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SPECIFIC, REVERSIBLE INACTIVATION OF YEAST β-HYDROXY-β-METHYLGLUTRARYL-CoA REDUCTASE BY CoA

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SUMMARY

A partially purified preparation of β-hydroxy-β-methylglutaryl-CoA reductase from baker's yeast is shown to be inactivated by incubation with low concentrations of free CoA. This inactivation is highly specific for the intact CoA molecule, is time-dependent, and is not reversed by the addition of excess substrate. Reversal is possible, however, by extensive dialysis. The apparent inactivating effect of thioesters such as acetyl-CoA and propionyl-CoA can be explained by the amount of free CoA liberated from the thioesters through enzymatic hydrolysis. β-Hydroxy-β-methyl-glutaryl-CoA reductase thus joins a group of enzymes (α-isopropylmalate synthase, homocitrate synthase and, possibly, N-acetylglutamate synthase) which have three features in common: they are associated with the mito-chondria, they control acetyl-CoA utilizing pathways, and they are inacti-vated by CoA.

HMG[‡]-CoA reductase (EC 1.1.1.34) is a key enzyme in the biosynthesis of sterols in yeast. In 1967, Kirtley and Rudney reported that the enzyme was progressively inactivated upon incubation with a number of CoA thio-esters including acetyl-CoA, acetoacetyl-CoA, and the substrate HMG-CoA (1). They found that free CoA also had a significant inactivating effect. We became interested in CoA-mediated inactivation as a possible regulatory device in yeast after we had observed that α-isopropylmalate synthase (EC 4.1.3.12) and homocitrate synthase (EC 4.1.3.21) were reversibly inacti-vated by CoA in a highly specific, time-dependent fashion (2, 3). These two enzymes catalyze the first committed step in the acetyl-CoA utilizing pathways leading to leucine and lysine, respectively. They are associated with the mitochondria (2, 4), a property shared by HMG-CoA reductase (5, 6). In this communication, we show that the time-dependent inactivation of

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[‡]Abbreviation used: HMG, β-hydroxy-β-methlyglutaryl.

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yeast HMG-CoA reductase by CoA is again a highly specific, reversible process, and that the inactivating effect of CoA thioesters, where it is observed, can be explained by the amount of CoA generated by the action of thioesterases present in the yeast preparations. CoA inactivation of mitochondrial enzymes controlling acetyl-CoA utilizing pathways thus appears to be a more general phenomenon.

MATERIALS AND METHODS

Organism. Commercial baker's yeast (Saccharomyces cerevisiae, grown by Anheuser-Busch, Inc., St. Louis, MO) was used throughout these studies.

Special Chemicals. CoA, HMG-CoA, acetoacetyl-CoA, 3'-dephospho-CoA, and (1-N^6-etheno)-CoA were from PL-Biochemicals. Acetyl-CoA and propionyl-CoA were synthesized from acetic anhydride and propionic anhydride by the method of Simon and Shemin (7).

Preparation and Assay of HMG-CoA Reductase. Following the purification procedure of Knappe et al. (8) up to the first ammonium sulfate fractionation, HMG-CoA reductase was purified to a specific activity of 17 nmol of NADPH oxidized min^{-1} mg^{-1}. Solubilization of the enzyme was achieved by toluene autolysis (8) and also by freezing the yeast cells at -15°C for at least 24 hrs, suspending them in cold 50 mM potassium phosphate buffer, pH 7.5, which contained 1.5 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, and 1 mM dithiothreitol, and passing the suspension through a French pressure cell twice at 16,000 pounds/in^2. The enzyme was assayed in the presence of 50 mM potassium phosphate buffer, pH 6.5, 4 mM dithiothreitol and 0.2 mM each of HMG-CoA and NADPH. The reaction was followed spectrophotometrically by measuring the decrease in absorbance at 340 nm at 23°. Correction was made for oxidation of NADPH in the absence of HMG-CoA.

RESULTS

Inactivation of HMG-CoA Reductase by CoA: Specificity and Reversibility. When partially purified HMG-CoA reductase was incubated with CoA, a decrease in activity was observed which was both time- and CoA-concentration-dependent (Figure 1). The enzyme responded rather specifically to CoA; 3'-dephospho-CoA was at least two orders of magnitude less effective than CoA, and neither (1,N^6-etheno)-CoA nor ADP had any effect at concentrations up to 1 mM. At each time point indicated in Figure 1, an aliquot of the incubation mixture was withdrawn and assayed in the presence of 0.2 mM HMG-CoA, a concentration more than 100 x K_m (8). It is noteworthy that incubation with such a large excess of the thioester substrate did not cause relief from inactivation, suggesting that CoA did not behave as a competitive product inhibitor.

