Changes in Peroxisomal Enzyme Activities and Heterogeneity of Catalase during 3′-Methyl-4-dimethylaminoazobenzene Hepatocarcinogenesis

Yohichi MOCHIZUKI, Masayuki ITABASHI, and Hiromitsu HAGA

Department of Pathology, Cancer Research Institute, Sapporo Medical College

SUMMARY

Change in activities of four peroxisomal enzymes (catalase, D-amino acid oxidase, L-α-hydroxy acid oxidase and urate oxidase) and the heterogeneity of catalase on DEAE-cellulose column chromatography in the rat livers during 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB) carcinogenesis and in the hepatomas induced by the carcinogen were studied. The activities of the four enzymes decreased during the 8th and 10th week of the carcinogenic course, and increased rapidly during the 13th week, with the exception of D-amino acid oxidase. Thereafter, the three enzyme activities were maintained at the same level of the 13th week. These changes of the three enzyme activities were in accordance with the degree of maturation of the cells. D-Amino acid oxidase activity was very low during the entire carcinogenic course, suggesting that the carcinogen itself affected this enzyme. Liver tumors induced by 3′-Me-DAB showed decreased activities of the four peroxisomal enzymes. Among these tumors, trabecular type I tumors showed moderate activities of the four enzymes. Many of less differentiated, trabecular type II tumors showed lack of D-amino acid oxidase, L-α-hydroxy acid oxidase and urate oxidase, and showed very low activity of catalase. Elution patterns of catalase on DEAE-cellulose column in the livers during the carcinogenesis were the same as those in livers of adult normal rats. Only the tumors alone showed an unadsorbed catalase fraction on the column resembling fetal livers and Morris hepatomas.

INTRODUCTION

Early studies of Greenstein and his associates (1) emphasized the view that tumor tissues in general had none or negligible activity of catalase; however, this view is no longer tenable in view of the development of studies of Morris hepatomas. In these hepatomas, poorly-differentiated fast-growing

hepatomas showed none or very low activities of catalase, while highly-differentiated slowly-growing tumors contained a relatively high activity of catalase, and the level of this enzyme activity was found to correspond well with the number of peroxisomes which are the intracellular organelles containing catalase (2). Furthermore, our previous observations on regenerating hepatocytes of partially hepatectomized rats (3) and those on the livers of fetal and postnatally growing rats (4) suggested that peroxisomes were few and catalase activity was low in immature hepatocytes and that they increased as the cells underwent maturation. In other words, the activity of catalase and the number of peroxisomes in the liver are considered to be well correlated with maturation and differentiation of the cells. In this respect, the changes in the enzyme and the organelles are expected to occur in hepatocytes which undergo cancerization, since the changes in differentiation, maturity and/or growth are considered as relevant traits of preneoplastic cells.

Several studies indicated that catalase activity of the liver decreased during carcinogenesis (5-9). However, these were made without consideration to the fact that catalase is one of the enzymes located in peroxisomes. Thus, in the present experiments, activities of catalase, D-amino acid oxidase, urate and L-α-hydroxy acid oxidase, all of which are peroxisomal enzymes of the liver, were examined biochemically on the liver tissues of rats during 3'-methyl-4-dimethylaminoazobene (3'-Me-DAB) carcinogenesis. In addition, the heterogeneity of catalase as revealed by DEAE-cellulose column chromatography was examined, based on the findings of changes in the heterogeneity profiles observed in Morris hepatomas (10).

MATERIALS AND METHODS

Male Wistar rats, weighing 170-200g were fed either a commercial diet (No. NMF, Oriental Yeast Co., Tokyo) or a diet containing 0.06% 3'-Me-DAB. The rats were sacrificed by decapitation on the 8th, 10th, 13th, 15th, 19th and 23rd week after the commencement of the carcinogen feeding, respectively. The livers were excised quickly, and homogenized with 10 volumes of cold distilled water. The tumors were taken from the livers of rats fed the carcinogen for 23 weeks.

