The Effects of Varied Energy Source and Detergent on the Growth of Sterol Mutants of *Saccharomyces cerevisiae*

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The effects of altered membrane sterol composition on the growth characteristics of sterol mutants of *Saccharomyces cerevisiae* were determined using various energy sources and the detergent Tergitol. These mutants do not synthesize the end-product sterol, ergosterol, but do not require exogenous sterol for growth. The sterol biosynthetic intermediates that are incorporated into the mutant membranes are related to alterations in growth characteristics. The C-24 transmethylation step (*erg6*) was shown to be critical to membrane integrity. Cells with this lesion were protected by the presence of Tergitol or glycerol in the medium. A double mutant (*erg6/2*), containing the $\Delta^8 \rightarrow \Delta^7$ isomerization lesion (*erg2*) and the C-24 transmethylation lesion (*erg6*), was highly sensitive to ethanol and Tergitol. These results corroborate permeability and membrane fluidity studies indicating that C₂₇ sterols are much less efficient than C₂₈ sterols in maintaining normal membrane structure and function.

INTRODUCTION

Sterols have been identified as important components of biological membranes influencing critical membrane functions such as transport and the activity of membrane-bound enzymes (Demel & de Kruffy, 1976; Cobon & Haslam, 1973; de Kruffy *et al.*, 1972). The precise manner in which sterols interact with proteins and other lipids within the membrane is not well understood. Electron spin resonance (e.s.r.) investigations using spin-labelled fatty acids which intercalate into the membrane have provided some information concerning these interactions (Berliner, 1976; Henry & Keith, 1971; Rottem *et al.*, 1970; Hsia *et al.*, 1971). For example, in model membrane systems cholesterol was found to have a condensing effect on lipid bilayers in the liquid crystalline state while having a liquefying effect on bilayers in the gel or crystalline state (Hsia *et al.*, 1971; Demel & de Kruffy, 1976). Extensive studies in this area using biological membranes have been hampered by the fact that biological membranes are scrupulously regulated in their lipid content (de Kruffy, 1975). This makes sterol substitution and the adjustment of membrane sterol concentration impossible in most systems. Thus, studies have been restricted to membranes such as those from guinea-pig erythrocytes (Kores *et al.*, 1972) and *Mycoplasma mycoides* (Rottem *et al.*, 1970; Rottem *et al.*, 1973) where variable sterol composition is possible.

Sterol mutants of *Saccharomyces cerevisiae* offer several advantages for evaluating the contribution of sterols to the structure and function of biological membranes. These mutants are unable to synthesize ergosterol, the end-product sterol, but require no exogenous sterol for growth. The altered sterol compositions of these mutants have been extensively characterized biochemically and genetically (Bard *et al.*, 1977; Barton *et al.*,

Table 1. *Presumptive enzyme defects and principal sterol intermediates accumulated in wild-type and sterol mutants of S. cerevisiae*

Strain	Blocked biosynthetic step	Sterol intermediates accumulated
A184D (wild-type)		Ergosterol (ergosta-5,7,22-trien-3 β -ol) Zymosterol (cholesta-8,24-dien-3 β -ol)
<i>erg2</i>	$\Delta^8 \rightarrow \Delta^7$ isomerization	Ergosta-8-en-3 β -ol Ergosta-8,22-dien-3 β -ol Ergosta-5,8,22-trien-3 β -ol
<i>erg3</i>	5(6) desaturation	Ergosta-7,22-dien-3 β -ol Ergosta-8,22-dien-3 β -ol Ergosta-7,22,24(28)-trien-3 β -ol Ergosta-8,22,24(28)-trien-3 β -ol
<i>erg5</i>	22(23) desaturation	Ergosta-5,7-dien-3 β -ol Zymosterol Ergosta-7,24(28)-dien-3 β -ol
<i>erg6</i>	C-24 transmethylation	Zymosterol Cholesta-5,7,22,24-tetraen-3 β -ol
<i>erg6/2</i>	$\Delta^8 \rightarrow \Delta^7$ isomerization and C-24 transmethylation	Zymosterol

1974; Barton *et al.*, 1975; Molzhan & Woods, 1972). The enzymic step in ergosterol biosynthesis that is presumed blocked and the sterol intermediates that are accumulated and incorporated into the membranes of each strain have been determined (Table 1). Using e.s.r. techniques the substitution of sterol intermediates for ergosterol has been shown (Kleinhans *et al.*, 1979) to alter radically the permeability of one sterol mutant to nickel. In addition, several sterol mutants have been shown to have altered permeability characteristics to various mono-, di- and trivalent cations and to crystal violet dye (Bard *et al.*, 1978). Subsequent e.s.r. investigations have correlated substituted sterol intermediates with changes in membrane fluidity (Lees *et al.*, 1979).

