CONTROL OF ERGOSTEROL BIOSYNTHESIS IN YEAST

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It has been established that the activity of HMG-CoA* reduction to MVA in animal is controlled by bile acids which are presumed to be the end products in cholesterol metabolism (Fimognari and Rodwell, 1965). Similar results were reported with Mycobacterium and Pseudomonas (Fimognari and Rodwell, 1965a). However, these organisms are known not to produce any sterol. With yeast which produces ergosterol, nothing has been reported about the control of ergosterol synthesis and about the further metabolism of ergosterol. This paper reports that the activity of HMG-CoA reduction in ergosterol synthesis of yeast undergoes a feed-back inhibition by acidic lipids which are formed from ergosterol.

MATERIALS AND METHODS

Saccharomyces cerevisiae (ATCC 12341) was grown under semi-anaerobic or strictly anaerobic conditions (Meyer and Bloch, 1963) depending on the purpose of experiments. According to the method of Klein (1955), the yeast cells grown semi-anaerobically were collected, resuspended (20 mg wet weight/ml) in the glucose-salts solution and shaken for 150 min. Preparation of the cell-free extracts was carried out by the method of Katsuki and Bloch (1967) with some modifications: The harvested cells were ground with an equal weight of the mixture of carborundum and Celite 535 (1:1) and the mixture

* Abbreviations used are as follows: HMG, 3-hydroxy-3-methylglutaric acid; MVA, mevalonic acid; NSF, nonsaponifiable fraction.

was treated with 0.1 M phosphate buffer, pH 7.0 (1 ml/1 g of wet cells). It was centrifuged at 5,000 x g for 1 hr and the supernatant obtained was again centrifuged at 80,000 x g for 1 hr. After the floating lipids were carefully removed, the supernatant was used as the cell-free extracts. Incubation with the cell-free extracts was carried out at 37°C for 1 hr with shaking. After the reaction, the incubation mixture was saponified by the conventional method.

1-^{14}C-Acetate (25 μCi/μmole), 2-^{14}C-MVA (5 μCi/μmole) and 3-^{14}C-HMG (2 μCi/μmole) were obtained commercially. 3-^{14}C-HMG-CoA was synthesized according to the method of Hilz et al. (1958). ^{14}C-Ergosterol was prepared by incubating 1-^{14}C-acetate with the yeast cells followed by purification on thin-layer plates (Bloch, 1957).

RESULTS AND DISCUSSION

Conversion to NSF with growing cells Under strictly anaerobic conditions, S. cerevisiae requires oleic acid and ergosterol for growth and the growth amount is limited by ergosterol in the presence of an adequate amount of oleic acid (Fig. 1). In order to investigat the control mechanism of ergosterol synthesis, the conversion of ^{14}C-acetate to NSF was examined with the cells which were grown at various concentrations of ergosterol under strictly anaerobic conditions. Fig. 1 shows that the conversion of ^{14}C-acetate to NSF decreased with increasing concentrations of ergosterol, while the conversion to fatty acids did not.

Conversion to NSF with cell-free extracts The results obtained above suggest that ergosterol might control its synthesis from acetate. Therefore, further experiments with the cell-free extracts were done to examine the incorporation of radioactivity into NSF from ^{14}C-acetate and from ^{14}C-MVA. Unexpectedly, ergosterol itself

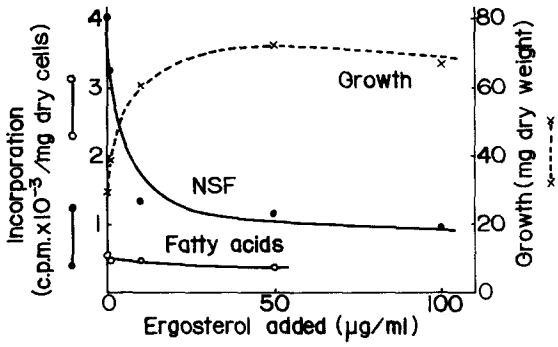

Fig. 1. Effect of ergosterol on the conversion of ^{14}C-acetate to NSF with growing cells. The medium contained in a total volume of 25 ml, 2.5 g of glucose, 170 mg of Difco yeast-nitrogen base, 8.0 mg of potassium oleate, 25 mg of Tween 80, 0.03 μmole of ^{14}C-acetate (1,400,000 c.p.m.), 1.25 mmoles of succinate buffer (pH 5.0), and indicated amounts of ergosterol. After inoculation it was incubated at 25°C for 48 hr under strictly anaerobic conditions.

showed no action on the incorporation of either precursor into NSF. On the other hand, the crude yeast lipids, which were extracted from the disrupted cells with chloroform-MeOH (2:1), showed a marked inhibition of the ^{14}C incorporation from ^{14}C-acetate but no action on that from ^{14}C-MVA.

For the separation of the inhibitory substance(s) from the crude yeast lipids, they were dissolved in ether and the solution was shaken with 1 M $NaHCO_3$ to separate the acidic lipids from the neutral ones. After separation, the $NaHCO_3$ solution was acidified with 6 N H_2SO_4 and it was shaken with ether. Each lipid fraction was obtained by evaporating the ether solutions. Most of the inhibitory activity was found in the acidic lipid fraction. As shown in Table I, the acidic lipids inhibited the incorporation of radioactivity into NSF from ^{14}C-acetate but not from ^{14}C-MVA. From these results, it is evident that the acidic lipids exerted their action at a step between acetate and MVA.

