CATALASE MULTIPLICITY IN NORMAL AND ACATALASEMIC MICE

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1. Introduction

Mouse catalase (H_2O_2 : H_2O_2 oxidoreductase EC 1.11.1.6) exists as a series of five multiple forms which are differentially distributed in the peroxisomes and cytoplasm of liver and kidney cells as well as in erythrocytes [1–3]. This situation is in direct contrast to that in most mammalian species, where the catalases do not exhibit native multiplicity [4], and have simple chemical and genetic properties. Chemical studies on beef liver catalase for example, have shown that the purified enzyme consists of four identical subunits [5] while other studies with acatalasemic humans have shown that a single structural gene is responsible for the synthesis of the enzyme [6]. These apparently divergent aspects between the properties of mammalian catalases may be reconciled, however, by recent evidence for an epigenetic basis of multiplicity for the five multiple forms of catalase [1], a result which suggests that the multiple forms are not "isozymes" in the recently defined sense of this term [7]. These views are supported by the present data which concern the subcellular and cellular localization of multiple forms of catalase from normal and acatalasemic mice.

2. Materials and methods

The strains of normal (Cs^a) and acatalasemic (Cs^b) mice previously described by Feinstein et al. [8] were used in this study*. The animals were young adult female mice between the ages of 6–8 weeks and were killed by exsanguination with the blood being collected in heparinized tubes. After centrifugation of the blood (1200 *g*, 10 min), the plasma and buffy coat were aspirated and discarded. The remaining erythrocytes were washed 3 times with isotonic saline and treated with an equal volume of 50 mM Tris-HCl (pH 7.4). The tissues were excised, washed in cold isotonic sucrose solution, homogenized in 10% sucrose/50 mM Tris-HCl (pH 7.4) (livers, 10% w/v; kidneys, 30% w/v), and fractionated according to the differential centrifugation procedure of Hogeboom [9]. The nuclei/whole cell fraction was discarded, and the resultant supernatant centrifuged to yield 3 fractions: large granules†; small granules†; and the supernatant. The granular fractions were sequentially extracted with buffers containing 50 mM Tris-HCl (pH 7.4) and a buffered solution containing Triton X-100 (0.2%). All preparations were treated with 10 mM dithiothreitol and centrifuged (100,000 *g*, 60 min) prior to electrophoresis.

Catalase activity was determined by a spectrophotometric method previously described [1] and is expressed in International Units/ml. Protein concentrations were determined using the Biuret method, with crystalline bovine serum albumin for standards [10].

Supernatant fractions were subjected to zone electrophoresis on vertical 12% starch gels at 4°, with Tris-glycine buffer (30 mM, pH 9.0). A voltage gradient of 20 V/cm was applied for 16 hr. Following

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† The large granule fraction consists of mitochondria, lysosomes, and peroxisomes with catalase being localized in the latter particles [11]: the small granules contain endoplasmic reticulum and cell membrane.

Table 1
Specific activities of normal and acatalasemic mouse erythrocyte and tissue catalase*.

Sample	Homogenate specific activity** (I.U/mg protein)	
	Normal strain (Cs^a)	Acatalasemic strain (Cs^b)
Liver	253	126
Kidney	85	24
Erythrocyte	20	(–)†

* Average of 5 separate experiments with female animals.
** Nuclei and whole cells removed.
† Activity undetectable by the technique used.

Table 2
Subcellular localization of soluble liver and kidney catalase activity in normal (Cs^a) and acatalasemic (Cs^b) female mice*.

Sample	Large granule catalase (%)	Small granule catalase (%)	Supernatant catalase (%)
Cs^a liver	49–56	1–3	43–49
Cs^b liver	58–66	1–3	33–41
Cs^a kidney	31–40	1–4	58–67
Cs^b kidney	33–42	1–4	55–61

* Results expressed as percentage of total soluble catalase activity in homogenate after nuclei and whole cells removed. Recovery 80–90%. The ranges of activity indicate the variation observed in 5 experiments.

Table 3
Comparative solubilization of large granular liver and kidney catalase from normal (Cs^a) and acatalasemic (Cs^b) mice*.

Sample	Aqueous released catalase activity (I.U. × 10^3)	Detergent released catalase activity (I.U. × 10^3)
Cs^a liver	8–11	14–17
Cs^b liver	3–6	14–17
Cs^a kidney	0.5–1	1–3
Cs^b kidney	0.05–0.1	0.1–0.4

* Results expressed in I.U.'s total catalase activity (× 10^3) recovered from large granules prepared from 10 ml of 10% homogenates. The ranges of activity indicate the variation observed in 5 experiments.

electrophoresis, the gels were sliced, stained for catalase activity and photographed [1].

3. Results and discussion

The specific activities of liver, kidney and erythrocyte catalase from normal and acatalasemic female mice are listed in table 1. Acatalasemic mice exhibit 50–70% of the liver catalase activity and 20–30% of kidney catalase activity of the normal strain whereas erythrocyte catalase is undetectable in the mutant strain. This result confirms previous work by Feinstein et al. [12] who have reported that acatalasemic mice (Cs^b strain) resemble acatalasemic humans with respect to erythrocyte catalase activity. The mutant mice however, exhibit reduced yet significant liver and kidney catalase activity whereas acatalasemic human tissue contained essentially zero activity. More recent studies have shown that catalase from acatalasemic mice is unstable and exhibits reduced catalatic activity compared to the wild type (Cs^a) [13, 14] and the difference in residual catalase activity between the tissues and erythrocytes of these mutant mice may be explained in terms of this fact and their differential localization. Liver catalase has a short half life (1½ days) and is therefore being continually synthesized and degraded [15, 16] thus ensuring a constant supply of the labile enzyme. Erythrocytes, however, lack the necessary protein synthetic apparatus and would rapidly lose their initial catalase activity.