The mutants used in this study are of importance since they were originally selected on the basis of their resistance to the polyene antibiotic nystatin. Very little evidence has appeared concerning the physiological effects of altered sterol composition in this or other systems. One such report (Thompson & Parks, 1974) indicated that a sterol mutant, *nys3*, of *S. cerevisiae* (allelic to the *erg3* mutant in this report) showed decreased fermentative growth and greatly decreased growth on ethanol (aerobic growth). Another report (Pierce *et al.*, 1978), using nystatin-resistant mutants of *Candida albicans*, indicated that the most highly resistant mutants had decreased growth rates and growth yields when compared with the wild-type strain. In this report differences in growth rate and growth yield among sterol mutants of *S. cerevisiae* grown on various energy sources and the effects of the non-ionic detergent Tergitol are reported.

METHODS

Organisms. The sterol mutants used in this investigation are derived from the wild-type strain of *Saccharomyces cerevisiae*, A184D, and have been described elsewhere (Bard *et al.*, 1977; Barton *et al.*, 1974; Barton *et al.*, 1975; Molzhan & Woods, 1972). Mutant *erg2* is blocked at the $\Delta^8 \rightarrow \Delta^7$ isomerization step, mutant *erg3* is blocked at the 5(6) desaturation step, mutant *erg5* is unable to desaturate the 22(23) position, and mutant *erg6* is unable to perform the C-24 transmethylation step in ergosterol biosynthesis (Table 1). Mutant *erg6/2* is a double mutant obtained by genetically combining the single mutants *erg6* and *erg2* into the same strain.

Media. Solid media consisted of 1% (w/v) Difco yeast extract, 2% (w/v) Bacto-peptone, 2% (w/v) glucose and 2% (w/v) agar (YEP/glucose agar). For some media, 2% (v/v) ethanol (YEP/ethanol) or 3% (v/v) glycerol (YEP/glycerol) was substituted for glucose; in others, energy sources were combined at the above concentrations. The non-ionic detergent Tergitol NP-40 (Sigma) was added at 1.5% (v/v) where indicated.

Determination of mean generation times (m.g.t.). Cells grown aerobically overnight on YEP/glucose agar

at 30 °C were inoculated into YEP/glucose broth. After overnight growth at 30 °C samples were removed and centrifuged (5000g). The cells were washed once in the medium to be tested and suspended in 20 ml of the same medium in a 250 ml nephelometer flask. Growth at 30 °C was monitored turbidimetrically using a Klett-Summerson colorimeter with a no. 66 red filter (640 to 700 nm). Sample sizes were adjusted to yield an initial absorbance of 0.04 to 0.06. Generation times were calculated from readings taken during the exponential phase (absorbance of 0.1 to 0.3). Growth of all liquid cultures was carried out in a rotary shaker.

Determination of growth yields. Cells were grown in an identical manner to that described for m.g.t. determination. The final suspension of washed cells was made in 250 ml Erlenmeyer flasks containing 40 ml of the medium to be tested. Sample sizes were adjusted to yield an initial absorbance of 0.04. The flasks were incubated at 30 °C for 72 h, and then the samples were harvested by centrifuging (5000g) and dried for 48 h at 62 °C. The dry weights were determined for each sample.

RESULTS AND DISCUSSION

The results (Table 2) presented in this report indicate the relative physiological effects of various sterol substitutions on growth rate (mean generation time) and growth yield. Mutants *erg2* and *erg5* grown on glucose (primarily fermentative growth) showed essentially no difference in rate or yield compared with the wild-type. Mutant *erg3* had a slightly decreased growth rate but a similar growth yield to the wild-type. The significantly decreased growth rates of mutants *erg6* and *erg6/2* indicated that membranes which have primarily C₂₇ sterols rather than C₂₈ sterols (Table 1) are deficient in their ability to grow under these conditions. However, growth yields indicated that mutant *erg6/2* was able to grow for a longer period than *erg6* under these conditions.

