Free Rad. Res., Vol. 26, pp. 343–350
Reprints available directly from the publisher
Photocopying permitted by license only

Multiplicity of Antioxidant Enzyme Catalase in Mouse Liver Cells

YI SUN

*Radiation Research Laboratory, 14 Medical Laboratories, The University of Iowa, Iowa City, Iowa 52242 and *Department of Molecular Biology, Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, Michigan 48105, USA*

Accepted by Prof. B. Halliwell

(Received 4 July 1996; In revised form 28 October 1996)

Multiplicity of catalase activity has been observed in crude homogenates from the tissue and cell lines of mouse liver by ethanol/Triton X-100/heat treatment. The five enzymatically active catalase bands were designated as CAT1, CAT2, CAT3, CAT4, and CAT5 with a nondenatured molecular mass of 270kDa, 258kDa, 229kDa, 210kDa, or 197kDa, respectively. Cultured mouse liver cell lines, mouse liver tissue homogenate, and pure mouse liver catalase showed only one catalase band (CAT1) after ethanol/Triton X-100 treatment at 4°C for 72 hr. The same treatment but incubated at 37°C for 72 hr yielded three bands (CAT2, CAT4, CAT5) in normal cell line, only one band (CAT5) in MNNG-transformed and SV40-transformed cells, two bands (CAT1, CAT4) in mouse liver tissue homogenates, and two bands (CAT1, CAT3) in pure mouse liver catalase. These five catalase bands were further biochemically characterized. The CAT1, CAT2, and CAT3 are sensitive to heat (68°C, 1 min), while CAT4 and CAT5 are rather heat resistant. The sensitivity to catalase inhibitors, such as aminotriazole, azide, or cyanide varies among the isoforms. Protease inhibitors could prevent the formation of CAT3 and CAT4, but not CAT5. Treatment with protease, however, removed all forms of catalase except CAT5. We conclude from this study that the appearance of different catalase bands is likely due to epigenetic modification of the protein, particularly proteolysis. The lowered catalase activity in transformed cells might also be attributable to the loss of two catalase isoforms.

Keywords: Antioxidant enzymes, catalase, isoforms, proteolysis, epigenetic modification

INTRODUCTION

Catalase (EC 1.11.1.6) is one of the primary antioxidant enzymes which decomposes H_2O_2 to form H_2O and O_2. Along with other antioxidant enzymes such as superoxide dismutase and glutathione peroxidase, catalase plays an important role in preventing cellular toxicity induced by reactive oxygen species. In the past two decades, the relationship between catalase and cancer has been the subject of numerous studies. Generally, catalase activity has been found to be low in tumor cells of both human and rodent origins (for review, see ref. 1). Low catalase levels in transformed and tumor cells may lead to the accumulation of H_2O_2[2] which is known to be mutagenic[3] and carcinogenic.[4,5] Using mouse liver cell as a model, we found that catalase activity was remarkably decreased after immortaliza-

*Corresponding address. Tel.: (313)-996-1959. Fax: (313)-996-7158.

tion and cellular transformation.[6] This lowered activity was due to a decreased amount of immunoreactive catalase protein which results from the transcriptional down regulation of the catalase gene. The involvement of the gene methylation was implicated in this transcriptional depression.[7,8]

Heterogeneity of catalase has been reported in bacterial cells,[9,10] mammalian cells,[11–17] and also involved in human diseases.[18–21] Since catalase heterogeneity is largely due to multiple types of epigenetic modification,[22] we wondered whether the lowered catalase activity in transformed cells could also be in part attributable to altered post-translational modifications, in addition to transcriptional depression of the gene.[8] We report here the finding of five enzymatically active catalase forms in mouse liver tissue and cell lines after ethanol/Triton X-100/heat treatment. The loss of several of these isoforms in transformed cells may also contribute to their lowered activities.

MATERIALS AND METHODS

Chemicals. Mouse liver catalase, human erythrocyte glutathione peroxidase, soybean trypsin inhibitor, phenylmethylsulfonyl fluoride (PMSF), leupeptin, pronase E, neuraminidase, bovine serum albumin, 3-amino-1,2,4-triazole (AT), sodium azide, bovine milk α-lactalbumin, bovine erythrocyte carbonic anhydrase, chicken egg albumin, bovine serum albumins and jack bean urease were purchased from Sigma (St. Louis, MO). Sodium cyanide, hydrogen peroxide, potassium ferricyanide, ferric chloride and phenol reagent solution were products of Fisher Scientific Co. Triton X-100 was from Mallinckrodt, Inc.

