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Properties of Catalase Activity in Vegetative and Sporulating Cells of Yeast *Saccharomyces cerevisiae*

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Properties of catalase activities have been examined in the intact cells of early stationary phase and cells 3 hr after transfer to sporulation medium in *Saccharomyces cerevisiae.* The catalase activities of the two cells had a broad optimal pH from 6 to 8. Catalase activity in the intact cells increased throughout a 4-hr period of the observation following transfer to sporulation medium. Almost all the catalase activity in vegetative cells was lost by the treatment at 60°C for 10 min. Catalase activities of both cells were inhibited by KCN, NaN_3, o-phenanthroline, and PCMB. The catalase activity of the vegetative cells was slightly more inhibited and inactivated than that of the sporulating cells by the inhibitors and by the treatment with HCl or NaOH.

Key words: catalase activity, vegetative cells, sporulation, *Saccharomyces cerevisiae*

Investigations into enzymatic activities [1–13] and uptake of chemicals [1,14–19] during ascosporulation have been made in *Saccharomyces cerevisiae.* It has been suggested that the change in the cellular property to provoke Ca^{2+} uptake is essential for the sporulation process in bacterial *Bacillus megaterium* [20–22]. Properties of ATPase activities have been studied in the cells of *S cerevisiae* [6] and *Saccharomycopsis fibuligera* [23]. Only a few reports have appeared [4,24,25] on the properties of the catalase activity in the intact cells of *S cerevisiae.*

This paper reports some properties of the catalase activity in intact cells and its change during early ascosporulation in *S cerevisiae.*

MATERIALS AND METHODS

Organism

Baker's yeast used throughout this work was a diploid strain of *Saccharomyces cerevisiae* fit for sporulation (kindly supplied by Mr. Yoshida, Oriental Yeast Co. Ltd., Osaka, Japan).

Abbreviations used: PCMB, p-chloromercuribenzoate; DNP, 2,4-dinitrophenol.

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Growth and Sporulation

Cells were grown in presporulation medium, which contained 5% glucose, 1% yeast extract, and 2% peptone, at 28°C for 24–48 hr, which is similar to the medium of Fowell [26]. The cells in the early stationary phase were harvested after centrifugation at 2,500g for 5 min and washed twice with sterile distilled water. The washed cells were transferred to the sporulation medium [26] according to the method of McClary et al [27]; the medium contained 0.1% glucose, 0.25% yeast extract, and 1% potassium acetate at a density of about 1.5×10^7 cells per ml. The sporulation culture was incubated with aeration at 28°C.

Measurement of Catalase Activity

Cells were washed three times with distilled water. Catalase activity was determined with a permanganate titration method [28] slightly modified by Kaziro [29]: about 75 mg (dry weight) of the washed cells in the 10 ml of 50 mM potassium phosphate buffer (pH 7.0, 0°C) was added to a solution containing 40 ml of 0.025 N hydrogen peroxide at 0°C and mixed; 10 ml of the reaction mixture is taken immediately after mixing, and at 10 min, 20 min, and 30 min, respectively, after mixing and put into 1 ml of 5% H_2SO_4, which terminates the reaction. The hydrogen peroxide was titrated with 0.02 N $KMnO_4$.

Measurement of Dry Weight

Dry weight of the cells was obtained after drying the cells at 80°C for 24 hr.

RESULTS

pH Dependency of the Catalase Activity

Catalase activity was observed at about 57 nmol and 110 nmol of H_2O_2 decomposed/min/mg cells in the cells harvested at the early stationary phase and in the cells harvested 3 hr after transfer to sporulation medium in *S cerevisiae*, respectively. The activity of the latter was about two times as high as that of the former. The catalase activity had a broad optimal pH and its curve was flat in a range of pH from 6 to 8 in the both cells, and at pH 5.5 it was slightly lower (Fig. 1). The shape of the pH dependency curve of the activity was similar to that of the catalase activity of cell homogenate in yeast *Kloeckera sp* [30]. The catalase activity in a cell-free extract of the cells harvested at the early stationary phase and of the cells harvested 2 hr following transfer to sporulation medium was about 2.9 μmol H_2O_2 decomposed/min/mg protein and about 6 μmol H_2O_2 decomposed/min/mg protein, respectively [3]. The catalase activity of the cell extracts of vegetative cells was much higher than that of the intact cells of *S cerevisiae* [31].

