Presence of a High-Molecular-Weight Form of Catalase in Enzyme Purified from Mouse Liver

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Ultracentrifugation studies of purified mouse hepatic catalase revealed that 5–7% of the total material consists of a form with a higher molecular weight than the bulk of the catalase. The two components were separated by sucrose-gradient centrifugation. Polyacrylamide-gel electrophoresis (in borate buffer) demonstrated that high-molecular-weight catalase is enriched in a more slowly migrating component, and sodium dodecyl sulphate/polyacrylamide gel-electrophoresis demonstrated that the molecular weight of the subunits of the high-molecular-weight material is identical with that of the subunits of the major form. These results suggest that high-molecular-weight catalase consists of subunits that are not markedly distinct from those present in the normal catalase tetramer.

Catalase (EC 1.11.1.6) is a ubiquitous enzyme found in most tissues of aerobic organisms (de Duve & Baudhuin, 1966; Deisseroth & Dounce, 1970). The exact role of catalase within intact organisms remains obscure, although recent results strongly support the argument that a relationship exists between hepatic catalase activity and lipid metabolism (Reddy & Krishnakantha, 1975).

Much is known about both the physical and chemical properties of catalases from a variety of sources (Deisseroth & Dounce, 1970). The native enzyme is a slightly ellipsoidal protein, consisting of four polypeptide chains (Schroeder *et al.*, 1969), each chain binding one molecule of protohaematin (Greenfield & Price, 1956). The molecular weights of several mammalian catalases have been tabulated, and range from 230000 to 250000 (Deisseroth & Dounce, 1970).

We have been interested in the possible role of catalase, as well as that of its substrate, H_2O_2 , in senescence (Baird & Samis, 1971; Nicolosi *et al.*, 1972; Samis *et al.*, 1972; Baird *et al.*, 1974, 1976; Massie & Baird, 1976). During the course of these studies hepatic catalase was purified from mouse liver, and examined by a variety of methods. In the present paper we give evidence that an active catalase molecule of higher than normal molecular weight exists in enzyme preparations purified from mouse liver.

Experimental

Materials

C57B1/6J male mice (2 months old) were used throughout the study, and were maintained as described previously (Baird & Samis, 1971). SDS* (electrophoresis purity) was purchased from Bio-Rad Laboratories, Richmond, CA, U.S.A. Benzidine and Coomassie Blue were purchased from Sigma, St. Louis, MO, U.S.A. All other reagents and materials were of the best grade available.

Methods

Animals were killed at 09:00h EST. Livers were excised and pooled and catalase was purified by the method of Price *et al.* (1962). Catalase activity was assayed by a spectrophotometric method essentially as previously described (Baird & Samis, 1971).

Sedimentation-velocity determinations were performed with a Beckman model E ultracentrifuge.

Purified catalase was applied to 5–15% (w/v) sucrose (in water) gradients and centrifuged in a Beckman L5-40 ultracentrifuge. Pertinent details of these and other methods are given in the appropriate Figure legends.

Polyacrylamide-gel electrophoresis of catalase was performed in borate buffer as described by Baird *et al.* (1976), or in 0.1% SDS as described in detail by Welton & Aust (1974). Peroxidatic activity

* Abbreviation: SDS, sodium dodecyl sulphate.

of material on the gels was detected by staining with benzidine (Welton & Aust, 1974). Benzidine was handled with extreme caution as it is known to be carcinogenic in mammalian systems. The peroxidase-stained gels were scanned at 615 nm in a linear gel transport with a Gilford 2400S spectrophotometer. We have found that such stains are stable when the gels are rinsed in water immediately after the appearance of the blue colour. Duplicate gels were stained for protein with Coomassie Blue (Fairbanks *et al.*, 1971) and scanned at 550 nm. Reference standards (with molecular weights in parentheses) were as follows: phosphorylase *a* (94000); bovine serum albumin (66500); pyruvate kinase (57000); aldolase (40000); lactate dehydrogenase (36000); lysozyme (14300); cytochrome *c* (11700).

Results and Discussion

The u.v.-visible spectrum of purified mouse liver catalase is presented in Fig. 1. We estimated that this material was greater than 99% pure (A_{407}/A_{276} 1.14), assuming that the criteria for purity of rat liver

Fig. 1. U.v.-visible scan of purified mouse hepatic catalase. Protein concentration was 300 μ g/ml in 0.02M-sodium phosphate buffer, pH 7.0.

Fig. 2. Tracings of representative scans of purified mouse hepatic catalase after (a) 16, (b) 26 and (c) 36 min of centrifugation in a Beckman model E ultracentrifuge. Samples were centrifuged at 56000 rev./min in 0.02M-sodium phosphate buffer, pH 6.8, and scanned at 407 nm. Protein concentration was 520 μ g/ml. Direction of force is represented by *g*.