Cell Culture and Sample Treatment. BNL CL.2 (liver embryonic cells), BNL 1NG A.2 (liver embryonic M-methyl-N-nitro-N-nitrosoguanidine transformed), and BNL SV A.8 (liver embryonic SV40 transformed) cells were obtained from the American Type Culture Collection. Cells were grown in Dulbecco's modified Eagles' medium with 10% calf serum (Hy-clone) as described earlier.[6] Balb/c mice (male, 2 month old) were used as a source of liver tissue. Liver was perfused to eliminate the contamination from blood catalase, as detailed previously.[6] Cell pellets or liver tissue were homogenized on ice in potassium phosphate buffer (0.05 M, pH 7.8) with a Tekmar (Cincinatti, OH), and then sonicated, again on ice in a Biosonik IV sonicator (Brownwill, San Francisco, CA). The sample treatment for catalase activity assay was performed as described by Cohen *et al.*[23] with slight modifications. Briefly, 21 μl of 95% ethanol was added to 200 μl of homogenate (20–30 mg protein/ml, assayed by Lowry *et al.*[24]) After incubation on ice for 30 min, 200 μl of 10% Triton X-100 was added and sample was incubated on ice for another 10 min. then diluted with homogenizing buffer to a final volume of 2.0 ml. The sample was then incubated either at 4°C or at 37°C for 72 hr., respectively, before being run on a native polyacrylamide gel.

Catalase Activity Gel Staining. Samples post ethanol/Triton X-100 treatment were run on 7.5% polyacrylamide slab gels with 5% stacking gels. The ferricyanide staining method for visualization of catalase activity on the gel was used as described by Woodbury *et al.*[25] with slight modification.[26] Briefly, after gel electrophoresis, the gels were washed in three changes of distilled water for a total of 45 min to remove the buffer from the gel's outside surface, and then soaked in 0.003% (v/v) H_2O_2 for 10 min. After twice rinsing with distilled water, the gels were stained with a fresh-made solution of 1% ferric chloride/1% potassium ferricyanide (w/v) until they became dark green with yellow activity bands. The gels were rinsed with water again, wrapped in Saran Wrap plus aluminum foil and stored at 4°C. Catalase inhibitors (azide, cyanide, and aminotriazole) were added in the gel washing step and incubated for a total of 45 min. For heat inactivation of catalase, samples were incubated at 68°C

for 1 min before loading on the gels. Protease inhibitors were added concurrently with ethanol-Triton and then incubated at 37°C for 72 hr., while protease was added into untreated sample and incubated at 37°C for 72 hrs.

Native Molecular Weight Analysis. The nondenatured molecular weight of the multiple forms of catalase was determined as described.[27] Briefly, samples and standard molecular weight markers were co-electrophoresed on a series of gels of various acrylamide concentrations (6–10%). The R_f value of each protein relative to the bromphenol blue tracking dye was measured for each gel. The R_f data were then plotted for each protein with the x-axis being gel concentration and y-axis 100 log (100 R_f). The slope versus molecular weight was plotted. The molecular weight of each catalase activity band was calculated by comparison with standard curves derived from the markers. Standard molecular weight marker proteins were stained for protein with Coomassie brilliant blue dye.