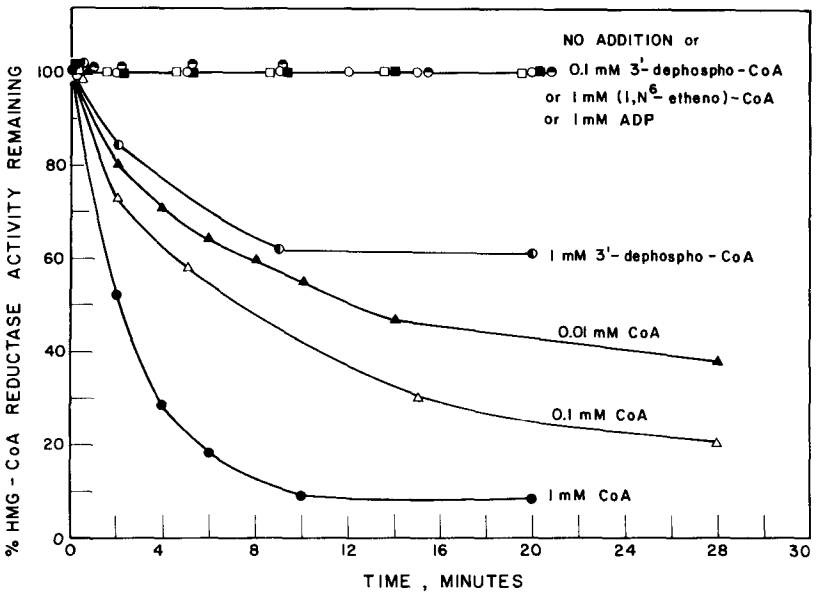

Figure 1. Extent and specificity of the time-dependent CoA inactivation of HMG-CoA reductase. The partially purified enzyme, dissolved in 50 mM potassium phosphate buffer, pH 7.0, containing 1 mM EDTA and 0.4 mM DTT, was incubated at 30°C with the indicated concentrations of CoA or its analogs. At the specified time intervals, starting with zero time, an aliquot of the incubation mixture was added to the standard assay mixture (resulting in a 20-fold dilution) and activity was determined as detailed in the Methods section. The final concentrations of CoA and its analogs in the assay mixture were insufficient to cause (product) inhibition; this was established in separate experiments.

While reactivation of CoA-inactivated HMG-CoA reductase was not obtained upon addition of substrates, it was possible to regain most of the activity upon prolonged dialysis (Table 1).

Effect of CoA Thioesters on HMG-CoA Reductase. Kirtley and Rudney observed loss of HMG-CoA reductase activity upon incubation of the enzyme with various CoA thioesters (1). To establish whether this effect was caused by the thioesters themselves or by free CoA liberated through hydrolysis of the thioesters, the experiments outlined in Figure 2 were performed. Of the four CoA thioesters tested by us, only acetyl-CoA and propionyl-CoA progressively inactivated HMG-CoA reductase. HMG-CoA was without effect under the conditions employed, and acetoacetyl-CoA showed a

Table 1. Reversibility of CoA Inactivation of HMG-CoA Reductase[a]

Dialysis time hours	HMG-CoA Reductase Activity (nmoles NADPH oxidized/min)	
	Control (No CoA treatment)	After prior CoA Inactivation
0	7.2 ± 0.8	< 0.4
11	8.0 ± 0.2	6.1 ± 0.2

[a]Partially purified HMG-CoA reductase was incubated for 30 min at 30° in 50 mM potassium phosphate buffer, pH 7.0, containing 1 mM EDTA and 0.4 mM dithiothreitol, in the presence or absence of 1 mM CoA. The solution was then dialyzed at 4° against 50 mM potassium phosphate buffer, pH 7.0, containing 1 mM EDTA and 0.02 mM dithiothreitol.