Thermal Stability of the Catalase Activity

Thermal stability for the catalase activity of vegetative cells in early stationary phase is shown in Figure 2. Almost all the activity was lost by the treatment at 60°C for 10 min. The ATPase activity was increased about three times as high by the treatment at 60°C for 10 min [6], whereas glucose uptake activity was completely lost by the same treatment in the intact cells of *S cerevisiae* [17].

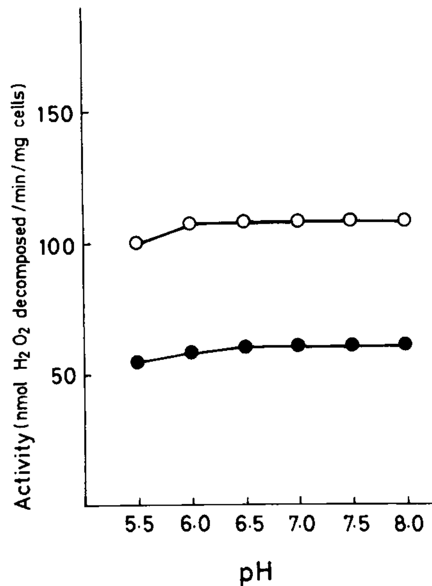

Fig. 1. pH dependency of catalase activity in vegetative and sporulating cells. Catalase activity in intact vegetative cells harvested at early stationary phase and sporulating cells harvested 3 hr after transfer to sporulation medium was measured at 0°C in a buffer containing hydrogen peroxide over the pH range 5.5–8.0 (pH 5.5 citrate phosphate buffer, pH 6.0–7.5 phosphate buffer, pH 8.0 Tris-HCl buffer). After the reaction was terminated by putting the cells into dilute H_2SO_4 solution, the decrease of hydrogen peroxide in the medium was determined by a titration with $KMnO_4$. Other conditions and procedures are described in the Materials and Methods section. (●), vegetative cells; (○), sporulating cells 3 hr after transfer to sporulation medium.

Effect of Inhibitors on the Catalase Activity

Table I shows the effect of various inhibitors on the catalase activity in the cells of early stationary phase and the cells harvested 3 hr following transfer to sporulation medium. The activity was inhibited by KCN, NaN_3, and o-phenanthroline in vegetative cells in the early stationary phase and sporulating cells harvested 3 hr following the transfer. The catalase activity of the vegetative cells was inhibited a little more strongly than that of the sporulating cells. Other inhibitors also affected the catalase activity in the cells at early stationary phase and in the cells harvested 3 hr after transferring into sporulation medium (Table II). The activity was inhibited by PCMB in both vegetative cells and sporulating cells, and the activity of the former was inhibited slightly more than that of the latter. DNP scarcely inhibited the activity, and a small part of the activity was lost by 5 M urea.

Effect of HCl Treatment on the Catalase Activity

Table III shows the effect of HCl treatment on catalase activity both in the cells of early stationary phase and in the cells harvested 3 hr following transfer to sporulation medium. A large part of the catalase activity was lost by the treatment at a concentration of 3×10^{-1} M HCl for 10 min at 25°C. The activity of the vegetative cells was lost by the HCl treatment somewhat more than that of the sporulating cells.

