Vol. 106, No. 2, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 31, 1982 Pages 435-441

A REQUIREMENT FOR ERGOSTEROL TO PERMIT GROWTH OF YEAST STEROL AUXOTROPHS ON CHOLESTANOL

RUSSELL J. RODRIGUEZ, FRED R. TAYLOR, and LEO W. PARKS

Department of Microbiology, Oregon State University
Corvallis, Oregon 97331-3804

Received April 12, 1982

SUMMARY: The ability of cholestanol (5α-cholestan-3β-ol) to support growth of two independently derived sterol auxotrophs, FY3 and GL7, has been examined. Growth on this stanol was precluded unless minute quantities of sterol were also available. Contaminating sterol in most cholestanol preparations or excess sterol in the inoculum used in growth studies could provide the required sterol in quantities capable of sustaining growth through an entire culture cycle. Evidence is presented for multiple functions of sterols in Saccharomyces cerevisiae.

The importance of sterol molecules in the maintenance of biological membranes has been the subject of much investigation (1,2,3). It is believed that the major role of sterols is as bulk membrane components which regulate fluidity. However,it has been suggested that sterols may also have a more specific function(s) (4). Evidence for this comes from work with Dermestes vulpinus (5) and Mycoplasma capricolum (4,6). In these studies a synergistic effect was observed between cholesterol and sitosterol, or cholesterol and lanosterol, a phenomenon referred to as a sparing effect. It is important to note that in each case cholesterol was not acting to initiate growth but rather to supplement growth. The yeast Saccharomyces cerevisiae has been used extensively to study the function(s) of sterols. Research has involved studying anaerobic growth of wild type cells in the presence of sterols (7), mutant strains blocked in the late stages of ergosterol biosynthesis (8,9), and sterol auxotrophs (10,11,12). To the best of our knowledge no data have been published demonstrating specific roles for sterols in yeast.

ABBREVIATIONS: HPLC, High performance liquid chromatography
TLC, Thin layer chromatography
GLC, Gas-liquid chromatography

0006-291X/82/100435-07$01.00/0

We have found that highly purified cholestanol does not support growth of the sterol auxotrophs FY3 and GL7, independently derived from Saccharomyces cerevisiae. These yeast can, however, be induced to grow on cholestanol by the addition of very small quantities of ergosterol. We have designated this phenomenon the "sparking" of growth.

MATERIALS AND METHODS. Yeast strains, media, and growth conditions. Saccharomyces cerevisiae strain FY3 was derived in this laboratory (10) and strain GL7 was obtained from Thomas Buttke (Mississippi Medical Center at Jackson). Growth medium was buffered with 50 mM potassium succinate to pH 5.5 and contained the following: 0.67% yeast nitrogen base (Difco), 2% dextrose; 0.002% of isoleucine, glutamate, serine, glutamine, histidine, tyrosine, and uracil; 0.003% of proline, glycine, alanine, and tryptophane; 0.004% of lysine, methionine, leucine, and adenine; 0.005% of valine and phenylalanine; 0.001% of aspartate and asparagine; and 0.015% of threonine and cysteine. For solid medium 1.5% agar was added. A mixture of palmitoleic acid-oleic acid (1:4, v/v) in tergitol-ethanol (1:1, v/v) was added to a final concentration of 100 µg/ml as a source of unsaturated fatty acid. Sterols were added in a mixture of tyloxapol-ethanol (1:1, v/v). In all experiments where cholestanol was used, it was added at 5 µg/ml. Inocula for growth experiments were derived by taking cells from single colonies and growing them in liquid medium containing cholesterol (10 µg/ml) to stationary phase. Cells from this culture were transferred to medium containing cholestanol and again grown to stationary phase. These cells were washed twice with medium devoid of sterol, resuspended to the same volume, and used as inocula (0.01 ml/5 ml). Incubations were done in a Scientific Industries Inc. temperature gradient incubator operated isothermally at 28-30°C with shaking. Growth was monitored with a Klett-Summerson spectrophotometer equipped with a green filter.

Purification of sterols. Ergosterol, cholesterol, cholestanol, and [4-^{3}H]cholestanol, prepared from [4-^{3}H]cholesterol by the method of Nace (13), were purified by HPLC using a preparative ultrasphere column (10 mm I.D. x 250 mm) and a solvent system previously described (14). Solvents were pumped through the column at 4 ml/min. Alternatively cholestanol was purified by argentation chromatography (15). Sterols were also purified by TLC in the solvent systems of Skipski and Barklay (16), and by recrystallization in hot methanol. Purity was determined by GLC and by HPLC (14,17).

