EPIGENETIC INTERCONVERSIONS OF THE MULTIPLE FORMS OF MOUSE LIVER CATALASE

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1. Introduction

Although the properties of mammalian catalase (hydrogen peroxide: hydrogen peroxide oxidoreductase, EC 1. 11. 1. 6) have been extensively investigated, a diversity of opinion remains evident in the current literature as to the extent and nature of the multiplicity of this enzyme. Differing numbers of catalase heteromorphs have been resolved from mammalian liver and erythrocyte extracts by groups of workers using techniques such as polyacrylamide [1, 2] and starch gel electrophoresis [3, 4], immunoelectrophoresis [5, 6], and ion-exchange chromatography [1, 5, 7–9]. In addition, catalase activities have been identified in both the cell sap and particulate fractions of liver and kidney with the major portion of the particulate activity being localized in the peroxisomes as both soluble and bound forms (for reviews see [4, 10]). Electrophoretically distinct catalases have been identified both in these separate fractions [3] and in different tissues [4, 6] from species such as rat and mouse; whereas other mammals such as sheep [4] and beef cattle [11] exhibit a uniform pattern of multiple forms throughout the different constituent tissues and subcellular fractions.

This complex multiplicity of catalase stands in contrast to the simple chemical and genetic picture of the enzyme. Chemical studies on beef liver catalase have shown that the enzyme consists of four identical subunits [12], and genetic studies, utilizing the inborn error "acatalasemia" in humans and mice, have indicated that a single structural gene is responsible for the total synthesis of the enzyme (for review see [13]).

This present communication attempts to reconcile these apparently divergent aspects by providing evidence for the existence of epigenetic modifications of a single form of soluble, granular, mouse liver catalase, resulting in a complex series of multiple forms which are characteristic of the cytosol.

2. Methods

Adult female mice (Quackenbush strain) were used in these experiments. The animals were killed by exsanguination and the livers excised and washed in cold isotonic sucrose solution. Homogenization was carried out in Potter-Elvejhem homogenizers using a number of solutions and liver concentrations. Sucrose homogenates (10–20%, w/v) were prepared in 10% sucrose/ 50 mM tris** HCl (pH 7.4) and fractionated according to the differential centrifugation procedure of Hogeboom [14]. The nuclei/whole cell fraction was discarded and the resultant supernatant centrifuged to yield three 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 centrifuged at 100,000 *g* for 60 min prior of electrophoresis.

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** *Abbreviations*: DTT: dithiothreotol; EDTA: ethylene diamine tetraacetic acid; tris: tris (hydroxymethyl) aminomethane.

† The large granule fraction consists of mitochondria, lysosomes, and peroxisomes with the catalase being localized in the latter; the small granule fraction contains endoplasmic reticulum and cell membrane [10].

Catalase activity was determined by a modification [15] of Beer's and Sizer's spectrophotometric method [16]. Hydrogen peroxide decomposition was followed by measuring the initial rate of decrease in absorbance at 240 nm in a 3 ml cuvette in a UNICAM (SP 800) spectrophotometer at 30°. The reaction mixture contained 50 mM tris HCl (pH 7.4), 10 mM hydrogen peroxide, and 1–10 μl of enzyme. Activity in I.U. was calculated utilizing the reported value for the extinction coefficient of hydrogen peroxide at 240 nm of 43.6 M^{-1} cm^{-1} [15]: I.U./ml = ΔA/min × 69 × (dilution factor).

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 electrophoresis, the gels were sliced, incubated in a 100 mM phosphate buffer (pH 6.0) for 30 min, stained for catalase activity by the method of Scandalios [17], and photographed. The zymograms shown in this paper are photographs of the original negatives (i.e. black and white transposed).

3. Results

Table 1 demonstrates that the soluble catalase activity in mouse liver is approximately equally distributed between the peroxisomes (large granule fraction) and the supernatant fraction. In addition, it is evident from these results that detergent solubilized catalase activity is almost exclusively associated with the peroxisomes, so that when total liver catalase distribution is considered, two-thirds is found to be located in this larger granular fraction. The small granules (endoplasmic reticulum) contain a small but significant proportion of the catalase activity in this tissue.