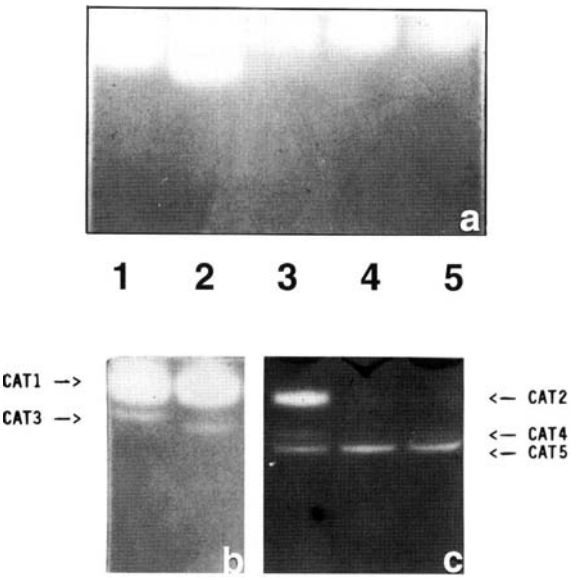

FIGURE 1 Visualization of multiple catalase bands after ethanol/Triton X100/heat treatment: Cell pellets were homogenized and sonicated and treated with ethanol/Triton X-100 as described in Materials and Methods. Samples were then incubated at 4°C (a), or at 37°C (b and c) for 72 hr. followed by gel electrophoresis and catalase activity staining as detailed in M & M. The lane arrangements in (a) are lane 1, 10 U of pure mouse liver catalase; lanes 2–5 are homogenates from liver tissue, normal liver cell line, MNNG-transformed and SV40-transformed cell lines with 45 μg protein per lane, respectively. Lane arrangement in (b & c) was the same as (**a**), with pure catalase and liver tissue homogenate in lanes 1 and 2 (**b**) and the liver cell lines in lanes 1, 2, and 3 (**c**). 30 μg protein from each sample was loaded.

RESULTS AND DISCUSSION

Definition of Five Enzymatically Active Isoforms of Mouse Liver Catalase

To examine possible involvement of epigenetic modifications in altered catalase activity in transformed cells, we first modified experimental conditions described by Masters *et al.*[17] to ensure the visualization of multiple catalase isoforms. Cell homogenates were treated with ethanol (which prevents inactivation of catalase by H_2O_2) and Triton X-100 (a non-ionic detergent used to stabilize the membrane), then incubated either at 4°C or at 37°C for 72 hr. As shown in Figure 1a, only one band was visualized in samples following incubation at 4°C which is similar to the pattern seen when the samples are not treated with ethanol/Triton X-100 (not shown). The order of catalase activity, from the highest to lowest, is liver tissue homogenate, normal liver cells, MNNG-transformed, and SV40-transformed cells, an observation consistent with catalase activity assay.[6] After incubation at 37°C, however, multiple bands were visualized with the number depending on the samples (Figure 1b and 1c). In pure mouse liver catalase (Fig. 1b, lane 1) and liver tissue homogenate (Fig. 1b, lane 2), two bands were seen. Normal liver cell line showed three bands (Fig. 1c, lane 1), while transformed liver cells produced only one band (Fig. 1c, lanes 2 & 3). Clear visualization of above-mentioned banding pattern on activity gels could only be achieved with samples treated in combination of ethanol, Triton X-100 and heat for 72 hr. Samples treated with heat (37°C) alone, Triton X-100 plus heat, or ethanol plus heat gen-

erated only very faint bands (data not shown). In addition, 72 hr incubation at 37°C was also required for generating two fast migrating bands. Only very faint bands corresponding to these two catalase isoforms were seen if samples were incubated for 48 hr (data not shown).

We next determined the molecular weight of these five enzymatically active catalase bands observed after ethanol/Triton X-100/heat treatment by electrophoresis on native acrylamide gels of different percentages (Figure 2). Five catalase isozymes was designated CAT1, CAT2, CAT3, CAT4, and CAT5 (Fig. 1b and 1c, as indicated) according to their molecular masses. Using the method described by Hedrick and Smith,[27] it was calculated that CAT1 has molecular weight of 270kDa; CAT2, 258kDa; CAT3, 229kDa; CAT4, 210kDa; and CAT5, 197kDa. Two bands in pure catalase sample were denoted CAT1 and CAT3; and in liver tissue homogenates, CAT1 and CAT4, respectively, and three bands in normal cell line were denoted CAT2, CAT4, CAT5, respectively. While one band in two transformed cells was denoted CAT5. Thus, in liver cell lines, high molecular weight form (CAT1) could be converted into low MW forms (CAT2, CAT4, CAT5 in normal cell lines and CAT5 only in two transformed lines) during high temperature treatment (37°C vs 4°C).

Biochemical Characterization of Catalase Isoforms

Since mouse catalase has only one structural gene,[16,28] and since without ethanol/Triton X-10/heat treatment only one form (CAT1) could be detected, multiplicity of mouse liver catalase must be due to epigenetic modifications. We therefore performed biochemical characterizations of these catalase isoforms.

