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TWO SPECIES OF CYTOCHROME P-450 INVOLVED IN ERGOSTEROL BIOSYNTHESIS OF YEAST

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SUMMARY: Discrimination of cytochrome P-450 involved in Δ^{22}-desaturation of ergosta-5,7-dien-3β-ol ($P\text{-}450_{22\text{-}DS}$) from that involved in lanosterol 14α-demethylation ($P\text{-}450_{14\text{-}DM}$) in ergosterol biosynthesis was investigated with microsomes of several strains of Saccharomyces cerevisiae. In mutant N22 which is partially defective in the Δ^{22}-desaturation, the 14α-demethylation was not blocked. In contrast, mutant SG1 which is known to lack the 14α-demethylation showed a significant activity of the Δ^{22}-desaturation. The Δ^{22}-desaturation activity was markedly increased upon aerobic adaptation of yeast cells but the 14α-demethylation was not affected. Buthiobate, a specific inhibitor of $P\text{-}450_{14\text{-}DM}$, and rabbit antibodies against $P\text{-}450_{14\text{-}DM}$ did not inhibit the Δ^{22}-desaturation activity at all. It is evident from the obtained observations that these phenomena are not explainable in terms of NADPH-cytochrome P-450 reductase. These results indicate that $P\text{-}450_{22\text{-}DS}$ is different from $P\text{-}450_{14\text{-}DM}$ in molecular species.

The involvement of cytochrome P-450 in the 14α-demethylation of lanosterol was established in ergosterol biosynthesis of Saccharomyces cerevisiae (1, 2). Thereafter, the cytochrome P-450 ($P\text{-}450_{14\text{-}DM}$) was purified (3) and characterized (4-6). Recently Hata et al. reported that Δ^{22}-desaturation of ergosta-5,7-dien-3β-ol is also catalyzed by an enzyme system containing cytochrome P-450 in S. cerevisiae (7). The existence of multiple molecular species of cytochrome P-450 involved in various kinds of monooxygenations has been well known in mammals (8,9) but not in yeast. The present investigation was undertaken from the interest as to whether the two cytochromes in yeast

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Abbreviations: $P\text{-}450_{14\text{-}DM}$, cytochrome P-450 involved in 14α-demethylation; $P\text{-}450_{22\text{-}DS}$, cytochrome P-450 involved in Δ^{22}-desaturation.

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are of the same molecular species or not. We report in this paper that cytochrome P-450 involved in the Δ^{22}-desaturation (P-$450_{22\text{-DS}}$) is of new molecular species different from P-$450_{14\text{-DM}}$.

MATERIALS AND METHODS

[28-^{14}C]Ergosta-5,7-dien-3β-ol (specific radioactivity, 620 mCi/mol) was prepared as reported (10).

Strains of Saccharomyces cerevisiae used were: nystatin-resistant mutant N22 and its parent strain M10 (10,11); nystatin-resistant mutant SG1 and its parent strain D587 (12,13); and wild-type strain ATCC 12341. The former four strains were grown semi-anaerobically in a growth medium (2% glucose/0.5% polypeptone/0.5% yeast extract/0.5% KH_2PO_4) without shaking at 28°C for 48 h. When the cells were to be adapted aerobically, 5 g of the wet cells were suspended in 500 ml of 0.1 M potassium phosphate buffer (pH 6.8) containing 2% glucose, and the suspension was vigorously shaken aerobically at 28°C for 2.5 h. The wild-type yeast cells were grown semi-anaerobically with gentle stirring in the same growth medium as above at 28°C for 15 h. Microsomes were prepared as reported (7).

Incubation for the determination of Δ^{22}-desaturation and 14α-demethylation activities was carried out in the same test tube, not separately. The former was assayed by determination of ^{14}C-incorporation from exogenously added [^{14}C]ergosta-5,7-dien-3β-ol into ergosterol and the latter by determination of conversion of endogenous non-labeled lanosterol present in microsomes into 4,4-dimethylzymosterol. The standard reaction mixture contained, in total volume of 1 ml, 16 nmol of [^{14}C]ergosta-5,7-dien-3β-ol (21,800 cpm), 0.5 μmol of NADPH, 10 μmol of glucose 6-phosphate, 0.1 unit of glucose-6-phosphate dehydrogenase, 0.2 μmol of EDTA, 1 μmol of KCN, 0.1 μmol of dithiothreitol, 100 μmol of potassium phosphate buffer (pH 7.4) and microsomes (4 mg of protein). [^{14}C]Ergosta-5,7-dien-3β-ol was dispersed in the mixture using 1 mg of Tween 20. The reaction was carried out with shaking at 30°C for 25 min. Then the mixture was saponified and nonsaponifiable lipids were extracted. The lipids were subjected to thin-layer chromatography (6) for separation into two fractions —— one (R_f 0.64) containing ergosta-5,7-dien-3β-ol and ergosterol, and the other (R_f 0.72) containing lanosterol and 4,4-dimethylzymosterol. Sterols were extracted from each fraction and analyzed. Sterols from the fraction with lower R_f value were analyzed by gas chromatography (7) and radioactivities in ergosterol and ergosta-5,7-dien-3β-ol fractions (ratio of retention times, 0.86 : 1.0) were measured. Sterols from the fraction with higher R_f value were analyzed by gas chromatography on an OV-101 glass-capillary column (10) and areas of peaks of lanosterol and 4,4-dimethylzymosterol (ratio of retention times, 1.0 : 1.05) were measured.

Antibodies against P-$450_{14\text{-DM}}$ and control γ-globulin were prepared as reported (6).