Table 2 demonstrates that most of the soluble liver and kidney catalase activity is differentially distributed between the peroxisomes (large granules) and the supernatant fractions in both strains. The small granules did not contribute any more than a few percent of the total activity. Soluble catalase activity in Cs^a liver is approximately equally distributed between the large granules and the supernatant whereas in Cs^b liver, the level of supernatant catalase is significantly lower than that of the peroxisomes. Soluble kidney catalase from both strains exhibits a consistently higher level of activity in the supernatant fraction.

In addition to soluble catalase, the peroxisomes contained further activity which was solubilized by detergent treatment. Table 3 lists the relative quanti-

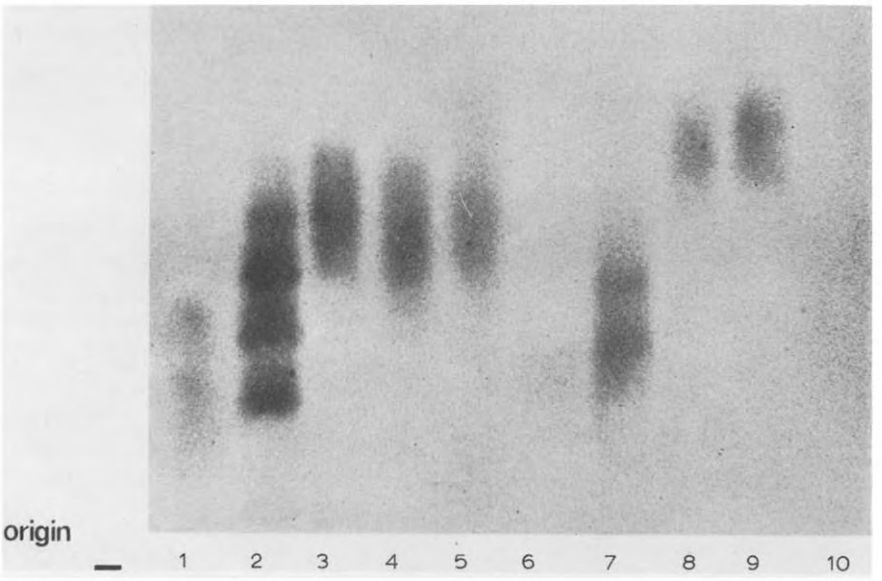

Fig. 1. Starch gel zymogram of liver, kidney and erythrocyte catalase multiple forms from normal (Cs^a) and acatalasemic (Cs^b) female mice. 1) Cs^a erythrocyte (5% w/v); 2) Cs^a liver supernatant (10% w/v); 3) aqueous extract of Cs^a liver large granules (10% w/v); 4) Cs^a kidney supernatant (20% w/v); 5) aqueous extract of Cs^a kidney large granules (30% w/v); 6) Cs^b erythrocyte (50% w/v); 7) Cs^b liver supernatant (25% w/v); 8) aqueous extract of Cs^b liver large granules (25% w/v); 9) Cs^b kidney supernatant (50% w/v); 10) aqueous extract of Cs^b kidney large granules (50% w/v).

ties of soluble and detergent solubilized catalase activity obtained from liver and kidney granule preparations. It can be seen that kidney peroxisomes from acatalasemic mice contain approx. 10% of the catalase activity of normal mice. Liver peroxisomes from Cs^b mice, however, exhibit considerably more activity and contain one half of the soluble activity and comparable levels of the detergent solubilized activity when compared with the normal strain. Considered together tables 2 and 3 confirm previous studies on the subcellular distribution of mouse tissue catalase [1, 3, 4] and illustrate that acatalasemic mice exhibit significant yet reduced levels of liver and kidney peroxisomal and cytoplasmic catalase activity.

Starch gel zymograms of liver, kidney, and erythrocyte catalase showing multiple forms from normal and acatalasemic mice are shown in fig. 1. Five forms are observed in normal mouse liver extracts which are differentially distributed between the peroxisomal and supernatant fractions. The more anodal migrating species (catalases 1 and 2) are observed in the aqueous peroxisomal extracts (slot 3) whereas catalases 2–5 are present in the supernatant fraction (slot 2). Catalase 5 activity is observed in erythrocyte lysates of Cs^a mice (slot 1) while both kidney peroxisomal and supernatant fractions contain mostly the faster migrating forms of activity (slots 4 and 5). The subcellular and cellular distribution of catalase multiple forms from acatalasemic mice (Cs^b) is similar to that of normal mice although activities are reduced and the multiple forms are electrophoretically distinct to those of normal mice. Although catalase activity for acatalasemic erythrocytes (slot 6) and kidney peroxisomal extracts (slot 10) is not apparent in fig. 1, prolonged incubations prior to histochemical staining reveal zones of activity corresponding to the slowest migrating form in slot 7 and the zone in slot 8, respectively.

Recently, an epigenetic basis of multiplicity for mouse liver catalase has been proposed and supported by evidence indicating interconvertibility of the five forms of activity [1]. Since catalase is a tetramer [5, 17], the presence of two types of subunits, one a native subunit (A) and the other a more basic epigenetic modification (A′) would allow for such

multiplicity: A4, A3A′, A2A2′, AA′3, A′4. The activity (tables 1–3) and multiplicity (fig. 1) data presented demonstrate that acatalasemia in mice effects the activity and electrophoretic migration of all of the multiple forms of catalase which provides evidence that a single structural gene is responsible for their synthesis and supports a recent proposal [1] that mouse catalase multiplicity results from an epigenetic modification of a single type of catalase subunit.

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