Response of catalase activity and membrane fluidity of aerobically grown *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* to aeration and the presence of substrates

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Intracellular catalase (EC 1.11.1.6) activity of permeabilized aerobically grown cells of *Schizosaccharomyces pombe* was insensitive to cell aeration and inhibition of protein synthesis, and was only mildly enhanced by the presence of glucose and ethanol via *de novo* protein synthesis. By contrast, the intracellular catalase activity of *Saccharomyces cerevisiae*, which, in freshly harvested cells, was two to three times lower than that in *Sch. pombe*, increased on aeration without substrates or with ethanol and was inhibited on aeration with glucose following cell permeabilization. The enhanced intracellular activity was due to *de novo* protein synthesis while the inhibitory effect of glucose, absent in *Sch. pombe*, was caused by one of the major glucose metabolites, succinate. The intact-cell catalase activity of both yeasts increased greatly during aeration. In *Sacch. cerevisiae*, this increase was again prevented by glucose. In parallel, export of catalase to the cell surface increased in both yeasts. This was especially conspicuous in *Sch. pombe* aerated in the presence of ethanol, and may represent a protective mechanism against the damaging effects of ethanol. The cell-surface-bound catalase activity was confirmed in isolated plasma membranes of both yeasts. The fluidity of the plasma membrane increased during aeration. This effect was further stimulated by the presence of glucose and to a lesser extent by ethanol. Both yeasts exhibited increased extracellular catalase activity during aeration which could not be caused entirely by cell lysis. In *Sch. pombe* this activity was strongly enhanced by the presence of ethanol.

Introduction

Animal cells suffer sizeable hydrogen peroxide induced damage at 10–100 µM-H₂O₂ (Block, 1991; Farber *et al.*, 1990). Higher peroxide concentrations (of the order of 10 mM) are necessary to affect plant (Price, 1990) and bacterial (Bol & Yasbin, 1990) cells, especially their membranes (Brandi *et al.*, 1989). Yeast cells can withstand exposure to 10–100 mM-H₂O₂, although some yeasts, e.g. *Rhodotorula glutinis*, exhibit inhibition of membrane transport at peroxide concentrations ≤ 5 mM (Janda *et al.*, 1990). Some yeasts, such as the methylo-trophic *Hansenula polymorpha*, even produce H₂O₂ concentrations up to 12 mM during growth on glucose plus methanol (Aminova *et al.*, 1991). One of the causes of resistance to H₂O₂ is the protection afforded by cell

catalases and/or peroxidases. Anaerobic yeast cells exposed to oxygen synthesize catalase by an O₂-induced and nutrient-dependent process (Hörtner *et al.*, 1982; Bissinger *et al.*, 1989; Wieser, 1991). Much less attention has been paid to changes occurring in fully oxygen-adapted cells on exposure to further stress. We studied two yeast species under conditions when aerobically grown, i.e. oxygen-adapted, cells were further aerobically incubated in the absence or presence of a carbon and/or energy source to examine whether the stress induced by aeration in various substrates evokes further changes in catalase content and/or membrane fluidity. We studied the catalase content of the fission yeast *Schizosaccharomyces pombe*, which is especially resistant to H₂O₂ since it lacks membrane lipid peroxidation (Janda *et al.*, 1993). This yeast offers several important advantages for studying effects of oxidative stress on plasma membrane energization and transport: easy preparation of a highly purified plasma membrane fraction with high

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H⁺-ATPase activity (Dufour *et al.*, 1988), reconstitution of the plasma membrane fraction into phospholipid vesicles capable of generating $\Delta\tilde{\mu}_{\text{H}^+}$ on addition of ATP (Mair & Höfer, 1988) and reproducible preparation of large amounts of protoplasts suitable for patch-clamp studies of plasma membrane ion channels (Vacata *et al.*, 1993). *Sacch. cerevisiae* was used as reference organism since most information on catalase content has been acquired in this species (Kaplan, 1962; Sels & Brygier, 1980; Martinez *et al.*, 1981).

