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EVIDENCE FOR TWO IMMUNOLOGICALLY DISTINCT ACETYL-COENZYME A SYNTHETASES IN YEAST

T. SATYANARAYANA, ADRIAN D. MANDEL and HAROLD P. KLEIN
Ames Research Center, NASA, Moffett Field, Calif. 94035 (U.S.A.)
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SUMMARY

1. In this investigation, some immunological properties of the yeast acetyl-CoA synthetase [acetate:CoA ligase (AMP), EC 6.2.1.1] elaborated under aerobic and non-aerobic conditions are presented.

2. The antibody produced by each enzyme is immunologically specific. No evidence was found for the presence of inhibitory agent(s) in either enzyme extract which inhibited the heterologous enzyme antibody reaction.

3. The enzyme purified from aerobic yeast was inhibited 85% by its homologous antiserum which inhibited activity of the enzyme from non-aerobic yeast by only 20%, while enzyme activity from non-aerobic yeast was inhibited 65% by its homologous antiserum, which inhibited activity from aerobic yeast by about 13%.

4. Data presented in this paper indicate the presence of two distinct acetyl-CoA synthetases in this strain of yeast.

INTRODUCTION

In earlier reports, we have presented evidence for the bimodal distribution of acetyl-CoA synthetase of *Saccharomyces cerevisiae* during aerobic growth [1]. The enzyme was localized in the microsomal fraction during the early stages of growth, but shifted to the mitochondrial fraction when the cells reached the stationary phase. On the other hand, the enzyme was always localized in the microsomal fraction of these cells when grown under non-aerobic conditions [2].

The enzyme of either microsomal or mitochondrial origin from aerobically-grown cells differed from the enzyme from non-aerobic cells in a number of ways [3]. Firstly, the K_m value for acetate of the "aerobic" enzyme was 10-fold lower, and for ATP 3–4-fold lower, than the corresponding values for the "non-aerobic" enzyme. The "aerobic," but not the "non-aerobic," enzyme also utilized propionate as a substrate. In addition, the "aerobic" enzyme was found to be regulated by long chain acyl-CoA esters, whereas the "non-aerobic" acetyl-CoA synthetase was unaffected [4]. These studies strongly suggested that the acetyl-CoA synthetase elaborated under the two growth conditions represented two different proteins.

This paper presents data which further support the contention that two distinct acetyl-CoA synthetase may be produced by this yeast.

METHODS

General Methods

Saccharomyces cerevisiae, strain LK2G12, was grown, harvested, disintegrated and fractionated as described earlier [4]. The "aerobic" enzyme was crystallized from the mitochondrial fraction of 48-h grown cells [5]. The "non-aerobic" enzyme was partially purified from 48-h-old cells grown in standing cultures (Satyanarayana, T. and Klein, H. P., unpublished).

The enzyme assay was carried out as described earlier [1]. Protein was determined by the method of Lowry et al. [6] using bovine serum albumin as a standard. All other chemicals used were of reagent grade.

Preparation of Antiserum

Anti-acetyl-CoA synthetase serum was prepared by injecting increasing amounts of the enzyme mixed with Freund's adjuvant (Difco) into the ear veins of rabbits, once every 5 days for 15 days. 7 days after the last injection, the rabbits were bled by heart puncture after overnight fasting. The clear serum collected after centrifugation was stored frozen. To eliminate any nonspecific effects on the enzyme activity, non-immune (i.e., pre-immunization) serum was collected in a similar way.

