

Acta Medica Okayama

Volume 33, Issue 3

1979

Article 8

JUNE 1979

Properties of erythrocyte catalase from heterozygotes for Japanese type acatalasemia.

Masana Ogata*

Junko Mizugaki†

*Okayama University,

†Okayama University,

Properties of erythrocyte catalase from heterozygotes for Japanese type acatalasemia.*

Masana Ogata and Junko Mizugaki

Abstract

The level of blood catalase activity in heterozygotes for Japanese type acatalasemia was demonstrated to be about half of normal levels by means of titration and spectrophotometric methods. A distribution plot of catalase activities in heterozygous blood was completely separate from that of normal blood. Comparative analysis of the partially purified erythrocyte catalase preparations obtained from normal and heterozygous individuals revealed no distinct differences between them regarding stability to heat, sodium dodecyl sulfate and some enzyme inhibitors or pH dependency. The erythrocyte catalase in heterozygotes for Japanese type acatalasemia contains about half the normal specific activity and as stable as that in normal individuals.

KEYWORDS: erythrocyte catalase, heterozygote for Japanese type acatalasemia, stability

*PMID: 40400 [PubMed - indexed for MEDLINE]

Acta Med. Okayama 33, (3), 205—211 (1979)

PROPERTIES OF ERYTHROCYTE CATALASE FROM HETEROZYGOTES FOR JAPANESE TYPE ACATALASEMIA

Masana OGATA and Junko MIZUGAKI

*Department of Public Health, Okayama University Medical
School, Okayama 700, Japan*

Received March 5, 1979

Abstract. The level of blood catalase activity in heterozygotes for Japanese type acatalasemia was demonstrated to be about half of normal levels by means of titration and spectrophotometric methods. A distribution plot of catalase activities in heterozygous blood was completely separate from that of normal blood. Comparative analysis of the partially purified erythrocyte catalase preparations obtained from normal and heterozygous individuals revealed no distinct differences between them regarding stability to heat, sodium dodecyl sulfate and some enzyme inhibitors or pH dependency. The erythrocyte catalase in heterozygotes for Japanese type acatalasemia contains about half the normal specific activity and as stable as that in normal individuals.

Key words: erythrocyte catalase, heterozygote for Japanese type acatalasemia, stability

An investigation of the family members of Japanese type acatalasemia revealed individuals having blood catalase activities intermediate between normal and acatalasemia, which were designated as hypocatalasemia by Takahara *et al.* (1). The analysis of the family pedigrees of acatalasemia and hypocatalasemia cases demonstrated that hypocatalasemia is the heterozygous carrier state of the acatalasemia gene (1). In Swiss type acatalasemia, contrary to the Japanese type, the activity level in blood catalase of heterozygotes was found to be variable and the activity range of heterozygotes merged with that of normal individuals (2).

In this experiment, some properties of catalase from heterozygotes for Japanese type acatalasemia were investigated by the determination of activity levels in blood, enzyme stability and pH dependency of partially purified erythrocyte catalase.

MATERIALS AND METHODS

Blood samples

Fresh blood samples of heterozygote individuals employed in this experiment were obtained from members of one of the families of Japanese type acatalase-

nia, GIO family, whose genotype and phenotype of the biochemical defect are known (3).

Determination of catalase activity

Catalase activity was determined by the permanganate titration methods using either hydrogen peroxide (4, 5) or perborate (6) as the substrate, and by spectrophotometry (7). Catalase activity in total blood was expressed as Kcat or perborate unit per gram of hemoglobin (PU/g Hb) or k per gram of hemoglobin (k/g Hb).

Preparation of erythrocyte catalase

Packed red cells, obtained from fresh blood samples with heparin as an anticoagulant, were washed three times with cold saline and hemolyzed with deionized water. For partial purification of catalase the following two methods were employed: (I) hemolysate was mixed with DEAE-cellulose (Whatman DE-52) and the catalase adsorbed on to the cellulose cake was eluted with sodium potassium phosphate buffer (pH 6.8) according to the method of Aebi *et al.* (2). (II) hemolysate was applied on to a Sephadex G-100 column and elution of catalase was carried out according to the previous report (8).

Examination of catalase stability

Heat stability test. A partially purified catalase preparation obtained by DEAE cellulose chromatography was used for the heat stability test. Immediately after incubation at various temperatures ranging from 25°C to 70°C for 10 min as described by Aebi *et al.* (2), the remaining activity was measured by the titration method using perborate as the substrate.

