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COMPARATIVE RESPONSES OF THE YEAST MUTANT STRAIN GL7 TO LANOSTEROL, CYCLOARTENOL, AND CYCLOLAUDENOL

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SUMMARY: Under anaerobic growth conditions the isomeric 4,4',14-trimethyl-cholestane derivatives lanosterol and, more efficiently, cycloartenol satisfy the sterol requirement of the yeast sterol auxotroph Saccharomyces cerevisiae strain GL7. Aerobic mutant growth is supported only by cycloartenol and not by lanosterol, suggesting different structural requirements for aerobic and anaerobic cells. It is proposed that the non-planar conformation imposed by the 9,19-cyclopropane ring of cycloartenol moderates the adverse membrane effects of the nuclear methyl groups at C-4 and C-14. Under both aerobic and anaerobic conditions cyclolaudenol, a C-24-methyl derivative of cycloartenol, is a significantly more effective sterol source for strain GL7 than cycloartenol. This result is in keeping with the predominance of C-24-methyl sterols (ergosterol) in wild-type yeast.

The nuclear methyl groups at C-4 and C-14 of lanosterol weaken sterol-phospholipid interactions in artificial and natural membranes (1,2). Lanosterol, unlike cholesterol, fails to change the fluidity of artificial membranes presumably because it is relatively mobile in the phospholipid bilayer (1). As a growth factor for the sterol-requiring Mycoplasma capricolum, lanosterol is also less effective than cholesterol (3). From past work we have concluded that the presence of the axial 14α-methyl group accounts in large measure for the different properties of cholesterol- and lanosterol-containing membranes (1,2).

In the preceding paper (4), lanosterol was compared with cycloartenol, the isomeric 9,19-cyclopropane analogue, as a growth factor for the sterol-requiring Mycoplasma capricolum and the effects of these two sterols on model membrane microviscosity were also examined. Replacement of the C-19 angular methyl group by the 9,19-cyclopropane ring was found to render the 4,4',14-trimethyl sterol molecule more effective for membrane function.

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(Apparently the conformation of the cycloartenol ring system is such as to minimize the detrimental effects of the methyl groups at the sterol α-face.) We now report studies comparing the competence of lanosterol and cycloartenol as sterol sources for a sterol-requiring Saccharomyces cerevisiae mutant, strain GL7. We demonstrate that cycloartenol is also more effective than lanosterol in supporting growth of the eucaryotic sterol auxotroph. Furthermore we find that cyclolaudenol, a cycloartenol derivative bearing a methyl group at C-24 of the aliphatic side chain, surpasses cycloartenol as a sterol supplement for the yeast mutant, the converse of the relationship observed in Mycoplasma (4).

MATERIALS AND METHODS. Saccharomyces cerevisiae strain GL7, defective in heme synthesis and 2,3-oxidosqualene-lanosterol cyclase (5), was kindly provided by Dr. D. B. Sprinson. The conditions for aerobic and anaerobic growth of the mutant have been described (6). Sterol-supported growth of the mutant was determined either spectrophotometrically (aerobic growth) or by dry weight measurements of stationary phase cells (anaerobic growth). Sterol analysis was performed by gas-liquid chromatography (GLC) of the non-saponifiable fractions (6). The separation and tentative identification of the partially demethylated cyclosterols was achieved by thin layer chromatography (TLC) (7). Proton Magnetic Resonance (PMR) spectra were recorded at 270 MHZ in $CDCl_3$ with tetramethylsilane as an internal reference.

Cholesterol and cholestanol were obtained from the Sigma Chemical Co. Cyclolaudenol was donated by G. Ourisson, cycloartenol and cycloartanol were the gifts of D. Arigoni, and cycloeucalenol was provided by P. Benveniste. Lanosterol was purified via the dibromide (8) and 24,25-dihydrolanosterol was provided by R. B. Clayton. The purity of all sterols was $\geq$ 95% as determined by GLC.

RESULTS. Aerobic growth. S. cerevisiae strain GL7, defective in squalene epoxide-lanosterol cyclization, requires an exogenous sterol supplement for growth (5) (Figure 1). Cholesterol satisfies this requirement as effectively as ergosterol (6). Lanosterol, the normal ergosterol precursor in yeast, does not support growth of the mutant (Figure 1A). In contrast, cycloartenol, the 9,19-cyclopropane isomer of lanosterol, supports mutant growth, albeit less effectively than cholesterol (Figure 1A). Similarly, negative results were obtained with 24,25-dihydrolanosterol and positive results with cycloartanol (24,25-dihydrocycloartenol) (results not shown). The presence of an additional methyl group at C-24 of cycloartenol (cyclo-

