DISCRIMINATION BETWEEN CHOLESTEROL AND ERGOSTEROL BY YEAST MEMBRANES

by

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Received June 25, 1976

Summary

The typical sterol of animal membranes (cholesterol) failed to support normal growth of yeast under anaerobic conditions when compared to the growth induced by the organism's natural sterol (ergosterol). This pathology was evident in strongly reduced numbers of cells, failure of budded cells to separate, and premature death. This study demonstrates that one or all of the $\Delta^{5,7}$-, Δ^{22}-, and 24ß-methyl groupings present in ergosterol are functionally significant.

Introduction

Substantial evidence indicates that sterols play their principal role as architectural components of membranes[1]. Regulation of sterol biosynthesis is also known to be highly dependent on phylogenetics. For instance, in the animal kingdom reduction of $\Delta^{5,7,24}$-sterols occurs to give cholesterol as the dominant and functional end-product of the pathway. On the other hand, in Ascomycetes and Basidiomycetes the $\Delta^{5,7}$-diene system is not reduced, the Δ^{24}-bond is methylated, and a Δ^{22}-bond is introduced giving ergosterol which is the 7,22-bisdehydro-24ß-methyl derivative of the animal sterol. Such biosynthetic differences could be entirely fortuitous having originally arisen by random mutation and then transmitted without change through succeeding generations, but it would be necessary to assume a low correlation between the structure of the sterol and its membraneous function. In agreement with this

[1] For a review of the evidence in biology as a whole see (1). The specific role of sterols in fungi as constituents of membranes is supported by the isolation of ergosterol from the mitochondria of Neurospora crassa (2) and the isolation of 24-dehydroergosterol from the protoplasmic membrane (3) of a yeast strain, Saccharomyces cerevisiae N.C.Y.C. 366, containing 24-dehydroergosterol as the dominant sterol (4).

explanation are the suppositions in the literature that yeast lacks a high degree of structural specificity for sterol and that different sterols either support anaerobic growth maximally or not at all[2]. In order to clarify this question we have made a quantitative study of the effects of cholesterol and ergosterol on the growth of yeast. Substantial differences favoring ergosterol were observed[3]. This indicates there is actually a functional significance to one or all of the $\Delta^{5,7}$-, Δ^{22}-, and 24β-methyl groups which exist in the natural sterol of yeast and by inference, the information supports a causal relationship between function and biosynthetic events.

Materials and Methods

A wild type diploid Saccharomyces cerevisiae (ATCC 18790) was cultured under anaerobic conditions (which prevents sterol biosynthesis) in a synthetically compounded yeast nitrogen base described by Andreasen and Stier (9) which was modified by the inclusion of only the following vitamins: biotin, calcium pantothenate, nicotinamide, pyridoxine-HCl and thiamine-HCl. Sterols (20 mg/l) were added in a suspension of Tween 80 (15 ml/l) which provided oleic acid. Growth was measured microscopically as well as through the use of a Coulter Counter, Model TA_{II}, equipped with a population accessory.

Results and Discussion

When grown on ergosterol, yeast increased in numbers rapidly reaching a stationary phase of ca. 100 million cells/ml in three days from an inoculum equivalent to 100 thousand cells/ml (Fig. 1A). Nearly all of the cells at stationary phase were single entities, while in the early phase of growth

[2] The idea of an "all-or-nothing" response originated with J. W. Proudlock et al. (5) who found that various sterols were either "active" or "inactive" in their ability to support anaerobic growth of yeast. Subsequently, viable aerobic mutants with altered sterol pathways, e.g., deletion of the 24-methyl transferase, were obtained (6,7). In addition ergosterol requiring mutants are known, and in one case it is reported, though without publication of data, that, since stigmasterol, sitosterol, and cholesterol also support growth, there is no stringent requirement for ergosterol (8).

[3] Our results are anticipated by the exploratory experiments of Andreasen and Stier (9) who state without further detail that cholesterol, while supporting growth of anaerobic yeast, did so "to a lesser extent than ergosterol". Structural effects have also been observed more recently (10) which demonstrate a definite decrease in both optimal and permissive growth temperatures of a yeast mutant when ergosterol was replaced by $\Delta^{8(9),22}$-ergostadien-3β-ol. In addition, the sterol substitution affected the activity of membrane-bound enzymes. For related physical evidence see (11,12).

