The Effects of Catabolite Derepression on the Accumulation of Steryl Esters and the Activity of β -Hydroxymethylglutaryl-CoA Reductase in *Saccharomyces cerevisiae*

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The transition from the fermentative mode of growth on glucose to the oxidative mode of growth on ethanol by *Saccharomyces cerevisiae* is accompanied by many changes collectively known as catabolite derepression. Catabolite derepression caused a progressive increase in the total content of cellular sterols, amounting to a fourfold increase after 8 h; the additional sterols existed almost entirely as esters of monoenoic fatty acids. The increase in sterol content could be explained by the large increase in the activity of β -hydroxymethylglutaryl-CoA reductase (EC 1.1.1.34), but a secondary factor in sterol accumulation may have been the slower rate of cell growth during ethanol oxidation. Subsequent anaerobic growth of derepressed cells resulted in the hydrolysis of steryl esters to free sterols, and a progressive depletion of cellular sterol content.

INTRODUCTION

The phenomenon of catabolite repression in yeast is well known (for review, see Linnane & Haslam, 1970). Exponential growth of cultures in the presence of high concentrations of rapidly fermentable sugars, such as glucose, results in cells which contain low activities of many hydrolytic and oxidative enzymes whose synthesis is repressed; derepression occurs when the fermentable sugar is exhausted, or if the cells are grown on a non-fermentable substrate such as ethanol.

Starr & Parks (1962) found that the synthesis of total sterol was not subject to catabolite repression in a wild-type diploid strain of *Saccharomyces cerevisiae*. Thompson *et al.* (1974) later investigated the sterol C₂₄ methyltransferase (EC 2.1.1.41) of a haploid strain of yeast, and again concluded that the sterol synthetic pathway is not controlled by catabolite repression. However, other reports provided evidence that in certain strains of *S. cerevisiae* sterol accumulation is greater under conditions of derepression than in the repressed state (Gal'tsova & Lyapunova, 1968; Johnson *et al.*, 1972; Rogers *et al.*, 1974; Astin *et al.*, 1977).

This report covers investigations into the role of catabolite repression in the biosynthesis of yeast sterols, and the relationship between free and esterified sterols. The activity of β -hydroxymethylglutaryl-CoA reductase (mevalonate:NADP⁺ oxidoreductase; EC 1.1.1.34) increased during catabolite derepression and paralleled the total content of cellular sterols, suggesting that it regulates sterol biosynthesis.

METHODS

Growth media. Liquid growth media contained Difco yeast extract (10 g l^{-1}), bacteriological peptone (5 g l^{-1}), *Saccharomyces* salts (Wallace *et al.*, 1968) and a source of energy, either glucose (1 or 5 %, w/v) or ethanol (1 %, v/v). Media and sugar solutions were autoclaved separately at 0.1 MPa for 15 min; ethanol was added after autoclaving. Slopes of solid media for the maintenance of yeast cultures contained Difco yeast extract (10 g l^{-1}), bacteriological peptone (5 g l^{-1}), Difco agar (20 g l^{-1}), glucose (5 %, w/v) and *Saccharomyces* salts.

Yeast strain. A prototrophic brewery strain of *S. cerevisiae*, designated AB1, was used in these studies and was supplied by Allied Breweries (Production) Ltd, Burton-on-Trent.

Culture conditions. Cultures were grown aerobically at 28°C on a rotary shaker (180 rev. min^{-1}). Starter cultures were grown for 18 h in medium containing glucose (5 %, w/v); from this 10 mg dry wt cells were inoculated into 1 l glucose medium (1 %, w/v). Samples were taken for measurements of ethanol, glucose, dry weight and lipid analyses at intervals between 10 and 22 h.

For the analysis of β -hydroxymethylglutaryl-CoA reductase activity in cells grown under various degrees of catabolite repression, 10 mg dry wt cells were inoculated into each of the following media and grown aerobically with shaking: (a) for 18 h on glucose (5 %, w/v), (b) for 18 h on glucose (1 %, w/v) and (c) for 48 h on ethanol (1 %, v/v) plus glucose (0.1 %, w/v).