A previous investigation (Parks *et al.*, 1978) has indicated that many sterol mutants are also deficient in their ability to grow on non-fermentable carbon sources. Mutants *erg6* and *erg6/2* were unable to grow on ethanol while the other mutants and the wild-type showed greatly decreased growth rates but only slightly lower growth yields. This indicated that these strains could continue to grow on ethanol for an extended period of time. The ability to utilize glucose in the presence of ethanol was tested in order to establish whether the inability of mutants *erg6* and *erg6/2* to respire ethanol was due to respiratory deficiency or to sensitivity to ethanol, or to a combination of both factors. Fermentative growth in the presence of ethanol (YEP/glucose/ethanol) indicated that mutant *erg6* was unaffected by ethanol, its growth rate and growth yield being equal to those shown on glucose alone. The growth rates and growth yields for the wild-type and mutants *erg2* and *erg5* also remained unchanged. Mutant *erg6/2* showed a significantly lower growth rate and growth yield on YEP/glucose/ethanol, indicating a sensitivity to ethanol coupled with a decreased ability or inability to metabolize ethanol.

The effect of the non-ionic detergent Tergitol was tested since other types of yeast membrane mutants require detergent for growth or have noticeable increases or decreases in growth capacity in the presence of detergents (M. Bard, unpublished results). Tergitol was chosen since it would not serve as a metabolic substrate or a source of fatty acids. Thus, the effects here would be limited to stabilization or destabilization of membranes with varying sterol content. All the strains tested showed decreased growth yields in the presence of Tergitol, with the exception of mutant *erg6* which showed a significantly increased growth rate and growth yield. The wild-type and mutant *erg2* showed no change in growth rate while the growth rates of the other three mutants were substantially decreased. The decreased growth yields noted for the wild-type and four of the mutants might have been due to a synergistic effect of Tergitol plus the ethanol that accumulated as the result of glucose fermentation. This appeared to be the case for mutant *erg3* and the wild-type and, to a lesser extent, for mutants *erg2* and *erg6/2* since growth yields were less on YEP/glucose/ethanol/Tergitol than on YEP/glucose/Tergitol or YEP/glucose/ethanol. Growth of mutant *erg6* on YEP/glucose/ethanol/Tergitol was equal to that on YEP/glucose/Tergitol, again indicating that this strain is insensitive to ethanol and has a stabilized membrane structure in the presence of Tergitol.

Table 2. *Mean generation times and growth yields of yeast sterol mutants grown on various energy sources and in the presence of Tergitol*

Mean generation times (m.g.t.) are expressed in min. Growth yields are expressed as mg dry wt ml⁻¹ after 72 h growth at 30 °C. For details of media, see Methods. Each result is the mean value $\pm$ standard error for three determinations, unless specified otherwise in parentheses.

Strain	YEP/glucose		YEP/ethanol		YEP/glucose/ethanol	
	m.g.t.	Yield	m.g.t.	Yield	m.g.t.	Yield
<i>erg2</i>	104 $\pm$ 4 (6)	7.6 $\pm$ 0.2	358 $\pm$ 25 (7)	6.0 $\pm$ 0.3	116 $\pm$ 4	7.4 $\pm$ 0.4
<i>erg3</i>	115 $\pm$ 6 (7)	7.9 $\pm$ 0.2	368 $\pm$ 24 (5)	5.8 $\pm$ 0.2	148 $\pm$ 8	7.5 $\pm$ 0.1
<i>erg5</i>	99 $\pm$ 8 (4)	6.7 $\pm$ 0.1 (5)	435 $\pm$ 24 (5)	5.5 $\pm$ 0.2	101 $\pm$ 4	7.1 $\pm$ 0.6
<i>erg6</i>	158 $\pm$ 2 (6)	1.9 $\pm$ 0.1 (6)	no growth	0.1 $\pm$ 0.02	168 $\pm$ 2	1.8 $\pm$ 0.1 (6)
<i>erg6/2</i>	151 $\pm$ 6 (5)	4.8 $\pm$ 0.1	no growth	0.6 $\pm$ 0.2	202 $\pm$ 10 (5)	1.4 $\pm$ 0.1 (4)
A184D (wild-type)	97 $\pm$ 3 (4)	7.9 $\pm$ 0.2	296 $\pm$ 35 (5)	5.8 $\pm$ 0.5	100 $\pm$ 5	8.0 $\pm$ 0.5
Strain	YEP/glucose/Tergitol		YEP/glucose/ethanol/Tergitol		YEP/glucose/glycerol	
	m.g.t.	Yield	m.g.t.	Yield	m.g.t.	Yield
<i>erg2</i>	102 $\pm$ 3	3.1 $\pm$ 0.1	144 $\pm$ 7 (4)	2.2 $\pm$ 0.4	112 $\pm$ 7	7.8 $\pm$ 0.2
<i>erg3</i>	179 $\pm$ 7	4.0 $\pm$ 0.2	174 $\pm$ 6	2.6 $\pm$ 0.1	108 $\pm$ 2	8.0 $\pm$ 0.1
<i>erg5</i>	121 $\pm$ 10 (4)	1.7 $\pm$ 0.1	137 $\pm$ 5	1.5 $\pm$ 0.1	128 $\pm$ 8 (4)	7.3 $\pm$ 0.1
<i>erg6</i>	135 $\pm$ 3	3.2 $\pm$ 0.1	129 $\pm$ 2	2.9 $\pm$ 0.1	137 $\pm$ 3	2.8 $\pm$ 0.1
<i>erg6/2</i>	270 $\pm$ 5	1.3 $\pm$ 0.4	443 $\pm$ 41 (6)	0.8 $\pm$ 0.1	167 $\pm$ 6	5.4 $\pm$ 0.4 (5)
A184D (wild-type)	90 $\pm$ 3	4.3 $\pm$ 0.2	123 $\pm$ 3	3.0 $\pm$ 0.03	96 $\pm$ 1	8.1 $\pm$ 0.2