Activities of catalase, D-amino acid oxidase, and urate oxidase were measured by the methods previously described (4). Activity of L-α-hydroxy acid oxidase was measured by the following procedure. 0.5ml of appropriately diluted homogenates were incubated at 37°C for 15 min in a reaction medium consisting of 0.15 ml of 0.05 M glycolic acid in 0.1 M pyrophosphate buffer (pH 8.3), 0.05 ml of flavine mononucleotide (10 mg/100 ml), 0.05 ml of 0.03% catalase, and 0.75 ml of 0.1 M pyrophosphate buffer (pH 8.3). The reaction

was terminated with 1 ml of cold 50% trichloroacetic acid. After centrifugation at 3,000 rpm for 10 min, to 1 ml of the supernatant was added 1 ml of 0.025% 2,4-dinitrophenylhydrazine at room temperature. After 20 min, 2 ml of 3 N NaOH solution was added and the absorbancy was measured on a Klett colorimeter using the No. 54 Filter. Activities of these enzymes were expressed on the basis of protein measured by the method of Lowry *et al* (11).

DEAE-cellulose column chromatography of catalase was done according to the procedure described previously (10).

RESULTS

Activities of Peroxisomal Enzymes during Carcinogenesis.

Fig. 1 shows the changes in the activities of four peroxisomal enzymes during carcinogenesis. Catalase activities in the 8th and 10th week showed a decrease of about 40% against normal values, followed by a considerable incerase thereafter. The activity in the 13th week was 80% of the normal values. The activities in the 15th and 19th week were comparable to that of the 13th week.

Activity of urate oxidase was slightly decreased in the 8th and 10th week, followed by a restoration to normal values in the 13th week. In the 15th and 19th week, the activity was slightly decreased, but no statistical significance was seen. Activity of L-α-hydroxy acid oxidase was 55% of the normal values in the 8th and 10th week, then it increased reaching the values of the lower limit of normal in the 13th week. Activities in the 15th and 19th week were similar to that in the 13th week. Activity of D-amino acid oxidase was markedly decreased, reaching 20% of the normal values in the 8th

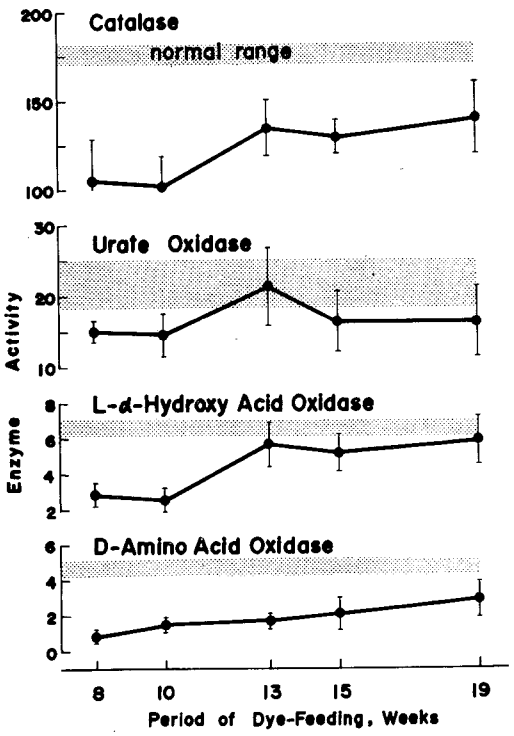

Fig. 1. Changes in activities of four peroxisomal enzymes in the course of 3′-Me-DAB carcinogenesis. Bars indicate standard deviation.

week. Then, the activity gradually increased but it did not reach the normal values even in the 19th week.

Activities of Peroxisomal Enzymes in Primary Hepatic Tumors.

