SELECTIVE INHIBITORY EFFECT OF TOXOHORMONE ON MICE HEPATIC CATALASE FORMS

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1. Introduction

Toxohormone, a factor isolated from tumors by Nakahara and Fukuoka in 1948 [1] and which depresses hepatic catalase activity when injected into mice, has been widely studied. Apart from its effect on hepatic catalase, it also effects the concentration of plasma iron and hepatic ferritin [2]. Toxohormone-like factors with the same biological characteristics have also been isolated from microorganisms with impaired respiration [3] and autolysates of normal yeast [4] and animal tissues [5].

Kampschmidt et al. [6] reported that such effects might be attributed to lipopolysaccharides from bacterial contaminants of raw materials, but there are differences between this and those types of preparations [7, 8]. Recently, Urushizaki [8] has found, by means of electrofocussing that they differ markedly in their action on hepatic catalase in rats.

Using this technique and following in part to Feinstein and Peraino [9], we have used toxohormone-like factor from 'petite' mutants of *Saccharomyces cerevisiae* to study its effect on the multiple forms of hepatic catalase in mice, specially in relation to its possible site of action.

2. Materials and methods

Toxohormone preparations were obtained by Nakagawa's method [10] from *Saccharomyces cerevisiae* with impaired respiration (strain T27). Male albino mice (Swiss), each weighing 20 g, were injected in the peritoneal cavity with a single dose of 30 mg of toxohormone in 0.25 ml of distilled water, 24 hr after they were deprived of food. Control mice received 0.25 ml of distilled water.

For electrofocussing five mice were used in each experiment. After killing them by decapitation, a piece of liver was removed and rinsed in cold distilled water or 0.25 M sucrose solution. 0.5 g of liver from each mouse placed in 10 ml of the liquid used for washing the liver were homogenized cold in a Potter–Elvehjem homogenizer for 2 min.

For estimating total catalase activity the homogenate prepared with water was sonicated for 5 min at 0°C (MSE 20 Kcycles, 40 W) and then centrifuged in the cold for 30 min at 48 000 *g*. The supernatant in each case was used for electrofocussing. For soluble catalase, 0.25 M sucrose solution was used and the homogenate centrifuged as above.

Mitochondrial catalase was measured in the mitochondrial fraction separated according to the technique described by Hogeboom [11]. The mitochondrial particles were suspended in cold distilled water, sonicated for 5 min and centrifuged at 48 000 *g* for 30 min.

Electrofocussing was carried out using ampholine pH 3–10 in an LKB apparatus for 70 hr at 2°C maintained by a closed refrigeration circuit. The amount of sample applied in each separation corresponded to 25 mg of protein determined according to

Lowry's method [12]. One ml fractions were collected, their pH determined (Beckman, Expand. SS-2) and catalase activity measured by the iodimetric method [13] and expressed in terms of reaction rate per minute [14], per ml in each fraction and per 25 mg of protein in total activity recovered from the column.

3. Results and discussion

Fig. 1 shows total and soluble hepatic catalase activity of normal animals expressed as a percentage of total activity recovered from the column. This separation pattern indicates five fractions in total hepatic catalase activity in mice, corresponding to the isoelectric points 5.0, 6.2, 6.7, 7.2 and 8.0. The low activity of soluble catalase at an isoelectric point of 6.2 corresponds to the highest activity of mitochondrial catalase, as shown in fig. 2.

Fig. 3 shows total hepatic catalase activity in toxohormone treated mice represented as the percentage of total activity recovered from the column. Table 1 shows the activities obtained for normal and toxohormone-injected mice and the percentage reduction in activity of the latter. When toxohormone is injected, total catalase activity of all fractions is decreased by up to 64%. However the greatest decrease in activity is in the fraction at i.p. 6.2 (cf. fig. 3), i.e. down to 10% of that of normal mice (table 1). This corresponds to an 83% decrease in activity of mitochondrial catalase caused by injecting toxohormone.

The fraction with an isoelectric point of 6.2 may be the same as that called a native subunit by Holmes and Masters [15]. These authors presented evidence for an epigenetic basis of multiplicity for the five multiple forms of catalase in mouse liver which they separated by zone electrophoresis on starch gels. They suggest these forms are not strictly isoenzymes but rather five tetramers formed by a native subunit and an epigenetic modification joined in different proportion. More recently Holmes [16], working with acatalasemic mice, and Jones and Masters [17] using 3-amino-1,2,4-triazole as inhibiting agent, confirm this concept.

The present study suggests, that toxohormone-like substances which lack activity in vitro, act by means of an unknown mechanism on the actual synthesis of the above mentioned native subunit. However, these substances have no effect on the catalase that has