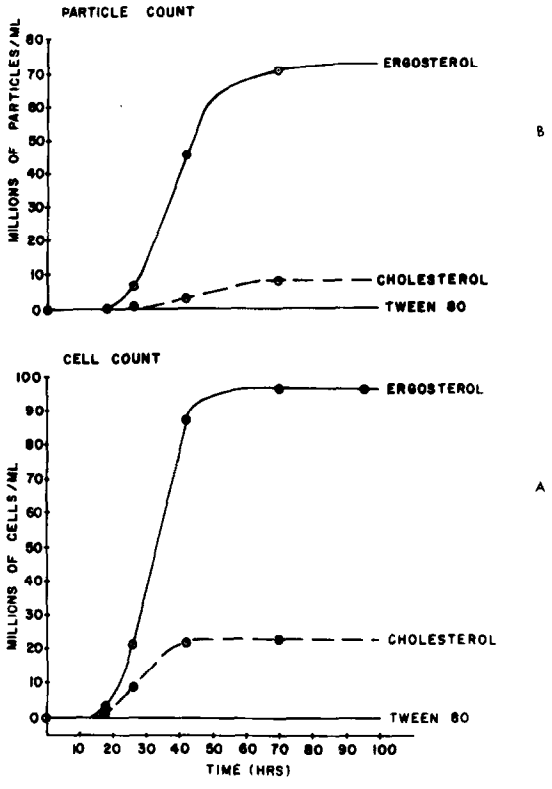

Fig. 1. Growth curves for anaerobically grown yeast in the presence of the sterols (20 mg/l) as indicated, Tween-80 (15 ml/l) was present in all cases. The curves indicated by "Tween-80" lacked sterol. "A" is a set of curves derived from microscopic counts of live cells which were separate and included buds one half or more as large as the parent. "B" is a set of curves constructed from Coulter Counter data for all particles with diameters greater than 3.2 μm.

budded and incompletely separated cells (aggregates) were present microscopically. The precise distribution at stationary phase is shown in Figures 2A and 2C which are derived from use of a Coulter Counter. The mode of the population was at a cell diameter of 6 μm and distribution of the population around the mode occurred in an approximately normal fashion.

When cholesterol was substituted for ergosterol, growth of individual cells was in fact induced, i.e., budding took place. However, as growth proceeded many of the daughter cells failed to mature and separate from the parent. This resulted in pseudomycelia, i.e., aggregates of from two to about five cells

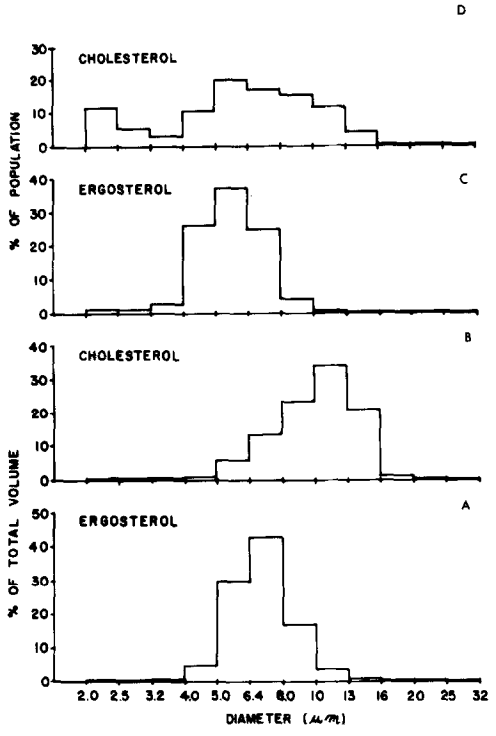

Fig. 2. Distributions of yeast particle sizes in the absence of oxygen and the presence of the sterols indicated at a conc. of 20 mg/l and of Tween-80 (15 ml/l). The diameter is that of a sphere with the volume of the particle measured. The "% of Total Volume" is the sum of volumes represented by particles with the various ranges of diameters shown divided by the total volume of all particles (A,B). The "% of Population" is the number of particles having volumes which correspond to the ranges of diameters shown divided by the total number of particles (C,D).

usually arranged linearly but occasionally in clumps (Fig. 3). Each succeeding bud tended to be smaller than the one preceding it in the line, and the juncture between them was often so substantial that in many there was little invagination. In a few cases they were almost rod-shaped. As time went on, the extent of budding increased without a subsequent increase in separation. This led to an increase in cell numbers (Fig. 1A) without a quantitatively parallel increase in particle (aggregate) numbers (Fig. 1B). The ability to bud was not, however, consistent with normal cell longevity, for an increasing number of apparently dead cells (usually opaque) was observed under phase con-