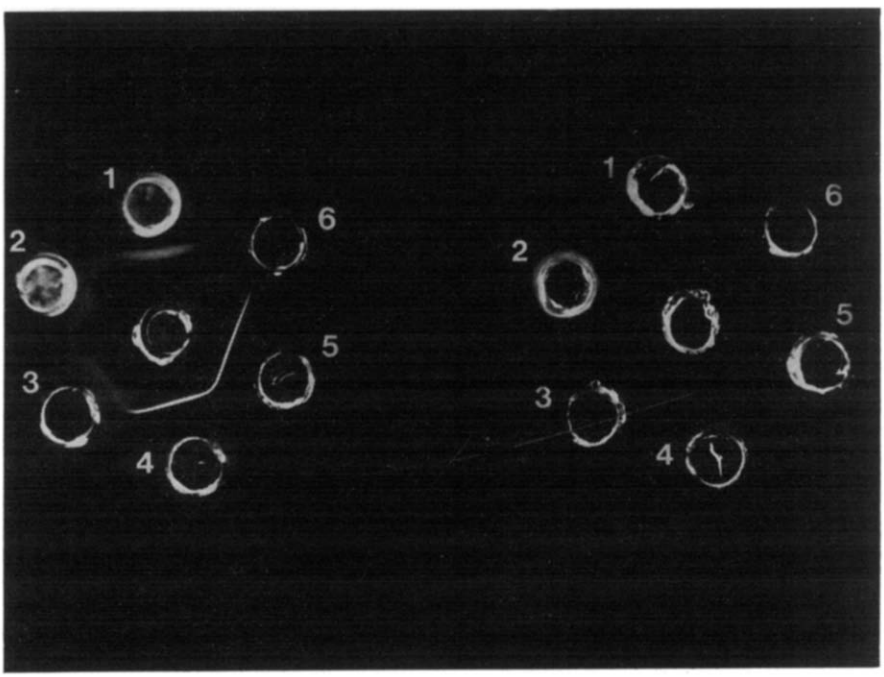

Fig. 1. Typical gel diffusion pattern with antiserum in center well and various fractions obtained during the purification of yeast acetyl-CoA synthetase from "aerobic" and "non-aerobic" cells. Center wells: antiserum against crystalline "aerobic" acetyl-CoA synthetase. Left side (aerobic): 1, crude extract; 2, mitochondrial fraction; 3, solubilized enzyme; 4, $(NH_4)_2SO_4$ fraction; 5, crystalline enzyme; 6, non-immune serum. Right side (non-aerobic): 1, crude extract; 2, microsomal fraction; 3 solubilized enzyme; 4, $(NH_4)_2SO_4$ fraction; 5, non-immune serum; 6, no addition.

For the preparation of antibodies against the "aerobic" acetyl-CoA synthetase, a total of 2.76 mg protein (spec. act. 47.9) was injected, while in the case of the "non-aerobic" preparation, a total of 8.28 mg protein (spec. act. 2.1) was used.

Immunodiffusion

Agar diffusion studies were carried out by the method of Ouchterlony [7] with 1.2% ionagar No. 2 in 0.1 M potassium phosphate buffer, pH 7.4, containing 0.9% KCl. Plates were developed at room temperature for 24 h.

Enzyme inhibition assays

The antiserum inhibition experiments were carried out by adding the antiserum (diluted with 0.1 M potassium phosphate buffer, pH 7.4, containing 0.9% KCl) to the standard enzyme assay reaction mixture [4]. Controls were routinely carried out using the same dilutions of non-immune serum.

RESULTS

The antiserum directed against the "aerobic" enzyme was tested on various fractions obtained during the purification of both the "aerobic" and "non-aerobic"

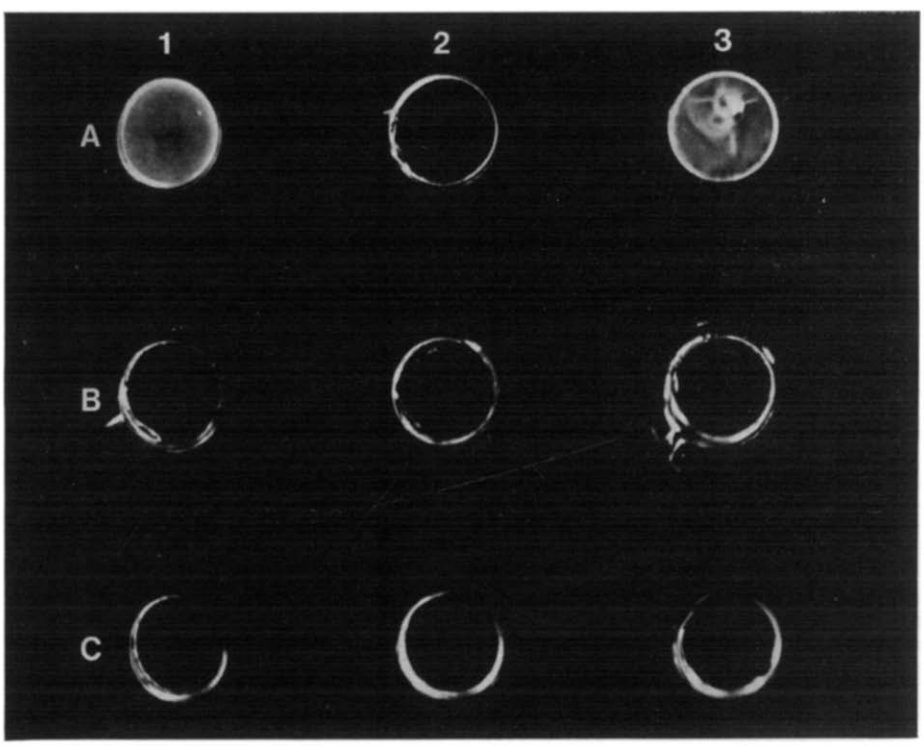

Fig. 2. Double diffusion study using aerobic acetyl-CoA synthetase antiserum in the center wells (Row B). Row A, well No. 1, "aerobic" enyzme, crude extract (spec. act. 0.7); No. 2, solubilized fraction, "non-aerobic" enzyme (spec. act. 0.6); No. 3, mixture of 1 and 2. Row C, well No. 1, aerobic enzyme, solubilized fraction (spec. act. 2.9); No. 2, non-aerobic enzyme, $(NH_4)_2SO_4$ fraction (spec. act. 2.1); No. 3, mixture of 1 and 2.