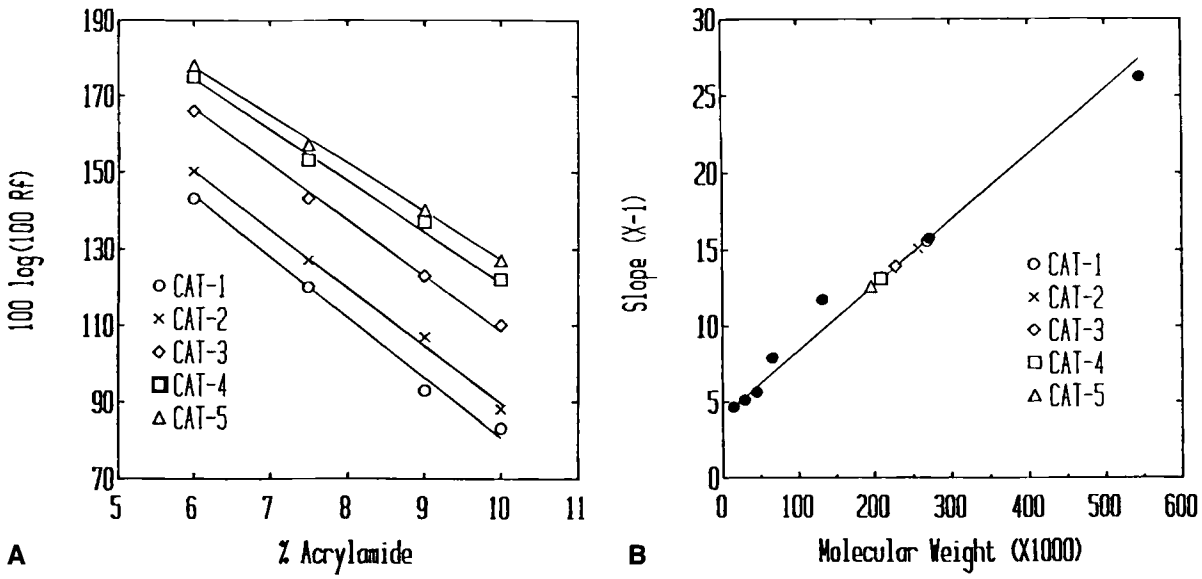

FIGURE 2 Determination of nondenatured molecular mass of catalase isozymes: (**a**) Effect of different acrylamide concentration on the mobility of CAT1-5. Samples were treated with ethanol/Triton X-100/heat for 72 hr at 37°C, loaded on various concentration of acrylamide gels, and activity stained as detailed in M & M. The migration of each catalase isoform (100 Rf) was plotted against percentage of acrylamide. (**b**) Determination of the molecular mass of the 5 catalases from the slopes determined in (a). The proteins used as molecular mass standard were as follows: a, bovine milk α-lactalbumin (14.2kDa); b, bovine erythrocyte carbonic anhydrase (29kDa); c, chicken egg albumin (45kDa); d, and e, bovine serum albumin (monomer, 66kDa; dimer, 132kDa); f, and g, jack bean urease (trimer, 272kDa; hexamer, 545kDa). The standard proteins were also run on different percentage of acrylamide gels, stained with Coomassie blue dye, and slope calculated as shown in (a). The slope was plotted against known molecule weight and was indicated by the solid circles. The slopes for CAT1-5 were then plotted and molecular weight for each isozyme calculated.

(1) Heat inactivation and catalase inhibitors treatment. The catalase isoforms in pure catalase (CAT1, CAT3) and in liver cell lines (CAT2, CAT4, CAT5) were examined for their sensitivity to heat and catalase inhibitors. As shown in Figure 3a, CAT1, CAT2 and CAT3 were heat sensitive and inactivated by heat at 68°C for 1 min. CAT4 and CAT5, however, were heat resistant. Catalase inhibitor treatment also showed different sensitivity among isozymes. As shown in Figure 3b, 1 mM sodium azide completely inhibited CAT1, CAT2, CAT3, moderately inhibited CAT4 and slightly inhibited CAT5. All isozymes were completely inhibited by 5 mM sodium azide (not shown). 1 mM sodium cyanide inhibited CAT3 and CAT4 completely and slightly inhibited CAT1, CAT2, and CAT5 (Fig. 3c). At the concentration of 5 mM, sodium cyanide completely inhibited CAT1, CAT2, CAT3 and CAT4 and somewhat inhibited CAT5 (not shown). The sensitivity of these isozymes to 3-amino-1,2,4,-triazole (AT) was also different. 10 mM AT inhibited CAT3 and CAT4 completely, and moderately inhibited CAT1, CAT2, and CAT5 (Fig. 3d). All isozymes were nearly completely inhibited when 30 mM AT was used (not shown). It has been previously shown that mycobacteria produced two classes of catalase, the heat liable T-catalase and heat stable M-catalase. The two classes can be distinguished by heating (which inactivates T form) and by treatment with aminotriazole (which inactivates M form).[9] CAT1, CAT2, and CAT3 are somewhat similar to T-catalase in mycobacteria (heat sensitive) and CAT4 is somewhat similar to M-catalase (sensitive to aminotriazole but resistant to heat).

