REDUCTION OF THE ACETYL COENZYME A CARBOXYLASE CONTENT OF *SACCHAROMYCES CEREVISIAE* BY EXOGENOUS FATTY ACIDS

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1. Introduction

The synthesis 'de novo' of long-chain fatty acids in yeast proceeds via malonyl–CoA as intermediate [1]. Owing to extensive studies of Lynen's group [2], the mechanism of this biosynthetic process is now well understood. Very little is known, however, about the control of fatty acid synthesis in yeast. With animal systems, much effort has been made to elucidate the regulatory mechanisms for coenzyme A carboxylase (acetyl–CoA:CO_2 ligase (ADP), EC 6.4.1.2), the enzyme which plays a critical role in the control of mammalian and avian fatty acid synthesis (see [3]).

The present report deals with studies on the regulation of acetyl–CoA carboxylase in *Saccharomyces cerevisiae* by exogenous fatty acids. The results of these studies have shown that addition of palmitic, palmitoleic, oleic, linoleic or linolenic acid to the culture medium gives rise to a decrease in the cellular content of this enzyme, and that the extent of this decrease is in good agreement with the extent of depression of fatty acid synthesis in whole cells caused by these fatty acids.

2. Materials and methods

Myristic, palmitic, oleic, linoleic and linolenic acid were purchased from Nakarai Chemicals (Kyoto, Japan). Palmitoleic, *cis*-vaccenic and arachidonic acid were obtained from Sigma (St. Louis, U.S.A.).

A wild type strain of *S. cerevisiae* (X 2180-1B [4]) was used in all experiments. The culture medium (YEPS medium) consisted of 0.7% Bacto-yeast extract (Difco, Detroit, U.S.A.), 0.7% Bactopeptone (Difco), 2% sucrose, 0.5% KH_2PO_4 and 0.5% K_2HPO_4. Unless otherwise stated, the medium contained in addition 1% Brij 58 (Kao–Atlas, Tokyo, Japan). Cells were grown with or without addition of fatty acids at 25°C for 12–16 hr with agitation at 72 reciprocations per min. Cells were harvested by centrifugation and washed three times with 0.1 M potassium phosphate buffer pH 6.5 containing 5 mM 2-mercaptoethanol and 1 mM EDTA. The following procedures were all carried out at 0–5°C. Five grams (wet wt.) of cells were suspended in 10 ml of the same buffer and disrupted with 15 g of glass beads (diameter, 0.45–0.50 mm) in a cell homogenizer (Braun, Melsungen, Germany) for 50 sec at high speed under cooling with liquid CO_2. The homogenate was centrifuged at 20 200 *g* for 15 min, and the resulting supernatant centrifuged at 105 000 *g* for 1 hr. One milliliter of the final supernatant was applied on a Sephadex G-50 column (1 × 13 cm) to remove low molecular weight materials. The protein fraction obtained was assayed for acetyl–CoA carboxylase activity by the [^{14}C] bicarbonate-fixation method. The assay was carried out at 37°C under the conditions described previously [5] except that preincubation was omitted and that citrate was not included in the reaction mixture. One enzyme unit is defined as that amount which catalyzes the carboxylation of 1 μmole of acetyl–CoA per min under the conditions used.

For immunochemical titrations, anti-acetyl–CoA carboxylase serum, which was kindly provided by Dr. M. Sumper [6], was added directly to the enzyme solution. The mixture was incubated at 20°C for 15 min and then allowed to stand at 0°C for 90 min. The surviving acetyl–CoA carboxylase activity was assayed without removing the precipitate, if any. The rate of fatty acid synthesis in whole cells was measured by

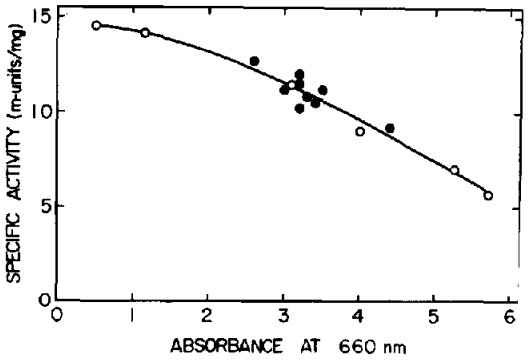

Fig. 1. Relationship between acetyl–CoA carboxylase activity and growth of *S. cerevisiae*. Growth was followed by measuring absorbance at 660 nm of the culture. Cells were grown in the YEPS medium without fatty acid in the presence (•) and absence (○) of Brij 58 and harvested at the densities indicated. Acetyl–CoA carboxylase was assayed as described in Materials and methods.