enzymes (Fig. 1). A single precipitin line was observed against crystalline "aerobic" acetyl-CoA synthetase, whereas serum obtained before immunization did not react with the enzyme. On the other hand, the fractions from the "non-aerobic" enzyme purification steps did not give any precipitin lines with this antiserum.

The presence of inhibitor(s) in "non-aerobic" extracts which might cause inhibition of precipitin formation was tested by mixing fractions of approximately the same specific activity from both aerobic and non-aerobic cells and using these on immunodiffusion plates. It was found (Fig. 2) that the simultaneous presence of "non-aerobic" acetyl-CoA synthetase and "aerobic" acetyl-CoA synthetase did not inhibit the precipitin reaction of "aerobic" acetyl-CoA synthetase using the homologous antiserum. In addition, antiserum prepared against partially purified "non-aerobic" acetyl-CoA synthetase gave several precipitin lines with its homologous "non-aerobic" preparations. On the other hand, the "aerobic" crystalline acetyl-CoA synthetase did not cross-react with "non-aerobic" acetyl-CoA synthetase antibody nor did it inhibit

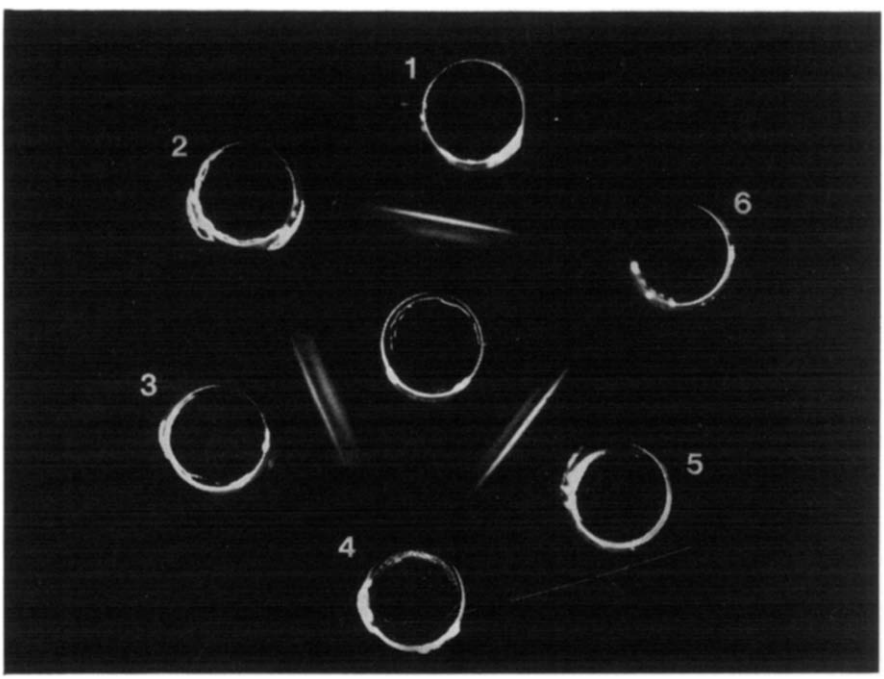

Fig. 3. Immunodiffusion plate with antiserum in the center well. Center well, antiserum directed towards "non-aerobic" (partially purified) preparation. 1, $(NH_4)_2SO_4$ fraction (non-aerobic); 2, aerobic crystalline acetyl-CoA synthetase; 3, mixture of 1 and 2; 4, aerobic crystalline acetyl-CoA synthetase; 5, $(NH_4)_2SO_5$ fraction (non-aerobic); 6, non-immune serum.

the precipitin reactions of the "non-aerobic" enzyme (Fig. 3). As was found before, the non-immune serum gave no precipitin lines.