It is noteworthy that in all heating and inhibitor experiments, pure human red blood cell glutathione peroxidase was loaded in lane 2, serving as a negative control to demonstrate that neither one of these five bands is glutathione peroxidase. We have previously observed that glutathione peroxidase could be visualized in this activity gel only when glutathione was present in staining step to provide substrate for the enzyme.[26] None of these

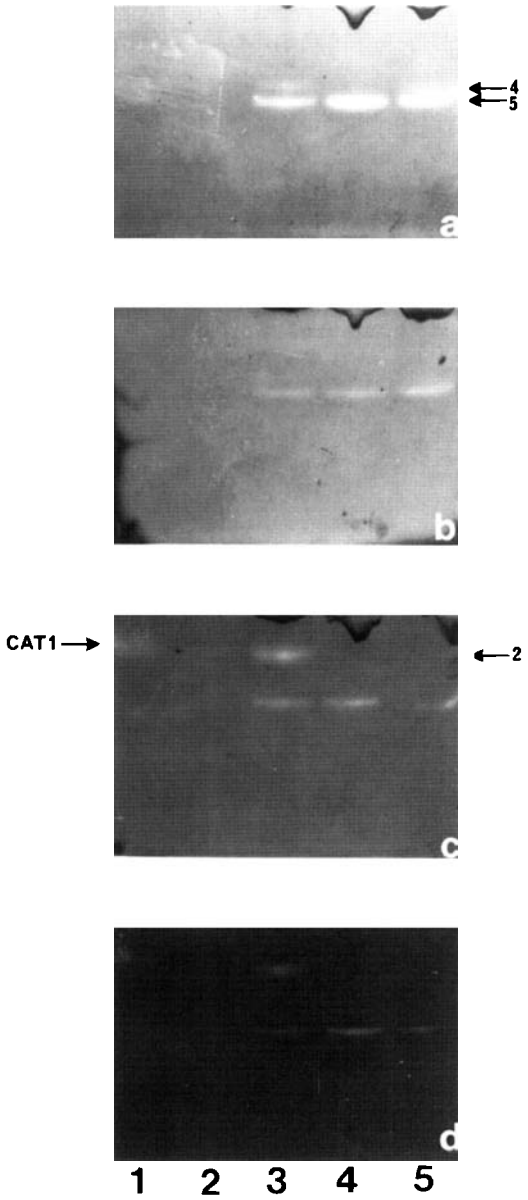

FIGURE 3 Five catalase isozymes show distinctive sensitivity to heat and catalase inhibitors: The samples were treated with ethanol/Triton X-100/heat for 72 hr, gel electrophoresized, and activity stained as described in M & M. For all gels, lane 1 contained 2.5 U of pure mouse liver catalase, lane 2 contained 25 mU of pure human red blood cell glutathione peroxidase, lanes 3–5 were 30 μg of liver cell lines: normal, MNNG-transformed and SV40-transformed, respectively. (**a**) Heat inactivation, samples were heated at 68°C for 1 min just prior to loading on the gel. (b–d) treatment with catalase inhibitors, 1 mM sodium azide (**b**); 1 mM sodium cyanide (**c**); 10 mM 3-amino-1,2,4-triazole (**d**) were included in the gel staining step for 45 min as detailed in M & M. The arrows and numbers 1–5 represent CAT1-5, respectively.

multiple bands is produced by potential H_2O_2-degrading activity of cytochrome c since the molecular weight of the cytochrome c is about 12 kDa, much smaller than any of the catalase isoforms. Furthermore, multiple bands could not be the catalase subunits since dissociation of active tetrameric form of catalase leads to loss of activity.[16] The nondenatured molecular weight of these multiple forms of catalase also support this assertion. Overall, these results indicated that multiple bands produced after ethanol/Triton X100/heat treatment of liver homogenates are the catalase isoforms. They are biochemically different from each other with distintive molecular mass and different sensitivity to heat and chemical inhibitors.