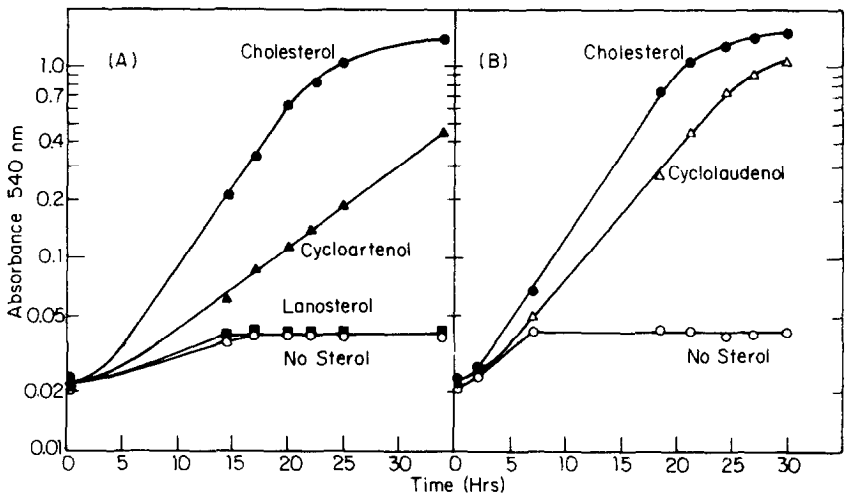

Figure 1 Effect of sterol supplements on the aerobic growth of strain GL7. Log phase yeast cells were used to inoculate flasks supplemented with various sterols. Growth was measured spectrophotometrically.

laudenol) significantly improved the efficacy of the cyclosterol in supporting growth of strain GL7 (Figure 1B).

The non-saponifiable fractions from strain GL7 grown on cholesterol and the cyclosterols were analyzed by GLC (not shown). While the spectrum of cholesterol-grown cells contained only peaks corresponding to 2,3-oxidosqualene and cholesterol, as expected from the characteristics of the mutant, the non-saponifiable fraction of cells grown on the 9,19-cyclosterols contained 2 or 3 additional sterols. Based upon their retention time on GLC and their Rf. values on TLC (7), the cyclosterol metabolites accounting for about 80% of the total sterols were tentatively identified as the 4-monomethyl and 4-desmethyl derivatives. The retention of the 9,19-cyclopropane ring in the demethylated products was confirmed by PMR (doublets centered at 0.39 and 0.14 p.p.m., $J = 4.1\ H_z$).

Anaerobic growth. In order to avoid complications arising from metabolic (presumably oxidative) demethylation, the sterol requirements for strain GL7 were next investigated under strictly anaerobic conditions. For anaerobically-grown strain GL7 this requirement is still absolute, but apparently less specific. Thus, lanosterol was relatively effective in

TABLE I. Effect of Sterol Supplements on the Anaerobic Growth of Strain GL7. The yeast mutant was grown anaerobically with various sterols as described in Materials and Methods. After 60-72 hrs., cells were washed and lyophilized for dryweight determinations.

Experiment	Supplement	Dry wt (mg)	Per cent
I	Cholesterol	122	100
	Lanosterol	84	69
II	Cholesterol	160	100
	Cycloartenol	144	90
III	Cholesterol	111	100
	Lanosterol	76	68
	Cyclolaudenol	114	103
IV	Cholestanol	110	100
	24,25-Dihydrolanosterol	136	124
	Cycloartanol	154	141

supporting anaerobic growth (Table 1), in contrast to the negative response of aerobic cells to this sterol. Anaerobic cells grew even better with cycloartenol (Table 1) and similarly, cycloartanol was superior to 24,25-dihydrolanosterol (Table 1). Cyclolaudenol, the C-24-methyl derivative of cycloartenol, supported anaerobic growth as well as cholesterol (Table 1). It was the most effective of the 4,4',14 trimethyl sterols tested.

The non-saponifiable fractions from anaerobically-grown cells contained only squalene and the added test sterol (GLC, not shown). Thus, the intact, unmodified cyclosterols were remarkably effective and lanosterol moderately active as sterol supplements for strain GL7 when grown anaerobically. Their utilization is not dependent on metabolic modification.

DISCUSSION. The question whether lanosterol can satisfy the sterol requirement of anaerobically-grown wild-type yeast has been investigated in several laboratories but the results have been conflicting (9-13). For

the yeast mutant strain GL7 our results seem clear cut. Lanosterol supports growth adequately though not optimally.

Under the same conditions, the lanosterol isomer cycloartenol is significantly more effective as a sterol supplement. It should be stressed that in the anaerobic cell both sterols *per se* are functionally competent; they are not metabolically modified. The superior response of a eucaryotic cell to cyclosterols complements results obtained with *Mycoplasma* and model membranes (4). Apparently, restraints due to the 9,19-cyclopropane ring impose conformational changes on the sterol structure which greatly lessen or moderate the otherwise adverse membrane effects of the nuclear methyl groups at C-4 and C-14 (both axial). The nature of these conformational changes and in particular how they effect the disposition of the axial 14α-CH_3 of cycloartenol compared to lanosterol are discussed in the preceding paper (4).