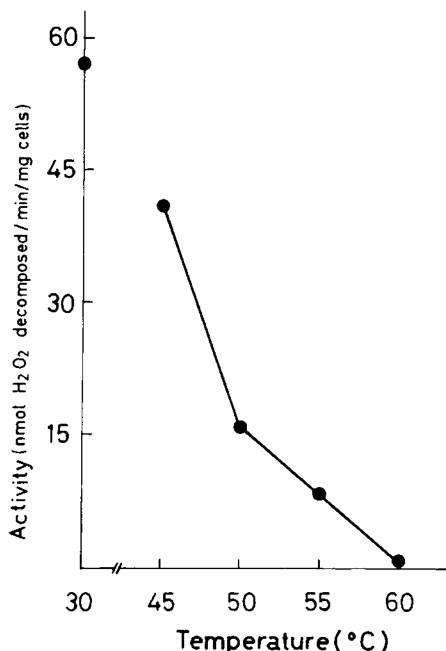

Fig. 2. Inactivation of catalase activity by heat treatment in vegetative cells. Cells harvested at early stationary phase were washed three times in distilled water. The washed cells were suspended in 10 mM potassium phosphate buffer (pH 7.0) and were treated with appropriate temperature for 10 min. The catalase activity of the cells as measured at 0°C in potassium phosphate buffer (pH 7.0) containing hydrogen peroxide. After the reaction was terminated by putting into dilute H_2SO_4 solution, the decrease of hydrogen peroxide in the medium was determined by the titration with $KMnO_4$. Other conditions and procedures are described under the Materials and Methods section.

Effect of NaOH Treatment on the Catalase Activity

Table IV shows the effect of NaOH treatment on catalase activity in the cells of early stationary phase and the cells harvested 3 hr after transfer to sporulation medium. The activity was completely lost by the treatment with 10^{-1} M NaOH at 25°C for 10 min. A considerable amount of the activity was lost by the treatment with 5×10^{-2} M and 3×10^{-2} M NaOH at 25°C for 10 min. The catalase activity of the vegetative cells was lost by the NaOH treatment somewhat more than that of the sporulating cells. Catalase activity was inactivated at such high concentrations of NaOH and HCl as described above, and it was more sensitive to NaOH than to HCl. It may be possible that the catalase activity was protected from inactivation by the cell wall and that catalase activity in latent form was activated by HCl or NaOH.

The Catalase Activity During Early Ascosporulation

The variation of the catalase activity following transfer to sporulation medium is shown in Figure 3. Up to 4 hr after the transfer, the activity exhibited its value more than two times as much.

DISCUSSION

Catalase activity in the intact cells of *S cerevisiae* was much lower than that in the cell-free extracts, and it increased by about two times 3 hr after transfer to

TABLE I. Effect of Inhibitors on Catalase Activity in Vegetative and Sporulating Cells*

Yeast cells	Inhibitor	Concentration (M)	H_2O_2 decomposed (nmol/min/mg cells)	Inhibition (%)
Before transfer to sporulation medium	None		57	0
		0^{-2}	3	95
	KCN	10^{-3}	25	56
		10^{-4}	56	2
		10^{-4}	5	91
	NaN_3	10^{-5}	26	54
		10^{-6}	48	16
		10^{-3}	20	65
	o-phenanthroline	5×10^{-4}	36	37
		10^{-4}	50	12
3 hr after transfer to sporulation medium	None		109	0
	KCN	10^{-2}	13	88
		10^{-3}	61	44
		10^{-4}	107	2
		10^{-4}	14	87
	NaN_3	10^{-5}	68	38
		10^{-6}	100	8
		10^{-3}	50	54
	o-phenanthroline	5×10^{-4}	77	29
		10^{-4}	101	7

*Catalase activity in intact vegetative cells and sporulating cells harvested 3 hr following transfer to sporulation medium was measured in the absence or presence of inhibitor at 0°C in potassium phosphate buffer (pH 7.0) containing hydrogen peroxide. After the reaction was terminated by putting into dilute H_2SO_4 solution, the decrease of hydrogen peroxide in the medium was determined by the titration with $KMnO_4$. Other conditions and procedures are described under the Materials and Methods.

sporulation medium (Fig. 3). It is not yet clear whether or not the increase in the catalase activity is related to sporulation, for the change of incubation conditions might affect the activity. It was reported that biosynthesis of catalase was induced by the aeration of anaerobic cells in *S cerevisiae* [32,33]. It was also reported that the increase in catalase activity was induced by methanol in yeast *Candida boidinii* [34]. Furthermore, the catalase activity was induced by n-alkane in yeast *C tropicalis* [35,36] and other *Candida* yeasts [37], and catalase synthesis was induced by hydrogen peroxide in *Escherichia coli* [38,39]. Catalase activity level was reported to be low in the glucose-grown cells of *C tropicalis* [40], and the induction of catalase activity by methanol was inhibited by glucose in *Kloeckera sp* [30]. Catalase activity was decreased after transferring methanol-grown cells into an ethanol medium in *C boidinii* [41]. Catalase activity has been known to be sensitive to glucose repression in *S cerevisiae* [42,43]. It was reported that acatalastic mutants of *S cerevisiae* lost almost all their capacity to sporulate and that sporulation seemed to depend on a sufficient level of catalase, which eliminated hydrogen peroxide produced in the cells [44]. This author has suggested previously that catalase protects enzymes needed for