Of authentic compounds tested so far, bile acids, such as deoxycholate, cholate and glycocholate, at 0.1 mM showed a strong in-

TABLE I

Inhibition of Conversion to NSF by Acidic Lipids

Radioactive substrate	Acidic lipids (1.0 mg)	Total incorporation	Inhibition
		c.p.m.	%
1-^{14}C-Acetate	-	52,900	
"	+	7,600	85.6
3-^{14}C-HMG-CoA	-	79,400	
"	+	10,400	86.9
2-^{14}C-MVA	-	77,400	
"	+	70,100	9.5

Reaction mixture (1.0 ml) contained 0.3 ml of cell-free extracts (7 mg protein); ATP, 3 mg; GSH, 2 mg; NADP, 0.5 mg; glucose-6-phosphate, 3 mg; crude coenzyme A (Sigma, grade II), 1.5 mg; $MgSO_4 \cdot 7H_2O$, 0.4 mg; $MnSO_4 \cdot 4H_2O$, 0.3 mg; Tween 80, 1 mg; 0.08 M potassium phosphate buffer, pH 7.0; and 1-^{14}C-acetate (550,000 c.p.m.) or 3-^{14}C-HMG-CoA (2,100,000 c.p.m.) or 2-^{14}C-MVA (200,000 c.p.m.) as indicated.

hibition of the conversion of ^{14}C-acetate to NSF (48 %, 35 % and 25 %, respectively). Unlike bile acids, some kinds of the artificial detergents, such as Triton X-100 and lauryl sulfate, inhibited the conversion not only of ^{14}C-acetate but also of ^{14}C-MVA.

Inhibition step In order to decide the inhibition step, the effect of the acidic lipids on the conversion of ^{14}C-HMG-CoA to NSF was examined. As shown in Table I, ^{14}C-HMG-CoA was converted to NSF, and the acidic lipids showed an 86.9 % inhibition of the conversion, but free ^{14}C-HMG was not converted to NSF. These results strongly suggest that the inhibition step is the reduction of HMG-CoA to MVA.

Purification of the acidic lipids from yeast The apparently low value of the inhibition by the acidic lipids from yeast compared to authentic bile acids was found to be due to the interfering action of other lipids contaminated in them. The acidic lipids were fractionated by column chromatography on silica gel. The inhibitor(s) was

eluted by ether-MeOH (75:25) after removing fatty acids by eluting with hexane-ether (50:50). For further purification, it was chromatographed on thin-layer plate of silica gel G with benzene-dioxane-acetic acid (60:30:2). Anisaldehyde spray (Kritchevsky et al., 1963) revealed four spots, Rf values of which were 0.65, 0.45, 0.40 and 0.30, respectively. Of the four components, 0.1 mg of third one (Rf, 0.40), which was obtained in the yield of 3.0 mg from 500 g of wet cells, showed 90 % inhibition of the conversion of ^{14}C-acetate to NSF. The other components also had inhibitory activities, which were not determined exactly owing to some contamination in them. Furthermore, the esterification of these components with diazomethane gave four spots in the solvent system of n-hexane-ethyl acetate (30:20) (Rf values: 0.73, 0.27, 0.18 and 0.09).

Conversion of ergosterol to the inhibitors In order to demonstrate that the inhibitors from yeast are normal metabolites, the conversion of ^{14}C-ergosterol to the inhibitors was examined. ^{14}C-Ergosterol (10 μmoles, 10,000 c.p.m.) was added to the growing

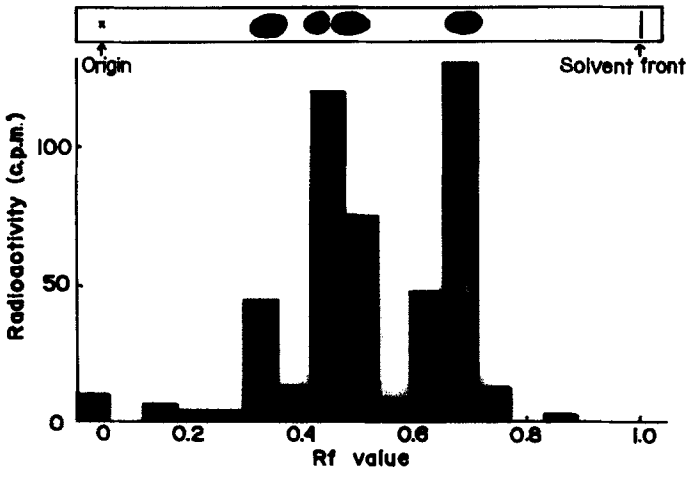

Fig. 2. Thin-layer chromatogram of the inhibitors derived from ^{14}C-ergosterol. Inhibitors (500 c.p.m.) were chromatographed with benzene-dioxane-acetic acid (60:30:2) as described in the text. Radioactivity in the 1 cm segments of the developed plate was counted.

culture of yeast and the mixture was incubated for 48 hr. The inhibitors were extracted from the cells according to the procedure described above and was subjected to thin-layer chromatography. Fig. 2 shows that the radioactivity on the thin-layer plate was located at the spots which were revealed with anisaldehyde spray. These results strongly support that the inhibitors are normal metabolites which are derived from ergosterol and control the biosynthesis of ergosterol in yeast.

The details of the experiments and the properties of the inhibitors will be reported in a subsequent paper.

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