The effect of anaerobic growth on the composition of free sterols and steryl esters was investigated by anaerobic growth for 31 h in a modular fermentation system (LHE series 500, L. H. Engineering Co., Stoke Poges, Bucks) at 28°C with an agitation rate of 700 rev. min^{-1} and a nitrogen input rate of 200 ml min^{-1} . Trace amounts of oxygen were removed from the nitrogen by passage through a gas train containing reduced vanadium sulphate (Meites & Meites, 1948).

Lipid analyses. For the analysis of total sterol and fatty acid composition, whole cells (100 mg dry wt) were saponified with potassium hydroxide (40 %, w/v, final concentration) by refluxing at 100°C for 1.5 h. Free and esterified sterols were extracted from freeze-dried cells (100 mg dry wt) with dimethylsulphoxide (5 ml) by the method of Bailey & Parks (1975). Total sterols after saponification and free and esterified sterols of freeze-dried cells were extracted with $3 \times 30\text{ ml}$ petroleum ether (b.p. 40 to 60°C) and the extracts were combined and washed with distilled water (40 ml). Extracts were then dried over anhydrous sodium sulphate for 1 h, evaporated to dryness in a rotary evaporator at room temperature, dissolved in diethyl ether and transferred to stoppered tubes. Samples were evaporated to dryness in a stream of nitrogen and taken up in 1 ml of spectroscopically pure hexane for steryl ester analyses, or in 0.5 ml hexane for total sterol analyses. Total sterol analyses were performed by gas-liquid chromatography (g.l.c.) on a $1.5\text{ m} \times 6.4\text{ mm}$ glass column containing 1 % SE30 absorbed on Gas-Chrom Q (100 to 120 mesh) at 240°C .

Samples containing both free sterols and steryl esters were separated into their component classes by column chromatography on activated alumina (5 g) as described by Bailey & Parks (1975). Free sterols were then analysed by g.l.c. Steryl esters were hydrolysed by refluxing with potassium hydroxide (20 %, w/v) in 80 % ethanol for 1 h (Sorbus & Holmlund, 1976); sterols were then extracted as previously and analysed by g.l.c.

Both total and esterified fatty acids were extracted and analysed by g.l.c. (10 % polyethylene glycol adipate on Gas-Chrom W, 100 to 120 mesh, at 190°C) as described by Proudlock *et al.* (1971).

Lipids were quantified by adding the following compounds prior to saponification or extraction with dimethylsulphoxide. Cholestan- 3β -ol was added for quantification of total and free sterols; 5-cholesten- 3β -ol laurate was the internal standard for esterified sterols. Pentadecanoic or heptadecanoic acid was used to quantify the fatty acids.

The efficiency of alumina absorption chromatography was examined by thin-layer chromatography. This was carried out on $250 \times 250\text{ mm}$ plates using 0.25 mm thick silica gel G. Plates were prepared, activated and developed according to the dual solvent system described by Schlotzhauer *et al.* (1977). Lipids were detected by spraying with berberine (0.1 %, w/v, in methanol/acetone, 1:1, v/v) and visualized under ultraviolet light. Authentic pure sterols and steryl esters were run beside the fractions from the alumina column and it was confirmed that a complete separation of free sterols from steryl esters had been achieved.

Enzyme assays. β -Hydroxymethylglutaryl-CoA reductase activity was measured in cell extracts by a modification of the method of Qureshi *et al.* (1976). Harvested and washed cells (80 %, v/v) were suspended in potassium phosphate buffer (100 mM, pH 7.0) plus EDTA (1 mM) and dithiothreitol (1 mM). Precooled glass beads (0.45 to 0.55 mm) equivalent to the cell wet weight were added and the cells were disrupted at 0 to 4°C using a Braun homogenizer for $2 \times 30\text{ s}$ at $4000\text{ rev. min}^{-1}$.