A majority of hepatic tumors induced by 3'-Me-DAB in this experiment were trabecular carcinomas. These tumors were classified into two types, type I (more differentiated tumors) and type II (less differentiated tumors), according to Ma and Webber (12). As shown in Table 1, the activities of each enzyme measured were considerably lower in trabecular type I tumors than the control values, and strikingly low in trabecular type II tumors. Activities of D-amino acid oxidase, L-α-hydroxy acid oxidase and urate oxidase were not measurable in many cases of the latter type of the tumors.

Table 1. *Activities of peroxisomal enzymes.*

	Catalase	D-Amino acid oxidase	L-α-Hydroxy acid oxidase	Urate oxidase
Trabecular type I tumors	46.05	0.345	2.417	9.449
	41.79	2.277	1.609	3.996
	28.39	1.193	2.222	1.976
	18.70	0.746	0.746	0.895
	14.29	0	0.198	0.190
Trabecular type II tumors	8.03	0.182	0.091	1.091
	7.81	0	0	0.297
	6.38	0	0	0
	5.93	0.198	0	0
	4.64	0	0	0
	1.96	0	0	0
Normal rat livers	173.46[a] ±7.53	5.144 ±0.893	5.365 ±0.763	21.320 ±2.068

Catalase activity: units/min/100 mg protein. D-Amino acid oxidase activity: pyruvic acid produced, μg/min/mg protein. L-α-Hydroxy acid oxidase activity: pyruvic acid produced, μg/min/mg protein. Urate oxidase activity: uric acid oxidized, μg/min/mg protein.

a) Mean ± standard deviation.

DEAE-cellulose Column Chromatography of Catalase.

Elution patterns of catalase on DEAE-cellulose column chromatography of normal rat liver, livers of rats fed 3'-Me-DAB, and the tumors induced by the carcinogen are shown in Figs. 2, 3, 4, and 5. The catalase of normal rats was adsorbed onto DEAE-cellulose. The predominant amounts of the en-

zymes were eluted with 0.1 M and 0.2 M NaCl. In the hepatic tumors, there was a moderate proportion of catalase not adsorbed onto DEAE-cellulose. The elution patterns of catalase in rats fed the carcinogen for 8 to 19 weeks were principally the same as that of normal liver.

DISCUSSION

A number of histological investigations on the hepatocarcinogenesis of 3′-Me-DAB in rats are available (13–23). According to these observations, the original hepatocytes degenerated and disappeared in the early stages. Subsequently, these changes were followed by a regenerative process leading to the development of hyperplastic nodules, as the regenerated small hepatocytes grew larger. Some areas of the hyperplastic nodules acquired hyperbasophilic properties. Cell proliferation occurred in these areas during the middle to the late stages, and these areas are considered as an ancestral population of later hepatomas (16, 23).

The changes in peroxisomal enzyme activities during the carcinogenesis in the present study seem to correspond to the histological changes mentioned above. The low activities of four peroxisomal enzymes in the 8th and 10th week of the carcinogenesis are considered to be due to the immature state of the renewed hepatocytes. The findings that all enzyme activities with the exception of D-amino acid oxidase increased in the 13th week might be ascribed to the maturation of the renewed hepatocytes. However, considering that the activities of catalase and D-amino acid oxidase did not reach normal

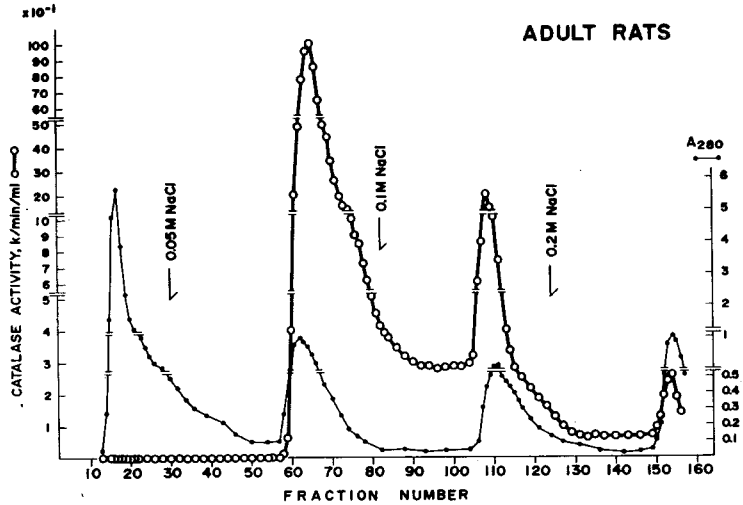

Fig. 2. DEAE-cellulose column chromatography of catalase in the liver of normal adult rat.

