INHIBITION OF ERGOSTEROL SYNTHESIS IN CELL-FREE EXTRACTS OF YEAST BY BILE ACID*

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Received June 24, 1970

SUMMARY

The inhibition of the conversion of ^{14}C-acetate into the non-saponifiable fraction by bile acid with the cell-free extracts of yeast was demonstrated not to be due to the non-specific detergent nature of it on the basis of the following observations: 1) The inhibition by various bile acids showed a greater variation than expected from their critical micellar concentrations. 2) The inhibition seemed specific to the reduction step of 3-hydroxy-3-methylglutaryl-CoA to mevalonate and was reversed by the addition of serum albumin, differently from the non-specific inhibition by synthetic surfactants, such as sodium lauryl sulfate and benzalkonium chloride.

Numerous papers have appeared which report that water-soluble lipids, such as free fatty acid, long chain acyl-CoA derivative, and bile acid, regulate lipogenesis by inhibiting various enzymes involved in it. As to the mechanism of the inhibition by these lipids, however, Taketa and Pogell suggested that the inhibition by palmityl-CoA might be due to its detergent action because various enzymes tested were non-specifically inhibited by it (1).

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* Abbreviations used are: DOC, 3α,12α-dihydroxycholanate (deoxycholate); HMG, 3-hydroxy-3-methylglutarate; MVA, mevalonate; NSF, nonsaponifiable fraction.

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In addition, Dorsey and Porter reported that the inhibition of pigeon liver fatty acid synthetase by palmityl-CoA is ascribed to its detergent action (2). On the basis of these observations, they posed a question against the view that palmityl-CoA or other acyl-CoA derivative plays a physiological role as a regulator of enzyme activity. Pande and Mead also offered a similar view regarding the effect of free fatty acid on some kinds of enzymes (3). Furthermore, as to bile acid, which is to be dealt with in this paper, Dietschy reported that DOC inhibited non-specifically the conversion of acetate into cholesterol, fatty acids, and CO_2 with rat intestinal slices (4). Based on these facts, he presumed that DOC simply inactivated the enzymes concerned. For disclosing the effect of these lipids on enzyme activity from the viewpoint of physiological significance, therefore, it is essential to clarify whether the effect is due to their detergent action or not.

Previously, we reported the evidence which supports that the activity of HMG-CoA reduction in ergosterol synthesis of yeast undergoes a kind of feedback inhibition by acidic lipids formed from ergosterol (5,6). This paper deals with the inhibition of the ergosterol synthesis in the cell-free extracts of yeast by bile acid. The results obtained suggest that the inhibition is not regarded as non-specific inactivation due to its detergent action.

MATERIALS AND METHODS

Saccharomyces cerevisiae (ATCC 12341) grown semi-anaerobically was harvested, resuspended in the glucose-salts solution, and was shaken for 150 min as described previously (6). Preparation of the cell-free extracts (80,000 x *g* supernatant) was carried out by the method described in the previous paper (6).

1-^{14}C-Acetate (25 μCi/μmole), 2-^{14}C-MVA (5.0 μCi/μmole), and 3-^{14}C-HMG (2.0 μCi/μmole) were obtained commercially. 3-^{14}C-HMG-CoA was synthesized according to the method of Hiltz et al. (7). All the bile acids employed were gifts of Shionogi Research Laboratory (Osaka). Each bile acid gave a single spot on thin-layer chromatography in a few solvent systems. Radioactivity was measured in a Nuclear Chicago liquid scintillation spectrometer.

RESULTS AND DISCUSSION

Inhibition of the conversion of ^{14}C-acetate into NSF by various bile acids Previously we reported that ergosterol synthesis in yeast was inhibited by some bile acids (5). In order to investigate further this problem, the relation between the extent of the inhibition by various bile acids and the virtue of their detergent actions were examined (Table I). As to the detergent nature of bile acid, Hofmann and Small reported that the critical micellar concentrations of dihydroxy bile acids are almost the same, and that monohydroxy bile acids do not form a micelle at 37°C under such experimental conditions as those shown in Table I (8). As can be seen from the table, however, the extent of the inhibition by dihydroxy bile acids showed a great variation of the range by a factor of about fifteen. In addition, 3α-hydroxycholanate, which should not form a micelle under these conditions, showed the strongest inhibition among the bile acids tested. These results show that the inhibition of the conversion of ^{14}C-acetate into NSF by bile acid is not due to its micelle forming action.