Under aerobic growth conditions these differences in response are even more striking. Lanosterol elicits no detectable growth of the mutant, whereas cycloartenol has substantial activity.[1] It is interesting to note (Figure 1, and Table 1) that relative to cholesterol, cycloartenol is a better sterol source under anaerobic than aerobic conditions (90% compared to ~ 45%). Why the same cells do not respond to lanosterol under aerobic conditions remains unexplained. Mutant strain GL7 (heme$^-$) lacks the P_{450} system for oxidative removal of the 14α-methyl group, a step that is ordinarily the first in the oxidative demethylation of lanosterol (15). In the present instance, this deficiency may preclude necessary modification

[1] Gollub *et al.* (5) mention, without offering an explanation, related observations on growth responses to lanosterol. Mutant strains derived from yeast strain 587 grow on lanosterol only under anaerobic conditions, while strains derived from yeast strain X2180 utilize lanosterol both aerobically and anaerobically. Conceivably, changes in oxygen availability alter the permeability or structure of the yeast cell wall, plasma membrane or internal organelles, and these differences may vary among strains. Alternatively growth under aerobic versus anaerobic conditions may involve more diverse or specific roles for membrane sterols. It should be noted that, in the instances investigated, lanosterol is not suitable for membranes of ordinarily aerobic, eucaryotic cells (14).

of the lanosterol structure elsewhere in the molecule. However in certain mutants or in the presence of inhibitors of 14α demethylation, yeast accumulates 4α,14α-dimethyl sterols (16-18).

As pointed out above, the yeast mutant strain GL7 utilizes cycloartenol for anaerobic growth without changing its structure. On the other hand yeast growing aerobically on cycloartenol modifies the cyclosterol substantially by removing either one or both of the methyl substituents at C-4. It is therefore not clear whether the aerobic growth we observe is due to unmodified cycloartenol or C-4 demethylation products.[2] As for the ability of the mutant strain GL7 to dealkylate cycloartenol with retention of the 9,19-cyclopropane bridge, our in vivo results confirm earlier studies by Anding et al. with cell-free extracts from wild-type yeast demonstrating the conversion of cycloartenol to 31-nor-cycloarten-3-one (7). The existence of yeast enzymes for metabolizing cyclosterols which are typical plant products is notable. Moreover, in yeast as in plants (19) the initial dealkylation of cycloartenol occurs at C-4, not at C-14. This seems to be the case both in wild-type yeast and when the 14α demethylation enzyme is non-functional because of the absence of the P_{450} system as in GL7 (5). Steric inaccessibility of the 14α-CH_3 group in cycloartenol rather than enzyme specificity appears to be responsible for the plant-type sequence of nuclear demethylation.

Under both anaerobic and aerobic conditions, cyclolaudenol, a C-24 methyl derivative of cycloartenol, was a substantially more effective sterol source for strain GL7 than cycloartenol. This result is in keeping with the predominance of C-24 alkyl sterols (ergosterol) in normal yeast. The response of yeast is however in notable contrast to the results reported for Mycoplasma capricolum, where cycloartenol is superior to cyclolaudenol as the sterol source (4). In the procaryotic cell the relative preferences for

[2] Cycloeucalenol, a 4,14-cycloartenol derivative sustains growth of strain GL7, an observation consistent with but not proving the possibility that the active molecule is partially dealkylated cycloartenol.

the two cyclosterols are reversed. Furthermore, in model membranes and mycoplasma membranes cyclolaudenol raised the microviscosity only minimally, in contrast to cycloartenol. Thus, while the relative fluidity changes caused by various sterols in model membranes may parallel the sterol competence for some sterol requiring cells such as Mycoplasma, this relationship does not hold for the yeast mutant.

Finally, we wish to comment on the sterol specificity of the mutant strain GL7 as compared to that of other yeast mutants and wild-type yeast. Anaerobic wild-type yeast (9-11) and yeast sterol auxotrophs (20) have been reported to prefer ergosterol markedly over cholesterol as a sterol source. In contrast strain GL7 grows as well on cholesterol as on ergosterol aerobically (6) and almost as well (85%) anaerobically. Interestingly, Nes et. al. (11) report that wild-type yeast maintained anaerobically on cholesterol eventually adapts to grow to 80% of the cell yield obtained with ergosterol. Strain GL7 may have already acquired this adaptation, perhaps as a result of a spontaneous or secondary mutation. At any rate, the ability of strain GL7 to grow well on sterols other than ergosterol makes this mutant an ideal system for studying structure-function relationships in a eucaryotic cell.

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