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EFFECT OF UNSATURATED FATTY ACIDS ON STEROL BIOSYNTHESIS IN YEAST

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Summary

Lipid-depleted yeast, grown anaerobically, contains only very low amounts of sterols. The hydroxymethylglutaryl-CoA reductase activity, the regulatory enzyme of sterol synthesis in yeast, is also low. Aeration of such cells in a buffer containing a carbon source induces hydroxymethylglutaryl-CoA reductase activity and increases sterol synthesis. The velocity of the increase depends on the carbon source present during the aeration period. Glucose and sugars that are easily converted to glucose were found to be most effective.

A supplement of unsaturated fatty acids during anaerobic growth causes a several-fold greater velocity of the enzyme induction and of sterol biosynthesis. Linolenic acid (30 μM) accelerated sterol biosynthesis about 7-fold. Activities of galactokinase and galactose-1-phosphate uridyltransferase, which are involved in the conversion of galactose to glucose, increased several-fold in the supplemented cells within 6 h of aeration, concomitantly with stimulation of sterol synthesis from galactose.

It is suggested that the stimulation of enzyme induction and sterol biosynthesis in fatty acid supplemented cells is due to a completion of the protein-synthesizing apparatus during cell growth. A markedly enhanced capacity of these cells to incorporate leucine into acid-precipitable protein supports this assumption.

Introduction

It is well known that in anaerobically grown yeast the syntheses of sterols, unsaturated fatty acids, cytochromes and other cell components are sup-

pressed and the cells are devoid of typical mitochondrial structures which are characteristic for aerobically grown cells (for a review see Ref. 1). When sterols or unsaturated fatty acids are provided during anaerobic growth, they will be readily incorporated into membranes [2,3] and the promitochondria have an extensively developed membrane system [4] and also a functional protein-synthesizing apparatus [5].

Aeration of anaerobically grown yeast cells results in the completion of typical mitochondrial functions without cell division [6] together with a drastic increase of the activity of enzymes which form the normal respiratory capacity of the cell [7,8]. Under the conditions of aeration the content of sterols in the cells also increases rapidly [9]. We have shown [10] that this rise of sterol formation is closely related to a rise of the activity of hydroxymethylglutaryl coenzyme A reductase, indicating a regulatory function of this enzyme in sterol biosynthesis not only in mammals [11] but also in yeast.

The development of functional organelles in the cell after transfer from anaerobic to aerobic conditions depends to a great extent on the carbon source and on the composition and quantity of lipids present during anaerobic growth. It has been shown that unsaturated fatty acids play an essential role in the biogenesis of these organelles, particularly mitochondria [12—17].

In this communication we report on the effects of various fatty acids on sterol biosynthesis, on the induction of hydroxymethylglutaryl-CoA reductase, galactokinase and galactose-1-phosphate uridyltransferase and on the protein-synthesizing capacity of yeast cells. A preliminary report of this work has appeared [18].

Materials and Methods

Chemicals. Hydroxymethyl[3-^{14}C]glutaryl-CoA (spec. act. 50 Ci/mol), D-[U-^{14}C]glucose (spec. act. 213 Ci/mol), and L-[1-^{14}C]leucine (spec. act. 50.6 Ci/mol) were obtained from New England Nuclear Corp., Boston, MA. Tergitol NP 40, hydroxymethylglutaric acid, mevalonolactone, fatty acids, and hydroxymethylglutaryl-CoA were from Sigma Chemical Co., St. Louis, MO, and Tween 80 and Al_2O_3 (Alcoa A-305) from Serva Feinbiochemica, Heidelberg, F.R.G.

Growth conditions for yeast. Baker's yeast (*Saccharomyces cerevisiae*, from Sandvoss, München) was grown anaerobically or aerobically and harvested as described before [10]. For growth with unsaturated fatty acids, the acid was dissolved in a small volume of ethanol and added to the growth medium which contained 0.4% Tergitol NP 40 as detergent. Control media contained ethanol and Tergitol alone. In the experiments with oleic acid, the acid was added in the form of Tween 80 to the growth medium.