(2) Treatment with protease inhibitors and protease. Since it seems likely that these enzymatically active catalase isozymes are proteolytic cleavage products of the larger form (CAT1), we treated samples with ethanol/Triton X-100 plus various protease inhibitors concurrently, and then incubated at 37°C for 72 hr. As shown in Figure 4, addition of 12 μg/mg sample protein of soybean trypsin inhibitor inhibited the formation of CAT3 in pure catalase (Fig. 4a, lane 1) and prevented degradation of CAT2 and CAT4 in two transformed cells (Fig. 4a lanes 4,5). It did not, however, prevent the conversion of CAT1 to CAT3, CAT4, and CAT5 in liver tissue homogenates (lane 2), nor the conversion of CAT1 to CAT2, CAT4, CAT5 in liver cell line (lanes 3–5). Addition of 20 μg of PMSF (phenylmethyl-sulfonylfluoride) or 0.3 μg leupeptin per mg sample protein inhibited CAT3 formation in pure catalase and prevented conversion of CAT1 to CAT2 and CAT4 in liver cell lines (Fig. 4 b&c, lanes 1, and 3–5), but did not inhibit conversion of CAT1 to CAT3 and CAT5 in liver tissue homogenates (Fig. 4c, lane 2). Finally, we examined effect of pure protease on the formation of these isozymes. Incubation of ethanol/Triton X-100-untreated samples with 25 μg of pronase E per mg sample protein at 37°C for 72 hr removed all forms of catalase except CAT5 (Fig. 4d). The

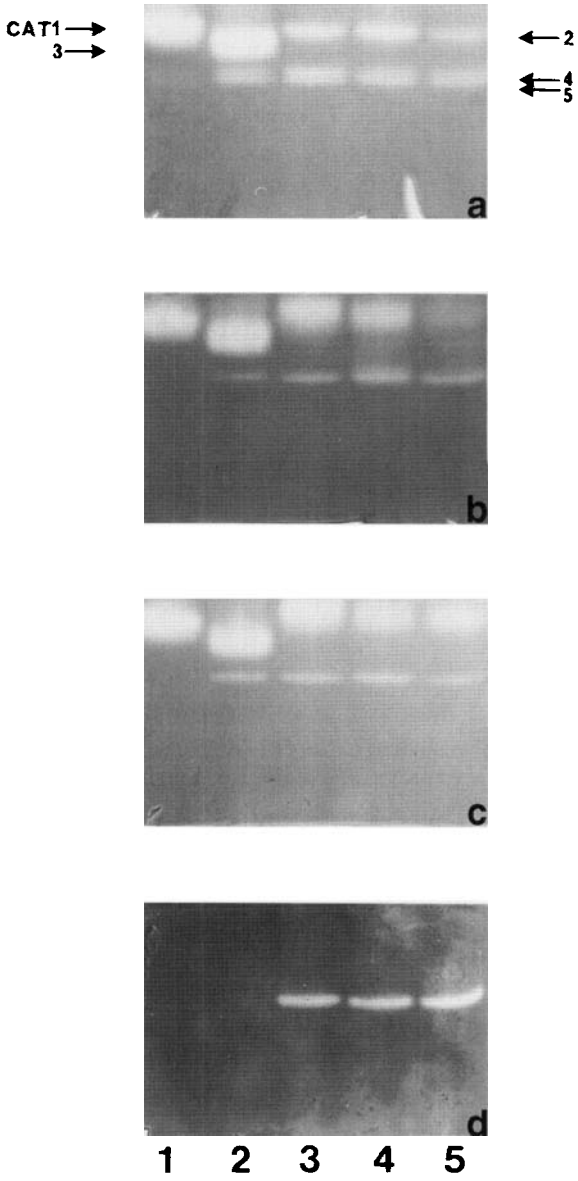

FIGURE 4 Effects of protease inhibitors and protease on the formation of multiple catalase bands: Different protease inhibitors were included in fresh ethanol/Triton X-100 treated samples and incubated at 37°C for 72 hr. For protease treatment, untreated samples were used and incubated with protease at 37°C for 72 hr. In all gels, lane 1 contained 10U of pure mouse liver catalase, lanes 2–5 contained homogenates from liver tissue homogenate, and three liver cell lines: normal, MNNG-transformed, and SV40-transformed with 60 μg of protein per lane, respectively. (**a**) 12 μg of trypsin inhibitor per mg of sample protein; (**b**) 20 μg of PMSF per mg sample protein; (**c**) 0.3 μg of leupeptin per mg sample protein; and (**d**) 25 μg of pronase E per mg sample protein. The arrows and numbers 1–5 represent CAT1-5, respectively.

