REGULATION OF ACETYL-CoA CARBOXYLASE OF SACCHAROMYCES CEREVISIAE

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Matsuhashi, Matsuhashi, Numa, and Lynen (1964) have purified the acetyl-CoA carboxylase of Saccharomyces cerevisiae, Lowenbrau strain, and have reported that this enzyme cannot be regulated (Matsuhashi, Matsuhashi, and Lynen, 1964). However, earlier reports from this laboratory have shown that fatty acid synthesis in extracts of S. cerevisiae, strain LK2G12, can be stimulated by a number of intermediates of both the Krebs cycle and the glycolytic pathway (White and Klein, 1965, 1966). Furthermore, it was shown that acetyl-CoA carboxylation is the rate-limiting step and that citrate, fructose-1, 6-diphosphate, and L-α-glycerophosphate can stimulate the activity of this enzyme. It is the purpose of this report to show that the acetyl-CoA carboxylase of this strain of S. cerevisiae can be stimulated by a large number of compounds. In addition, some information is presented about the mechanism involved in these stimulations.

S. cerevisiae was cultivated, harvested, disintegrated, and fractionated as previously described (White and Klein, 1965). The source of acetyl-CoA carboxylase was a 0-35% ammonium sulfate fraction of a dialyzed high-speed supernatant. This fraction was stable for several months when stored at -70°C. The source of fatty acid synthetase was a "light particle fraction" of this yeast (Klein et al., 1967).

Table 1 shows the effect of several intermediates of the Krebs cycle and of the glycolytic pathway. Many of these, as well as a number of related compounds, are seen to stimulate the acetyl-CoA carboxylase.

TABLE 1

Effect of Various Compounds on Acetyl-CoA Carboxylase Activity

Substance tested	Concentration of substance tested ($\times 10^{-3}$ M)		
	32	64	96
Fructose-1,6-diphosphate	4.7	---	10.2
α-glycerophosphate	4.0	---	---
β-glycerophosphate	2.6	3.9	4.2
6-phosphogluconate	3.0	3.8	---
Fructose-6-phosphate	1.3	---	3.1
Glucose-6-phosphate	1.7	---	1.8
Carbamyl-phosphate	1.7	---	0.9
Acetyl phosphate	0.9	---	1.6
Glycerol-1,2-diphosphate	0.8	---	---
DL-glyceraldehyde	1.4	---	0.9
DL-glyceraldehyde-3-phosphate	0.9	---	1.4
Glycerate	1.6	1.0	---
Dihydroxyacetone	---	1.2	1.2
Citrate	7.2	9.8	---
Isocitrate	---	7.0	8.0
α-Ketobutyrate	5.0	7.0	---
Glutarate	2.4	5.0	---
α-Ketoglutarate	---	3.9	3.0
Glyoxylate	1.3	---	---
Trans-aconitate	---	1.8	3.7
Adipate	---	2.9	3.4
Fumarate	2.3	3.9	---
Ascorbate	---	1.6	2.0
L-(+)-Lactate	2.5	3.2	---
D-(-)-Lactate	2.0	2.7	---
Tricarballylate	1.3	2.2	2.6
Cis-aconitate	1.0	1.7	---
DL-Glutamate	---	1.9	1.7
L-Aspartate	---	1.5	0.8
Malonate	---	1.0	1.7

Each sample contained in a final volume of 0.5 ml of: 0-35% ammonium sulfate fraction containing 0.24 mg protein, 20 μmoles potassium phosphate buffer, pH 7.0, 5 μmoles ATP, 2.5 μmoles $MnCl_2$, 0.5 μmole AcCoA, 20 μmoles $KHC^{14}O_3$ (10^8 CPM/μmole), and activating substances as indicated. Samples were preincubated for 15 min. at 30°C with all reagents except bicarbonate. The reaction was started by the addition of bicarbonate and samples incubated for 30 min. at 30°C. The reaction was stopped by the addition of 6N HCl. $C^{14}O_2$ incorporated into malonyl-CoA was measured as malonic acid after alkaline hydrolysis of the CoA ester and isolation by paper chromatography. Control values = 1.0.

Stimulation of fatty acid synthesis by α-glycerophosphate in animal systems has been ascribed to relief of palmityl-CoA inhibition of acetyl-CoA

carboxylase (Howard and Lowenstein, 1964, 1965). Such a mechanism does not appear plausible for α-glycerophosphate stimulation of the yeast enzyme, since Rasmussen and Klein (1967) have shown that enzyme preparations stimulated by this substance lack acyl-CoA compounds. These investigators have, therefore, suggested that α-glycerophosphate and citrate stimulate yeast acetyl-CoA carboxylase by a common mechanism. In animal preparations stimulation of acetyl-CoA carboxylase by citrate and isocitrate has been shown to involve an allosteric modification resulting in a significant increase in molecular weight (Gregolin et al., 1966; Matsuhashi, Matsuhashi, and Lynen, 1964; Numa et al., 1966; Vagelos et al., 1963). In order to test whether stimulation of the yeast system changes sedimentation behavior, the ammonium sulfate fraction was subjected to density gradient centrifugation studies. Sucrose, which is generally used for such gradients, inhibited the enzyme by as much as 80%. However, Ficoll, melibiose, arabinitol, melezitose, mannitol, and sorbose were inert. Since mannitol was readily available, density gradient studies were performed with this substance.

