)	21.7 ± 4.0	1.09 ± 0.17
Light mitochondrial (L)	21.7 ± 3.6	6.64 ± 0.82
Microsomal (P)	9.0 ± 2.3	0.44 ± 0.11
Supernatant (S)	31.2 ± 2.6	0.98 ± 0.14
Recovery	91.0 ± 2.3	–

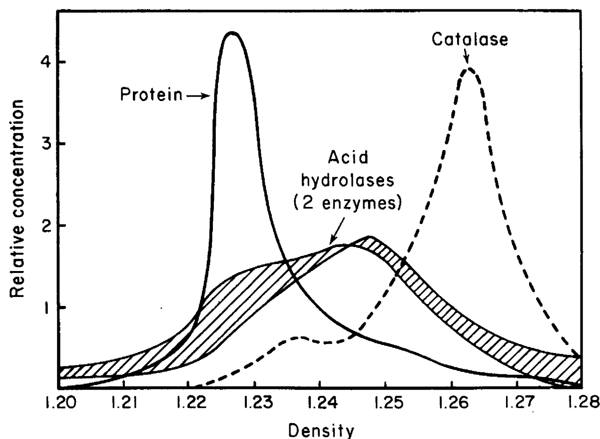

Fig. 1. Mitochondrial fraction (M + L) from rat liver, equilibrated in linear gradient of sucrose in D_2O (0.85 to 1.58 M over 4.1 cm). The particles were included homogeneously in the gradient and the curves indicate the ratio of the final to the initial concentration of the measured components. The two acid hydrolases are acid phosphatase (left) and acid deoxyribonuclease (right).

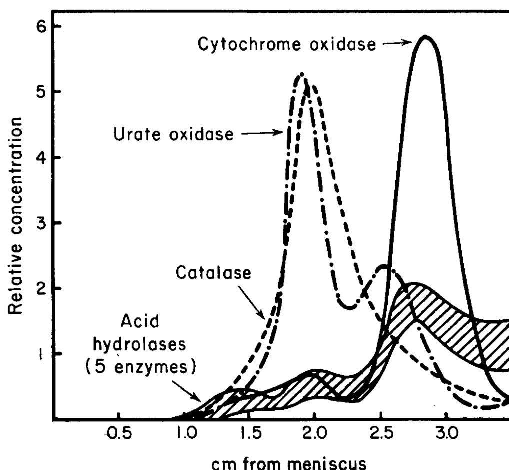

Fig. 2. Mitochondrial fraction (M + L) from rat liver, equilibrated in gradient of glycogen in 0.5 M sucrose (0 to 0.23 g/ml over 4.1 cm). The gradient was initially linear but became distorted in the course of centrifugation. The results are expressed as in Fig. 2, but as a function of the position in the tube. The five acid hydrolases are acid phosphatase, β-glucuronidase, cathepsin, acid ribonuclease and acid deoxyribonuclease. Their distribution curves were all included within the shaded area.

experiment of Fig. 1, the distribution of catalase resembles closely that observed previously for urate oxidase in a similar gradient[13] and differs significantly both from that of the proteins, which belong mostly to the mitochondria, and from that of the lysosomal hydrolases. The similarity between catalase and urate

oxidase shows up in an even more striking fashion in the glycogen gradient, which allows an almost complete separation of these two enzymes from the lysosomes (Fig. 2).

Several other experiments of this type were performed, in gradients of sucrose in water and of glycogen in sucrose solutions of various concentrations. In all cases, the distribution of catalase was similar to that of urate oxidase, though slightly shifted towards the region of lower density. These results have shown that the median density of the two particulate enzymes increases linearly with the density of the sucrose solution to which they are exposed. On the other hand, mitochondria and lysosomes exhibit a different behaviour, obeying a more complex function.

Activation experiments

The observation[9,12] that particulate catalase is partly latent has been confirmed. As shown in Table 2, the free activity measured on homogenates is essentially equal to the activity which is recovered in the supernatant after high-speed centrifugation, suggesting that under these conditions the activity associated with particles is entirely masked. However, some activity, corresponding to 10 – 20 % of the total activity present, is always released from the particles when the latter are further isolated. Complete release can be accomplished by the addition of Triton X-100 or digitonin to the suspensions. The concentration of digitonin necessary to unmask the catalase activity quantitatively is distinctly higher than that sufficient for complete release of the lysosomal hydrolases. Catalase also differs from the latter enzymes by the fact that exposure of the particles to media of low tonicity (down to 5 mM sucrose) causes almost no release of activity. In contrast with catalase, urate oxidase is fully accessible to its substrate under the conditions of its assay, which, however, is carried out at 37°C.