The homogenate was incubated with Triton X-100 (0.5 %, v/v) for 60 min at 0°C then centrifuged at 1500 g for 2 min. The pellet (P_{1500}) was set aside and the supernatant was centrifuged at 8000 g for 10 min; the resultant supernatant (S_{8000}), pellet (P_{8000}) and pellet obtained from the initial centrifugation (P_{1500}) were

stored at -17°C prior to the assay of enzyme activity. Activity of β -hydroxymethylglutaryl-CoA reductase was more than 95 % of the initial activity after storage at -17°C for 5 months.

β -Hydroxymethylglutaryl-CoA reductase activity was determined spectrophotometrically by the rate of decrease in absorbance at 340 nm caused by the oxidation of NADPH. The reaction mixture contained, in 1 ml, dithiothreitol (5 mM), potassium phosphate buffer (pH 7.0, 100 mM), NADPH [150 μM (P-L Biochemicals), prepared in sodium carbonate buffer (100 mM, pH 10.6)], β -hydroxymethylglutaryl-CoA [150 μM (Sigma or Calbiochem), prepared in acetate buffer (200 mM, pH 4.6)] and Triton X-100 (0.5 %, v/v). Reactions were started by the addition of β -hydroxymethylglutaryl-CoA and the decrease in absorbance at 340 nm was measured using a Pye Unicam SP1800 double-beam spectrophotometer. Endogenous oxidation of NADPH was corrected for by initiating in parallel a control incubation lacking β -hydroxymethylglutaryl-CoA. All assays were in triplicate.

To test the possibility that the observed activities were affected by proteolysis, an homogenate derived from derepressed cells was also prepared in the presence of pepstatin (5 μM ; obtained from Dr R. Beynon, University of Liverpool) dissolved in 10 % (v/v) methanol (Saheki & Holzer, 1975), or phenylmethanesulphonyl fluoride (2 mM; Sigma) dissolved in 95 % (v/v) ethanol (Pringle, 1975).

Other analytical procedures. Protein was estimated by the method of Lowry *et al.* (1951), after the extract had been dialysed overnight against water to remove dithiothreitol. A calibration curve was prepared using bovine serum albumin (Calbiochem, Type V). Dry weights of cells were determined volumetrically by centrifuging a sample of cells in calibrated thick-walled graduated glass capillary tubes (Astin *et al.*, 1977). Ethanol was assayed using the g.l.c. method described by Cooper (1971). Glucose was assayed using the glucose oxidase method (Perid kit, Boehringer).

RESULTS

Preliminary investigations of a large number of brewing strains of *S. cerevisiae* (unpublished results) indicated the possibility that sterol synthesis is subject to catabolite repression because the total sterol content of many strains after growth on medium containing ethanol (1 %, w/v) was at least three times that found after growth on glucose (5 %, w/v). A representative strain, ABl, was chosen for the study.

Cells growing on medium containing glucose (1 %, w/v) exhibit diauxic behaviour with respect to growth and physiology (for review, see Linnane & Haslam, 1970). This provides a convenient system for the investigation of the transition between repressed and derepressed states. In the present work this technique was used to monitor cellular sterol and sterol ester contents throughout the transition. Figure 1 shows a typical experiment in which glucose (1 %, w/v) was first fermented to ethanol, allowing the initial rapid phase of growth, followed by a lag in growth and then a second slower growth phase in which the oxidation of ethanol provided energy.

Sterols began to accumulate from the time (14 h) at which glucose was exhausted and oxidative metabolism started. This established the relationship between catabolite derepression and sterol accumulation. As sterols in *S. cerevisiae* may be free or esterified (Johnson *et al.*, 1972; Bailey & Parks, 1975; Nurminen *et al.*, 1975; Hossack *et al.*, 1977), both forms were determined throughout the course of the experiment. The sterol ester component was responsible for the increase in total sterol, whereas the free sterol remained virtually unchanged throughout the experiment (Fig. 1).

