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The level and stability of residual catalase in cultured acatalasemic skin fibroblasts.

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Abstract

In an attempt to determine the level and heat stability of residual catalase in somatic cells of acatalasemic Japanese, skin fibroblasts from an acatalasemic subject were cultured, and the catalase activity of the cultured fibroblasts was compared with that of cultured normal fibroblasts. Catalase activity was determined using an oxygen electrode. The residual catalase activity in cultured acatalasemic fibroblasts was 10% of the normal. The heat stability at 55 degrees C of residual catalase in the acatalasemic fibroblasts was similar to that of normal fibroblasts.

KEYWORDS: tissue culture, skin fibroblast, actalasemia, catalase

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The Level and Stability of Residual Catalase in Cultured Acatalasemic Skin Fibroblasts

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In an attempt to determine the level and heat stability of residual catalase in somatic cells of acatalasemic Japanese, skin fibroblasts from an acatalasemic subject were cultured, and the catalase activity of the cultured fibroblasts was compared with that of cultured normal fibroblasts. Catalase activity was determined using an oxygen electrode. The residual catalase activity in cultured acatalasemic fibroblasts was 10% of the normal. The heat stability at 55°C of residual catalase in the acatalasemic fibroblasts was similar to that of normal fibroblasts.

Key words : tissue culture, skin fibroblast, acatalasemia, catalase

Acatalasemia, which was first described by Takahara (1) in 1948, is a rare congenital abnormality which is characterized by the lowest activity of catalase in the blood. The disorder has been found in, besides Japan, Switzerland (2) and Peru (3). Kitamura *et al.* (4) showed that catalase activity was deficient in cultured skin fibroblasts obtained from Japanese patients with acatalasemia. Krooth *et al.* (5, 6) reported the lack of catalase activity in cultured fibroblasts of Japanese with acatalasemia by a spectrophotometric assay (7). Kimoto *et al.* (9) reported that skin fibroblasts, which were cultured from acatalasemic Japanese and transformed by SV40, had no catalase activity.

However, Sadamoto (8) detected trace catalase activity in cultured fibroblasts of acatalasemic Japanese using perborate and manometric methods. Aebi *et al.* (10) also demonstrated the presence of residual catalase activity in cultured Swiss acatalasemic skin fibroblasts.

The purpose of the present study was to compare the activity and heat stability of residual catalase in cultured skin fibroblasts of a Japanese acatalasemic patient with the activity and stability of normal fibroblasts. An oxygen electrode was used for the assay of catalase activity.

Materials and Methods

Skin specimens (approximately 1 cm^2) obtained by biopsy from an acatalasemic patient with oral gangrene and from two normal individuals served as materials. The fibroblasts which grew out from the skin were observed under a phase-contrast microscope. The tissue was cultured in Harnden's growth medium (11) according to the techniques described by Sadamoto (8). Acata-

lasemic fibroblasts from 3 large bottles and normal fibroblasts from one bottle were harvested with a rubber policeman. The fibroblasts were washed three times with 0.9% NaCl solution. The washed fibroblasts were suspended in 1.0 ml of 0.01 M phosphate buffer (pH 6.8), and sonicated in a Sonifire (Branson Co., Conn. U.S.A.).

Catalase activity was assayed (12) using an oxygen electrode (13). Appropriate amounts of sonicated homogenates of cultured fibroblasts and 3.5 ml of the reaction mixture composed of 2.5 mM H_2O_2 in 0.01 M phosphate buffer (pH 6.8) were put into a vessel with an oxygen electrode (Kyusui Kagaku Co., Tokyo, Japan). The time-course of oxygen evolution was recorded, and the amount of oxygen evolved in the first 30 sec after addition of the homogenate was used as an index of catalase activity. Protein concentrations in cultured skin homogenates were determined by a protein-dye binding method (14).

Results

Typical oxygen evolution curves are shown in Fig. 1. Activities of residual catalase obtained from acatalasemic fibroblasts of the tenth and eleventh generations were similar to each other. Catalase activity in the cultured normal fibroblasts averaged 303 μmoles O_2/min per mg of protein for two generations. Residual catalase activity in acatalasemic fibroblasts averaged 30.3 μmoles O_2/min per mg of protein, showing 10% of the activity in normal fibroblasts (Table 1).