SDS stability test. Partially purified catalase prepared by DEAE cellulose fractionation was mixed with sodium dodecyl sulfate (SDS) solution of various concentrations ranging from 0.5 mM to 3.5 mM and incubated for 30 min at 25°C. Immediately after incubation, the remaining activity was measured by the titration method with perborate as substrate.

Inhibition tests. Enzyme inhibitors used for this test were aminotriazole (AT), formamide (FA), guanidine (G), hydroxylamine (HA) and urea (U) at final concentrations of 5×10^{-4} M, 5×10^{-1} M, 8.5×10^{-2} M, 2.5×10^{-5} M and 3.75×10^{-1} M, respectively. A partially purified catalase preparation obtained by Sephadex G-100 column chromatography was incubated at 37°C with one of the enzyme inhibitors described above for 10 min (FA, G and HA), 15 min (U) or 30 min (AT). Then the remaining catalase activity was determined immediately by the titration method using hydrogen peroxide as the substrate.

pH dependency test. Partially purified catalase preparation obtained by Sephadex G-100 column chromatography was added directly to the reaction mixture consisting of 3% hydrogen peroxide and 0.1 M phosphate buffer at various pH ranging from 5 to 10 at 37°C and catalase activity was measured by the titration method.

RESULTS

Total catalase activity in blood. Activity measurements of total catalase in hemolysates from normal and heterozygous individuals in Japan are illustrated

in Fig. 1. An average activity of 5.08 ± 0.43 Kcat was found in 36 samples from normal individuals. The activities measured in 5 samples from heterozygotes were 2.24 ± 0.27 Kcat. There is no overlap between the distribution plots of catalase activities. Thus a distinction between normal individuals and heterozygotes for Japanese type acatalasemia can be made by the determination of blood catalase levels.

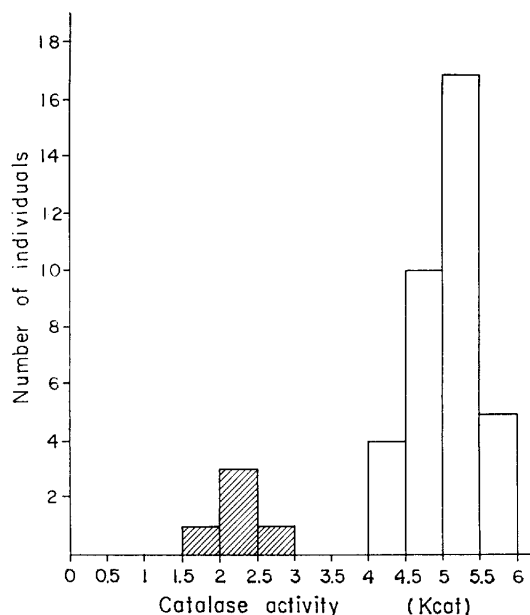


Fig. 1. Distribution of catalase activity in hemolysates of normal and heterozygote for Japanese type acatalasemia
 □ normal, ▨ heterozygote

In a separate experiment using three different assay methods, the average catalase activity in hemolysates from heterozygotes for Japanese type acatalasemia was shown to be about half of the normal level irrespective of assay method,

TABLE 1. CATALASE ACTIVITY IN HEMOLYSATES OF HETEROZYGOTES FOR JAPANESE TYPE ACATALASEMIA

	Permanganate titration method		Spectrophotometry
	Perborate (PU/g Hb)	Hydrogen peroxide (Kcat)	Hydrogen peroxide (k/g Hb)
Normal	2988.9 ± 413.1 (n=3)	4.88 ± 0.17 (n=3)	321.16 ± 60.80 (n=3)
Heterozygote	1243.8 ± 174.4 (n=3)	1.99 ± 0.17 (n=3)	106.22 ± 18.08 (n=3)
(M $\pm$ SD)			

and the difference between normal and heterozygous samples in each assay method was significant ($p < 0.05$) by Welch's test (9) (Table 1).

Heat stability. The relative activities of normal and heterozygous catalase which remained after incubation at various temperatures ranging from 25°C to 70°C for 10 min are illustrated in Fig. 2. No distinct difference was observed between the heat stability of catalase of normal and heterozygous individuals.

SDS stability. After incubation with various concentrations of SDS ranging from 0.5 mM to 3.5 mM, the remaining relative activities of normal and heterozygous catalase are illustrated in Fig. 3. The SDS stability of catalase from normal and heterozygous subjects is about the same.