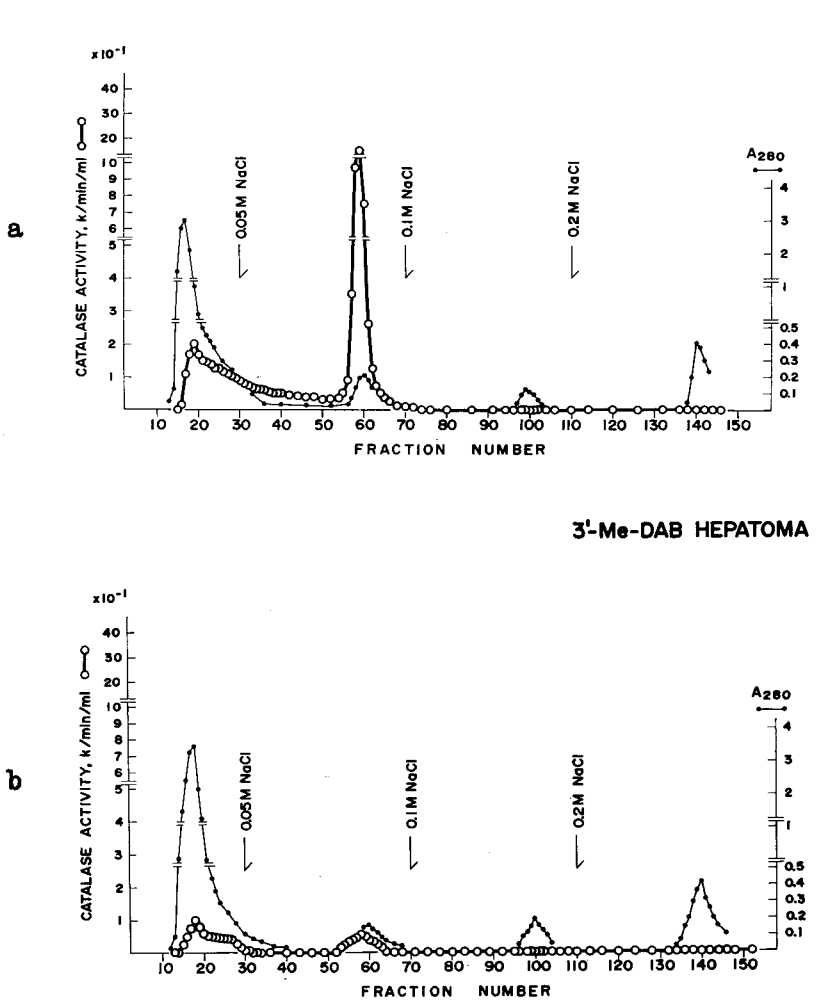

Fig. 3. DEAE-cellulose column chromatography of catalase in 3'-Me-DAB induced hepatomas.
a: slightly-differentiated hepatoma.
b: poorly-differentiated hepatoma.

values in the 13th week, the maturation of the renewed hepatocytes might be incomplete enzymatically. In the 15th week, activity of urate oxidase was slightly decreased, and L-α-hydroxy acid oxidase and catalase did not show a further increase. These observations suggest that hyperplastic lesions which appeared frequently in the 15th week have low activities of peroxisomal enzymes. In fact, histochemical investigations of catalase revealed that there