TABLE II. Effect of Inhibitors on Catalase Activity in Vegetative and Sporulating Cells*

Yeast cells	Inhibitor	Concentration (M)	H_2O_2 decomposed (nmol/min/mg cells)	Inhibition (%)
Before transfer to sporulation medium	None		58	0
	PCMB	10^{-4}	21	64
		5×10^{-5}	37	36
		10^{-5}	50	14
	DNP	10^{-3}	52	10
		10^{-4}	58	0
	Urea	5	47	19
		3	58	0
3 hr after transfer to sporulation medium	None		111	0
		10^{-4}	51	54
	PCMB	5×10^{-5}	83	25
		10^{-5}	100	10
	DNP	10^{-3}	103	7
		10^{-4}	111	0
	Urea	5	97	13
		3	110	1

*Catalase activity in intact vegetative cells and sporulating cells harvested 3 hr after transfer to sporulation medium was measured in the absence or presence of inhibitor at 0°C in potassium phosphate buffer (pH 7.0) containing hydrogen peroxide. After the reaction was terminated by putting into dilute H_2SO_4 solution, the decrease of hydrogen peroxide in the medium was determined by the titration with $KMnO_4$. Other conditions and procedures are described in the Materials and Methods section. PCMB, p-chloromercuribenzoate; DNP, 2,4,-dinitrophenol.

sporulation from inactivation by hydrogen peroxide formed in the cells [3] as hydrogen peroxide inactivation of *Pseudomonas* cytochrome oxidase (EC 1.9.3.2) was protected by catalase [45]. Dingman and Stahly [46] reported that catalase activity increased at two periods corresponding with the transition from log phase to stationary phase and with the appearance of spores, that the synthesis of catalase was neither induced by H_2O_2 or O_2 nor depressed by glucose, and that vigorous aeration had no effect on the growth rate but almost completely prevented the sporulation in *Bacillus larvae*.

It has been reported that catalase is localized in microbodies [40, 47–49]. Patent catalase activity (catalase activity is detectable in the intact cells) and cryptic catalase activity, which can be revealed by cell treatment with chemicals, have been known in *S cerevisiae* [24]. Kaplan [25] suggested that patent catalase was localized in the cell surface. Thus, it is assumed that the patent catalase is different from that localized in microbodies and that both catalases might participate in the sporulation by protecting the sporulation enzymes from hydrogen peroxides. The patent catalase was thermostable and only a slight inactivation of its activity was observed by thermal treatment at 60°C for 10 min [24]. Almost all the catalase activity of intact cells used here was lost by the treatment at 60°C for 10 min (Fig. 2). This result seems to lower the possibility that the catalase activity is a nonenzymatic decomposition of H_2O_2. Intra-

TABLE III. Inactivation of Catalase Activity by HCl Treatment in Vegetative and Sporulating Cells*

Yeast cells	HCl treatment (M)	H_2O_2 decomposed (nmol/min/mg cells)	Inhibition (%)
Before transfer to sporulation medium	None	56	0
	3×10^{-1}	8	86
	2×10^{-1}	16	71
	10^{-1}	31	45
	5×10^{-2}	40	29
3 hr after transfer to sporulation medium	None	108	0
	3×10^{-1}	43	60
	2×10^{-1}	72	33
	10^{-1}	86	20
	5×10^{-2}	94	13

*Cells harvested at early stationary phase or cells 3 hr following transfer to sporulation medium were washed three times with distilled water. The washed cells were suspended in various concentrations of HCl at 25°C for 10 min, collected by centrifugation, and washed three times with distilled water. The catalase activity of the resultant cells was measured at 0°C in potassium phosphate buffer (pH 7.0) containing hydrogen peroxide. After the reaction was terminated by putting into dilute H_2SO_4 solution, the decrease of hydrogen peroxide in the medium was determined by the titration with $KMnO_4$. Other conditions and procedures are described in the Materials and Methods section.