Conditions for enzyme induction and sterol synthesis. The conditions were the same as those described previously [10]. In experiments with labeled glucose, 0.01 mCi D-[U-^{14}C]glucose was added per 250 ml 0.1 M phosphate buffer (pH 6.2) containing 1% glucose.

Determination of sterols and enzyme assays. Determination and extraction of sterols was the same as described before [10]. Enzyme activities were measured in crude cell extracts. These were prepared by grinding 300 mg (wet

wt.) yeast cells with 900 mg Alcoa A-305 for 3 min. The resulting paste was extracted for 3 min with 0.05 M phosphate buffer (pH 7.2), followed by centrifugation at 3000 × *g* for 10 min. Hydroxymethylglutaryl-CoA reductase activity was assayed according to Shapiro et al. [19] with some modifications [20]. Galactokinase was assayed as described by Heinrich and Howard [21], and galactose-1-phosphate uridyltransferase according to Mayes and Hansen [22].

Incorporation of [1-^{14}C]leucine into cell protein. 100 mg (wet wt.) yeast cells grown under aerobic or anaerobic conditions were suspended in 50 ml cold growth medium containing 1% glucose. 3-ml samples of the cell suspension were shaken for 2 min at 30°C and then amino acid incorporation was initiated by addition of 0.03 ml L-[1-^{14}C]leucine. At various time intervals, 0.1-ml aliquots were pipetted into 2 ml 5% trichloroacetic acid containing 10 mM L-leucine, and stored overnight at 4°C. The samples were then heated at 85°C for 20 min and after cooling poured on Millipore filters (pore size 0.45 μm), washed three times with 5% trichloroacetic acid containing 5 mM L-leucine, followed by two washes with 70% ethanol. After drying the filter papers in air radioactivity was counted in a toluene-based scintillation fluid.

Protein determination. Protein was determined by the method of Lowry et al. [25], using bovine serum albumin as standard.

Results

Induction of sterol biosynthesis

Yeast cells grown under anaerobic conditions contain only very small amounts of sterols [10]. Approx. 0.3—0.5 mg sterols per g cells dry wt. was usually found in such cells. When anaerobically grown yeast was transferred to

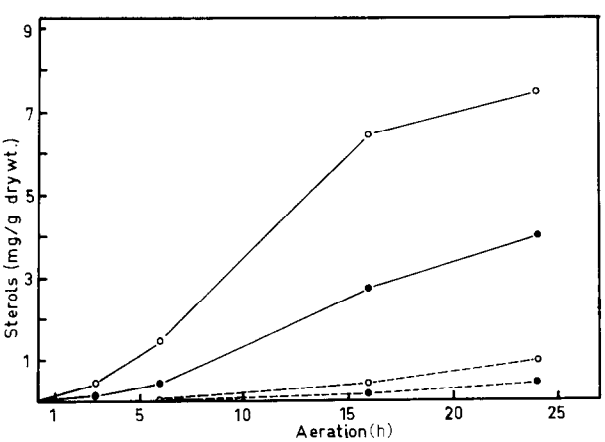

Fig. 1. Induction of sterol synthesis in yeast cells by various carbon sources. Yeast was grown anaerobically for 24 h on glucose, harvested, resuspended in buffer containing 1% of the carbon source and aerated. The sterol content in the cells was determined at various time intervals. Values are newly formed sterols with corrections for sterol synthesis without carbon source in the aeration buffer. ○———○, glucose; ●———●, galactose; ○- - - - -○, ethanol; ●- - - - -●, acetate.