Starch gel zymograms of mouse liver catalase obtained from different subcellular fractions by the utilization of a variety of aqueous extraction media are shown in fig. 1. The supernatant fraction (slot 1) showed a complex series of 8–9 bands which when treated with DTT, simplifies in pattern to 4 regions of activity (slot 2). In contrast, when the large and small granular fractions were extracted with buffer, a single band of catalase activity of higher anodal electrophoretic mobility was observed (slots 3 and 4). The detergent solubilized catalase activity from the large granule fraction gave a similar multibanded pattern to that of the supernatant fraction (slot 5).

Table 1
Subcellular distribution of mouse liver catalase.

Catalase activity	Large granules	Small granules	Supernatant
Aqueous released catalase*	28	< 1	26
% Total aqueous catalase	52%	1%	47%
Detergent released catalase*	25	< 1	2**
Total catalase activity*	53	1	28
% Total activity	65%	1%	34%

These results are given as the mean values of three experiments.

* Units of activity are expressed in I.U. ($\times 10^{-3}$) obtained from 10 ml of a 10% liver homogenate.

** Supernatant activity increased by 10% (approx.) in presence of Triton X-100.

Homogenization of mouse liver in buffer without sucrose released both supernatant and soluble peroxisomal catalase, however, the resultant electrophoretic pattern in this case resembled that of the supernatant, only (cf. slots 1 and 6). Extraction in the presence of TX-100 increased the activity but did not alter the pattern (slot 7). When DTT and EDTA were incorporated independently, a multibanded pattern resulted similar to that observed for slot 2 (slots 8 and 9). If DTT, EDTA and TX-100 were present in combination, however, a pattern similar to that of a peroxisomal aqueous extract resulted (slots 10 and 11).

4. Discussion

Studies on the turnover of mammalian liver catalase have indicated that this enzyme has a short half life (∼1½ days), is synthetized in the rough ER, and is subsequently released into the soluble phase or incorporated into the peroxisomes [10, 18–21]. This synthetic sequence results in a differential distribution of catalase activity within the liver cells, and this subcellular localization may vary widely between species. Rat, sheep, cattle, horse, and guinea-pig livers, for example, have 60–95% of their catalase activity localized in the soluble phase, whereas mouse