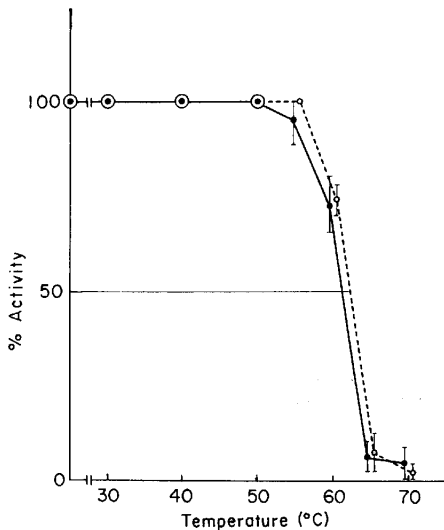


Fig. 2

Fig. 2. Heat stability of partially purified erythrocyte catalase —●— normal ($M \pm SD$; $n=3$), ---○--- heterozygote for Japanese type acatalasemia ($M \pm SD$; $n=3$)

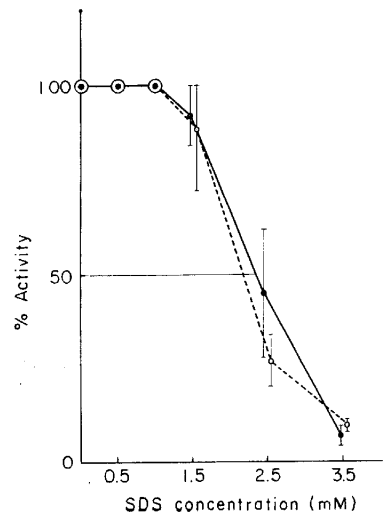


Fig. 3

Fig. 3. SDS stability of partially purified erythrocyte catalase —●— normal ($M \pm SD$; $n=3$), ---○--- heterozygote for Japanese type acatalasemia ($M \pm SD$; $n=3$)

Enzyme inhibition. The remaining relative activities of normal and heterozygous catalase after incubation with various enzyme inhibitors (aminotriazole, formamide, guanidine, hydroxylamine and urea) are shown in Table 2. There are no distinct differences between the effects of these inhibitors on normal and heterozygous catalase preparations.

TABLE 2. STABILITY OF PARTIALLY PURIFIED ERYTHROCYTE CATALASE AGAINST ENZYME INHIBITORS

Enzyme inhibitor	Remaining catalase activity (% of the original activity)	
	Normal	Heterozygote
	(n=2)	(n=2)
Aminotriazole	45.6 ± 16.7%	42.6 ± 2.9%
Formamide	91.8 ± 2.4%	93.7 ± 3.4%
Guanidine	85.9 ± 5.2%	87.9 ± 3.2%
Hydroxylamine	12.9 ± 10.1%	11.8 ± 8.0%
Urea	96.9 ± 2.0%	96.5 ± 1.6%

(M ± 1/2 range)

pH dependency. The relative activities of normal and heterozygous preparations under various pH conditions were shown in Fig. 4, in which the relative activity at pH 7.0 was taken as unity. The pH activity curve of catalase from heterozygous individuals was similar to that from normal individuals under both acid and alkali conditions.

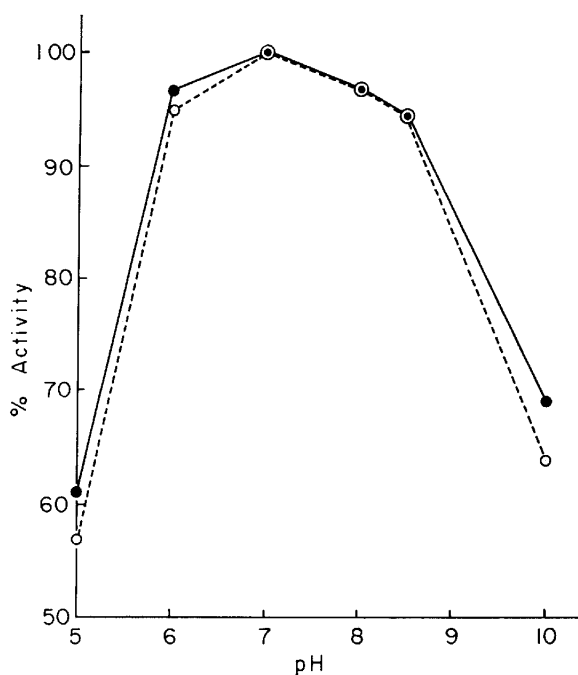


Fig. 4. pH dependency of partially purified erythrocyte catalase —●— normal (Mean; n=2), ---○--- heterozygote for Japanese type acatalasemia (Mean; n=2)

DISCUSSION

Catalase activities in blood of individuals who are genetically heterozygous were about half of the normal level as measured by the titration method using hydrogen peroxide or perborate as the substrate and spectrophotometry and no overlap with normal values was observed. The present data agree with earlier observations that heterozygotes for Japanese type acatalasemia have catalase activity intermediate between normal and homozygous catalase (1).