Furthermore, we have demonstrated in a separate experiment using about 40 species of bile acids that a specific structure of bile acid is necessary for the inhibition (9).

Table I

Inhibition of the Conversion of ^{14}C-Acetate into NSF by Various Bile Acids.

Bile acid[1)]	$I_{1/2}$ (M)[2)]	Relative[3)] Value
3α,7α,12α-Trihydroxycholanate	1.55×10^{-4}	0.71
3α,6α-Dihydroxycholanate	1.10×10^{-4}	1.0
3α,7α-Dihydroxycholanate	1.51×10^{-5}	7.3
3α,12α-Dihydroxycholanate (DOC)	1.10×10^{-4}	(1.0)
7α,12α-Dihydroxycholanate	1.17×10^{-4}	0.94
3α,6β-Dihydroxycholanate	3.14×10^{-5}	3.5
3α,7β-Dihydroxycholanate	7.98×10^{-6}	14
3α-Hydroxycholanate	6.40×10^{-6}	17
6α-Hydroxycholanate	2.90×10^{-4}	0.38
7α-Hydroxycholanate	8.05×10^{-6}	14
12α-Hydroxycholanate	1.51×10^{-4}	0.73
3β-Hydroxycholanate	3.67×10^{-3}	0.03

Reaction mixture (1.0 ml) contained ^{14}C-acetate (2.33×10^5 c.p.m.), 5 μmoles of ATP, 2 μmoles of GSH, 0.1 μmole of CoA, 1 μmole of NADP, 10 μmoles of glucose-6-phosphate, 2 μmoles of $MgSO_4 \cdot 7H_2O$, 1 μmole of $MnSO_4 \cdot 4H_2O$, 80 μmoles of potassium phosphate buffer (pH 7.0), 0.3 ml of the cell-free extracts (7 mg protein), and the bile acid as indicated. Incubation was carried out at 37°C for 60 min with shaking. After the reaction, the incubation mixture was saponified by the conventional method and NSF was counted for its radioactivity. ^{14}C-Incorporation without bile acid was 1.83×10^4 c.p.m. under these conditions.

1) Each bile acid was added in a form of sodium salt. With sodium salt of monohydroxy bile acid, it was added after being dispersed in the solution with the aid of Tween 80 (final concentration, 1 mg/ml). Tween 80 gave no effect on the conversion of ^{14}C-acetate into NSF as well as on the inhibition by DOC.
2) The concentration necessary for a 50% inhibition.
3) Reciprocal of the relative $I_{1/2}$ value of a given bile acid to that of DOC as a standard.

Effect of DOC on several metabolic activities with the cell-free extracts In order to ascertain whether the observed inhibition is specific for a certain enzyme or not, the effects of DOC on the conversions of ^{14}C-HMG-CoA, ^{14}C-MVA, and of ^{14}C-acetate, into NSF