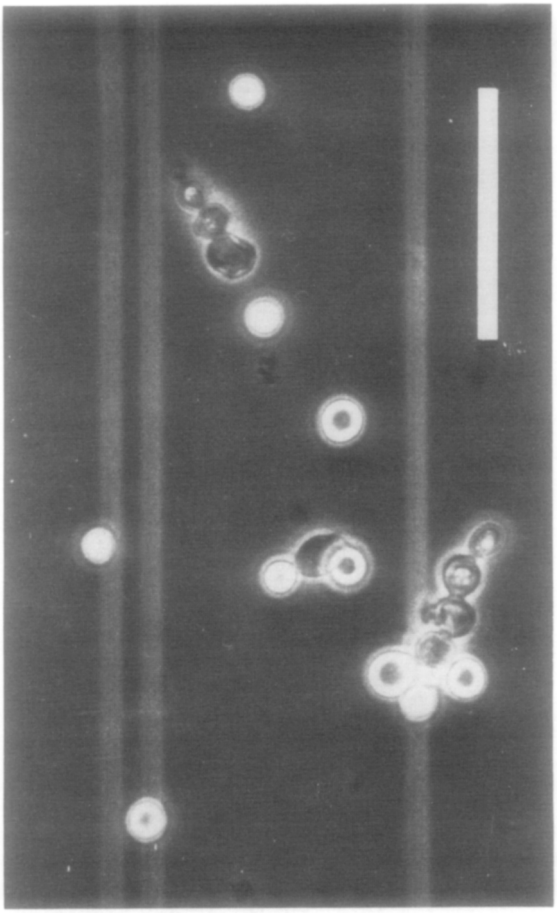

Fig. 3. A phase contrast photomicrograph of anaerobically grown yeast cells in the presence of cholesterol (20 mg/l) and Tween-80 (15 ml/l) after 72 hrs. The white bar is 20 μm in length. Cultures grown on ergosterol for the same period of time were composed only of individual cells (such as the five simple ones shown in this photomicrograph) and a few budded cells but lacked any of the aggregates or dead cells shown here.

trast microscopy as time proceeded (Fig. 3). The incidence of dead cells was greatest among aggregates. The cholesterol-induced pathology was also reflected in the visual count of live cells (brightly transparent with dark organelles) which only rose to ca. 25 million cells/ml after three days (Fig. 1A). Daughter cells one half or more as large as the parent, whether or not separated, were counted as individual cells. The population of unsep-

arated aggregates was of course much less than of the individual cells and on the average there were about 3 to an aggregate which would give a peak population of about 8 million aggregates/ml. The experimental counts (Fig. 1B) agreed with this, and the number found represents only 12% of the aggregates or particles which resulted from growth-support by ergosterol (Fig. 1B). The cholesterol-induced aggregation is further seen in the abnormal size-distribution of particles (Fig. 2D). While the mode is the same as with ergosterol-grown cells, the incidence of very much smaller and larger particles is dramatically increased, and the distribution is biphasic. A peak at ca. 2.25 μm, absent in the ergosterol-grown cells, we tentatively associate with small, dark particles seen microscopically which lack obvious cellular structure and probably represent fragmentation of dead cells. The increase in large particles, which correlates with the visual observation (Fig. 3), can be seen more clearly when the percentage of the total volume is plotted against the diameter, because the contribution of a large particle to the percentage of the total volume will be greater than that of a smaller particle due to the geometric relationship ($V=4/3\ \pi r^3$) of the radius to volume. In the cholesterol-grown cells (Fig. 2B) the mode of the volume is at 11 μm while in the ergosterol-grown cells (Fig. 2A) it is at 7 μm.

The results unequivocally show that yeast membranes can discriminate between cholesterol and ergosterol and indicate the inclusion of the $\Delta^{5,7}$-diene system, Δ^{22}-bond and 24β-methyl group in the organism's natural sterol constitute a fine-tuning of the structure-function relationship.

Acknowledgement

Supported by NIH Grants Nos. AM-12172 and GM-20526. We are indebted to Dr. L. W. Parks for advice on methods of growing yeast anaerobically and for stimulating theoretical conversations.

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