buffer containing a carbon source and aerated, synthesis of sterols was initiated. Cells did not grow under these conditions (resting cells [10]). The kinetics of sterol formation with different carbon sources are shown in Fig. 1. Some sugars, such as mannose, fructose or maltose, were about as effective as glucose, while other compounds were less effective or ineffective (Table IA). During the first hours of aeration resting cells can synthesize small amounts of sterols also in the absence of an added carbon source (Table IA). Carbon for these sterols is probably derived from endogenous material still present in the anaerobic cells. The data in the figures are corrected for this sterol synthesis without added carbon source. In the presence of [U-^{14}C]glucose during aeration, radioactivity was incorporated into the newly formed sterols (Fig. 2), thus indicating that glucose continually provides substrate for sterol biosynthesis. While radioactivity in whole cells (per g dry wt.) increased only very little, high incorporation rates were found in the digitonin-precipitable sterol fraction. Since both the amount of sterols and the radioactivity in the newly formed sterols increased in parallel, it is evident that the specific activity of the newly synthesized sterols remains constant throughout the entire aeration period.

Effect of unsaturated fatty acids on the biosynthesis of sterols

When yeast was grown under anaerobic conditions but with a supplement of unsaturated fatty acids in the culture medium and sterol synthesis in these cells was initiated by aeration, the sterol synthesis proceeded with a several-fold greater velocity and a higher sterol level in the cells was reached. As can be seen from Fig. 3, sterol synthesis in the supplemented cells starts very rapidly upon aeration. This enhancement was also found when compounds other than glucose served as a carbon source (Table I (B)). With galactose, acetate (see Fig. 3), ethanol, pyruvate and glycerol an appreciable sterol synthesis was observed.

TABLE I

INDUCTION OF STEROL SYNTHESIS IN YEAST

Cells were grown anaerobically for 24 h on glucose without (A) or with (B) 0.1% oleic acid, harvested, resuspended in buffer containing 1% of the carbon source and aerated for 6 h. The sterol content in the cells was then determined. Values are corrected for sterol synthesis without carbon source in the aeration buffer.

Carbon source (1%) present during aeration	Sterols (mg/g dry wt.)	
	(A)	(B)
None	0.3—0.5	0.4—0.6
Glucose	2.0	13.0
Mannose	2.2	11.5
Fructose	2.5	13.5
Maltose	2.3	12.8
Galactose	0.7	2.9
Acetate	0.3	2.5
Ethanol	0.3	2.4
Pyruvate	<0.2	1.3
Glycerol	<0.2	0.9
Succinate	<0.2	<0.2
Ribose	<0.2	<0.2
Xylose	<0.2	<0.2

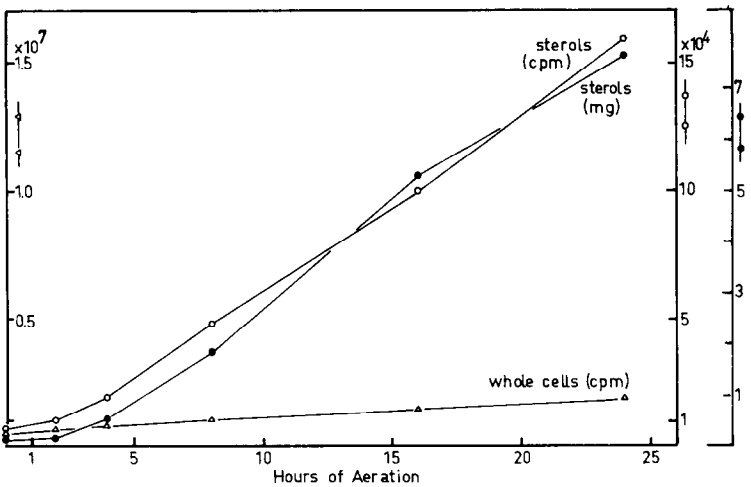

Fig. 2. Incorporation of radioactivity from [U-^{14}C]glucose into newly synthesized sterols and whole cells. Yeast was grown anaerobically for 24 h on glucose, harvested, resuspended in buffer containing 1% glucose plus 10 μCi [U-^{14}C]glucose and aerated. Radioactivity in the sterol fraction was estimated in an aliquot of the digitonine precipitate. Values are given per g dry wt. of yeast.