In previous studies of Swiss type acatalasemia, the detection of heterozygotes was also based on the relative level of catalase activity in blood by the titration method using perborate as the substrate and the activities obtained for heterozygotes ranged between 65% and 85% of normal levels with considerable overlapping of the two groups. A reevaluation of catalase level of heterozygotes for Japanese type acatalasemia was carried out using spectrophotometry or the titration method with hydrogen peroxide as the substrate and it was found that catalase activities in blood of individuals who ought to be genetically heterozygous all fell within the normal range.

Comparison of the level of catalase activities in blood of heterozygotes reveals a distinction between Japanese type acatalasemia and Swiss type acatalasemia.

In Japanese type acatalasemia, the partially purified catalase preparations from heterozygotes and normal individuals were compared by means of stability tests to heat, SDS and some enzyme inhibitors and of pH dependency test (Fig. 2, 3, 4 and Table 2). The catalase molecule of heterozygotes for Japanese type acatalasemia is as stable as normal catalase.

Aebi *et al.* (2) reported that in Swiss type acatalasemia, on the other hand, the difference between partially purified catalase preparations from normal and heterozygous individuals could be established on the basis of heat stability and electrophoresis. Properties of catalase found in heterozygotes for Swiss type acatalasemia were intermediate between those of the normal and homozygous enzymes.

The above observations indicate that two types of heterozygotes for acatalasemia are distinguishable; one is the Japanese type having intermediate catalase activity but normal stability and the other is Swiss type having approximately normal catalase activity but intermediate stability. The difference between the Japanese and Swiss type acatalasemias is that the properties of heterozygous catalase resembles those of homozygous catalase in the Swiss case (10).

Acknowledgments. Authors express their thanks to Professor, Shigeo Takahara for his interest in this work and to Mr. Yasuo Kuroda for his technical assistance.

REFERENCES

1. Takahara, S., Hamilton, H. B., Neel, J. V., Kobara, T. Y., Ogura, Y. and Nishimura, E. T.: A new genetic carrier state. *J. Clin. Invest.* **39**, 610-619, 1960.
2. Aebi, H., Wyss, S. R., Scherz, B. and Gross, J.: Properties of erythrocytes catalase from homozygotes and heterozygotes for Swiss-type acatalasemia. *Biochem. Genet.* **14**, 791-807, 1976.
3. Ogura, Y., Onoda, M., Sano, K. and Takahata, M.: Report on two families of acatalasemia discovered in dental clinics (in Japanese). *Kokubyo gakkai Zasshi* **32**, 313-318, 1965.
4. von Euler, H. and Josephson, K.: Über Katalase I. *Justus Liebig's Ann. Chem.* **452**, 158-181, 1927.
5. Bonnichsen, R. K., Chance, B. and Theorell, H.: Catalase activity. *Acta Chem. Scand.* **1**, 685-708, 1947.
6. Matsubara, S., Suter, H. and Aebi, H.: Fractionation of erythrocyte catalase from normal, hypocatalatic and acatalatic humans. *Humangenetik* **4**, 29-41, 1967.
7. Aebi, H.: Catalase. In *Methods of Enzyme Analysis*, ed. H. U. Bergmeyer, Verlag Chemie, Weinheim, 3rd ed., Vol. 1., pp. 673-684, 1974.
8. Ogata, M., Tomokuni, K., Watanabe, S., Osaki, H., Sadamoto, M. and Takahara, S.: Residual catalase in the blood of Japanese acatalasemia. *Tohoku J. Exp. Med.* **107**, 105-114, 1972.
9. Welch, B. L.: The generalization of "Student's" problem when several different population variances are involved. *Biometrika* **34**, 28-35, 1947.
10. Ogata, M. and Mizugaki, J.: Properties of residual catalase in the erythrocytes of Japanese-type acatalasemia. *Hum. Genet.* **48**, 329-338, 1979.

Erratum

An error appeared in the article by Hatase, *et al.* published in the April issue (Vol. 33 No. 2). On page 73 and back cover page of the Contents "STIMURATORY" in the title should read "STIMULATORY".