Other workers have suggested that the component sterols of the two fractions differ; in particular, zymosterol is predominantly esterified and ergosterol is mainly in the free form (Nurminen *et al.*, 1975; Bailey & Parks, 1975; Hossack *et al.*, 1977). Results presented in Table 1 agree with these general conclusions, and show the response of individual sterols and their esters to derepression. Throughout the experiment, zymosterol was totally esterified and comprised 20 to 25 % of the total sterol esters. Ergosterol was the predominant free sterol; its percentage of the free sterols increased as the cells became derepressed, whereas its percentage of the esterified sterols decreased during derepression. 24:28-Dehydroergosterol was a major component of both free sterols and sterol esters; during derepression, its percentage of the free sterols decreased and its percentage of the esterified sterols

Fig. 1. Changes in the content of cellular sterols and steryl esters during diauxic growth on glucose medium. *Saccharomyces cerevisiae* strain AB1 was grown in medium containing glucose (initially 1%, w/v). At intervals, samples were withdrawn and cell dry weight ($\square$), the concentration of glucose ($\triangle$), the concentration of ethanol ($\blacksquare$) and the content of free sterols ($\circ$) and of steryl esters ($\bullet$) were measured (see Methods).

Table 1. Sterol composition of yeast cells during diauxic growth

Cells were grown as described in Fig. 1 and free sterols and steryl esters were determined as described in Methods. Catabolite derepression started at 14 h when glucose was exhausted. Episterol was detected in trace amounts [$<0.15 \mu\text{g (mg dry wt)}^{-1}$] in free sterols and steryl esters at 12 and 16 h, but was not detected at 20 and 22 h.

Sterol	Free sterol content [$\mu\text{g (mg dry wt)}^{-1}$] after incubation for:				Steryl ester content [$\mu\text{g (mg dry wt)}^{-1}$] after incubation for:			
	12 h	16 h	20 h	22 h	12 h	16 h	20 h	22 h
Zymosterol	0	0.25	0	0	0.75	2.74	5.78	5.42
Ergosterol	1.61	1.88	2.61	3.78	0.75	2.51	4.11	5.06
24:28-Dehydroergosterol	1.33	1.22	1.01	1.27	1.48	7.16	10.54	20.57
Fecosterol	0.49	0.53	0	0	0.43	1.54	0	0
Lanosterol	0.28	0.38	0.66	1.24	0.16	1.11	1.55	5.87
Total	3.71	4.26	4.28	6.29	3.57	15.06	21.98	36.92

increased. Fecosterol was a relatively minor component of both free sterols and steryl esters throughout the experiment. Lanosterol was also a minor component of the sterols except at the very beginning and towards the end of the experiment when its percentage of the total and, particularly, of the free sterols increased.

The fatty acid composition of steryl esters appears to resemble the fatty acid content of the whole yeast cell (Madyastha & Parks, 1969). Furthermore, fatty acid synthesis is subject to catabolite repression (Johnson *et al.*, 1972) as is the desaturase reaction (Brown & Johnson, 1970). Both the chain length and the degree of unsaturation of the fatty acid moieties of the steryl esters increased during derepression (Fig. 2).

As *S. cerevisiae* is incapable of sterol synthesis under anaerobic conditions (Andreason & Stier, 1953), the fate of the steryl esters in derepressed cells was determined during subsequent anaerobic growth. Table 2 shows that the steryl esters decreased under conditions of

Fig. 2. Changes in the fatty acid composition of steryl esters during diauxic growth on glucose medium. Cells were grown as in Fig. 1 and the fatty acid composition of the steryl esters was determined as described in Methods. The percentage of unsaturated fatty acids (○) and the ratio of fatty acids with carbon chain lengths equal to or greater than 16 to those fatty acids with less than 16 carbon atoms (●) are shown. The arrows indicate the time at which glucose was exhausted.

Table 2. *Sterol composition of derepressed yeast cells during subsequent anaerobic growth*

Cells were grown for 22 h on 1 % (w/v) glucose medium as described in Methods. After harvesting and resuspending in fresh 5 % (w/v) glucose medium, the cells were grown anaerobically and free sterols and steryl esters were determined as described in Methods.