Cells of both *Sch. pombe* and *Sacch. cerevisiae* exhibit an intact-cell (patent) catalase activity and an intracellular (cryptic) activity revealed upon cell permeabilization (Kaplan, 1962). In addition, we found that both species secrete catalase into the medium. We studied the relationships of all three types of activity, changes in these activities brought about by prolonged aeration, and the role of membrane permeability in patent catalase activity. We also examined the effect on catalase activities of substrates exerting catabolite repression/inactivation (glucose) or energizing the cells and thought to alter membrane permeability (ethanol).

Methods

Organism and growth. The yeast strains used were *Saccharomyces cerevisiae* K, derived from distillery yeast (CCY 21-4-60), and *Schizosaccharomyces pombe* 972h⁻ (NCYC 1824). Both *Sch. pombe* and *Sacch. cerevisiae* were stored on agar plates containing 1% (w/v) yeast extract, 2% (w/v) bacto-peptone and 2% (w/v) glucose. Both strains were grown in culture flasks on a reciprocal shaker at 30 °C until the transition point between exponential and stationary phase was reached (20 h for *Sacch. cerevisiae* K, 22 h for *Sch. pombe*). *Sacch. cerevisiae* was cultured in a synthetic medium according to Sigler *et al.* (1981), *Sch. pombe* in a minimal medium as described by Gutz *et al.* (1974) which contained 2.5% (w/v) glucose as carbon source. Harvested cells were washed twice with double-distilled water and resuspended at 5% cell concentration (fresh wt/vol.), taking into account the additional volumes to be added in supplying substrates (glucose, ethanol) or cycloheximide. The cell suspensions were then aerated on magnetic stirrers and samples taken every 1–2 h.

For determination of cell dry weight, 1 ml aliquots of aqueous suspensions were dried overnight at 95 °C. Live cell counts during and after aeration were determined by plating tests; protein content in suspension was assayed by the Lowry method.

Cell permeabilization was performed according to Kaplan (1954) using n-butanol/ethanol (3:1, v/v) or iso-butanol/ethanol (3:1, v/v).

Glucose levels in yeast suspensions were assessed using Glucophan glucose detection strips (Spolana, Czechoslovakia), the presence of ethanol was determined by gas chromatography on a Perkin Elmer Model 900 chromatograph using a stainless steel column (91 cm × 3 mm) packed with Porapak Q-S 100/200 mesh. The N₂ carrier gas flow rate was 25 ml min⁻¹, column temperature 135 °C, injector temperature 165 °C, flame ionization detector temperature 150 °C, injected volume 2.0 ml.

Measurement of catalase activity. Catalase activity was determined by measuring the evolution of O₂, caused by the catalase-specific splitting of H₂O₂ to H₂O and O₂, using a Clark-type oxygen electrode (Beckman). Errors due to oxygen consumption through respiration

were minimized by addition of antimycin A a short time before the addition of peroxide. Control experiments with secreted catalase showed that antimycin A did not inhibit catalase during the short time of its application.

To distinguish between patent (intact cell) and extracellular (supernatant) catalase activity, both measurements were performed immediately after separating the cells from the medium by centrifugation at 3000 g for 5 min. The reaction mixture contained 60–100 mM-potassium phosphate buffer (pH 7.0), 4% (fresh wt/vol.) yeast suspension for whole-cell measurements, 0.5% suspension for cryptic activity measurements, or supernatant from 5% suspension for extracellular activity measurement. The reaction was started by addition of 20 mM-H₂O₂ and was recorded for 0.5–1 min at 25 °C. The initial linear trace segments were used to calculate reaction rates and hence the catalase activity.

Purified plasma membranes from *Sch. pombe* were prepared as described by Dufour *et al.* (1988), with slight modifications introduced by Mair & Höfer (1988); those from *Sacch. cerevisiae* were purified according to Serrano (1988). Membranes were kept at –80 °C. Immediately after thawing, catalase activity was measured in 50 µl samples containing 3–3.7 mg protein ml⁻¹ in 3 ml phosphate buffer (pH 7.0). Inhibition with 1 mM-sodium azide was used to distinguish catalase activity from any nonenzymic H₂O₂ splitting.