Materials. Ergosterol, cholesterol, amino acids, nucleotide bases, and detergents were purchased from Sigma. Cholestanol was purchased from Applied Science, Sigma, and Cal-Biochem. Highly purified cholestanol was a generous gift from Dr. Henry Kircher. HPLC equipment was from Beckman. [4-^{3}H]cholesterol was from ICN. All reagents were from either Mallinckrodt or Amachem and were redistilled prior to use.

RESULTS AND DISCUSSION. Cholestanol supplementation. The sterol auxotrophs FY3 and GL7 have been used in a number of studies to determine the effect of different sterols on growth and cellular metabolism (10,11,12). In one of these studies (12) cholestanol was shown to support growth of GL7 to the same degree as cholesterol. We have found that, when highly purified, cholestanol is unable to support the growth of FY3 or GL7. Results presented

here indicate that the contradiction between these two studies is due to the purity of the cholestanol and to the sterol content of inoculum cells.

Cholestanol obtained from commercial sources was capable of supporting growth of FY3 and GL7 to the same degree as ergosterol and cholesterol, even after purification by TLC and recrystallization. However, when cholestanol was purified by HPLC or argentation chromatography it was unable to satisfy the sterol requirement of FY3 and GL7. Analysis of TLC purified cholestanol by GLC showed a single peak which co-eluted with cholesterol. HPLC analysis revealed that each sample of cholestanol, with the exception of that supplied by Dr. Henry W. Kircher, possessed a compound which absorbed ultraviolet light at 210 nm and eluted in a volume similar to cholesterol. Cholestanol does not absorb ultraviolet light at 210 nm and elutes in a much greater volume than cholesterol (elution volume determined with [^{3}H]cholestanol) (14). Both components of the cholestanol were isolated to purity by preparative HPLC, quantitated by GLC, and assayed for their ability to support growth of FY3. While FY3 was capable of growth on the compound which absorbed ultraviolet light, it was unable to grow on the purified cholestanol. HPLC or TLC (argentation) purified cholestanol was used for the remainder of this study.

In addition, we observed that FY3 or GL7 cells grown to stationary phase on ergosterol or cholesterol were able to grow when subcultured into medium containing cholestanol, regardless of whether or not the inoculum was washed free of residual sterol (Figure 1). When these cholestanol-grown cells were subinoculated into fresh medium containing cholestanol no growth was observed (Figure 1). It is important to note that cells transferred from a culture grown on ergosterol or cholesterol to medium devoid of sterol were unable to grow. One explanation for these observations is that when these auxotrophs are grown on ergosterol or cholesterol they accumulate excess sterol which is able to support growth in the presence of cholestanol but not in its absence. Since the excess sterol is a finite quantity it can only support a certain number of cell doublings. Therefore if the inoculum from a sterol grown culture is limited to a small number of cells, the number of doublings

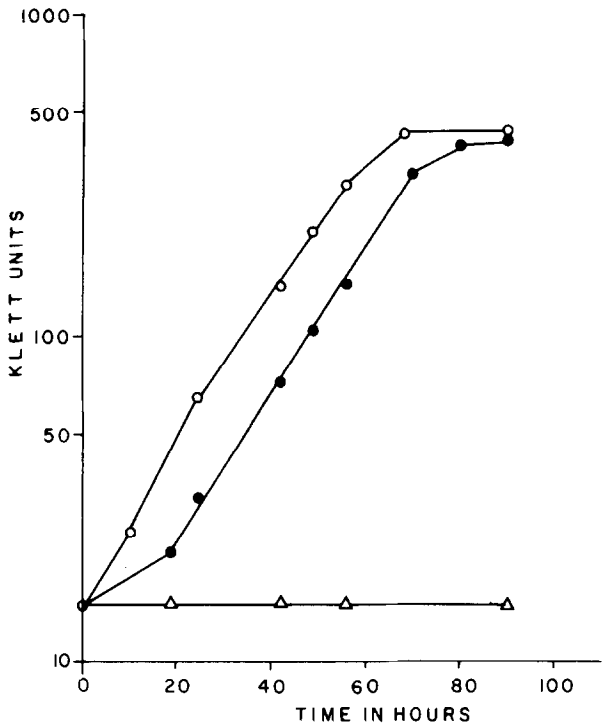

Figure 1). Growth of FY3 on ergosterol, 5 µg/ml, (○), subsequent transfer of ergosterol grown cells to medium containing cholestanol (●), and subinoculation of cholestanol-grown cells into medium containing cholestanol (△).

made possible by the residual sterol would be insufficient to increase culture turbidity. This was found to be the case when the inoculum consisted of 10^3 cells rather than 10^5 cells.