Sterol	Free sterol content [$\mu\text{g (mg dry wt)}^{-1}$] after anaerobic incubation for:			Steryl ester content [$\mu\text{g (mg dry wt)}^{-1}$] after anaerobic incubation for:		
	0 h	22 h	31 h	0 h	22 h	31 h
Zymosterol	0	0	0	5.93	1.39	0.90
Ergosterol	4.00	3.39	2.08	6.72	0	0
24:28-Dehydroergosterol	2.09	1.21	0.81	13.84	0.73	0.57
Fecosterol	1.07	0	0	1.98	0	0
Lanosterol	1.62	2.02	0.96	2.21	0.82	0.53
Total	8.78	6.62	3.85	30.68	2.94	2.00

anaerobic growth, in agreement with the results of Hossack *et al.* (1977), Aries & Kirsop (1978) and Taketani *et al.* (1978). Significant quantities of two components with retention times greater than that of lanosterol were noted in the free sterol fraction after anaerobic growth, but no attempt was made to identify or quantify these unknown sterols. Zymosterol was not present in the free sterol fraction either before or after anaerobic growth, but ergosterol was found only as the free sterol after anaerobiosis.

Solubilization and assay of β -hydroxymethylglutaryl-CoA reductase

The possibility that homogenization had not completely revealed β -hydroxymethylglutaryl-CoA reductase activity in the extracts was investigated by treating fractions with the detergent Triton X-100. Triton X-100 disrupts membranes and forms mixed micelles containing detergent together with membrane proteins and lipids. The detergent binds to the membrane proteins by hydrophobic interactions, and during solubilization it maintains the correct orientation of an amphiphilic protein.

Preliminary studies showed that enzyme activity was enhanced not only by preincubation of the various fractions with Triton X-100, but that a higher specific activity was also obtained

Fig. 3. Effects of Triton X-100 on β -hydroxymethylglutaryl-CoA reductase activity. The cell-free supernatant fraction of yeast homogenate (S_{8000}) was prepared and assayed in the presence of different concentrations of Triton X-100 at 30 °C as described in Methods. The bars represent the range of specific activities and the points are the average of three determinations. Specific activities are expressed in nmol NADPH oxidized min⁻¹ (mg protein)⁻¹.

Table 3. *Effects of Triton X-100 on the β -hydroxymethylglutaryl-CoA reductase activity in different fractions of a yeast homogenate*

Yeast cells were grown for 20 h on glucose (1 %, w/v) medium, harvested, homogenized, incubated with or without Triton X-100 and then fractionated as described in Methods. β -Hydroxymethylglutaryl-CoA reductase activities were determined with or without Triton X-100 (0.5 %, v/v) in the assay medium. Activities are expressed as nmol NADPH oxidized min⁻¹.

Fraction	Triton X-100 in pre-incubation (% v/v)	No Triton X-100 in assay		Triton X-100 in assay	
		Activity (nmol min ⁻¹)	% of total activity	Activity (nmol min ⁻¹)	% of total activity
S_{8000}	0.5	5200	85.6	6298	83.8
P_{8000}	0.5	539	8.9	638	8.5
P_{1500}	0.5	337	5.5	584	7.7
Combined	0.5	6076	100	7520	100
S_{8000}	0	3651	74.6	5898	77.2
P_{8000}	0	797	16.3	1130	14.8
P_{1500}	0	444	9.1	608	8.0
Combined	0	4892	100	7636	100

if Triton X-100 was included in the assay incubation. The specific activity of the particulate S_{8000} fraction in the presence of different concentrations of Triton X-100 is shown in Fig. 3. Maximum activity was obtained in the presence of 0.5 % (v/v) Triton X-100. Moreover, there was only a 10 % loss of specific activity after storing for 27 h with Triton X-100 (0.5 %, v/v) at 0 °C.