Measurement of membrane fluidity. The fluidity of the plasma membrane was determined by measuring the steady-state fluorescence anisotropy of the reporter probe trimethylammonium diphenyl-hexatriene (TMA-DPH) (Jähnig, 1979; Block, 1991) at 25 °C on a Hitachi 650-10 fluorescence spectrophotometer. The probe, dissolved in dimethylformamide, was added to a yeast suspension (40–100 µg dry wt ml⁻¹) to a final concentration of 0.5 µM. The emission and excitation wavelengths were 450 and 360 nm, respectively. Membrane fluidity was expressed as the order parameter *S*

$$S = (r/r_0)^{1/2}$$

where *r*₀ is the theoretical limiting anisotropy (0.395 for TMA-DPH) in the absence of rotational motion, and *r* is the steady-state anisotropy measured in the membrane. The parameter *S* reflects the orderliness of membrane phospholipids (Cogen *et al.*, 1973; Van der Meer *et al.*, 1986). In a fully ordered membrane *S* = 1.0.

Short-term effects of the presence of glucose, ethanol and H₂O₂ on membrane fluidity were determined by measuring *S* for 30–60 min following the addition of the substances to cultures. Long-term effects were measured over 6 h periods of aeration in the presence or absence of these substances. Glucose or ethanol (200 mM) was added at the beginning of aeration. At the cell densities used here, some of the substrate remained until the end of aeration. H₂O₂ was added in 20 mM doses at 1 h intervals during aeration.

Results

Cryptic catalase activity

The cryptic catalase activity revealed after cell permeabilization was 5–8 µmol O₂ (mg dry wt)⁻¹ min⁻¹ in aerobic *Sch. pombe* cells, two to three times higher than in aerobic *Sacch. cerevisiae* cells (2–4 µmol O₂ (mg dry wt)⁻¹ min⁻¹). The large cryptic activity of *Sch. pombe* was not affected by up to 6 h aeration whereas in *Sacch. cerevisiae* aeration caused a 25% rise in cryptic catalase activity, which could be prevented by cycloheximide (Fig. 1a, b).

Fig. 1. Development of cryptic catalase activity in *Sch. pombe* during 6 h aeration in water (a), 200 mM-glucose (c) and 200 mM-ethanol (e), and in *Sacch. cerevisiae* in water (b), glucose (d) and ethanol (f). Data are means of four experiments; bars indicate SEM. Open symbols, without cycloheximide; filled symbols, in the presence of 3 mM-cycloheximide; $\times$, glucose-containing medium was removed before cell permeabilization.

Fig. 2. Development of patent catalase activity (normalized values) during 6 h aeration of *Sch. pombe* in water (a), 200 mM-glucose (c) and 200 mM-ethanol (e), and of *Sacch. cerevisiae* in water (b), glucose (d) and ethanol (f). Open symbols, without cycloheximide; filled symbols, in the presence of 3 mM-cycloheximide. The data shown are representative of four experiments. For value normalization and the statistical evaluation of the difference see Table 1 (*Sch. pombe*) and Table 2 (*Sacch. cerevisiae*).

Aeration in the presence of 200 mM-glucose caused a temporary (2–3 h) increase in the cryptic catalase activity of *Sch. pombe* which was prevented by cycloheximide, whereas in *Sacch. cerevisiae* glucose caused a gradual decline of cryptic activity to 10–20% of the original level within 2–3 h (Fig. 1c, d). This inhibition of intracellular catalase activity in *Sacch. cerevisiae* had the following features: (a) it occurred in cells permeabilized with n-butanol but not in cells permeabilized by iso-butanol; (b) it took place during the permeabilization, i.e. with intracellular catalase in its native form – supernatant from glucose-containing culture added to already permeabilized cells had no effect; (c) it occurred with both

glucose and fructose but was not caused directly by the sugars because sugar added during permeabilization had no effect; (d) removal of the sugar-containing medium before cell permeabilization prevented the inhibition (Fig. 1d); (e) it was not mediated by intracellular acidification since, at the intracellular pH of glucose-metabolizing cells (5.5–6.0), the partial inhibition of catalase was completely reversible (data not shown); (f) ethanol had no such effect. Hence, the inhibition was obviously caused by a glycolytic metabolite which accumulated during aeration in sugar-containing medium and which was inactive in the presence of iso-butanol. Permeabilization of the cells in the presence