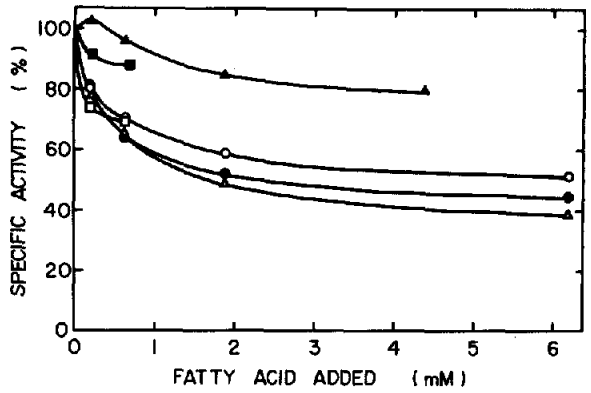

Fig. 2. Effects of exogenous fatty acids on the specific activity of acetyl–CoA carboxylase. Various fatty acids were added to the culture medium at the concentrations indicated; (▪-▪-▪) myristic acid; (□-□-□) palmitic acid; (•-•-•) palmitoleic acid; (○-○-○) oleic acid; (▲-▲-▲) *cis*-vaccenic acid; (△-△-△) linoleic acid. The specific activity found for cells grown without fatty acid was 10.8–11.8 m-units/mg.

incorporation of radioactive acetate into fatty acids as follows. Cells grown with or without exogenous fatty acids were washed with the respective culture medium and suspended in the same medium containing 1 mM sodium [1-^{14}C] acetate (0.1 mCi/mmole, the Radiochemical Centre, Amersham, England) to yield 20 mg wet wt. of cells per ml. Incubation was carried out at 25°C with agitation as described above. After 15, 30 and 60 min, 5 ml of the culture was removed and mixed with 0.6 ml of 50% trichloroacetic acid. The cells were collected by centrifugation and washed three times with 5 ml of water. Saponification and extraction of fatty acids were conducted according to the procedure described by Matsuhashi et al. [7], and the radioactivity was measured in the scintillator solution of Patterson and Greene [8]. Protein was determined by the method of Lowry et al. [9] with bovine serum albumin as the standard.

3. Results

As shown in fig. 1, the specific activity of acetyl–CoA carboxylase was a function of growth. In all the following experiments, cells were harvested during the mid-logarithmic phase of growth when the absorbance at 660 nm of the culture was about 3. At this cell density, the specific activity of acetyl–CoA carboxylase was fairly reproducible, ranging from 10 to 13 m-units per milligram of protein. Addition of 1% Brij 58 to the YEPS medium did not affect the relationship between specific activity and growth phase, although the detergent caused a slight delay of growth.

Fig. 2 represents the effects of various fatty acids added to the culture medium on the specific activity of acetyl–CoA carboxylase. Palmitoleic, oleic and linoleic acid were the most effective, bringing about a 50–60% reduction of the enzyme activity at the highest concentration used. Myristic acid and *cis*-vaccenic acid were less effective. High concentrations of myristic acid and palmitic acid could not be tested due to their insolubility. In other experiments (not shown), linolenic acid at 6.2 mM effected a 51% reduction, and arachidonic acid at 4.4 mM a 23% reduction.

In an attempt to examine whether the reduced acetyl–CoA carboxylase activity is due to a decrease in the enzyme content of cells or in the catalytic efficiency per enzyme molecule, immunochemical titrations were carried out with the use of specific antibody. The enzyme preparations used were obtained from the cells employed in the experiment shown in fig. 2. The results of the immunochemical titrations are illustrated in fig. 3. Despite the fact that the preparations derived from cells grown with

