REGULATION OF STEROL BIOSYNTHESIS IN YEAST: INDUCTION OF 3-HYDROXY-3-METHYLGLUTARYL-CoA REDUCTASE BY GLUCOSE

J. Berndt, M. Boll, M. Löwel and R.Gaumert

Institut für Biochemie der Gesellschaft für Strahlen- und Umweltforschung, Zentralabteilung, München, Germany

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SUMMARY:

Anaerobically cultured yeast cells have a very low HMG-CoA reductase activity and a low sterol content. When these cells are transfered to phosphate buffer containing 1.2 % glucose and held under aerobic conditions, the specific activity of the HMG-CoA reductase increases up to sixfold within 8 hrs. The increase in the reductase activity is paralled by an increase in the sterol content. This induction of HMG-CoA reductase in resting yeast cells is inhibited by cycloheximide indicating that a de novo synthesis of enzyme protein is mediated by glucose under aerobic conditions. It appears that the regulation of sterol synthesis in yeast is closely connected with the aerobic glucose metabolism.

The reduction of HMG-CoA[x)] to mevalonic acid by HMG-CoA reductase (EC.1.1.1.34) represents the first biosynthetic reaction unique to polyisoprenoid biosynthesis. HMG-CoA-reductase is the rate limiting and regulating enzyme in hepatic sterol biosynthesis and its activity fluctuates in response to alterations in the physiological state of the animal (1,2). Only very few data, however, are available on the regulation of sterol biosynthesis in yeast. In Sacchermomyces cerevisiae STARR and PARKS (3) were able to show that sterol formation increases sharply when anaerobically grown cells are transfered to aerobic conditions in buffer containing glucose. Possibly this increase in sterol formation coincides with an increase of the specific activity of HMG-CoA reductase (4).

In this communication we report the induction of HMG-CoA reductase in yeast by glucose under aerobic conditions. A close connection was observed between the activity of the enzyme and sterol formation.

METHODS

Bakers yeast (SANDVOSS, Munich) was used in these studies. The yeast

x) HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A, HK, hexokinase, G6PDH, glucose-6-phosphate dehydrogenase

was washed four times with 0.02 M potassium phosphate buffer, pH 7.3, (500 x g, 5 min) in the cold and directly used as an inoculum. For the cultivation of anaerobic cells, 1-liter Erlenmeyer flasks were completely filled with the following medium: 0.2 % yeast extract (Difco), 1 % glucose, 0,1 % NH_4Cl, 1.4 % KH_2PO_4. After flushing the flasks with purified nitrogen gas for 45 min the medium was inoculated and the cultures were incubated at 30°C for 60-70 hrs., giving a yield of approximately 2 g yeast (wet wt.) per liter of medium. The cells were washed twice as above.

For the experiments, 1 g of cells (wet wt.) was incubated in 300 ml 0.1 M potassium phosphate buffer (pH 6.2) with 1.2 % of glucose (or other carbon sources as specified) at 30°C under aerobic conditions (vigorous shaking in air). After the period of incubation, cells were washed as above.

Cell free extracts were prepared by grinding 500 mg of cells with 1.5 g of Aluminiumoxid Alcoa A 305 (5) in a mortar for 3 min in the cold. They were then extracted for another 3 min with 3 ml of 0.02 M potassium phosphate buffer (pH 7.3) and centrifuged at 30.000 x g for 30 min.

Specific activity of HMG-CoA reductase was assayed as previously described (6), up to 0.3 mg of protein were used.

Sterols were extracted from yeast as follows: 100 mg of yeast (wet wt.) and 300 mg of Alcoa were grinded for 3 min in a mortar, then 0.25 ml of water were added and the mixture was extracted three times with 2 ml of a ethanol/acetone (1+1) mixture and the extracts adjusted to 10 ml. An aliquot of this extract was incubated for 30 min at 37°C with 0.1 ml of 10 N KOH and neutralized with 2 N HCl. Sterols were precipitated overnight with digitonine (0,2 % solution) and estimated using the method described by Stadtman (7) with a cholesterol standard. Glucose-6-phosphate dehydrogenase and hexokinase activities were measured according to Bergmeyer (8).

RESULTS AND DISCUSSION

When yeast cells are cultured under anaerobic conditions (which retards sterol synthesis) with glucose as carbon source, the specific activity of HMG-CoA reductase is found to be very low (see Fig. 1). As sterols can only be synthesized via the HMG-