Stimulation of sterol synthesis occurred only when unsaturated fatty acids were present during growth of the cells. No increase in the synthesizing capacity was seen when unsaturated fatty acids were added at the beginning of the aeration in buffer. When cells were grown aerobically with an unsaturated fatty acid supplement, there was no stimulation of sterol synthesis upon aeration of these cells (data not shown).

Specificity of the effect of unsaturated fatty acids

The stimulation of sterol formation was specific for unsaturated fatty acids.

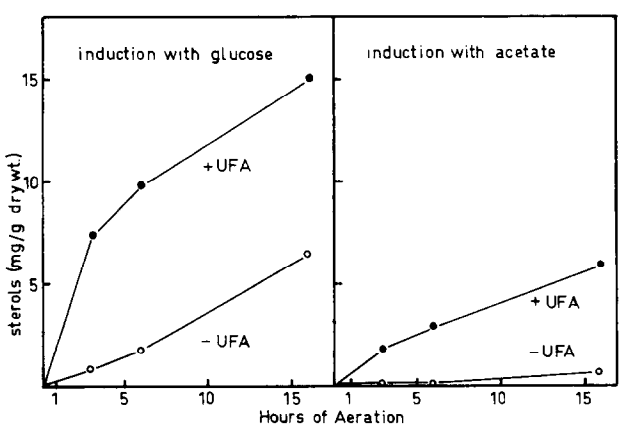

Fig. 3. Effect of oleic acid on the induction of sterol synthesis. Yeast was grown anaerobically for 24 h on glucose with or without 0.1% oleic acid (Tween 80). The cells were harvested, resuspended in buffer with 1% glucose or 1% acetate and aerated. Values are corrected for sterol synthesis without carbon source in the aeration buffer. UFA, unsaturated fatty acids.

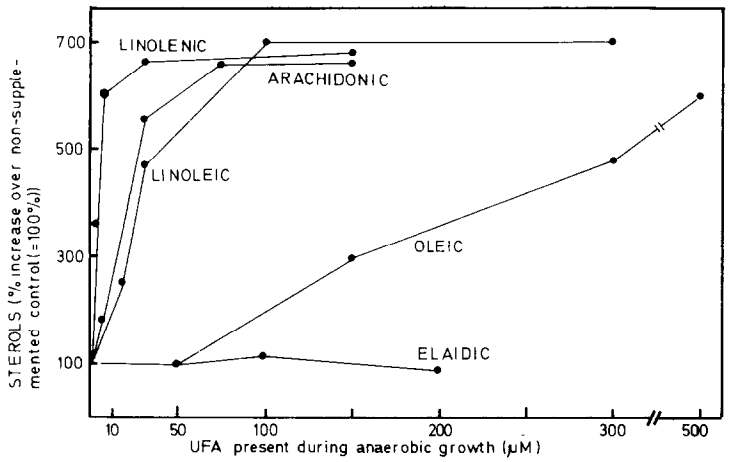

Fig. 4. Effect of different fatty acids on the induction of sterol synthesis. Cells were grown anaerobically for 24 h on glucose in the presence of different amounts of one of the unsaturated fatty acids (UFA), harvested, resuspended in buffer containing 1% acetate and aerated for 16 h. Control cells were grown without an unsaturated fatty acid supplement and the sterol content of these cells after aeration for 16 h in buffer with acetate was set 100%.