Table 1. Relative changes in patent catalase activity of *Sch. pombe* during 6 h aeration under different conditions and the contribution of surface and intracellular activity to patent activity

To eliminate differences in absolute catalase levels between individual batches the values are referred to the patent catalase activity in water at 0 h aeration (set at 1.0). The actual values measured varied between 0.1 and 0.5 $\mu\text{mol O}_2$ (mg dry wt)⁻¹ min⁻¹. Values are the means of four determinations $\pm$ SEM. *P* is the significance of the difference estimated by Student's *t*-test; *P*₀, difference from 0 h aeration; *P*_C, difference from control after 6 h aeration. NS, Not significant (corresponds to *P* $\geq$ 0.1).

	Before aeration	After 6 h aeration with additions shown			
		None	Cycloheximide (3 mM)	Glucose (200 mM)	Ethanol (200 mM)
Total patent activity	1.0	1.90 $\pm$ 0.21 <i>P</i> ₀ < 0.0025	1.92 $\pm$ 0.12 <i>P</i> _C (NS)	1.63 $\pm$ 0.17 <i>P</i> _C (NS)	3.69 $\pm$ 0.45 <i>P</i> _C < 0.005
Intracellular activity	0.77 $\pm$ 0.05 (77%)	1.14 $\pm$ 0.09 (60%) <i>P</i> ₀ < 0.01	1.23 $\pm$ 0.09 (64%) <i>P</i> _C (NS)	1.09 $\pm$ 0.07 (67%) <i>P</i> _C (NS)	2.14 $\pm$ 0.28 (58%) <i>P</i> _C < 0.01
Surface activity	0.23 $\pm$ 0.05 (23%)	0.76 $\pm$ 0.09 (40%) <i>P</i> ₀ < 0.0025	0.68 $\pm$ 0.09 (36%) <i>P</i> _C (NS)	0.54 $\pm$ 0.07 (33%) <i>P</i> _C < 0.05	1.55 $\pm$ 0.28 (42%) <i>P</i> _C < 0.025

of a variety of glucose metabolites (pyruvate, lactate, fumarate, succinate) showed the inhibitor to be succinate (Sigler & Höfer, 1991).

In both *Sch. pombe* and *Sacch. cerevisiae* 200 mM-ethanol in the aerated culture caused a 15–25% increase in cryptic activity after 6 h, which was prevented by cycloheximide (Fig. 1e, f).

Intact-cell (patent) catalase activity

The patent catalase activity of both yeast species exhibited considerable variation between different batches grown under standardized conditions. However, the temporal trends caused by aeration or addition of substrates or cycloheximide were very similar in all batches tested. To facilitate statistical evaluation, the enzyme activity values were normalized to a level set at 1.0, at 0 h aeration. The patent catalase activity of *Sch. pombe*, which made up 3–6% of the total cryptic activity at this time, approximately doubled during aeration irrespective of the presence or absence of cycloheximide (Fig. 2a). The patent activity of *Sacch. cerevisiae* (about 10% of the cryptic level at 0 h aeration) rose by 70–80% during aeration and the increase was reduced by cycloheximide (Fig. 2b).

As shown by Sels & Brygier (1980) the whole-cell catalase activity of yeast cells consists of a surface-localized catalase, and an intracellular catalase enclosed within a diffusion barrier (the plasma membrane) with only a limited permeability for the peroxide. The relative proportion of the two contributory activities can be determined by selectively inhibiting the surface enzyme by acid pH.

As seen in Table 1 the doubling of patent activity in

Sch. pombe during aeration without substrates reflected an increase in both surface and intracellular activity. The rise in patent activity observed under these conditions was not prevented by cycloheximide. Aeration in the presence of glucose reduced the increase in patent activity as compared with the control. Cycloheximide again has no effect (Fig. 2a, c, e, Table 1). The ratio of intracellular to surface catalase activity was not changed when the cells were aerated in phosphate buffer at pH 7 instead of in water (data not shown). In the presence of ethanol the patent catalase activity of *Sch. pombe* increased strongly over 6 h aeration (200–300%) but the proportion of surface activity in the total patent activity remained the same as in the absence of substrate (Table 1).