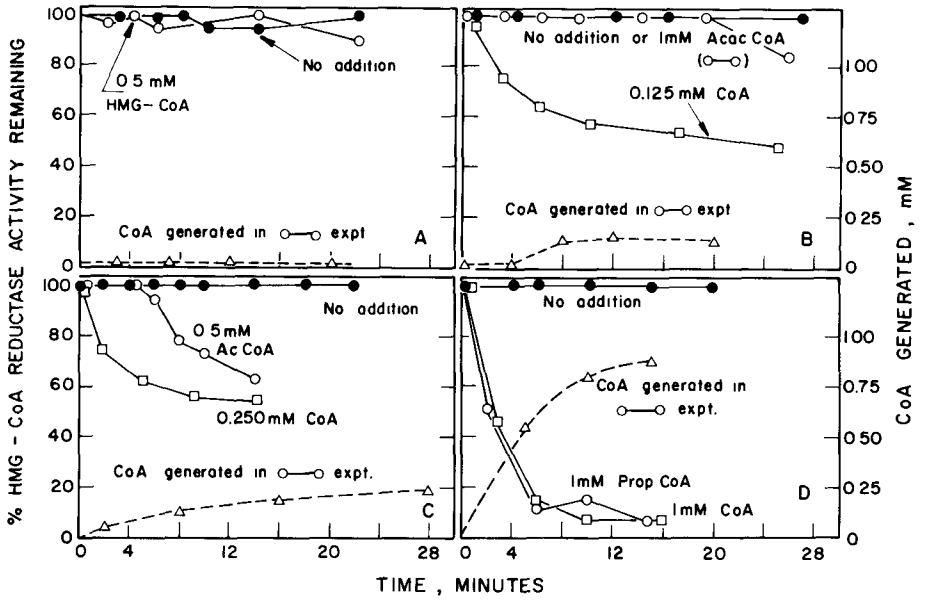

Figure 2. Relationship between the inactivating effect on HMG-CoA reductase of certain CoA thioesters and the generation of free CoA during incubation with such thioesters. Partially purified HMG-CoA reductase, dissolved in 50 mM potassium phosphate buffer, pH 7.0, containing 1 mM EDTA, was incubated with HMG-CoA (panel A), acetoacetyl-CoA (panel B), acetyl-CoA (panel C), or propionyl-CoA (panel D). At the indicated times, aliquots were withdrawn and assayed for activity (open circles). Controls are symbolized by closed circles. Also shown in each panel is the time course of the formation of free CoA during each incubation (dashed lines with open triangles) as measured in aliquots by reaction with 5,5'-dithiobis-(2-nitrobenzoate). A molar extinction coefficient of 13,600 (412 nm) was employed. The values were corrected for the presence of sulfhydryl groups in the enzyme preparation by performing similar incubations without the CoA thioesters. Finally, in the experiments represented by panels B, C, and D, HMG-CoA reductase was incubated (in separate experiments) with the maximum concentrations of CoA generated during incubation with acetoacetyl-CoA, acetyl-CoA and propionyl-CoA (0.125 mM, 0.250 mM, and 1 mM, respectively). The lines with the open squares show the time course of inactivation by these CoA concentrations.

small effect only after 25 minutes of incubation. The effects of acetyl-CoA and propionyl-CoA could be correlated with the rate at which these compounds were hydrolyzed by our yeast preparation. As shown in panels C and D, Figure 2, about half of the acetyl-CoA and nearly all the propionyl-CoA was hydrolyzed during the course of the experiment. The amount of CoA generated in each case was sufficient to explain the inactivation apparently caused by the two thioesters. In fact, it appeared that high concentrations of acetyl-CoA provided partial protection against CoA inactivation. This was confirmed in a separate experiment in which HMG-CoA reductase was incubated simultaneously with 1 mM acetyl-CoA and 0.1 mM CoA. Under these conditions, the extent of inactivation after 10 min was only about half of that obtained with 0.1 mM CoA alone. Protection against CoA inactivation was also provided by acetoacetyl-CoA (panel B, Figure 2). No CoA was generated in the experiment with HMG-CoA, and no inactivation was observed.

Both the rate and the extent of inactivation of HMG-CoA reductase by CoA varied somewhat from experiment to experiment, decreasing with the age of the enzyme. This variation may reflect a partial desensitization of the enzyme toward CoA with time, an effect also observed with α-isopropylmalate synthase.

DISCUSSION

There are now three enzymes in yeast for which specific, reversible inactivation by CoA has been demonstrated: HMG-CoA reductase (this paper), α-isopropylmalate synthase (2, 3), and homocitrate synthase (2). A fourth one may be N-acetylglutamate synthase (9). All of these appear to be located in the mitochondria. Also, all have important regulatory roles in their respective pathways. We believe that the emerging pattern further supports the notion, advanced previously (2, 10), that CoA inactivation has regulatory significance, in the sense that relatively high intramitochondrial levels of CoA may serve as a signal for relatively low levels of acetyl-CoA and may cause a temporary shutdown of various enzymes controlling biosyn-

thetic, acetyl-CoA utilizing pathways. The result would be a channeling of the remaining acetyl-CoA into the citrate cycle, since citrate synthase is not susceptible to CoA inactivation (2).

An important aspect of this work has been the demonstration that inactivation of HMG-CoA reductase, which appeared to be caused by CoA thioesters, was in reality due to free CoA generated from the thioesters by (enzyme-catalyzed) hydrolysis. Somewhat surprisingly, we did not see any change in activity when our HMG-CoA reductase preparation was pre-incubated with HMG-CoA, a compound which caused significant inactivation in the experiments of Kirtley and Rudney (1). We are inclined to believe that their HMG-CoA solution contained an appreciable amount of free CoA or that their enzyme preparation was especially rich in HMG-CoA hydrolyzing activity.

HMG-CoA reductase catalyzes a two-step reduction from a thioester to a primary alcohol. There is evidence that the intermediate aldehyde, mevaldate, remains bound to the enzyme (8, 11, 12), and it is believed that, as a result of the first half-reaction and replacement of the first NADP with NADPH, an enzyme-NADPH-CoA-mevaldate complex is formed which is rapidly converted to mevalonate and NADP (13, 14). Qureshi et al. (13) have proposed that enzyme-bound CoA, generated on the enzyme surface from HMG-CoA, acts as an activator of the second half-reaction by preventing the release of mevaldate from the enzyme. This activating effect of CoA is part of the catalytic reaction sequence and can apparently only be recognized when the reaction is dissected into its two major parts. It is therefore not in conflict with the inactivating role of CoA described here. It will be of great interest to establish the chemical mechanism of CoA inactivation and, in particular, to find out whether the inactivating CoA binds to the catalytic site of HMG-CoA reductase or to a separate site; the latter was observed with α-isopropylmalate synthase from yeast (10).

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