Saturated fatty acids (lauric, plamitic and stearic acid) were completely ineffective or inhibited anaerobic growth (palmitic and stearic acid). A number of unsaturated fatty acids were tested for their ability to activate sterol formation. Fig. 4 summarizes the results of these experiments. An unsaturated fatty acid was present in different concentrations during anaerobic growth and the increase in the velocity of sterol formation against a control (cells grown without a supplement) was measured after aeration for 16 h with acetate as a carbon source. Acetate was used as a carbon source in these experiments since unsupplemented cells produced only minimal amounts of sterols during aeration (Fig. 3) while with supplemented cells the effect of the different unsaturated fatty acids could be well distinguished. Comparing oleic, linoleic and linolenic acid, the ability to stimulate sterol formation increased with increasing number of double bonds in the fatty acid molecule. Arachidonic acid was about as effective as linoleic acid. Elaidic acid, the *trans* isomer of oleic acid, could not replace oleic acid. This indicates a specificity of the effect for the *cis* form of the unsaturated fatty acids (see also Ref. 4).

Effect of unsaturated fatty acids on the induction of hydroxymethylglutaryl-CoA reductase

We have shown [10] that the activity of hydroxymethylglutaryl CoA reductase, the regulatory enzyme of sterol synthesis in mammals [11] and in yeast [10], was very low in anaerobically grown yeast cells and that it increased several-fold when the cells were aerated in buffer in the presence of a carbon source. As can be seen from Figure 5, this induction is accelerated and higher activity levels are reached when the cells are grown with a supplement of oleic acid. This increase in enzyme activity was paralleled by an increase of sterol formation (Fig. 3). With acetate as the carbon source the enzyme is now substantially induced (Fig. 5) and sterol synthesis begins (Fig. 3).

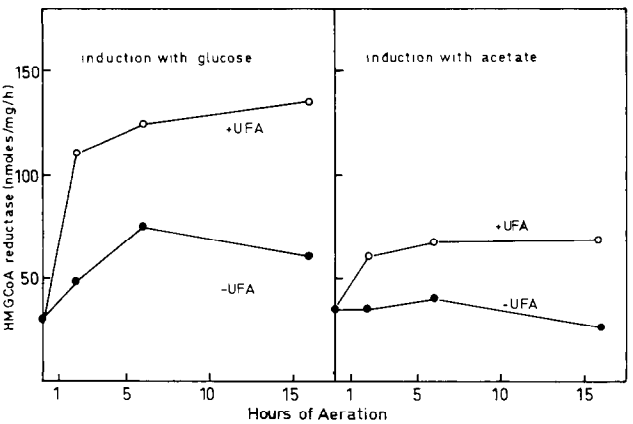

Fig. 5. Effect of oleic acid on the induction of hydroxymethylglutaryl-CoA reductase. Yeast was grown anaerobically for 24 h on glucose with or without 0.1% oleic acid, harvested, resuspended in buffer containing 1% glucose or 1% acetate and aerated. The activity of the enzyme was determined in a crude extract. UFA, unsaturated fatty acids.

Cycloheximide inhibited induction of hydroxymethylglutaryl-CoA reductase [10]. As shown in Table II, the increased synthesis of the enzyme in the supplemented cells was also prevented by cycloheximide. This indicates that fatty acids stimulate the synthesis of newly formed enzyme protein.

Effect of unsaturated fatty acids on the induction of enzymes of the galactose metabolism and on protein synthesis

With galactose as carbon source a considerable but lower sterol synthesis than with glucose was found (Table I, Fig. 1). In the conversion of galactose to glucose galactokinase and galactose-1-phosphate uridyltransferase are involved. These enzymes are induced during the aeration period, galactose-1-phosphate uridyltransferase with a lag period of about 6 h (Fig. 6). This lag period was paralleled by a similar lag period of the corresponding sterol synthesis (Fig. 1). When the yeast was supplemented with oleic acid during anaerobic growth, a much faster increase of the activity of both enzymes during aeration of the cells was observed (Fig. 6). Moreover, higher activity levels were reached and the lag period of the transferase disappeared. Concomitantly, sterol synthesis

TABLE II

INDUCTION OF HYDROXYMETHYLGLUTARYL-CoA REDUCTASE IN YEAST

Cells were grown anaerobically for 24 h on glucose with or without 0.1% oleic acid, harvested, resuspended in buffer containing 1% glucose and cycloheximide (0.1 μg/ml) where indicated and aerated for 2.5 h. The enzyme activity was determined in a crude extract as described in Materials and Methods.