The effect of Triton X-100 on the recovery of β -hydroxymethylglutaryl-CoA reductase activity in the supernatant fraction (S_{8000}), and the role of the detergent in solubilization is indicated in Table 3. Whole homogenate was divided into two and incubated for 1 h at 0 °C in the presence or absence of Triton X-100 (0.5 %, v/v), and then centrifuged according to the regime outlined in Methods. The activity of each fraction was determined with or without Triton X-100 (0.5 %, v/v) in the assay medium. Table 3 shows that preincubation with Triton X-100 resulted in a significant increase in the total measurable enzyme activity, particularly in the supernatant fraction (S_{8000}). Inclusion of Triton X-100 (0.5 %, v/v) in the assay medium increased the activity of all fractions, particularly when the detergent was absent during the preincubation. However, the total cumulative activity of all fractions

Table 4. *Effects of catabolite repression on the specific activity of β -hydroxymethylglutaryl-CoA reductase*

Cells were grown on media containing the carbon sources indicated for 18 h (media containing glucose) or 48 h (medium containing glucose and ethanol). The cells were harvested, homogenate was prepared and fractionated, and the β -hydroxymethylglutaryl-CoA reductase activities of the P_{1500} , S_{8000} and P_{8000} fractions were determined in the presence of Triton X-100 (0.5 %, w/v) during the preincubation and the assay as described in Methods. Specific activities are given as the arithmetic mean $\pm$ standard error for two separate preparations assayed in triplicate, and represent the total activities for the combined P_{1500} , P_{8000} and S_{8000} fractions. In all experiments more than 75 % of the total activity was associated with the S_{8000} fraction. The specific growth rates K (h^{-1}) were calculated from: $K = 2.303 (\log x_2 - \log x_1) / (t_2 - t_1)$, in which x_1 and x_2 are the cell concentrations at times (h) t_1 and t_2 , respectively.

Carbon source		Specific growth rate (h^{-1})	Specific activity [$nmol\ min^{-1}$ ($mg\ protein)^{-1}$]
Glucose (% w/v)	Ethanol (% v/v)		
5.0	0	0.336	13.2 ± 2.6
1.0	0	*	36.0 ± 9.7
0.1	1.0	0.113	105.0 ± 26.0

* Growth on glucose (1 %, w/v) was diauxic as in Fig. 1 and cells were harvested 6 h after the glucose was exhausted at which time partial catabolite repression had occurred.

was unaffected by the preincubation provided that Triton X-100 was present during the assay.

The possibility that the action of proteases may be creating artefacts was investigated by including protease inhibitors throughout the preparation procedure. The yeast slurry was divided into four and treated with (1) pepstatin, (2) phenylmethanesulphonyl fluoride, (3) pepstatin plus phenylmethanesulphonyl fluoride or (4) untreated (control). The slurries were then homogenized and treated with Triton X-100 (0.5 %, v/v) for 1 h at 0 °C. Treatment with phenylmethanesulphonyl fluoride and pepstatin was repeated every 2 h due to the hydrolysis of the former, the reversible nature of inhibition of the latter and because of the disruption of protease–polypeptide inhibitor complexes (Pringle, 1975). The homogenates were centrifuged and the supernatant (S_{8000}) was assayed in the presence of 0.5 % (v/v) Triton X-100. The analyses of enzyme activity showed no significant differences between the control and those samples treated with protease inhibitors.

The effect of different degrees of catabolite repression on the activity of β -hydroxymethylglutaryl-CoA reductase was investigated as follows. Cells of strain AB1 were grown in duplicate on media containing 5 % (w/v) glucose or 1 % (w/v) glucose or 1 % (v/v) ethanol plus 0.1 % (w/v) glucose. The yeast slurries were homogenized and incubated with Triton X-100, centrifuged to obtain the three fractions and analysed in the presence of Triton X-100 as already described. Table 4 shows that the activity of β -hydroxymethylglutaryl-CoA reductase was inversely related to the degree of catabolite repression and growth rate.