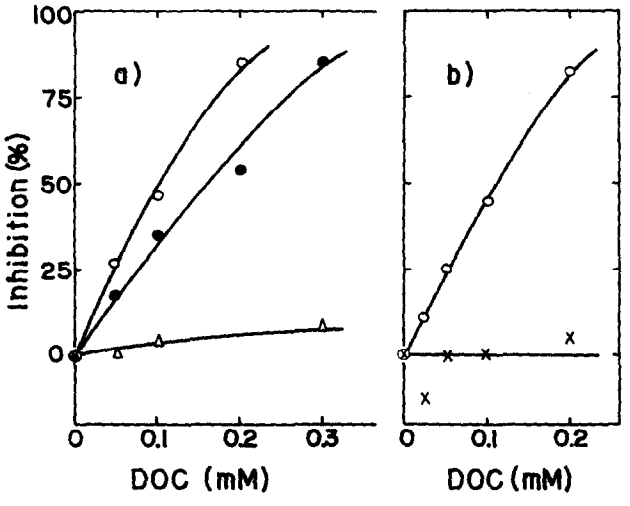

Fig. 1-a. Effect of DOC on the conversions of ^{14}C-acetate, ^{14}C-HMG-CoA, and of ^{14}C-MVA into NSF. Incubation with ^{14}C-acetate (—○—) was carried out in the same manner as shown in Table I. Incubation with ^{14}C-HMG-CoA (—●—) or ^{14}C-MVA (—△—) was carried out at 37°C for 20 min after preliminary incubation of the cell-free extracts with DOC at 37°C for 60 min. The constitution of their final reaction mixtures was the same as that in the experiment with ^{14}C-acetate, except for the use of ^{14}C-HMG-CoA (3.21 x 10^4 c.p.m.) or ^{14}C-MVA (1.75 x 10^5 c.p.m.) instead of ^{14}C-acetate, and the omission of CoA. ^{14}C-Incorporations from these substrates into NSF in the absence of DOC were 1.83 x 10^4 c.p.m. (^{14}C-acetate), 1.32 x 10^3 c.p.m. (^{14}C-HMG-CoA), and 4.56 x 10^4 c.p.m. (^{14}C-MVA).

Fig. 1-b. Effect of DOC on the conversion of ^{14}C-acetate into fatty acid fraction and NSF. Reaction was carried out in the same manner as shown in Table I. ^{14}C-Incorporation into fatty acid fraction in the absence of DOC was 6.20 x 10^3 c.p.m.
—×—, fatty acid fraction; —○—, NSF.

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were examined (Fig. 1-a). The conversion of ^{14}C-HMG-CoA was inhibited by DOC to an extent of approximately the same degree as that with ^{14}C-acetate. On the other hand, DOC scarcely inhibited the conversion of ^{14}C-MVA into NSF. These results suggest that the inhibition step is the reduction of HMG-CoA to MVA. Fig. 1-b shows the effect of DOC on fatty acid synthesis. As can be seen from this figure, the conversion of ^{14}C-acetate into fatty acid fraction was not inhibited by 0.2 mM DOC which showed a nearly 90% inhibition of its conversion into NSF. These results suggest

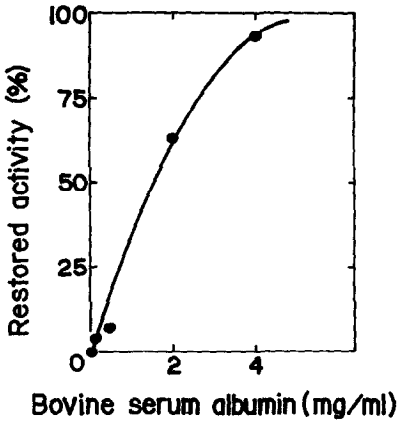

Fig. 2. Release of the DOC inhibition of the conversion of ^{14}C-acetate into NSF by bovine serum albumin. After preliminary incubation of the reaction mixture (shown in the legend to Table I with the omission of ^{14}C-acetate) and 0.125 mM DOC at 37°C for 60 min, reaction was carried out at 37°C for 60 min by the addition of ^{14}C-acetate and bovine serum albumin as indicated.

that the bile acid does not inhibit non-specifically various kinds of enzymes but exerts the action rather specifically on the particular enzyme(s) under certain conditions.

Reversion of the inhibition The results obtained above show that the inhibition by bile acid is not solely due to their detergent action. For further demonstration of it, the reversion of the inhibition by bovine serum albumin was attempted. As shown in Fig. 2, the inhibition of the conversion of ^{14}C-acetate into NSF by 0.125 mM DOC was reduced by increasing the amount of albumin and was almost completely released by the addition of about 4 mg of it. From these results, the inhibition by DOC was demonstrated not to be due to an irreversible inactivation of the enzyme.

Effect of synthetic surfactant on the conversion of ^{14}C-acetate into NSF For the comparison with the inhibition by bile acid, the inhibition experiments by sodium lauryl sulfate and benzalkonium chloride, i.e., synthetic anionic and cationic surfactants, respectively, were carried out. The experiments showed

that these surfactants inhibited the conversion of ^{14}C-acetate as well as ^{14}C-MVA into NSF in the cell-free extracts and that the inhibition was not reversed by the addition of albumin. These results show that the inhibition by bile acid is different from the one by the synthetic surfactants.

The results presented in this paper show that the action of a metabolite with detergent action in metabolism is not always due to its non-specific detergent action but suggest a possibility that it has a physiological significance.

ACKNOWLEDGMENTS: The authors are indebted to Dr. J. Nagai for his helpful discussion and encouragement, and to Dr. S. Hayakawa, Shionogi Research Laboratory, for the supply of the bile acids. This work was supported in part by a Scientific Research Grant from the Ministry of Education of Japan.

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