Anaerobic growth	nmol/mg protein per h	
	without oleic acid	with oleic acid
Anaerobic cells	32	34
Aerated cells	52	120
Aerated cells plus cycloheximide	28	35

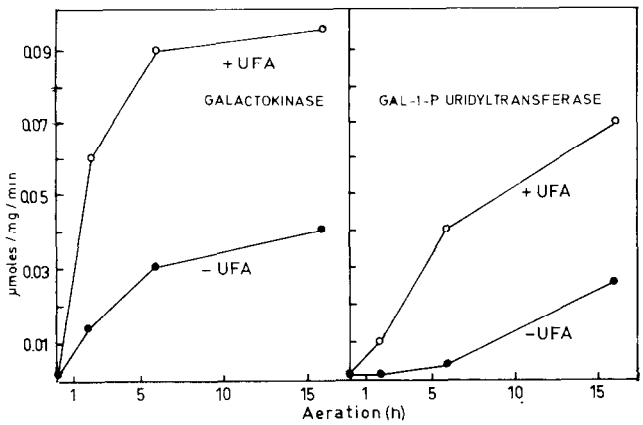

Fig. 6. Effect of oleic acid on the induction of galactokinase and galactose-1-phosphate uridyltransferase. Yeast was grown anaerobically for 24 h on glucose with or without 0.1% oleic acid, harvested, resuspended in buffer with 1% galactose and aerated. The activity of the enzymes was determined in a crude extract. UFA, unsaturated fatty acids.

was enhanced about 4-fold within 6 h of aeration (Table I). Apparently, a close relationship exists between the activity of the galactose-converting enzymes and the efficiency of galactose to serve as carbon source in sterol synthesis.

Unsaturated fatty acids not only had a stimulatory effect on the induction of several enzymes and on sterol biosynthesis, but also on the whole protein-synthesizing apparatus of the cell. 0.1% oleic acid supplement in the growth medium during anaerobic growth of the yeast markedly enhanced the capacity of these cells to incorporate leucine into trichloroacetic-acid precipitable protein (Fig. 7). On the other hand, aerobically grown cells with or without unsaturated fatty acid supplement had a much greater protein-synthesizing capacity.

Discussion

The regulatory function of hydroxymethylglutaryl-CoA reductase is due to the fact that the reaction catalyzed by this enzyme is irreversible [23] and that its product, mevalonic acid, thus can be used only for the sequence leading to squalene and further to sterols. The specific activity of this enzyme is low in anaerobically grown yeast. The induction of the reductase is probably among the first events in the biosynthesis of sterols. Thus, during aeration of the cells in the presence of a carbon source the activity of hydroxymethylglutaryl-CoA reductase increases, and this increase is paralleled by an increase in sterol biosynthesis (Figs. 1 and 5). The carbon source in the aeration buffer obviously determines the velocity of the increase in the enzyme activity and hence of the sterol synthesis. The highest velocities were found with glucose, fructose, mannose and maltose (Table I). We assume that glucose itself is the inducing compound for the induction of hydroxymethylglutaryl-CoA reductase. Other sugars, such as fructose, mannose or maltose, may be so effective because they are easily converted into glucose by the yeast cell. As we have shown previously [10], 0.05% glucose in the aeration buffer is sufficient to induce the

enzyme. Inhibition of this induction by cycloheximide proves that a de novo synthesis of enzyme protein takes place. Since radioactivity from labeled glucose was found in the newly synthesized sterols (Fig. 2), the carbon source present during aeration may function (probably after conversion to glucose) as inducer for hydroxymethylglutaryl-CoA reductase and also, after conversion to acetyl-CoA, as substrate for sterol synthesis.

