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ON THE HETEROGENEITY OF CATALASE IN RAT LIVER AND EXPERIMENTAL HEPATOMAS OF RATS AND MICE

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SUMMARY

Two forms of catalase (H_2O_2:H_2O_2 oxidoreductase, EC 1.11.1.6) activity, soluble and lipid-bound, exist in the total homogenate as well as in mitochondria, microsomes and plasma membranes of mice liver, rat liver and hepatomas.

The lipid-bound catalase activity is revealed by the treatment of the tissue with *n*-butanol.

An inverse correlation between morphological, immunochemical characteristics (reflecting the extent of malignancy) of the hepatomas studied and the activity of the two forms of catalase was found.

The nature of two forms of catalase activity is discussed. One infers that experimental data available do not favour the view of their being isozymes.

INTRODUCTION

Catalase (H_2O_2:H_2O_2 oxidoreductase, EC 1.11.1.6) is to be considered as one of the most puzzling enzymes due to its versatility of function and to the drastic drop of its activity upon conversion of a normal tissue to a malignant one.

We concentrated on the problem of whether all catalase activity resides in the water-soluble fraction where the enzyme is usually measured, or whether there might exist some other form of catalase more tightly bound to subcellular structures. KAPLAN[1–4] studied the effect of several chemical agents, including butanol, on yeast and came to the conclusion that, apart from the water-soluble catalase, cells contain a hidden (latent) form of the enzyme. 25–30% of the total catalase activity of human erythrocytes was found to be associated with the stroma[5]. A series of papers on isozymes of catalase was published by NISHIMURA *et al.*[6–8] and PATTON AND NISHIMURA[9].

Our previous investigations published in Russian[10,11,12] were concerned with different forms of catalase existing in animal tissue. In the present paper we summarized our findings and report some additional data on the matter.

MATERIALS AND METHODS

Normal rat and mouse liver, mouse regenerating liver (taken 48 h after partial hepatectomy), 2-week-old embryonic mouse liver, a number of ascites and solid transplantable mouse hepatomas (differing in growth rate)[13] as well as ascites rat hepatomas (Zajdela) were studied.

The tissue studied (100 mg) was minced and ground in the cold in a mortar with 15 ml 0.15 M phosphate buffer (pH 7). After 1-h extraction at 0°, the homogenate was centrifuged for 10 min at 1500 × *g* (2°). The supernatant represented soluble catalase activity. To release the catalase bound to the lipid-containing subcellular structures, the procedure of MORTON[14] was applied. The tissue was ground under the above conditions for 10 min with the mixture composed of equal volumes of *n*-butanol and phosphate buffer and then centrifuged for 10 min at 2000 × *g*. Traces of butanol were removed by extraction with ether. This fraction contained the total catalase activity. Bound catalase activity was calculated by subtraction of the value corresponding to the soluble catalase from the total activity. The activity of catalase was determined according to EULER AND HELLER[15].

Plasma membranes from rat liver were isolated by the method of TAKEUCHI AND TERAYAMA[16], plasma membranes from ascites cells were prepared according to the method of NEVILLE[17] as modified by EMMELOT AND BOS[18]. Purity of plasma membrane preparations was tested by electron microscopic analysis. Absence of DNA, determined according to the method of DISCHE[19] after fractionation by the method of SCHMIDT AND THANHAUSER[20], and glucose-6-phosphatase (measured according to the method of CORI AND CORI[21]) from plasma membrane preparation served as indication that they contained no nuclear or microsomal contaminations. Succinate dehydrogenase activity (measured according to the method of SLATER AND BONNER[22]) could not be detected, so contamination with mitochondria was considered unlikely.

Methods of isolation of mitochondria and microsomes were described previously[12].

All the figures in the tables represent the mean values of no less than 3–4 experiments.

RESULTS

A significant part of the catalase activity in normal liver, regenerating liver, embryonic liver as well as in hepatomas turned out to be hidden in the lipoprotein complexes and only came into the solution provided these complexes were broken down.

Table I represents the data concerning the catalase activity in mouse transplantable hepatomas, originally induced by *o*-aminoazotoluene, which differed from each other not only morphologically but also by the growth rate and antigen composition. The most simplified tumours as to the antigenic composition and the most advanced in malignancy among the hepatomas studied proved to be: (a) the ascites form of hepatoma 22a which contains but 1 of 7 organ specific antigens characteristic of normal liver and (b) the solid form of the same hepatomas 22a, which revealed 2 organ specific antigens, being composed of anaplastic undifferenti-

TABLE I

ACTIVITY OF SOLUBLE CATALASE AND BOUND CATALASE IN LIVER AND DIFFERENT TRANSPLANTABLE MOUSE HEPATOMAS

Activity is expressed in μmoles H_2O_2 decomposed per mg protein or mg dry wt.