In *Sacch. cerevisiae*, aeration in water enhanced the surface-localized part of the patent activity fourfold, the intracellular part changing little (Table 2). The presence of glucose prevented this increase in patent activity (Fig. 2d) and in fact caused a slight drop in the intracellular activity contribution (Table 2). In the presence of glucose plus cycloheximide (Fig. 2d) the patent activity decreased, which suggested a relatively rapid turnover of the enzyme. In cells aerated in a glucose-containing buffer (pH 7) (Table 2) the patent catalase activity did not exhibit a similar inhibition. The lowering of surface catalase activity in cells aerated with glucose in comparison with cells aerated in water may therefore be partially due to extracellular acidification causing irreversible inhibition of the surface-localized enzyme (pH₀ declined to about 3 after 6 h). In cells aerated with ethanol the patent activity doubled during the 6 h aeration, largely owing to *de novo* protein synthesis (Fig. 2f). The intracellular/surface activity ratio was about 1:1 (data not shown), similar to cells aerated in water

Table 2. *Relative changes in patent catalase activity of Sacch. cerevisiae during 6 h aeration under different conditions and the contribution of surface and intracellular activity to patent activity*

To eliminate differences in absolute catalase levels between individual batches the values are referred to the patent catalase activity in water at 0 h aeration (set at 1.0). The actual values measured varied between 0.2 and 0.4 $\mu\text{mol O}_2$ (mg dry wt) $^{-1}$ min $^{-1}$. Values are means of four determinations $\pm$ SEM. *P* is the significance of the difference estimated by Student's *t*-test; *P*₀, difference from 0 h aeration, *P*_C, difference from control after 6 h aeration. NS, Not significant (corresponds to *P* $\geq$ 0.1).

	Before aeration	After 6 h aeration with additions shown				
		None	Cycloheximide (3 mM)	Glucose (200 mM)	Glucose (200 mM) + buffer	Ethanol (200 mM)
Total patent activity	1.0	1.77 $\pm$ 0.09 <i>P</i> ₀ < 0.0005	1.37 $\pm$ 0.05 <i>P</i> _C < 0.005	1.05 $\pm$ 0.15 <i>P</i> _C < 0.0025	1.95 $\pm$ 0.13 <i>P</i> _C (NS)	1.99 $\pm$ 0.03 <i>P</i> _C < 0.0005
Intracellular activity	0.81 $\pm$ 0.08 (81 %)	0.87 $\pm$ 0.07 (49 %) <i>P</i> ₀ (NS)	1.08 $\pm$ 0.07 (79 %) <i>P</i> _C < 0.05	0.72 $\pm$ 0.09 (69 %) <i>P</i> _C (NS)	1.11 $\pm$ 0.07 (57 %) <i>P</i> _C < 0.025	0.99 $\pm$ 0.04 (50 %) <i>P</i> _C (NS)
Surface activity	0.19 $\pm$ 0.08 (19 %)	0.90 $\pm$ 0.07 (51 %) <i>P</i> ₀ < 0.0005	0.29 $\pm$ 0.07 (21 %) <i>P</i> _C < 0.0005 <i>P</i> ₀ (NS)	0.33 $\pm$ 0.09 (31 %) <i>P</i> _C < 0.001 <i>P</i> ₀ (NS)	0.84 $\pm$ 0.06 (43 %) <i>P</i> _C (NS)	0.99 $\pm$ 0.04 (50 %) <i>P</i> _C (NS)

Table 3. *Changes in the membrane-order parameter S during 6 h aeration of Sch. pombe and Sacch. cerevisiae cells in water, in the presence of 200 mM-glucose or 200 mM-ethanol, and with additional pulses of 20 mM-H₂O₂ at 1 h intervals*

The values (mean $\pm$ SEM) are means from five independent experiments, each of which included 10 measurements. $\Delta S_{(0 \rightarrow 6)}$, difference between *S* values at 0 and 6 h. The significance of each difference according to Student's *t*-test was *P* $\leq$ 0.0005.