Table 2. Latency of catalase in rat-liver homogenates. Total activity refers to measurements made on whole homogenate with addition of Triton X-100. Free activity refers to similar measurements carried out without the addition of Triton X-100 and in the presence of 0.25 M sucrose. Soluble activity is total activity of supernatant isolated after centrifuging the homogenate for 30 min at 100 000 × *g*.

Expt.	% of total activity	
	Free activity	Soluble activity
1	23.6	21.7
2	19.8	16.3
3	20.9	21.9
4	30.5	31.9

DISCUSSION

In its centrifugal behaviour, particulate catalase differs clearly from mitochondria in all the systems investigated and it may safely be concluded that these particles have little or no catalase activity. The enzyme sediments together with the lysosomes in conventional centrifugal fractionation experiments, but can be largely separated from the latter particles by means of density gradient isopycnic centrifugation. Also, while resembling the lysosomal hydrolases in being largely latent in preparations of intact particles, it is not released as easily as these enzymes by means of digitonin and is not liberated by exposure of the particles to hypotonic media. Therefore, a major localization of catalase in the lysosomes can also be ruled out.

On the other hand, catalase shows a striking correlation with urate oxidase in a number of different fractionation systems. In particular, it shares with this enzyme the property of accumulating in the denser regions in aqueous sucrose gradients and in the lighter regions in glycogen – 0.5 M sucrose gradients, a consequence of the unique linear relationship linking the density of their host-particles to that of the sucrose solution in the medium. As shown before[16,18], this relationship indicates that the particles are permeable to sucrose and do not respond osmotically to changes in sucrose concentration. In the case of catalase, this feature is confirmed by the lack of osmotic release of the enzyme.

In view of these observations, it seems very likely that catalase is associated with the urate oxidase-containing particles, which have been recently identified with the so-called "microbodies"[15]. However, it must be pointed out that, in contrast with urate oxidase, catalase is always found in soluble form to the extent of at least 20 % in hepatic homogenates and that the density distribution curve of the particulate enzyme is shifted slightly but significantly in the direction of lower densities, with respect to that of urate oxidase[16]. It must also be remembered that structure-linked latency has not been demonstrated so far for the latter enzyme.

One explanation could account for the distribution differences without introducing the assumption that the enzymes belong to different particles or that they are associated together, but in varying proportion, in the same particles. It is possible that homogenization causes the rupture of a number of microbodies and the consequent dissociation of catalase, which is released in soluble form. from urate oxidase which remains attached to an insoluble residue of higher density than the intact particle. Supporting this hypothesis is the fact that the denser fractions, which are very rich in urate oxidase and relatively poor in catalase, contain, in addition to intact microbodies, a large number of the multilamellar or multicanalicular cores, which characterize these bodies, in free form[15]. This observation suggests that urate oxidase may be part of this core, whereas catalase would be present in the soluble phase of the microbodies.

This interpretation is apparently contradicted by the fact that catalase displays structure-linked latency, whereas urate oxidase does not. However, catalase, owing to its very high activity, was measured at 0°C, whereas urate oxidase has always been assayed at 37°C for the opposite reason. It remains to be seen whether differences in latency would be observed if the enzymes could be determined under identical conditions. It must also be pointed out that if the acces-

sibility of two enzymes to their respective substrate is restricted by the same structural barrier, diffusion of the substrate may be limiting for a very active enzyme and not for one of low activity.

Accordingly, it is provisionally concluded that catalase is largely, and perhaps completely, associated with urate oxidase in the microbodies of rat liver. The same is probably true for D-amino acid oxidase, whose distribution in density gradients is identical with that of catalase[14,16]. The physiological role of these bodies raises intriguing problems. The only known link between the three enzymes so far identified in them is hydrogen peroxide, which is formed by two of them and utilized by the third one. However, it is difficult to imagine how such a tenuous link could form the basis of a physiological function.

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