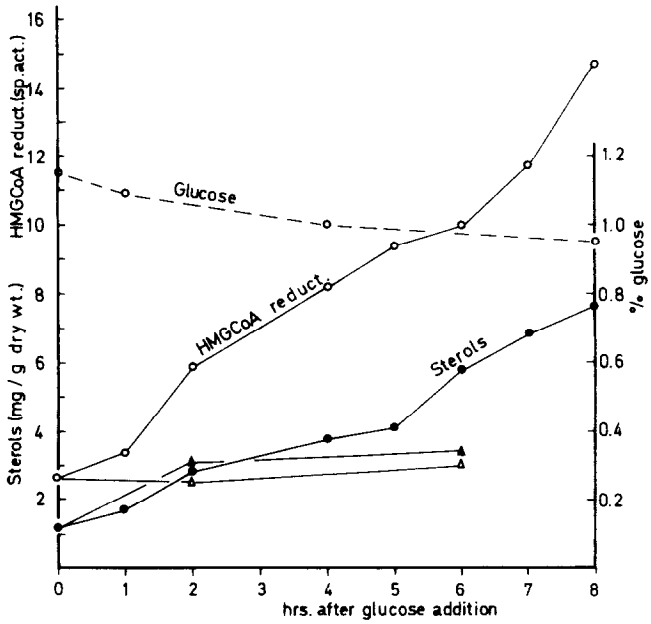

Fig. 1 Induction of HMG-CoA reductase in resting yeast cells by glucose under aerobic conditions. Yeast was cultured anaerobically (see methods) and at zero time suspended in phosphate buffer containing 1.2 % glucose and incubated under aerobic conditions. At the stated time intervals samples were removed and the specific activity (nmoles 3^{14}-C-HMGCoA converted to mevalonic acid per mg of protein per hr.) of the enzyme and the content of digitonine precipitable sterols were determined (see methods). Control experiments (Δ——Δ HMG-CoA reductase, ▲——▲ sterols) were without glucose. Also shown is glucose concentration in the incubation mixture.

CoA reductase reaction, the sterol content is thus also low (3, and Fig. 1).

When these anaerobically grown cells are suspended in potassium phosphate buffer (6.2) with 1.2 % of glucose and held under aerobic conditions for various periods of time, the specific activity of HMG-CoA reductase increases up to sixfold within 8 hours (Fig. 1). The amount of glucose present decreases only very slowly during the incubation period. In the control experiment (without glucose) the specific activity of the enzyme does not change. There is no measurable growth of the yeast cells (increase in turbidity) during the time of incubation with glucose. These results clearly demonstrate that HMG-CoA reductase is induced under aerobic conditions in resting yeast cells by glucose.

The increase in the HMG-CoA reductase activity is paralleled by an increase in the sterol content (Fig. 1). In the control without glucose, a small increase of the sterol content occurs when the cells are incubated under aerobic conditions (Fig. 1). This probably is due to a small amount of squalene preformed in the anaerobic cultured yeast cells, which have a very low, but measurable HMG-CoA reductase activity. Shifting from anaerobic to aerobic conditions, this squalene is then converted to sterols by a reaction sequence including steps which require oxygen.

In addition to HMG-CoA-reductase two enzymes of the carbohydrate metabolism have been assayed under the conditions of the experiments:

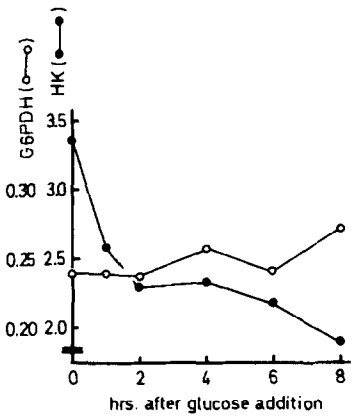

Fig. 2 Specific activities of hexokinase and glucose-6-phosphate dehydrogenase in resting yeast cells. Conditions are the same as in Fig. 1. Specific activity is expressed as umoles of substrate converted per minute per mg of protein.

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hexokinase and glucose-6-phosphate dehydrogenase. As shown in Fig. 2 G6PDH activity remains practically unchanged, while hexokinase activity decreases during longer periods of aerobiosis.

In Table 1 the effect of cycloheximide on the glucose mediated HMG-CoA reductase induction is shown. As can be seen, comparatively low concentrations (100 µg per 300 ml) of cycloheximide will almost completely inhibit the induction. Although the incubation medium (buffer + glucose) does not contain any nitrogen source, a de novo synthesis of the enzyme must occur. Evidently endogenous nitrogen sources are used for this purpose.

TABLE 1

Effect of cycloheximide on HMG-CoA reductase induction in resting yeast cells. The cells were incubated in the buffer-glucose medium containing the stated amounts of cycloheximide under aerobic conditions. After a period of 6 hrs. cells were harvested and the specific activity of HMG-CoA reductase was estimated as described in the methods section.

Cycloheximide, μg in 300 ml incubation medium	HMG-CoA reductase (nmoles per mg per h)	% inhibition
0	15.5	0
2.5	14.3	8
10	9.2	41
25	5.8	63
100	2.3	85
500	2.2	85
1500	1.8	88

Other carbon sources such as acetate, pyruvate or ethanol failed to stimulate HMG-CoA reductase activity within a 6 hour incubation period.

In yeast cells HMG-CoA reductase has been found to be located in the membraneous structure of the mitochondrion (4). Anaerobically grown yeast cells do not have complete mitochondria, but when these cells are transfered to aerobic conditions, the mitochondria develop very rapidly (9,10). Although the precise role of sterols in yeast is not clear, there seems to exist a close connection between the induction of HMG-CoA reductase as a regulatory enzyme in sterol synthesis and the development of complete mitochondria.

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