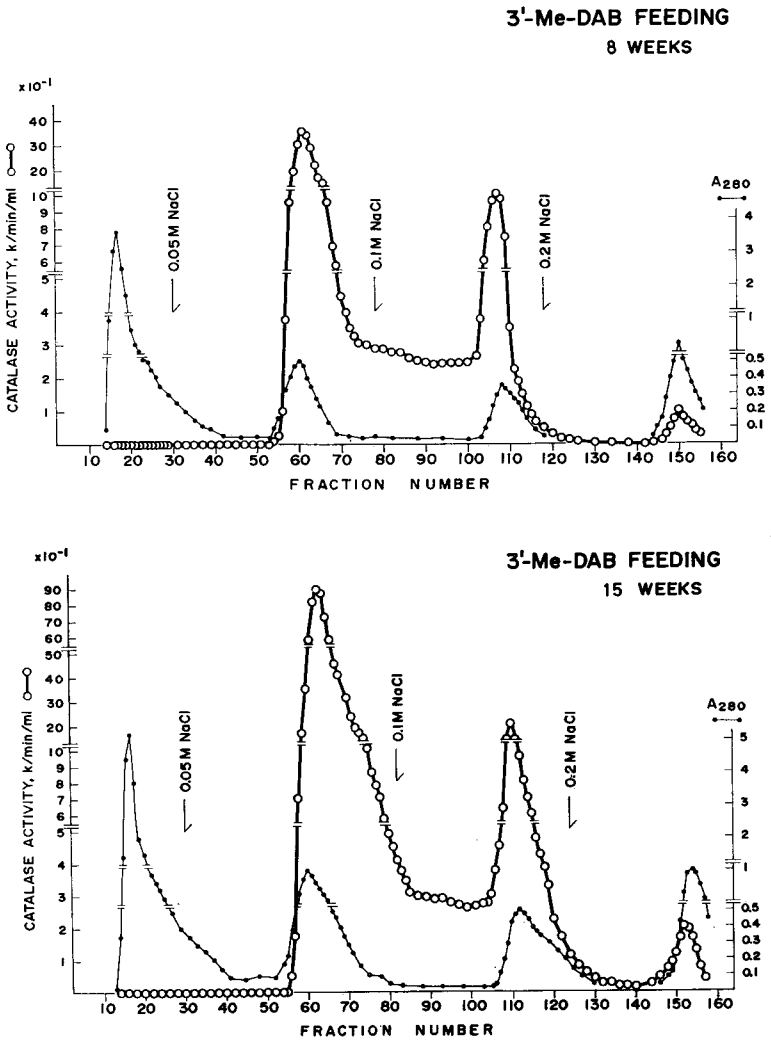

Fig. 4. DEAE-cellulose column chromatography of catalase in the livers in the 8th and the 15th week of the carcinogenic course.

were some hyperplastic lesions in which catalase activity was very low (24). In the late stages of carcinogenesis, one must carefully evaluate the results obtained biochemically, because the heterogeneity of cell populations appears. In the 19th week, the enzyme activities were expected to decrease because the hyperplastic nodules were increased in number and size. However, activities of catalase, urate oxidase, and L-α-hydroxy acid oxidase in the 19th week were actually similar to those in the 15th week. It is assumed that

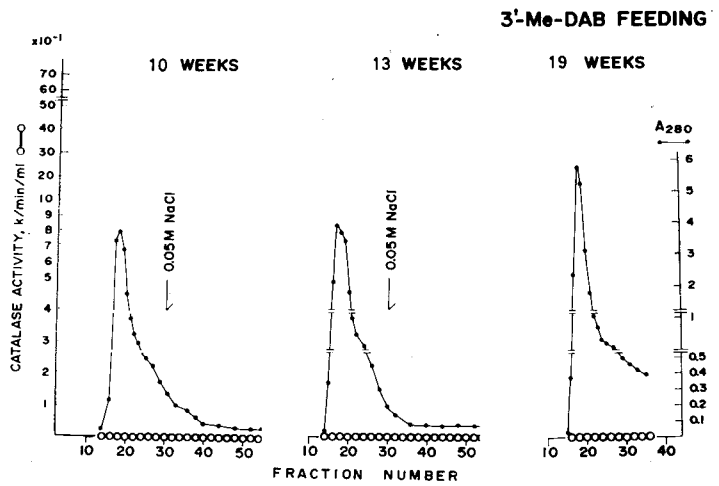

Fig. 5. DEAE-cellulose column chromatography of catalase in the livers of the 10th, the 13th, and the 19th week of the carcinogenic course.