Yeast	Time (h)	<i>S</i> (no H ₂ O ₂ pulses)			<i>S</i> (with H ₂ O ₂ pulses)		
		Water	Glucose	Ethanol	Water	Glucose	Ethanol
<i>Sch. pombe</i>	0	0.888 $\pm$ 0.0051	0.882 $\pm$ 0.0067	0.885 $\pm$ 0.0045	0.909 $\pm$ 0.0031	0.890 $\pm$ 0.0039	0.895 $\pm$ 0.0029
	6	0.865 $\pm$ 0.0029	0.847 $\pm$ 0.0050	0.858 $\pm$ 0.0034	0.875 $\pm$ 0.0034	0.854 $\pm$ 0.0028	0.854 $\pm$ 0.0028
	$\Delta S_{(0 \rightarrow 6)}$	0.023	0.035	0.027	0.034	0.044	0.041
<i>Sacch. cerevisiae</i>	0	0.855 $\pm$ 0.0022	0.852 $\pm$ 0.0022	0.855 $\pm$ 0.0026	0.853 $\pm$ 0.0019	0.854 $\pm$ 0.0022	0.856 $\pm$ 0.0022
	6	0.839 $\pm$ 0.0031	0.831 $\pm$ 0.0022	0.837 $\pm$ 0.0019	0.837 $\pm$ 0.0017	0.832 $\pm$ 0.0019	0.823 $\pm$ 0.0022
	$\Delta S_{(0 \rightarrow 6)}$	0.016	0.021	0.018	0.016	0.022	0.023

without buffer, indicating that the acidification caused by ethanol did not lead to an irreversible inhibition of the surface-localized enzyme (the pH declined to about 3.8 after 6 h; irreversible acid inhibition of catalase occurred only below pH 3.5; data not shown).

Purified plasma membrane fractions from both yeasts exhibited catalase activity. In *Sch. pombe* with 50 mM-H₂O₂ the activity was relatively high, 1.6 $\mu\text{mol O}_2$ (mg protein) $^{-1}$ min $^{-1}$, while in *Sacch. cerevisiae* it was considerably lower, 0.2 $\mu\text{mol O}_2$ (mg protein) $^{-1}$ min $^{-1}$. This was probably due to activity losses during preparation of the membrane fraction. In both cases activity was completely inhibited by 1 mM-sodium azide.

Membrane fluidity under various conditions

The observed increase in the intracellular activity during aeration could have been due to increased permeability of the plasma membrane to H₂O₂ and O₂. As membrane

fluidity is believed to affect permeability to small uncharged molecules (Jones & Greenfield, 1987) we measured changes in membrane fluidity during aeration. The plasma membrane fluidity of untreated cells of *Sch. pombe* (*S* = 0.87–0.90) was at the upper limit of the values found in *Sacch. cerevisiae* (*S* = 0.83–0.87). Table 3 shows that cell aeration was accompanied by significant plasma membrane fluidization in both species. Kinetic measurements (data not shown) revealed that the fluidity occurred for the most part within the first 30 min. The statistical significance of the differences between $\Delta S_{(0 \rightarrow 6)}$ values for individual variants was below the *P* < 0.05 level (*P* $\approx$ 0.1) and the membrane fluidization due to aeration thus appears to be independent of the presence of either H₂O₂ or substrates (glucose or ethanol). This statistical insignificance notwithstanding, the results seem to indicate that the fluidity of *Sch. pombe* plasma membranes is generally more sensitive to aeration and that the effect is potentiated in the presence of substrate.

H₂O₂ additions during aeration had different effects on *Sch. pombe* and on *Sacch. cerevisiae*. Whereas in *Sch. pombe* H₂O₂ promoted membrane fluidization in all cases (cf. ΔS in Table 3), in *Sacch. cerevisiae* it had no clear-cut additional fluidizing effect.