results indicated that the formation of these multiple catalase isoforms was mainly due to partial proteolysis and could be largely prevented by the addition of protease inhibitors.

We report here the finding of 5 enzymatically active catalase bands in pure mouse liver catalase, and homogenates from the liver tissue and cell lines after ethanol/Triton X-100/heat treatment. These five isozymes have been biochemically characterized and found to be largely different from each other. This is the first report, to our knowledge, to detect reproducibly five isoforms in crude liver tissue/cell homogenates after a special treatment and to characterize their biochemical properties. Two lines of evidence suggest that the conversions of CAT1 to other isoforms are mainly due to partial proteolysis: (a), protease inhibitors largely prevented isozyme formation, while protease degraded all the isoforms except CAT5; (b), the formation of five enzymatically active isoforms was expedited by incubation at 37°C, a process which favors protease activity. The possible oxido-reductive inter conversion in the formation of these five isozymes can be ruled out since the presence of dithiothreitol, a common reductant reagent, during or after band formation did not change band patterns (data not shown). The possible involvement of catalase glycosylation was also excluded since when neuraminidase, and enzyme shown to remove sialic acid residues from catalase[17] was used, no change in gel banding pattern was observed, although catalase activity was somewhat inhibited (data not shown). Overall, all the data available support that partial proteolysis of catalase is the major cause of multiplicity in our treated samples. The observation that protease could not remove CAT5 and protease inhibitors could not prevent its formation indicate that CAT5 is protease-resistant. The formation of CAT5 might be the results of a combination of the multiple epigenetic modifications.

The major finding of our work is that proteolysis of a single catalase can lead to the formation of multiple forms which still retain enzymatical activity, but have different biochemical properties. A major question that remains is what is the physiological relevance, if any, of the proteolytic modification of catalase. It is possible that proteolysis only occurs during cell injury,[29] cell death, or is simply an artifact due to improper storage. Future studies should address this issue.

Acknowledgment

I thank Dr. Larry W. Oberley at the University of Iowa for stimulating discussion.

References

[1] Y. Sun (1990). Free radicals, antioxidant enzymes, and carcinogenesis. *Free Radicals in Biology and Medicine*, **8**, 583–599.

[2] T. P. Szatrowski and C. F. Nathan (1991). Production of large amounts of hydrogen peroxide by human tumor cells. *Cancer Research*, **51**, 794–798.

[3] J. MacCann, E. Choi, E. Yamasakim and B. N. Ames (1975). Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. *Proceedings of National Academy of Science*, **72**, 5135–5139.

[4] P. A. Cerutti (1985). Prooxidant states and tumor promotion. *Science*, **227**, 375–381.

[5] W. A. Pryor (1976). The role of free radical reactions in biological system. In: Free Radicals in Biology, (ed. W. A. Pryor) Academic Press, New York, vol. 1, pp1–50.

[6] Y. Sun, L. W. Oberley, J. H. Elwell and E. Sierra-Rivera (1989). Antioxidant enzyme activities in normal and transformed mouse liver cells. *International Journal of Cancer*, **44**, 1028–1033.

[7] Y. Sun, L. W. Oberley, T. D. Oberley, J. H. Elwell and E. Sierra-Rivera (1993). Lowered antioxidant enzymes in spontaneously transformed embryonic mouse liver cells in culture. *Carcinogenesis*, **14**, 1457–1463.

[8] Y. Sun, N. H. Colburn and L. W. Oberley (1993). Depression of catalase gene expression after immortalization and transformation of mouse liver cells *Carcinogenesis*, **14**, 1505–1510.

[9] L. Wayne and G. A. Diaz (1986). Double staining method for differentiating between two classes of mycobacterial catalase in polyacrylamide electrophoresis gels. *Analytical Biochemistry*, **157**, 89–92.