the maturation of hepatocytes in the hyperplastic nodules takes place. In fact, a considerable number of hyperplastic nodules gained a moderate activity of catalase in the late stages, when the enzyme activity was examined histochemically (24). Thus, the changes in peroxisomal enzyme activities with the exception of D-amino acid oxidase in the carcinogenic course are considered to be well correlated with the degree of maturity and differentiation of hepatocytes.

The changes in the activity of D-amino acid oxidase were different from those in other peroxisomal enzymes. The activity was very low throughout the entire course of the carcinogenesis. It is tempting to suggest that 3'-Me-DAB has an inhibitory effect on the enzymes, but further studies will be required to establish this possibility.

The trabecular liver tumors induced by 3'-Me-DAB may be classified into trabecular type I tumors (more differentiated tumors) and trabecular type II tumors (less differentiated tumors), although the degree of differentiation of these tumors was low in general (12, 25). Trabecular type II tumors showed a lack of or very low activities of peroxisomal enzymes, while trabecular type I tumors showed low or moderate activities. Thus, the peroxisomal enzyme activities are assumed to be well correlated with the degree of differentiation of tumors. Among the four enzymes, catalase activity was in good accord with the degree of differentiation of tumors. The close correlation between the catalase activity and the degree of differentiation of tumors was also observed in Morris hepatomas (2). As to other perox isomal

enzymes, a certain discrepancy between the enzyme activities and the degree of differentiation of the tumors is present, although the discrepancy is not as outstanding as in Morris hepatomas. It might be assumed that activities of D-amino acid oxidase, L-α-hydroxy acid oxidase, and urate oxidase have no correlation with the degree of differentiation of tumors.

Although the isozyme of catalase in the mammalian liver has not been determined as yet, it has been clarified that there is a certain heterogeneity of the enzyme (26–33). Our previous investigations of pI-heterogeneity and the heterogeneity on DEAE-cellulose column chromatography of catalase in the livers of fetal, newborn, adult, and partially hepatectomized rats (34), and Morris hepatomas (10), have revealed that the heterogeneity patterns in Morris hepatomas closely resemble those in the livers of fetal and newborn rats, but is evidently different from those in the livers of adult rats. Similar heterogeneity patterns of the enzyme were also reported on human fetal livers and human hepatomas (35). In the present experiment with DEAE-cellulose column chromatography, the non-adsorbed components of catalase were demonstrated in the primary hepatomas. This finding strongly suggests that the presence of non-adsorbed components of catalase onto DEAE-cellulose column and high pIs of the enzyme are one of characteristics of liver tumors.

In the preneoplastic stages, however, the heterogeneity patterns of catalases were quite similar to those in adult livers. Although the liver tissues in the 15th and 19th week of the carcinogenic course contained hyperplastic lesions and hyperbasophillic areas, non-adsorbed components of catalase were not detected. It is interpreted that the sensitivity of the procedure may be insuficient to demonstrate the changes in the hyperplastic lesions or that the changes may have not occurred.

Holmes and Masters (30) considered that the heterogeneity of catalase was due to the epigenetic modification of catalase molecules, and Jones and Masters (33) reported that this modification was due to sialation and desialation of catalase molecules. In tumors, the sialation mechanism may be impaired. However, taking into account our previous findings (10) in which the pIs of catalase in Morris hepatomas were higher than those of the desialated catalase in the adult livers of the experiments of Jones and Masters (33), high pI forms of catalase in Morris hepatomas can not be satisfactorily explained to be the result of desialation of catalase alone.

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