Extracellular (supernatant) catalase activity

In both yeast species, catalase activity was determined in the medium after cell separation. When related to a medium volume corresponding to a certain dry weight, the activity equalled or exceeded the patent activity [$\sim 300 \text{ nmol O}_2 (\text{mg dry wt})^{-1} \text{ min}^{-1}$ in *Sch. pombe*, $400\text{--}700 \text{ nmol O}_2 (\text{mg dry wt})^{-1} \text{ min}^{-1}$ in *Sacch. cerevisiae* after 6 h aeration]. The simplest explanation for its appearance would be cell lysis but plating tests revealed no perceptible cell lysis during aeration. We determined the temporal changes in the specific activity of catalase in the supernatant along with corresponding extracellular protein levels. The protein level rose on average from 20 to $250 \mu\text{g protein} (\text{mg dry wt})^{-1}$ in *Sch. pombe* and from 20 to $600 \mu\text{g protein} (\text{mg dry wt})^{-1}$ in *Sacch. cerevisiae*, and the specific activity of extracellular catalase, i.e. the activity referred to a unit amount of extracellular protein, continuously increased during aeration. This hints at a directed export of catalase from the cells distinct from non-specific leakage of proteins.

The extracellular catalase activity in both species was inhibited during aeration in the presence of glucose, obviously owing to extracellular acidification (Sigler & Höfer, 1991) because no inhibition was found in buffered suspensions at pH 7. Unlike the membrane-bound catalase activity, the extracellular activity in *Sacch. cerevisiae* was similarly inhibited by ethanol and glucose. In *Sch. pombe*, on the other hand, ethanol had a stimulatory effect. Extracellular catalase in *Sch. pombe* is thus considerably more acid resistant than the *Sacch. cerevisiae* enzyme, and may also play a role in ethanol detoxification.

Discussion

Our results showed an extraordinary stability of catalase activity in stationary cells of *Sch. pombe*. In *Sacch. cerevisiae* two major types of catalase have been reported, cytosolic catalase T and peroxisomal catalase A (Seah & Kaplan, 1973; Seah *et al.*, 1973). Both enzymes are under positive control by haem (Hörtner *et al.*, 1982), which requires molecular oxygen for its synthesis (Wieser *et al.*, 1991). Catalase T levels are under cAMP-mediated nutrient control and increase on starvation (Bissinger *et al.*, 1989). They are also enhanced following heat shock (Wieser *et al.*, 1991). Our data on the susceptibility of the overall catalase activity of *Sacch. cerevisiae* to glucose, aeration and cycloheximide are in agreement with these

results. The intracellular catalase was inactivated when cells were aerated in the presence of glucose or fructose and permeabilized with n-butanol irrespective of whether protein synthesis was blocked. Lowering of catalase activity in glucose-grown cells exposed to drying or heat was noticed as early as 1919 (von Euler and Blix, 1919) and was ascribed to the effect of the aldehyde group of glucose. We found that it is caused by succinate, which is one of the major by-products of glucose dissimilation in *Sacch. cerevisiae* (Sigler *et al.*, 1980; Sigler & Höfer, 1991). This inhibition could also be responsible for the disappearance of catalase activity during the lag phase of growth of baker's yeast on glucose (Seah *et al.*, 1973). The reason for the lack of inhibition by succinate when cells were permeabilized with iso-butanol is not clear.

By contrast, the cryptic catalase activity of *Sch. pombe* seemed to be largely impervious to aeration and also to reduced oxygen supply during growth (data not shown). The presence of substrates during aeration had either a transient (glucose) or marginal (ethanol) stimulatory effect. In contrast to *Sacch. cerevisiae*, the intracellular activity was not inactivated by glucose metabolites during permeabilization.