Two markers were employed in the density gradient studies: one of these, the lighter of the two, was 19S gamma globulin (the generous gift of Dr. H. Fudenberg, University of California Medical Center). The heavier of the two markers was the fatty acid synthetase of this organism. This enzyme has been shown to have a sedimentation coefficient of 47S (Klein et al., 1967). As can be seen from Figure 1, most of the gamma globulin, under the conditions used here, was obtained in the third fraction, while the peak of synthetase activity occurred in the seventh and eighth tubes. Under these conditions, the peak of carboxylase activity routinely occurred in the third fraction, indicating a sedimentation coefficient of approximately 20S. Furthermore, preparations preincubated for periods up to 120 minutes with 4×10^{-2} M α-glycerophosphate or with 4×10^{-2} M citrate, and then subjected to density gradient centrifugation in the presence or absence of these activators showed no apparent change in sedimentation behavior. These results suggest that the

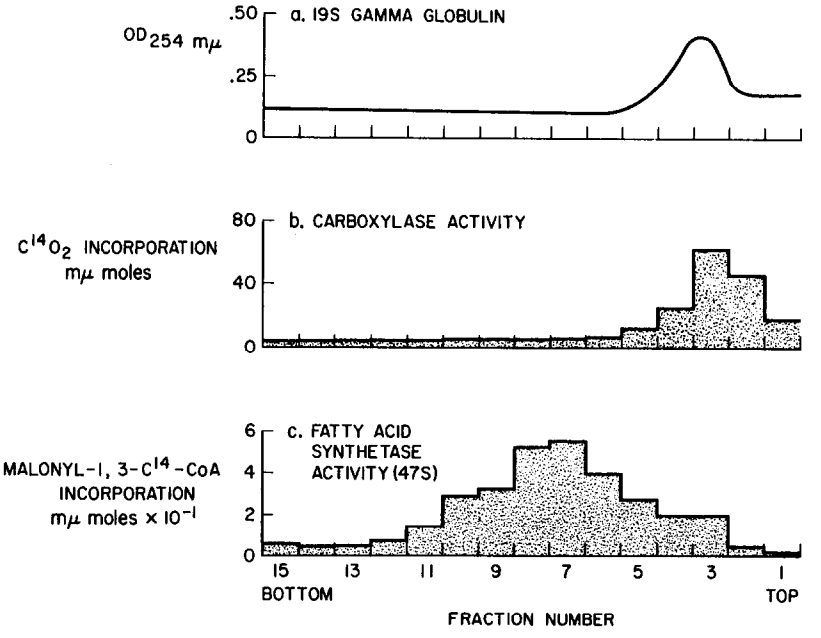

Fig. 1. Density gradient centrifugation of 19S gamma globulin, 0-35% ammonium sulfate fraction, and fatty acid synthetase. (a) 19S gamma globulin. 19S gamma globulin was layered on a 5-20% mannitol density gradient in 0.002 M tris-Mg buffer, pH 7.0, containing 0.002 M dithiothreitol. The gradient was centrifuged for 60 min. at 50,000 RPM in a Spinco SW-50L swinging bucket rotor at 5°C. The optical density of the gradient was read at 254 mμ and recorded by means of an Isco gradient collecting device. (b) Carboxylase activity. 0.1 ml of 0-35% ammonium sulfate fraction containing 2.6 mg protein was mixed with an equal volume of tris-Mg buffer described above, and treated as above. 15 fractions were collected by means of an Isco gradient collecting device. The fractions were assayed for carboxylase activity as described under Table 1. (c) Fatty acid synthetase activity. 0.1 ml of "light particle fraction" containing 1.0 mg protein was mixed with an equal volume of the buffer described above, and treated as above. The fractions were assayed for synthetase activity. Each sample contained, in a final volume of 1.0 ml, the following: 0.3 ml of gradient material, 20 μmoles potassium phosphate buffer, pH 6.3, 1 μmole NADPH, 0.1 μmole acetyl-CoA and 17.5 mμmoles malonyl-1,3-C^{14}-CoA (1.5×10^7 CPM/μmole). Samples were incubated for 30 min. at 30°C. Fatty acids synthesized were extracted in chloroform-methanol, and counted.

mechanism of stimulation of the yeast acetyl-CoA carboxylase is different from that of the animal enzymes. It also differs from the wheat germ acetyl-CoA carboxylase, which has been reported to be unaffected by citrate, α-glycerophosphate, and related compounds (Burton and Stumpf, 1966). Activation of the yeast enzyme may result in a change in tertiary structure not involving a gross molecular weight change. An extremely slight change in

sedimentation behavior of the enzyme, such as has recently been shown in the case of aspartate transcarbamylase (Schachman, 1967), is also possible.

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