Sonicated homogenates of cultured fibroblasts were incubated at 55℃ for 30 or 60 min to determine the heat stability of catalase. Catalase activities of homoge-

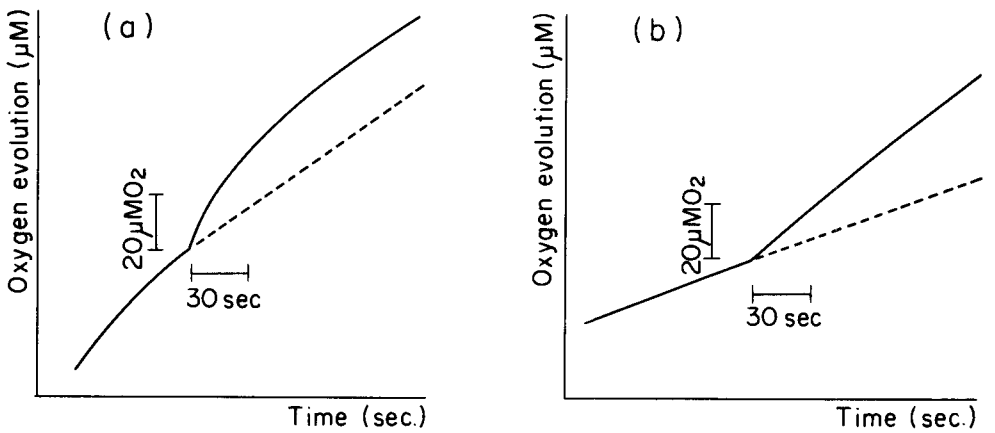

Fig. 1 Oxygen evolution from hydrogen peroxide catalyzed by catalase in cultured skin fibroblasts from acatalasemic (a) and normal (b) subjects. Final concentrations of protein in the fibroblasts of normal and acatalasemic subjects were 0.058 mg/ml and 0.55 mg/ml, respectively. ——, with enzyme solution; ------, without enzyme solution.

Table 1 Catalase activity in cultured skin fibroblasts from normal and acatalasemic subjects[a]

	Generation	No.[b]	Oxygen evolution (nmol O_2/min per ml)	Protein concentration (mg/ml)	Specific activity (nmol O_2/min per mg protein)
Normal fibroblasts	14th	4	1165.3 ±145.6	4.13	282.2 ±35.3
	16th	3	2013.5 ±192.0	6.18	323.7 ±31.1
Acatalasemic fibroblasts	10th	1	116.5	4.38	26.6
	11th	3	322.7 ±74.5	9.49	34.0 ±7.9

a: Values are expressed as means ±SD.
b: Number of experiment.

Table 2 Effect of heat treatment on catalase in homogenates of cultured skin fibroblasts from normal and acatalasemic subjects[a]

Time of treatment (min)	Treatment	Normal		Acatalasemic	
		μmol O_2/min per mg protein	Ratio to control	μmol O_2/min per mg protein	Ratio to control
30	Heated	39.9	1.16	9.8	0.88
	Control	34.3		11.2	
60	Heated	49.9	1.27	10.5	0.94
	Control	39.3		11.2	

a: Homogenates were heated in a water bath regulated at 55°C for 30 or 60 min. Protein concentrations were: normal fibroblasts, 1.30 mg/ml, and acatalasemic fibroblasts, 6.94 mg/ml.

nates before and after the heat treatment were measured as described for the measurement of residual catalase in the blood of acatalasemic Japanese (15). Since the amount of acatalasemic enzyme available was limited, the results of a single experiment are presented in this report. As shown in Table 2, the catalase activity of acatalasemic homogenates decreased slightly after heat treatment, while the activity of normal homogenates did not decrease.

Discussion

The residual catalase activity in cultured skin fibroblasts varies slightly depending on the assay method employed. Krooth *et al.* (5, 6) reported the presence of residual catalase in cultured skin fibroblasts from Swiss acatalasemic patients, while catalase activity in cultured skin fibroblasts from Japanese acatalasemic patients was not detected (5, 6). The spectrophotometric catalase assay method used by Krooth *et al.* (7) is less sensitive than other methods for measuring catalase activity, and it might not be sensitive enough to detect catalase activity in the fibroblasts from patients with the Japanese type of acatalasemia.

Sadamoto found, using a manometric method, that the residual catalase activity in the cultured skin fibroblasts of acatalasemic Japanese was 2.2% of the normal (8). The activity determined in the present study was 10% of the normal. In the present experiment, oxygen evolved during the initial 30 sec was measured using an oxygen electrode, while Sadamoto measured oxygen evolution for 10 min. As shown in Fig. 1, the reaction rate of residual catalase decreased with time. The reason for this decrease is unknown at the present. However, the difference in the incubation time might be one of the reasons for the higher residual activity in the present study than that reported by Sadamoto.

We have reported that the residual catalase activities in the vermiform appendix (16) and blood (15) of patients with acatalasemia were 2.7% and 0.18%, respectively, of the catalase activities of normal subjects. To compare the residual catalase activities of different sources, it will be necessary to use one standard method such as that used in the present study.

A preliminary result of the present study indicated that the heat stability of the residual catalase at 55℃ was not so different from that of the normal enzyme. Further studies concerning properties of residual catalase in acatalasemic fibroblasts, including heat stability and isoelectric point, are in progress.

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