Tissue	*Soluble catalase*		*Bound catalase*		*Bound catalase / Soluble catalase*	
	per mg protein	*per mg dry wt.*	*per mg protein*	*per mg dry wt.*	*per mg protein*	*per mg dry wt.*
Normal mouse liver C_3HA	462 (419–743)	166 (120–295)	1216 (1030–1552)	287 (177–493)	2.6	1.7
Hepatoma 38	208 (192–220)	29 (24.8–30.6)	1495 (1448–1785)	151 (82–257)	7.1	5.2
Hepatoma 48	175 (92–231)	28 (25–30)	1766 (1645–1925)	207 (155–267)	10	7.4
Solid hepatoma 22a	131 (90–208)	27 (24–30)	630 (485–903)	82 (55–133)	4.8	3
Ascites hepatoma 22a	64 (47–73)	— —	165 (130–244)	— —	2.5	—

ated cells. Unlike the above tumours, hepatomas 38 contained 5 antigens; hepatomas 48, 4 antigens. Both of them are composed of relatively differentiated cells resembling hepatocytes[13].

As seen from Table I, there exists an inverse correlation between the morphological and immunochemical characteristics of the hepatomas studied on the one hand, and their catalase activity on the other. It is noteworthy that a pronounced drop in the total catalase activity occurs only in the most malignant hepatomas 22a (solid and ascites forms). This phenomenon applies to both soluble and lipid-bound catalase activity to an equal extent, so the bound catalase/soluble catalase ratio maintains the normal value. As to the relatively differentiated hepatomas 38 and 48, the activity of their bound catalase form is reduced to a lesser extent than that of

TABLE II

ACTIVITY OF SOLUBLE CATALASE AND BOUND CATALASE IN NORMAL AND OTHER KINDS OF MOUSE LIVER

Activity is expressed in μmoles H_2O_2 decomposed per mg protein or mg dry wt.

Tissue	*Soluble catalase*		*Bound catalase*		*Bound catalase / Soluble catalase*	
	per mg protein	*per mg dry wt.*	*per mg protein*	*per mg dry wt.*	*per mg protein*	*per mg dry wt.*
Normal liver	462 (419–743)	166 (120–295)	1216 (1030–1552)	287 (177–493)	2.6	1.7
Embryonic liver	243 (213–287)	149 (122–176)	1261 (967–1555)	316 (199–433)	5.1	2.1
Regenerating liver	715 (520–1210)	269 (268–270)	2684 (1480–3560)	837 (612–1063)	3.7	3.1
Liver of mouse with transplantable hepatoma 22a	284 (134–419)	112 (82–228)	1100 (856–1172)	255 (137–318)	3.9	2.2

soluble catalase when calculated on the basis of dry weight and shows no deviation from the normal level on calculation per mg protein. As a result the bound catalase/soluble catalase ratios for hepatomas 38 and 48 drastically increase (Table I). The correlation between the total activity and the growth rate of tumours has been found by MILLER AND GOLDFEDER[23] as well.

To find out whether the inhibition of both forms of catalase activity is characteristic of malignant growth, the embryonic and regenerating livers were studied. According to our results (Table II), the drop of the catalase activity in the embryonic mouse liver is mainly confined to the soluble form.

The ability of a tumour developing in an organism to inhibit the activity of catalase in the host liver has been reported by many authors[6,24–26]. According to our observations, the activity of soluble catalase in the liver of tumour-bearing mice was found to be reduced indeed. However, the activity of bound catalase in liver of the same mice remains at the normal level, in spite of the fact that a very malignant fast-growing hepatoma was transplanted to them (Table II). As to the regenerating liver, the total catalase activity turned out to be even higher than in resting normal liver, mainly at the expense of bound catalase. Thus one can infer that drastic inhibition of both soluble catalase and bound catalase activity seems to be specific for fast-growing hepatomas.

Since some of the results of our experiments are expressed in terms of catalase activity per mg protein, and treatment of the phosphate extract with butanol caused a significant loss of protein from the extract, one could argue that in this case the additional activity ("bound" catalase) is only apparent. However, special experiments intended to clarify the issue showed that this was not the case (see Table III). We compared the phosphate tissue extracts and the extracts obtained by treatment of the same tissue with a mixture of either phosphate buffer and butanol, or phosphate buffer and deoxycholate. In some cases we made a point to prepare these extracts so that they contained equal or close to equal protein concentrations. As is seen from Table III, butanol and deoxycholate mitochondrial extracts (Expt. 1) were found to have twice as much catalase activity as the phosphate extract in spite of equal protein concentrations.