DISCUSSION

The present work indicates that catabolite derepression causes a large increase in the cellular content of steryl esters in a brewing strain of *S. cerevisiae*. Steryl esters also accumulate in response to respiratory adaptation (Nagai *et al.*, 1977; Taketani *et al.*, 1978) or during the stationary phase of the cell growth cycle in yeast (Bailey & Parks, 1975). Our results agree with those of Taketani *et al.* (1978) that the amount of free sterol remains almost unchanged even when the steryl ester content increases as much as fivefold.

The study of cell and model membranes indicates that the ratio of membrane sterol to phospholipid is maintained within narrow limits (Demel & De Kruffy, 1976), and it is

presumed that the free sterols in yeast are predominantly located in cell membranes. Steryl esters are not incorporated into cell membranes (Rouser *et al.*, 1971; Demel & De Kruffy, 1976) and their accumulation during catabolite derepression indicates that the total amount of sterol being produced is greater than that required for membrane assembly, and that the excess is esterified for storage. The suggestion by Nagai *et al.* (1977) that all yeast sterols can be esterified is in agreement with our results.

The present work also confirms that the steryl esters are rapidly converted back to free sterols when sterol synthesis is prevented by anaerobiosis, in agreement with the results of Taketani *et al.* (1978). An anaerobic yeast sterol esterase has been demonstrated by Wheeler & Rose (1973) which may be responsible for the conversion.

Zymosterol was not found in the free sterol fraction either during derepression, when large amounts of zymosteryl esters were present in the cell, or during anaerobiosis, when the zymosteryl esters were broken down. This suggests that zymosterol is not readily incorporated into cell membranes, but is either esterified or converted to fecosterol by the C₂₄ methyltransferase. The latter enzyme does not increase in activity during catabolite derepression (Thompson *et al.*, 1974), and the accumulation of zymosteryl esters and of free and esterified lanosterol suggests that under these conditions this enzyme limits the rate of fecosterol formation. Similarly, the very large amounts of 24:28-dehydroergosterol and its esters found in derepressed cells suggest that the activity of the 24:28 dehydrogenase also limits the formation of ergosterol. In contrast to the yeast strains studied by Aries & Kirsop (1978), strain AB1 did not contain detectable amounts of episterol at the end of catabolite derepression or during its subsequent anaerobic growth.

Bailey & Parks (1975) showed that zymosteryl esters are not methylated by the C₂₄ methyltransferase *in vitro*, and it seems likely that the other steryl esters are unavailable for direct biosynthetic modification. Under anaerobic conditions, the steryl esters appear to be converted to the free sterol, of which zymosterol and fecosterol are then completely converted to ergosterol and 24:28-dehydroergosterol, but lanosterol remains as such because of the absence of molecular oxygen.

The accumulation of sterols during catabolite derepression may be partly due to the slower growth rate of yeast during the secondary oxidative growth phase on ethanol. The reduction in growth rate on ethanol medium would allow the accumulation of sterols if the rates of sterol synthesis per mg dry weight of cells are maintained.

To quantify β -hydroxymethylglutaryl-CoA reductase it was necessary to use the detergent Triton X-100 to disrupt membranes and remove potential permeability barriers between the enzyme and substrates. It was also shown that Triton X-100 increased the β -hydroxymethylglutaryl-CoA reductase activity of the supernatant fraction; it is unlikely that this effect is solely due to the disruption of permeability barriers, and is presumably caused by a modification of the enzyme's microenvironment by the detergent.

Enzyme activities were compared in the presence of optimum concentrations of Triton X-100 (0.5 %, v/v). The activity in ethanol-grown cells was eight times higher than that of cells grown on 5 % (w/v) glucose, while cells grown on 1 % (w/v) glucose for 18 h, which were at the beginning of the second oxidative phase of diauxic growth, had an intermediate activity. Thus the activity of β -hydroxymethylglutaryl-CoA reductase is controlled by catabolite repression. Since the increase in β -hydroxymethylglutaryl-CoA reductase parallels the increase in total cellular sterol content, it is proposed that the enzyme is important in controlling sterol synthesis in yeast during catabolite derepression.

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