The effect of antisera on enzyme activity was also tested (Figs 4 and 5). At a dilution of 1:10 000 of the "aerobic" antiserum, the crystalline "aerobic" enzyme was

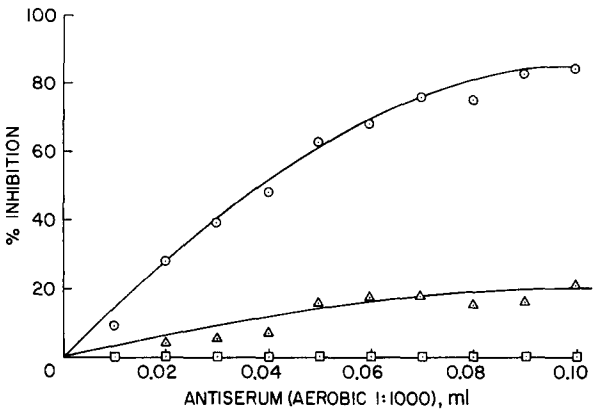

Fig. 4. Effect of "aerobic" enzyme antiserum on acetyl-CoA synthetase activity. Circles, "aerobic" (crystalline) enyzme; triangles, "non-aerobic" acetyl-CoA synthetase ($(NH_4)_2SO_4$ fraction); squares, non-immune serum with "aerobic" or "non-aerobic" acetyl-CoA synthetase. The incubation system contained, in 1 ml; CoA (0.1 μmole); ATP (10 μmoles); glutathione (10 μmoles); $MgCl_2$ (10 μmoles); potassium phosphate, at pH 7.4 (100 μmoles); neutralized hydroxylamine (200 μmoles); acetate (10 μmoles, "aerobic"; 100 μmoles, "non-aerobic" assays), antiserum or non-immune serum (as indicated). The reaction was started by the addition of the enzyme, then incubated for 20 min at 37 °C.

inhibited about 85%, while the "non-aerobic" acetyl-CoA synthetase was inhibited only about 20%. At a dilution of 1:10, the "non-aerobic" acetyl-CoA synthetase was inhibited about 65% by the homologous antiserum preparation whereas the "aerobic" acetyl-CoA synthetase was inhibited only 13%. No precipitation was visible either in the presence of "aerobic" or "non-aerobic" antisera under these conditions. In all cases, non-immune serum stimulated both enzyme activities (by about 28%) and the values shown here are corrected for this effect.

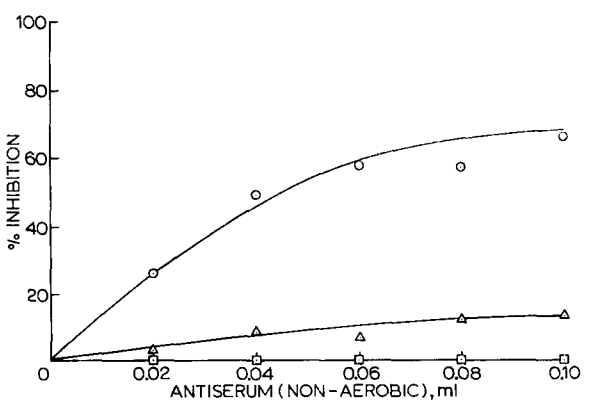

Fig. 5. Effect of "non-aerobic" enzyme antiserum on acetyl-CoA synthetase activity. Circles, "non-aerobic" enzyme ($(NH_4)_2SO_4$ fraction); triangles, "aerobic" (crystalline) enzyme; squares, non-immune serum with "aerobic" or "non-aerobic" acetyl-CoA synthetase. The incubation system as indicated under Fig. 4.

DISCUSSION

Evidence provided in this paper clearly established the presence of two acetyl-CoA synthetases, one elaborated under "aerobic" conditions, the other under "non-aerobic" conditions. The antibody directed towards one protein precipitated and inhibited the activity of homologous, but not the heterologous, enzyme. The presence of inhibitory agent(s) in heterologous antiserum or enzyme was ruled out in these experiments.

An interesting property observed is the effect of antibody on enzyme activity. The "aerobic" enzyme was inhibited to a maximum extent of 85% by the homologous antiserum. In a previous study [4], we found that the long chain acyl-CoA ester, palmityl-CoA, also inhibited "aerobic" acetyl-CoA synthetase activity by about 85%. The simultaneous presence of "non-aerobic" enzyme in "aerobic" preparations was ruled out in the earlier study [4], so no ready explanation is available to account for the fact that 100% inhibition is never achieved in the presence of either palmityl-CoA or of the homologous antibody. A similar situation was encountered for inhibition of the "non-aerobic" enzyme activity by the homologous antiserum.

It is of interest to note that there is a small inhibition of either enzyme activity by the heterologous antiserum. The slight cross-reactivity may be taken as an indication that there may be some similarity in the structure of the two proteins. Whether this is at the active site or in regions related to it or whether the two enzymes share a common peptide chain [8], remains to be elucidated.

ACKNOWLEDGEMENT

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