Sparking of growth on cholestanol by addition of ergosterol. The data suggesting that accumulated excess sterols may be responsible for growth of FY3 and GL7 on a stanol which by itself was non-supportive of growth led us to investigate a phenomenon we refer to as sparking of growth. An example of sparking is the ability of an auxotroph to grow on purified cholestanol when minute quantities of ergosterol are added. The low levels of ergosterol in themselves did not support growth. Figure 2 represents the determination of the minimal concentration of ergosterol capable of supporting growth of FY3. At ergosterol concentrations greater than 5 µg/ml, growth rates and cell yields were similar. However, at lower concentrations both parameters of growth diminished and at 10 ng/ml no increase in culture turbidity was

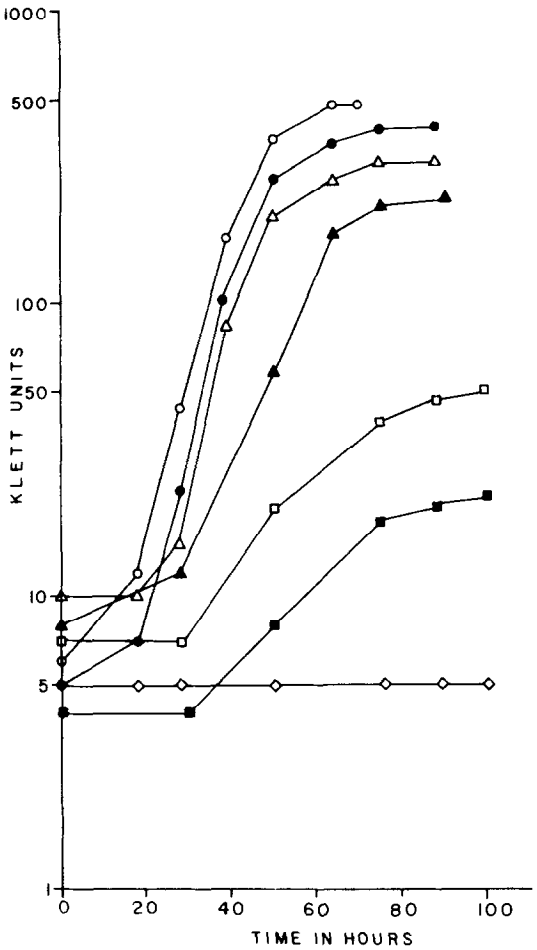

Figure 2). Growth of FY3 in medium containing the following concentrations of ergosterol: 5 and 10 µg/ml (0), 1 µg/ml (●), 0.5 µg/ml (Δ), 0.25 µg/ml (▲), 0.1 µg/ml (□), 0.05 µg/ml (■), and 0.01 µg/ml (◇).

detected. When cells were inoculated into media containing 100, 50 or 10 ng/ml of ergosterol and 5 µg/ml of cholestanol, growth rates and cell yields were equivalent to those of cultures grown with 5 µg/ml of ergosterol (Figure 3). The only difference between cultures grown on 5 µg/ml of ergosterol and those sparked to grow on cholestanol by ergosterol were increased lag times prior to the onset of growth. These lag times, which increased as the ergosterol concentration decreased, were due to an increase in the amount of cholestanol relative to ergosterol, causing a decrease in ergosterol availability. The fact that 10 ng/ml of ergosterol supports growth of FY3 only in the presence of cholestanol indicates that while cholestanol is unable to support growth by itself, it is capable of satisfying, in part, the sterol