A second non-fermentable energy source which would not be expected to have an adverse effect on membrane structure or function was tested. Preliminary studies showed that mutants *erg6* and *erg6/2* could not grow on glycerol and the other strains had a greatly decreased capacity to utilize it. On glucose plus glycerol medium, the growth rates and yields for mutants *erg2*, *erg3*, *erg6/2* and the wild-type were equal to those obtained on glucose medium. Mutant *erg6* was protected by the glycerol in YEP/glucose/glycerol medium, showing an increased growth rate and growth yield. However, mutant *erg5* showed a decreased growth rate but the same yield as found with YEP/glucose. This result remains unexplained.

With the exception of an overall decrease in the ability to grow on non-fermentable energy sources and the adverse synergistic effects of ethanol plus Tergitol, mutant *erg2* and the wild-type grew with media additives with the same efficiency as noted on YEP/glucose. Thus, the $\Delta^8 \rightarrow \Delta^7$ isomerization block, with the concomitant accumulation of sterol intermediates (Table 1), had little effect upon the growth properties of this strain. The 5(6) desaturation lesion of mutant *erg3* resulted in increased sensitivity to ethanol and Tergitol. Mutant *erg5* showed decreased growth with Tergitol but was relatively unaffected by ethanol. The C-24 transmethylation step (*erg6*) was of much greater consequence in terms of membrane integrity as the growth rate of this mutant on glucose was severely reduced and it was unable to grow on non-fermentable energy sources. Ethanol had no adverse effect on the membranes of mutant *erg6*, while Tergitol and glycerol stabilized them permitting faster and more extensive growth. The combination of the C-24 transmethylation block and the $\Delta^8 \rightarrow \Delta^7$ isomerization lesion in the same strain (mutant *erg6/2*) produced a synergistic effect. This strain showed increased sensitivity to ethanol and Tergitol individually and an extreme sensitivity to ethanol and Tergitol together. This increased sensitivity over that noted in mutant *erg6* can be related to the accumulation of zymosterol as the principal membrane sterol rather than the combination of zymosterol and cholesta-5,7,22,24-tetraen-3 β -ol found in mutant *erg6*.

Previous results have shown that mutants *erg6* and *erg6/2* are more sensitive to cations and dyes than other sterol mutants (Bard *et al.*, 1978) and that mutant *erg6/2* is more permeable to nickel than wild-type yeast (Kleinhans *et al.*, 1979). The present results corroborate the impaired membrane function indicated by these previous studies pointing out that the *erg6* lesion and the combination of the *erg6* and *erg2* mutations are particularly disruptive of normal membrane function. Preliminary results have indicated that mutants *erg6* and *erg6/2* show 50 % and 170 % increases in doubling times, respectively, when grown on YEP/glucose at 35 °C. The wild-type and mutants *erg2*, *erg3* and *erg5* showed no changes in growth rate at 35 °C. This investigation also corroborates results previously obtained which indicated that some sterol mutants are reduced in their capacity to grow aerobically (Thompson & Parks, 1974) and some sterol mutants show generally decreased growth rates (Pierce *et al.*, 1978). In this study growth rates and sensitivity to various medium additives can be related to specific membrane sterol composition.

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