TABLE IV. Inactivation of Catalase Activity by NaOH Treatment in Vegetative and Sporulating Cells*

Yeast cells	NaOH treatment (M)	H_2O_2 decomposed (nmol/min/mg cells)	Inhibition (%)
Before transfer to sporulation medium	None	58	0
	10^{-1}	0	100
	5×100^{-2}	8	86
	3×10^{-2}	35	40
3 hr after transfer to sporulation medium	None	110	0
	10^{-1}	0	100
	5×10^{-2}	44	60
	3×10^{-2}	86	22

*Cells harvested at early stationary phase or cells 3 hr following transfer to sporulation medium were washed three times with distilled water. The washed cells were suspended in various concentrations of NaOH at 25°C for 10 min, collected by centrifugation, and washed three times with distilled water. The catalase activity of the resultant cells was measured at 0°C in potassium phosphate buffer (pH 7.0) containing hydrogen peroxide. After the reaction was terminated by putting into dilute H_2SO_4 solution, the decrease of hydrogen peroxide in the medium was determined by the titration with $KMnO_4$. Other conditions and procedures are described in the Materials and Methods section.

cellular catalase was completely inactivated by the treatment about 64°C for 10 min [50]. The pH dependency curve of the catalase activity was flat within a pH range of from 6 to 8 (Fig. 1). Seah and Kaplan [51] reported that there were two peaks of catalase activity, a broad one from pH 6 to 7 and the other near pH 9.5, in purified catalase of *S cerevisiae*. The pH dependency curve of catalase activity was reported to be flat between pH 6.0 and 8.5, to be almost flat between pH 5.6 and 8.3, and a broad pH optimum from pH 5.5 to 9.0, in cell homogenate of yeast *Kloeckera sp*

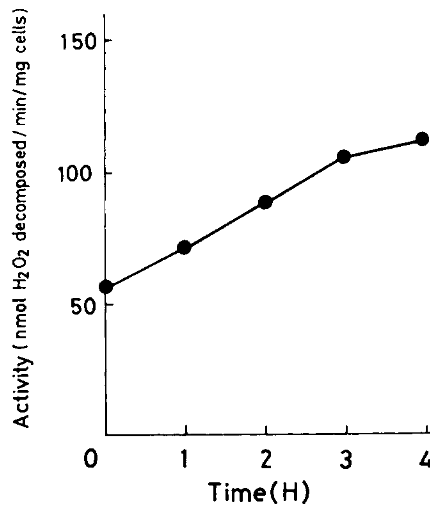

Fig. 3. Catalase activity in intact cells after transfer to sporulation medium. Cells in early stationary phase were transferred to sporulation medium and harvested every hour. The catalase activity of the harvested cells was measured at 0°C in potassium phosphate buffer (pH 7.0) containing hydrogen peroxide. After the reaction was terminated by putting into dilute H_2SO_4 solution, the decrease of hydrogen peroxide in the medium was determined by the titration with $KMnO_4$. Other conditions and procedures are described under the Materials and Methods section.

[30], in the purified catalase of bacterial *Proteus mirabilis* [52], and in cell extracts of *B larvae* [46], respectively. Purified catalase of *Drosophila melanogaster* had a broad pH optimum from pH 6.5 to 7.5 [53]. Concerning the possession of a broad optimum at around neutral pH for the catalase activity, one of the typical properties of catalase, there is a resemblance between the intact cells of *S cerevisiae* and those organisms.

Yasuhara et al [30] reported that the catalase activity of cell homogenate in yeast *Kloeckera sp* was completely inhibited by 10^{-3} M NaN_3; 10^{-4} M NaN_3 inhibited the catalase activity in the intact vegetative cells of *S cerevisiae* by 91% (Table I). Thus the both catalase activities seem to be inhibited by NaN_3 to the same degree. The catalase activity of the cells harvested 3 hr following transfer to sporulation medium was slightly less inhibited by various inhibitors (Tables I, II) and slightly less inactivated by the treatment with HCl or NaOH (Tables III, IV) than the catalase activity of the vegetative cells. Therefore, the catalase activity might possibly be dependent on the change of membrane properties for sporulation.

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