The formation of sterols from carbon sources other than glucose as shown in Fig. 1 had a lag period of several hours. This indicates that the enzymes necessary for the conversion of each carbon source into a suitable precursor (glucose?) for the sterol synthesis must first become induced. This was shown when the sterol synthesis was induced by galactose. Here a correlated time course was found in the increase of the sterol content (Fig. 1) and the increase of galactokinase and galactose-1-phosphate uridyltransferase activities (Fig. 6), which are involved in the conversion of galactose to glucose.

The presence of unsaturated fatty acids during anaerobic growth of the yeast had a profound effect on the induction of enzymes and on the biosynthesis of sterols. Hydroxymethylglutaryl-CoA reductase as well as galactokinase and galactose-1-phosphate uridyltransferase were much faster induced (Figs. 5 and 6), and the lag period of the latter disappeared. Consequently, in fatty acid supplemented cells there was an up to 8-fold increase in sterol biosynthesis over that in unsupplemented cells (Fig. 3, Table I(B)). This stimulatory effect of fatty acids depends to a high degree on the chain length and number of double bonds in the fatty acid molecule (Fig. 4).

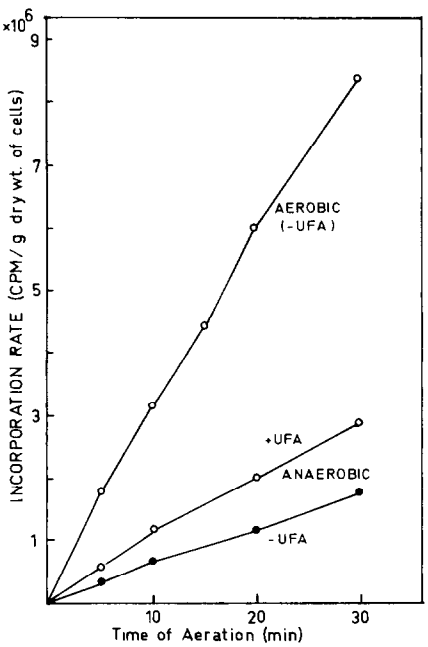

Fig. 7. Incorporation of L-[U-^{14}C]leucine into acid precipitable protein in yeast grown under different conditions. Cells were grown on glucose under aerobic conditions for 16 h, anaerobic cells were cultured for 24 h with or without a supplement of 0.1% oleic acid. The incorporation of labeled leucine into cell protein was carried out as described in Materials and Methods. UFA, unsaturated fatty acids.

Using lipid auxotroph yeast mutants, Haslam et al. [24] recently have shown that unsaturated fatty acids play a fundamental role in the completion of the mitochondrial as well as of the plasma membrane system. With unsaturated fatty acid depleted cells these authors have found a reduced ATP transport via the adenine nucleotide transporter and consequently a decrease in the concentration of intramitochondrial ATP required for macromolecular synthesis. Moreover, in supplemented cells the active transport (leucine and maltose uptake) was drastically increased [24]. The stimulatory effect of oleic acid on the protein-synthesizing capacity of anaerobically grown yeast cells reported here (Fig. 7) may thus well be due to a further completion of the membrane system in the cell and hence to an improved protein synthesis. Consequently, the induction of hydroxymethylglutaryl-CoA reductase, galactokinase and galactose-1-phosphate uridyltransferase is much faster in the fatty acid supplemented cells (Figs. 5 and 6). It should be pointed out, however, that aerobically grown yeast without fatty acid supplement has a much higher protein-synthesizing capacity (Fig. 7), indicating the importance of oxygen in the biogenesis of unsaturated fatty acids during the formation of fully functional cells.

The induction of the sterol biosynthesis during aeration as mentioned here provides an ideal model for the study of the distribution of newly formed sterols inside the yeast cell into the different compartments. This is currently under investigation.

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