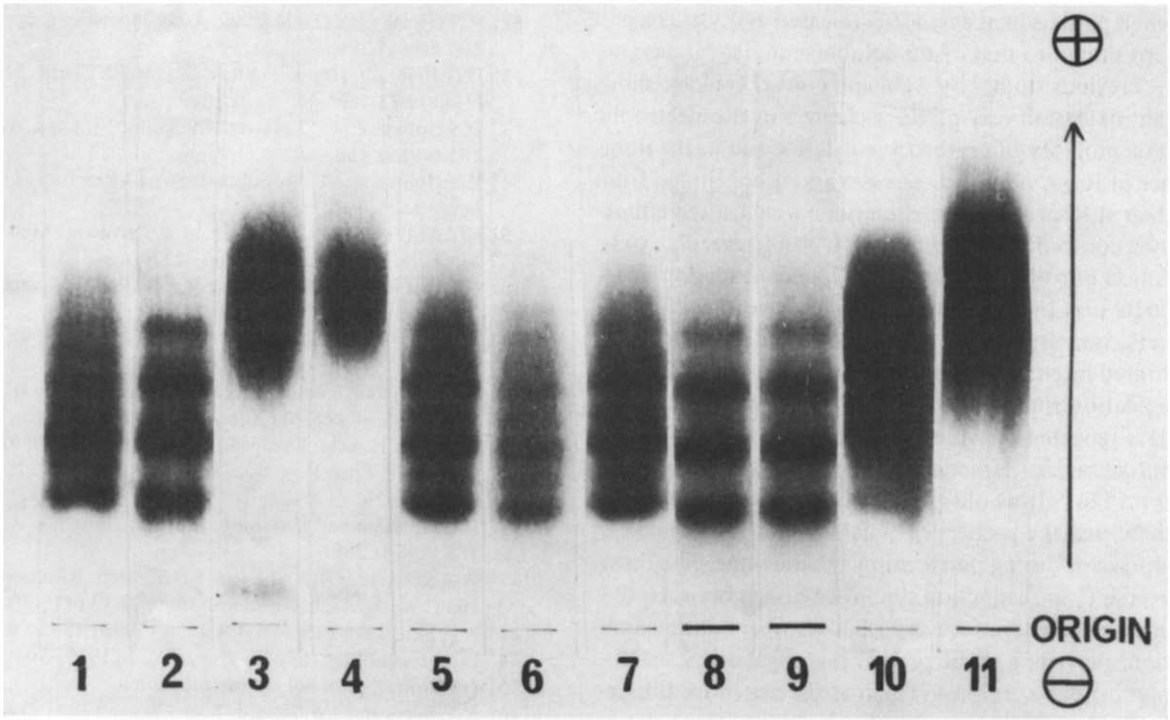

Fig. 1. Zymograms of mouse liver catalase activity obtained from different subcellular fractions by utilizing a variety of aqueous extraction media.

(1) Supernatant catalase from sucrose homogenate;
(2) (1) treated with 25 mM DTT;
(3) aqueous extract of large granules;
(4) aqueous extract of small granules;
(5) detergent extract of aqueous extracted large granules;
(6) supernatant of aqueous homogenate;
(7) supernatant of aqueous homogenate with TX-100;
(8) supernatant of aqueous homogenate with DTT;
(9) supernatant of aqueous homogenate with EDTA;
(10) supernatant of aqueous homogenate with DTT and EDTA;
(11) supernatant of aqueous homogenate with DTT, EDTA and TX-100.

has 60–80% of its activity present within the peroxisomes [4, 22–24, table 1]. These results would seem to reflect differences in the properties of the enzyme (e.g. solubility, turnover number) both between species and between different intracellular pools within the same animal.

In the case of mouse liver, it is apparent from the present results that the catalase activity of the cytosol is comprised from a complex series of multiple forms, whereas the original source of synthesis (ER) and the soluble peroxisomal catalase exist as a single species of higher anodal migration. Furthermore, in regard to the nature of these sequential interrelationships, it is of interest to note that treatment of the supernatant fraction with a chelating agent such as EDTA, or with the reducing agent DTT, simplified the complex pattern to four regions of strong activity, which with the soluble granular catalase constitute a regularly spaced series of five isoenzymes. In view of these facts, then, an explanation of this multiplicity would seem to be provided by the epigenetic modification of the catalase subunits. Since catalase is a tetramer [12, 26] the presence of two types of subunits, one a native subunit (A), and the other a more basic epigenetic modification (A^1) would allow for a sequence of five isoenzymes: A_4, A_3A^1, $A_2A_2^1$, AA_3^1, A_4^1 [25]. Additional support for this interpretation is provided by the fact that the extraction of liver homogenates in

the presence of EDTA, DTT, and Triton X-100 prevents this epigenetic interconversion from taking place when peroxisomal catalase is released and yields a pattern similar to that of the soluble granular catalase.

Previous studies by Aebi and coworkers have shown that oxidation may produce changes in the electrophoretic mobility of erythrocyte catalase and in the number of forms observed. These workers concluded from their studies that these alterations were caused either by a conformational change or by an irreversible oxidation of sulphydryl groups to higher oxidation products [9]. In addition Heidrich has investigated the heterogeneity of beef liver catalase and has demonstrated interconversions of five catalase forms with oxidation and reduction [2], and Heidrich and Hannig have reported that the native enzyme from the "light mitochondrial" fraction of beef liver exists as a single form [11]. It would appear from all these studies, then, that the properties of catalase may change on storage or during purification. At the same time, however, a clear distinction should be drawn between the artefactual alterations described above, and the conditions prevailing in the present investigation. Special precautions were taken (such as the use of fresh tissue extracts, the involvement of reducing agents to detect reversible redox changes, and the rapid analysis of samples) to obviate any such influences, and hence the distinctions in characteristic properties of the catalase between the different subcellular fractions in these studies is considered to present the native situation.

In conclusion then, the results in this communication indicate that an epigenetic modification of the catalase in mouse liver occurs when the soluble granular enzyme is released into the subcellular fraction. This observation allows the complex heterogeneity of catalase to be reconciled with the determination by a single structural gene, and contributes towards an understanding of the differential properties of catalase between separate species and subcellular fractions. The exact nature of this epigenetic modification has not yet been fully defined, but is currently under investigation in this laboratory.

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