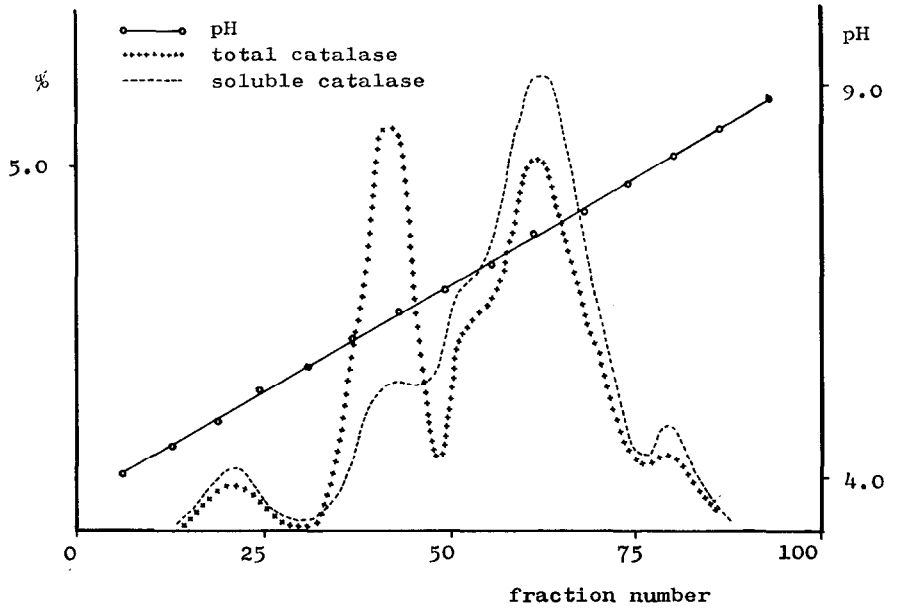

Fig. 1. Total and soluble catalase activity as percentage of total activity recovered from the column (non injected mice).

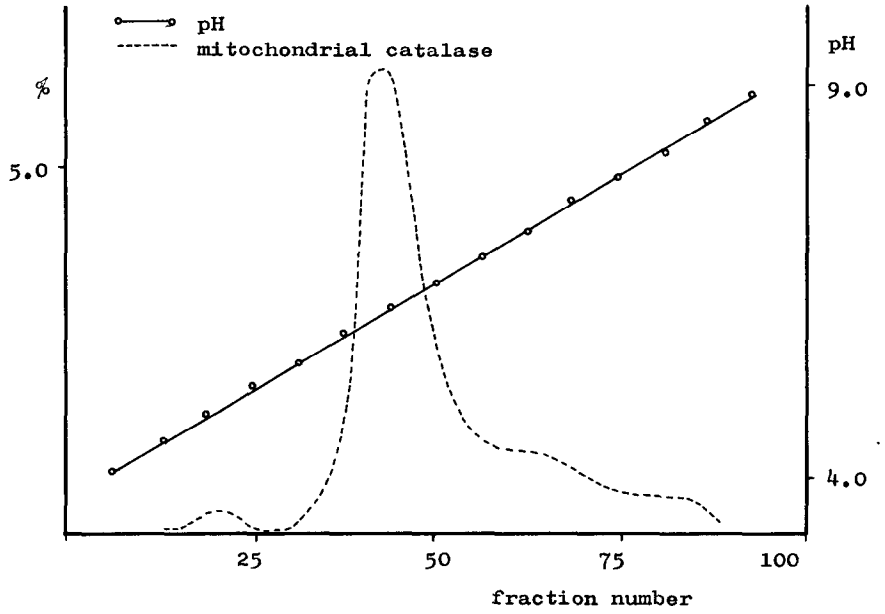

Fig. 2. Mitochondrial catalase activity as percentage of total activity recovered from the column (non injected mice).

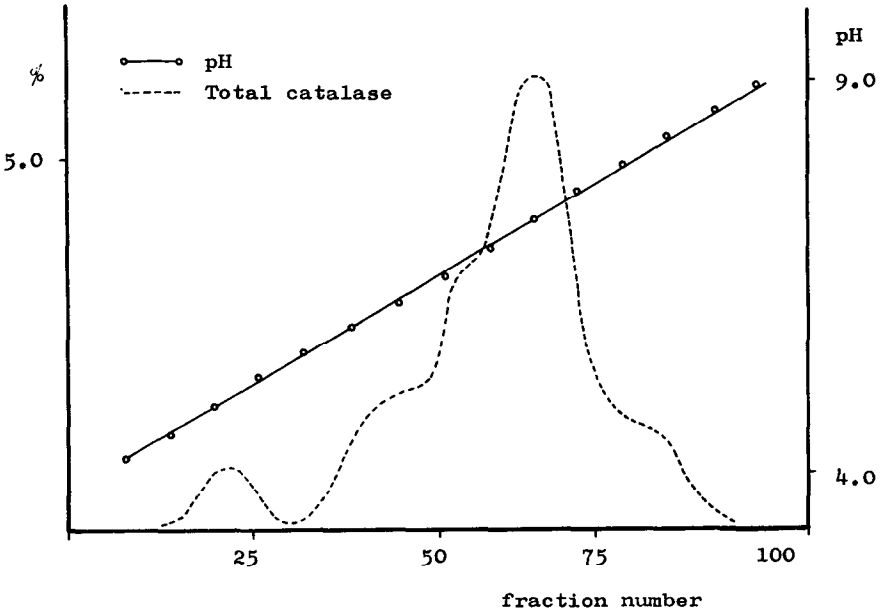

Fig. 3; Total catalase activity as percentage of total activity recovered from the column (mice injected with toxohormone preparations).

Table 1
Total, isoelectric point of 6.2 fraction in total and mitochondrial catalase activity, expressed as reaction rate/minute/25 mg of protein, recovered from the column and percentage of reduction after injection with toxohormone preparations.

	Activity		Percentage of reduction
	Normal mice	Toxohormone treated mice	
Total catalase	9.90	3.60	64
Fraction (i.p. 6.2) in total catalase	1.90	0.19	90
Mitochondrial catalase	39.00	7.0	83

Values are means of three experiments.

already been synthesized and that which has been epigenetically modified that appears to be very low in the liver of mice injected with toxohormone due to the turnover of the enzyme itself.

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