TABLE III

CATALASE ACTIVITY TISSUE EXTRACTS

Activity is expressed in μmoles H_2O_2 decomposed per mg protein

Expt.	*Cell subfraction*	*Extracts of tissue*					
		Phosphate		*Butanol*		*Deoxycholate*	
		Protein (mg)	*Activity*	*Protein (mg)*	*Activity*	*Protein (mg)*	*Activity*
1	Mitochondria of mice liver	21.2	1800	19.2	4790	21.2	3891
2	Homogenate of hepatoma 22	38.5	98	17.5	485	38.5	220
3	Homogenate of normal mice liver	115	774	59	3228	52	1260
4	Homogenate of normal mice liver	78	487	50.5	2480	—	—

In the butanol extract (Expt. 2) the concentration of protein was only half of that in the phosphate extract; nevertheless the catalase activity was nearly 5-fold that of the phosphate extract.

To find out whether the two forms of catalase (free and bound to the lipid-containing subcellular structure) do exist, we carried out in some experiments an exhaustive (up to 20 times) extraction of the homogenate with the phosphate buffer, each time vigourously grinding the mixture in a mortar. Only then was the residue treated with *n*-butanol. The bulk of the water-soluble catalase activity was extracted at once in the first volume of the phosphate buffer. Extractions following the first one could release additional but unsignificant amounts of catalase. However, the treatment of the tissue residue with *n*-butanol, after the exhaustive extractions with the phosphate buffer were completed, released catalase activity which exceeded by a factor of 4–5 that which appeared on the last extraction with the phosphate buffer. Treatment of the tissue residue with butanol released an additional catalase activity regardless of the way the data were calculated, per mg protein or per g of the original tissue.

We have previously[12] reported the existence of two forms of catalase activity

TABLE IV

ACTIVITY OF SOLUBLE CATALASE AND BOUND CATALASE IN SUBCELLULAR STRUCTURE OF MICE LIVER AND HEPATOMA 22a

Activity is expressed as μmoles H_2O_2 decomposed per mg protein.

Cell subfraction	*Liver*		*Hepatomas*			
	Soluble catalase	*Bound catalase*	*Bound catalase / Soluble catalase*	*Soluble catalase*	*Bound catalase*	*Bound catalase / Soluble catalase*
Mitochondria	3203 (2600–3936)	4029 (3570–4580)	1.25	624 (416–833)	2987 (2223–4200)	4.78
Microsomes	663 (560–991)	1323 (900–2000)	2.00	600 (477–900)	1407 (1230–1500)	2.34
Plasma-membranes	1747* (1107–2290)	4520* (3446–5796)	2.57	76**	108**	1.42

* Rat liver.
** Hepatoma Zajdela.

(in different quantitative ratios) in all subcellular structures studied. In addition, we have measured both activities in the plasma membranes of rat hepatocytes and ascites hepatomas Zajdela. Like the other structures, plasma membranes were found to possess both forms of catalase activity, quantitatively very close to those of mitochondria which are known to have the highest content of catalase. In the plasma membranes from hepatomas a drastic drop in both kinds of catalase activity was observed more pronounced in the bound form: the bound catalase/soluble catalase ratio reduced from a value of 2.57 (liver plasma membranes) to 1.42 (Table IV).

DISCUSSION

Heterogeneity of tissue catalase has been recognised by many authors but the interpretations of the phenomenon were different. Some authors have considered this heterogeneity an indication of the existence of catalase isozymes, while others believed that the same form of catalase might be bound to subcellular structures differently.

KAPLAN[1–4], studying yeast catalase, infers that there exist two forms of catalase, patent and cryptic. The patent form seems to be reversibly bound to the plasma membranes of yeast cells, while the cryptic catalase is localised inside the cells. Both forms are in equilibrium. Activity of the cryptic form is revealed provided the cells are lysed. After treatment of yeast cells with butanol, which destroys the cell structure, a 60-fold increase of the catalase activity was observed[2]. According to Kaplan, the patent catalase, unlike the cryptic one, is thermostable. In addition, the sensitivity of the "latent" catalase to the action of UO_2 turned out to be 500 times as high as that of the patent one.

On the other hand, mitochondria from rat liver were found to contain 4 types of catalase[28], differing from each other by localization, thermolability, rate of [^{14}C]leucine incorporation and chromatographic behaviour. These types of catalase were recognised as isozymes. NISHIMURA *et al.*[8] reported the existence of catalase isozymes in rat liver and in human red cells with different biochemical and immunochemical properties. The existence of isozymes of catalase in rat and mouse liver was reported by HOLMES AND MASTERS[29] as well.

According to our observations, there exist two forms of catalase in rat liver and hepatomas, soluble and lipid-bound. Comparison of their properties (thermolability, sensitivity to the inhibitory action of KCN, pH optima)[12] favours the view of the existence of but one molecular kind of catalase rather than the assumption that these forms are isozymes. If the latter possibility were correct, bound catalase activity, for instance, released from lipoprotein complexes of all subcellular structures and tissues should behave equally as to thermolability and sensitivity with respect to inhibitors; however, this was not the case. We believe that some peculiarities of soluble catalase and bound catalase activities might be due to different amounts and kinds of proteins, which they are bound to after extraction. But we cannot rule out the possibility of the existence of isozymes in each form (water-soluble and lipid-bound) of catalase.

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