RESULTS AND DISCUSSION

The Δ^{22}-desaturation activity of mutant N22 was about one-tenth of that of its parent strain M10 both in the semi-anaerobically-grown and aerobically-adapted cells (Table 1). This observation supports our previous report (10) suggesting that the mutant is blocked at the Δ^{22}-desaturation in ergosterol biosynthesis, though leaky to some extent, based on the analysis of its sterol composition in comparison with that of the parent strain. In contrast, the

Table 1. Ergosta-5,7-dien-3β-ol Δ^{22}-desaturation and lanosterol 14α-demethylation activities of microsomes from four S. cerevisiae strains.

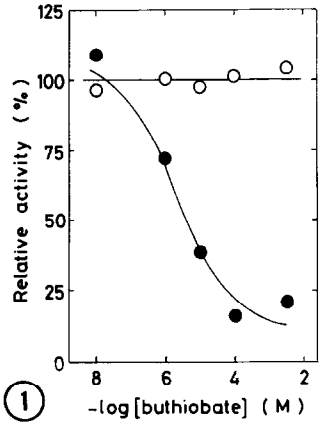
Strain	Growth conditions	Activity (pmol/min/mg protein)	
		Δ^{22}-Desaturation	14α-Demethylation
N22 (mutant)	Semi-anaerobic	0.3	85.0
	Aerobically adapted	2.8	69.7
M10 (parent)	Semi-anaerobic	7.4	65.7
	Aerobically adapted	25.1	48.6
SG1 (mutant)	Semi-anaerobic	1.2	0
	Aerobocally adapted	14.4	0
D587 (parent)	Semi-anaerobic	14.6	8.8
	Aerobically adapted	28.3	7.1

14α-demethylation activity of mutant N22 was rather higher than that of the parent strain (Table 1), indicating that P-450$_{14\text{-DM}}$ and its reductase is not deficient. On the other hand, the 14α-demethylation activity of mutant SG1 was completely deficient owing to the catalytically inactive P-450$_{14\text{-DM}}$ (13). Notwithstanding it, the Δ^{22}-desaturation activity of mutant SG1 was significant, though lower than that of the parent strain D587. This indicates that mutant SG1 contains high activity of the Δ^{22}-desaturation in spite of its complete lack of P-450$_{14\text{-DM}}$. This is consistent with the observation by Pierce et al. using intact yeast cells (14) that the Δ^{22}-desaturation occured in a mutant defective in the 14α-demethylation.

Table 1 also shows that the Δ^{22}-desaturation activity in all yeast strains used was increased by the aerobic adaptation, whereas the 14α-demethylation activity was not affected by the adaptation.

Buthiobate (S-n-butyl S'-p-tert-butylbenzyl N-3-pyridyldithiocarbonimidate), a specific inhibitor of P-450$_{14\text{-DM}}$ (15,16), which inhibited lanosterol 14α-demethylation of the microsomes from a wild-type yeast, S. cerevisiae ATCC 12341, showed no inhibitory effect on the Δ^{22}-desaturation of the same microsomes (Fig. 1).

Antibodies raized in a rabbit against P-450$_{14\text{-DM}}$ (6) strongly inhibited lanosterol 14α-demethylation activity of the wild-type yeast but it did not inhibit the Δ^{22}-desaturation at all (Fig. 2).



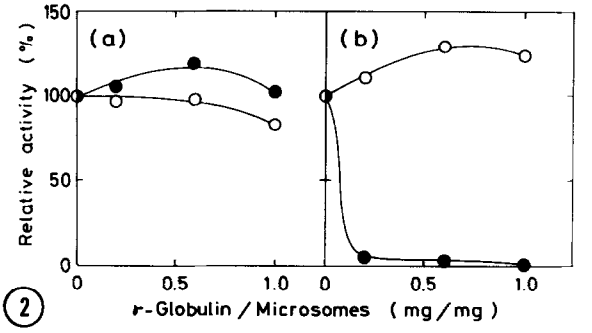


Fig. 1. Effects of buthiobate on ergosta-5,7-dien-3β-ol Δ^{22}-desaturation and lanosterol 14α-demethylation activities. The two activities of microsomes from semi-anaerobically-grown cells of S. cerevisiae ATCC 12341 were assayed as described in MATERIALS AND METHODS in the presence of indicated concentrations of buthiobate. ○, Δ^{22}-desaturation; and ●, 14α-demethylation.

Fig. 2. Effects of anti-P-450$_{14\text{-DM}}$ γ-globulin on the Δ^{22}-desaturation and 14α-demethylation activities. Microsomes from semi-anaerobically-grown cells of S. cerevisiae ATCC 12341 were preincubated with indicated amounts of anti-P-450$_{14\text{-DM}}$ γ-globulin (●) or control γ-globulin (○) at 30°C for 15 min. The two activities were then assayed as described in MATERIALS AND METHODS. (a), Δ^{22}-desaturation; and (b), 14α-demethylation.

It is evident from the following observations that these phenomena are not explainable in terms of NADPH-cytochrome P-450 reductase (EC 1.6.2.4) involved in the 14α-demethylation and Δ^{22}-desaturation: The contents of the reductase in microsomes of the mutants were comparable to, or rather higher than, those of their parent strains (13 and unpublished data). The level of the reductase was not increased by the aerobic adaptation of the cells (unpublished data). In addition, the reductase activity was not affected by buthiobate (16) or by anti-P-450$_{14\text{-DM}}$ γ-globulin.

In consequence, the lines of evidence described above indicate that the cytochrome P-450 responsible for the Δ^{22}-desaturation (P-450$_{22\text{-DS}}$) is of a novel molecular species distinct from P-450$_{14\text{-DM}}$. Although purification and precise characterization of P-450$_{22\text{-DS}}$ has not yet been performed, the multiplicity of cytochrome P-450 found in yeast, a primitive eukaryote, is interesting from the viewpoint of comparative biochemistry.

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