Increased export of catalase activity to the cell surface during aeration of *Sch. pombe* was apparently independent of *de novo* protein synthesis and was not much affected by glucose. In the presence of ethanol, which can energize transport processes in this yeast (Höfer & Nassar, 1987) the supply of catalase to cell surface was considerably stimulated. This process may protect against the possible deleterious action of ethanol on the cells. Thus, the surface catalase may oxidize ethanol (Handler & Thurman, 1988) to protect cell structures, especially the plasma membrane. An increase in plasma membrane lipid peroxidation and free radical production in animal tissues after an acute dose of ethanol has been reported by several authors (Di Luzio & Hartman, 1967; Müller & Sies, 1987; Nordman *et al.*, 1985; Rouach *et al.*, 1987; Reinke *et al.*, 1988). Moreover, Knecht *et al.* (1990) succeeded in demonstrating the *in vivo* formation of the α -hydroxyethyl free radical metabolite of ethanol in deermice. The participation of this radical in membrane lesions cannot be ruled out. Since this radical is also capable of reducing haem-containing proteins (Bors *et al.*, 1989) such as catalase, the increased delivery of catalase to the cell surface and into the extracellular medium might also serve to replace damaged molecules. *Sch. pombe* produces low amounts of ethanol and, as a member of the wine flora, is expected to be highly resistant to ethanol. Indeed, according to Koukou *et al.* (1990) ethanol inhibits the growth of this yeast only at concentrations above 7.5%, mainly owing to the high content of 18:1 fatty acids in the membrane, and to a high rate of phospholipid biosynthesis. Catalase activity

at the cell surface may contribute to the ethanol tolerance of this yeast.

Sels & Brygier (1980) reported that in *Sacch. cerevisiae* glucose repression during growth inhibited the delivery of catalase to the cell surface. In ethanol-grown cells the proportion of surface catalase was markedly higher. However, we found the proportion of surface activity to be comparatively small even when cells were grown at nonrepressive glucose concentrations.

The effect of ethanol on *Sch. pombe* patent catalase activity was in distinct contrast to that observed in *Sacch. cerevisiae*, where blockage of protein synthesis in the presence of ethanol abolished the increase in patent catalase activity. Ethanol dissimilation in suspensions of both yeasts caused extracellular acidification (pH_o 3.4–3.8) approaching that found with glucose (pH_o 3). In *Sacch. cerevisiae* both substrates inhibited the extracellular catalase almost completely. In *Sch. pombe*, however, the extracellular catalase activity was stimulated by ethanol.

The notion that catalase may be exported from yeast cells selectively poses the question of cells secreting a haem-containing protein of some 240 kDa (catalase T; Seah & Kaplan, 1973) into the external medium. The cell wall permeability in growing cells of *Sacch. cerevisiae* allows the passage of molecules up to 250 kDa (De Nobel & Barnett, 1991). Our finding of catalase activity in purified plasma membrane fractions from both yeasts indicates that its localization in the cells need not be confined to peroxisomes and the cytoplasm. Its association with the plasma membrane may be connected with its appearance in the outer medium.

Another difference between *Sch. pombe* and *Sacch. cerevisiae* was found in the fluidity of the plasma membrane. The plasma membrane of *Sch. pombe* was on the whole more rigid but glucose and ethanol had a stronger effect on fluidity. It was also more fluidized by H₂O₂ than the plasma membrane of *Sacch. cerevisiae*. Aeration of both *Sch. pombe* and *Sacch. cerevisiae* cells in water fluidized the cell membrane and ethanol had an additional fluidizing effect which persisted throughout aeration. Surprisingly, aeration in the presence of glucose caused an even stronger fluidization. No such glucose-induced membrane fluidization has to our knowledge been reported in yeast. In fact, low extracellular pH was found to lead to membrane rigidization during growth of *Sacch. cerevisiae* (Singh *et al.*, 1990). Glucose-induced membrane fluidization has been reported to occur in pancreatic islet cells which perform glucose-triggered insulin secretion (Cortizo *et al.*, 1990). In these cells, membrane disordering was not found to be accompanied by any significant changes in the fatty acid composition or in the transition temperature, i.e. no gross alterations in membrane lipid composition.

Our experiments demonstrated an increase in membrane fluidity during cell aeration and a concomitant increase in intact-cell catalase activity consisting of enzyme exported to the cell surface and of activity due to H₂O₂ diffusion into the cytoplasm. However, no direct relationship between these two processes could be found in the sense of a proportional increase of passive plasma membrane permeability and fluidity.

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