[10] P. C. Loewen and J. Switala (1987). Multiple catalases in *Bacillus subtilis*. *Journal of Bacteriology*. **169**, 601–3607.

[11] R. S. Holme and C. J. Masters (1969). On the tissue and subcellular distribution of multiple forms of catalase in the rat. *Biochimistry Biophysics Acta*, **91**, 488–490.

[12] R. S. Holmes and C. J. Masters (1972). Species specific features of the distribution and multiplicity of mammlian liver catalase. *Archieve Biochemistry Biophysics*. **148**, 217–223.

[13] R. S. Holmes and C. J. Masters (1978). Genetic control and ontogeny of microbody enzymes: A review. *Biochemistry Genetics*. **16**, 171–190.

[14] G. L. Jones and C. J. Masters (1976). On the turnover and proteolysis of catalase in tissues of the guinea pig and acatalasemic mice. *Archieve Biochemistry Biophysics*, **173**, 463–471.
[15] D. Crane, R. S. Holmes and C. J. Masters (1982). Proteolytic modification of mouse liver catalase. *Biochemistry Biophysics Research Communication*, **104**, 1567–1572.
[16] M. E. Percy (1984). Catalase: an old enzyme with a new role? *Canadian Journal of Biochemistry Cell Biology*, **62**, 1006–1014.
[17] C. Masters, M. Pegg and D. Crane (1986). On the multiplicity of the enzyme catalase in mammalian liver. *Molecular Cellular Biochemistry*. **70**, 113–120.
[18] D. de Craemer, L. Van Maldergem and F. Roels (1992). Hepatic ultrastructure in congenital total lipodystrophy with special reference to peroxisomes. *Ultrastructure Pathology*. **16**, 307–316.
[19] E. A. Wiemer, M. Out, A. Schelen, R. J. Wanders, R. B. H. Schutgens, H. V. D. Bosch and J. M. Tager (1991). Phenotypic heterogeneity in cultured skin fibroblasts from patients with disorders of peroxisome biogenesis belonging to the same complementation group. *Biochemistry Biophysics Acta*, **1097**, 232–237.
[20] S. Yajima, Y. Suzuki, N. Shimozawa, S. Yamaguchi, T. Orii, Y. Fujiki, T. Osumi, T. Hashimoto and H. W. Moser (1992). Complementation study of peroxisome-deficient disorders by immunofluorescence staining and characterization of fused cells. *Human Genetics*, **88**, 491–499.
[21] M. Schrader, E. Baumgart, A. Volkl and H. D. Fahimi (1994). Heterogeneity of peroxisomes in human hepatoblastoma cell line HepG2. Evidence of distinct subpopulations. *European Journal of Cell Biology*, **64**, 281–294.
[22] C. Masters and D. Crane (1990). Isozymes and the micro organization of organellar structure and function. *Progress in Clinical Biology Research*, **344**, 101–122.
[23] G. Cohen, D. Dembiec and J. Marcus (1970). Measurement of catalase activity in tissue extracts. *Analytical Biochemistry*, **34**, 30–38.
[24] O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randal (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265–275.
[25] W. Woodbury, A. K. Spencer and M. A. Stahmann (1971). An improved procedure using ferricyanide for detecting catalase isozymes. *Analytical Biochemistry*, **44**, 301–305.
[26] Y. Sun, J. H. Elwell and L. W. Oberley (1988). A simultaneous visualization of the antioxidant enzymes glutathione peroxidase and catalase on polyacrylamide gels. *Free Radical Research Communication*, **5**, 67–74.
[27] J. L. Hedrick and A. J. Smith (1968). Size and charge isomer separation and estimation of molecular weights of proteins by disc gel electrophoresis. *Archieve Biochemistry Biophysics*, **126**, 155–164.
[28] H. A. Hoffman and C. K. Grieshaber (1974). Genetic studies of murine catalase, liver and erythrocyte catalase controlled by independent loci. *Journal of Heredity*. **65**, 277–279.
[29] S. Gulati, A. K. Singh, C. Irazu, J. Orak, P. R. Rajagopalan, C. T. Fitts and I. Singh (1992). Ischemia-reperfusion injury: biochemical alterations in peroxisomes of rat kidney. *Archieve Biochemistry Biophysics*, **295**, 90–100.