Fig. 3. Relationship between $s_{20,w}$ and molecular weight for purified mammalian catalases and subunits of catalase generated by various treatments

Data were taken from Deisseroth & Dounce (1970). The stars ($\star$) represent the s value for high-molecular-weight catalase and normal mouse liver catalase.

Fig. 4. Sucrose-density-gradient centrifugation of mouse hepatic catalase

Purified catalase (0.1 ml, 4.55 mg/ml) was applied to a 5–15% (w/v) sucrose gradient. The material was centrifuged for 7.5 h, at 40000 rev./min, in a 50.1 rotor in a Beckman L5-40 ultracentrifuge. Fractions (10 drops) were collected and 0.1 ml of 0.02M-sodium phosphate buffer, pH 6.8, was added to each fraction, and the fractions were analysed for A_{407} (●) and total units of catalatic activity (▲).

catalase established by Price *et al.* (1962) are applicable to mouse catalase.

Centrifugation of this material in the model E ultracentrifuge (0.8 *A* unit) revealed the presence of

two components (Fig. 2). The major component had $s_{20,w}$ 11.1S, and the minor component had $s_{20,w}$ 14.8S. The minor component accounted for 5–7% of the total material.

We have plotted (Fig. 3) the data collected by Deisseroth & Dounce (1970) relating corrected s values and molecular weights of native catalases as well as for subunits of catalase. We estimated by extrapolation that the molecular weight of the major component of mouse hepatic catalase was 235000, and that of the high-molecular-weight catalase was 345000. This value, of course, assumes certain similarities between the two catalase molecules, and may be in error.

The two components were readily separated on a sucrose gradient (Fig. 4), in terms of both A_{407} and catalatic activity. Since the ratio between the absorbance of the Soret band (407nm) and that of the protein band (276nm) in purified mouse hepatic catalase is nearly unity (Fig. 1), the A_{407} is a useful estimate of protein content. We determined from the results of numerous sucrose gradients that the specific enzyme activity (enzyme activity/ A_{407}) of both the major form of catalase and high-molecular-weight catalase is identical. Therefore we suggest that the haematin content of both forms is similar, although we have not directly measured the haematin content of high-molecular-weight catalase.

Purified enzyme, as well as the material under the two peaks resolved by centrifugation (Fig. 4), were subjected to both SDS/polyacrylamide-gel electrophoresis and gel electrophoresis in borate buffer. Separation of the purified material on the basis of size and charge (borate buffer) revealed the presence of two components with peroxidatic activity (Fig. 5a). Electrophoresis of the material under the two peaks resolved on a sucrose gradient demonstrated that the material under the major peak (Fig. 5b) corresponds to the more rapidly migrating material in the purified catalase, and the material in the minor peak is enriched in more slowly migrating material (Fig. 5c). Separation of the material in the purified catalase, as well as the material in both peaks in the presence of 0.1% SDS, reveals the presence of two components (Fig. 6). The more rapidly migrating component has mol.wt. 60000 and the more slowly migrating minor component has mol.wt. 120000. We suggest that the 120000-mol.wt. component is a contaminant,

Fig. 5. Non-dissociating polyacrylamide-gel electrophoresis of purified mouse hepatic catalase activity before and after fractionation on a sucrose gradient

Origin of the run was at the cathode (0). Gels were stained for peroxidatic activity with benzidine. (a) Purified catalase; (b) major component (see Fig. 3); (c) high-molecular-weight catalase (more rapidly sedimenting component, see Fig. 3).

inasmuch as it does not possess any detectable peroxidatic activity. Therefore high-molecular-weight catalase appears to consist of subunits that are identical with those present in normal tetrameric catalase.

We have not determined whether high-molecular-weight catalase possesses some function *in vivo*, or is generated during the purification process. There are conditions under which ox liver catalase aggregates into higher-molecular-weight species (Itoh *et al.*, 1962). However, spontaneous aggregation into high-molecular-weight catalase most likely does not occur, as identical electrophoretic results are obtained whether performed in the presence or absence of dithiothreitol. Further, dilution of the material, followed by ultracentrifugation, does not alter the percentage of the total material accounted for by high-molecular-weight catalase. Finally, high-molecular-weight catalase is not present in catalase preparations purified from CFN rat liver.

Other studies from our laboratory (Baird *et al.*, 1976) indicate that the response of mouse liver catalase activity to the pharmacological agent allyl-isopropylacetamide is different from that observed in rats. High-molecular-weight catalase appears to be another anomalous feature of mouse liver catalase.

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Fig. 6. SDS/polyacrylamide-gel electrophoresis of purified mouse hepatic catalase before and after fractionation on a sucrose gradient

Origin of the run was at the cathode (0). Gels were stained with Coomassie Blue. (a) Purified catalase; (b) major component (see Fig. 3); (c) high-molecular-weight catalase (more rapidly sedimenting component, see Fig. 3).

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