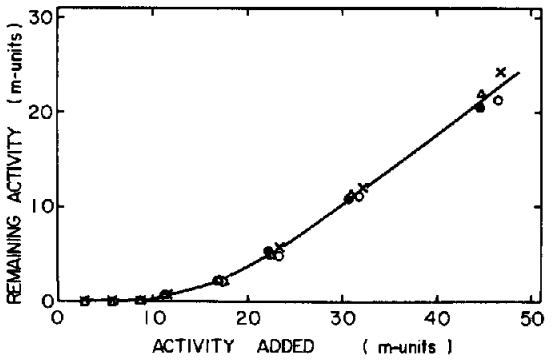

Fig. 3. Immunochemical titrations of acetyl–CoA carboxylase from cells grown with or without exogenous fatty acids. The enzyme preparations used were derived from cells grown without fatty acid (X-X-X), and from cells grown with 6.2 mM palmitoleic acid (●-●-●), oleic acid (○-○-○) and linoleic acid (△-△-△); the specific activities were 10.1, 4.5, 5.1 and 3.8 m-units/mg, respectively. Increasing amounts of the enzyme preparations were added to a fixed amount of anti-acetyl–CoA carboxylase serum (0.7 mg of protein). The surviving enzyme activity was determined as described in Materials and methods.

exogenous fatty acids exhibited much lower enzyme activity than did the preparation derived from control cells, the amount of enzyme activity inactivated by a fixed amount of antibody was identical regardless of the source of the preparations. This finding indicates that the catalytic efficiency per enzyme molecule is the same for all the preparations, and therefore that the reduction of acetyl–CoA carboxylase activity brought about by exogenous fatty acids is ascribed to a decrease in the enzyme content of cells.

The effects of exogenous fatty acids on the rate of fatty acid synthesis in *S. cerevisiae* was next examined by measuring [^{14}C] acetate incorporation into fatty acids. Cells grown with or without palmitoleic, oleic or linoleic acid were further incubated with [^{14}C] acetate in the presence of the respective fatty acid. As shown in table 1, the rate of [^{14}C] acetate incorporation into fatty acids was reduced by the exogenous fatty acids. The extent of this reduction was in good agreement with the extent of decrease in the acetyl–CoA carboxylase content of cells.

Table 1
[^{14}C] acetate incorporation into fatty acids by cells grown with or without exogenous fatty acids.

Fatty acid added	[^{14}C] acetate incorporation (cpm)		
	15 min	30 min	60 min
None	1700	4190	15320
Palmitoleic acid	1120	2810	8050
Oleic acid	690	2500	4490
Linoleic acid	1080	2020	9610

Fatty acids were added to the culture medium at a concentration of 6.2 mM. [^{14}C] acetate incorporation into fatty acids was measured as described in Materials and methods. The values are expressed as the radioactivity incorporated per 5 ml of the culture.

4. Discussion

The experiments described in the present paper have shown that the acetyl–CoA carboxylase content of *S. cerevisiae* is reduced by addition of certain fatty acids to the culture medium. The lowered acetyl–CoA carboxylase content content can account for the observed decrease in the rate of [^{14}C] acetate incorporation into fatty acids by whole cells resulting from the addition of exogenous fatty acids. Thus, under these conditions, the rate of fatty acid synthesis in *S. cerevisiae* appears to be regulated by changes in the cellular content of acetyl–CoA carboxylase rather than by inhibition of this enzyme. In relation to this finding, it is to be noted that a wild type strain as well as a fatty acid auxotrophic strain of *S. cerevisiae* is capable of incorporating fatty acids from the culture medium into cells [10].

It has been reported that fatty acid synthesis in *Lactobacillus plantarum* is markedly depressed by *cis*-vaccenic, palmitoleic and lactobacillic acid, which represent the major unsaturated and cyclopropane fatty acids of this organism, as well as by oleic acid, which has a close structural similarity to *cis*-vaccenic acid [11]. This effect is accounted for by depressed acetyl–CoA carboxylase activity, although there is no direct evidence for a reduction of the enzyme content. On the other hand, linoleic acid and linolenic acid, which are hardly detectable in these cells, exhibit little or no effect. In *S. cerevisiae*, however, even linoleic acid and linolenic acid, which are not usually

found in appreciable amounts in this organism [12], bring about a reduction of the enzyme content. Recent studies have demonstrated that human skin fibroblasts grown with fetal calf serum contain less acetyl–CoA carboxylase than cells grown with lipid-deficient serum [13]. The effect of lipids in the serum is replaced by fatty acids. Analogous effects of triglycerides and fatty acids have been observed also with rat hepatocytes maintained in a lipid- and protein-free synthetic medium [14].

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