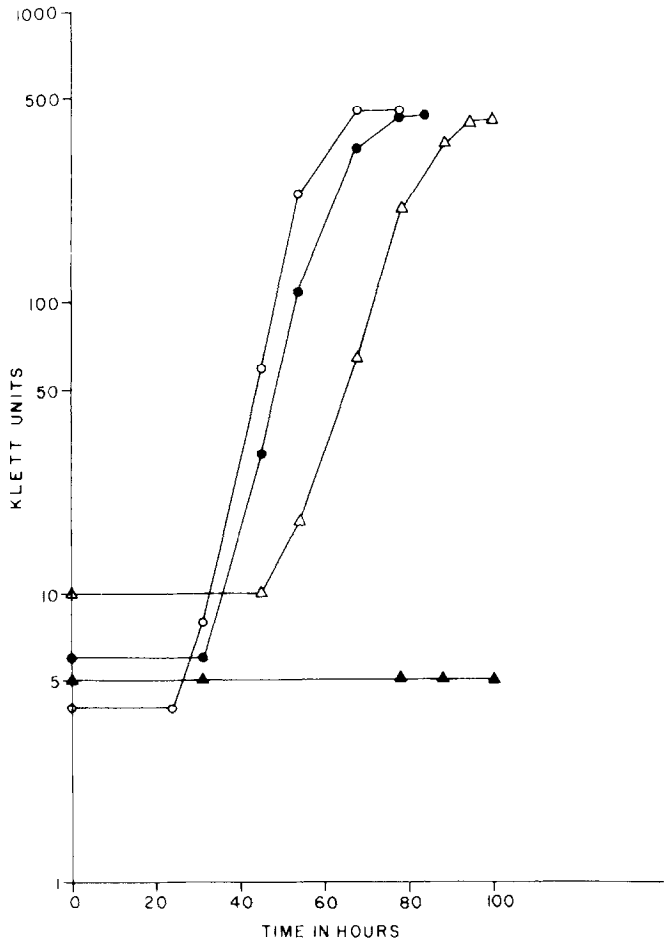

Figure 3). Sparking the growth of FY3 on cholestanol by 0.1 µg/ml (○), 0.05 µg/ml (●), and 0.01 µg/ml (△) of ergosterol. The ability of cholestanol to support growth is also shown (▲).

requirement. Also, since there is a low but definite level of ergosterol required for growth, it suggests that these supplements are satisfying different requirements. It is important to note that higher concentrations of ergosterol are capable of satisfying both classes of functions since cells can grow on it alone. It is conceivable to divide the "function" of sterols in yeast cells into two major classes, one which can be satisfied by cholestanol or ergosterol and another which cannot be satisfied by cholestanol. The function(s) satisfied by cholestanol or ergosterol are considered to involve bulk membrane properties such as fluidity. We suggest that the function(s) satisfied by ergosterol and not cholestanol are much more specific but have not yet been identified. We refer to this phenomenon as the "sparking" of

growth because it differs from previously reported observations (4). In our experiments ergosterol acts to initiate and maintain growth rather than modify growth. With this system it will now be possible to determine the effectiveness of other sterols and stanols in satisfying the requirements of each class of functions.

ACKNOWLEDGEMENTS

We wish to thank Connie Zook for typing this manuscript. This work was supported in part by Grant PCM-7827609 from the National Science Foundation and by Public Health Service Grant AM-05190 from the National Institutes of Health. R. J. Rodriguez was the recipient of an N. L. Tarter Fellowship. This is technical paper number 6330 of the Oregon Agricultural Experiment Station.

REFERENCES

1. Parks, L. W. (1978) Crit. Rev. Biochem. 6, 30-1341.
2. Demel, R. A. and Kruyff, B. D. (1976) Biochim. Biphys. Acta 457, 109-132.
3. Nes, W. R. (1974) Lipids 9, 596-615.
4. Dahl, J. S., Dahl, C. E. and Bloch, K. (1981) J. Biol. Chem. 256, 87-91.
5. Clark, A. J. and Bloch, K. (1959) J. Biol. Chem. 234, 2583-2588.
6. Dahl, J. S., Dahl, C. E. and Bloch, K. (1980) Biochem. 19, 1467-1472.
7. Nes, W. R., Sekula, B. C., Nes, W. D. and Adler, J. H. (1978) J. Biol. Chem. 253, 6218-6225.
8. McLean-Bowen, C. A. and Parks, L. W. (1981) J. Bacteriol. 143, 1325-1333.
9. Thompson, E. D. and Parks, L. W. (1974) Biochem. Biophys. Res. Commun. 57, 1207-1213.
10. Taylor, F. R. and Parks, L. W. (1980) Biochem. Biophys. Res. Commun. 95, 1437-1445.
11. Taylor, F. R. and Parks, L. W. (1981) J. Biol. Chem. 256, 13048-13054.
12. Buttke, T. M. and Bloch, K. (1981) Biochem. 20, 3267-3272.
13. Nace, H. R. (1951) J. Amer. Chem. Soc. 73, 2379.
14. Rodriguez, R. J. and Parks, L. W. (1982) Anal. Biochem. 119, 200-204.
15. Kircher, H. W. (1974) Lipids 9, 625.
16. Skipski, G. P., Smolowe, A. F., Sullivan, R. C. and Barklay, M. (1965) Biochim. Biophys. Acta 106, 386-396.
17. Neal, W. D. and Parks, L. W. (1977) J. Bacteriol. 129, 1375-1378.