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SUBUNIT SPECIFICITY OF THE TWO ACETYL-CoA SYNTHETASES OF YEAST AS REVEALED BY AN IMMUNOLOGICAL APPROACH

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Summary

1. In the present paper, the two acetyl-CoA synthetases (acetate:Coenzyme A ligase (AMP-forming), EC 6.2.1.1) elaborated under aerobic or nonaerobic conditions are further differentiated by an immunological approach.

2. The subunit of the aerobic isozyme was prepared and found to be homogeneous by disc gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) and by ultracentrifugal studies. An $s_{20,w}$ of 3.6 and an apparent molecular weight of 80 500 ± 500 were calculated for this subunit.

3. The subunit was precipitated by antibody prepared against the aerobic enzyme. Antibody prepared against the subunit also reacted in precipitin tests with the subunit, but not with the native enzyme. The latter antibody nevertheless inhibited the native enzyme but not the nonaerobic isozyme.

Introduction

In *Saccharomyces cerevisiae*, available evidence has so far indicated the presence of two distinct acetyl-CoA synthetases, depending upon the supply of oxygen during growth. The two isoenzymes differ in their subcellular localization [1], affinity constants for substrates [2], inhibition by long-chain acyl-CoA compounds [3] and their reaction to antibodies elicted to one or the other of the isozymes [4].

Based on the estimated molecular weights of the aerobic enzyme and of its subunit, as well as the Hill interaction coefficient in the presence of palmitoyl-CoA, it was proposed that this enzyme, in its natural form, might be a trimeric protein [5]. Furthermore, since the nonaerobic enzyme appears to be close to

four times the molecular weight of the known subunit of the aerobic enzyme, it seems possible that the two enzymes differ only in their polymeric structure. Other studies on the kinetics of formation for the two enzymes further suggested the possibility of conversion of the nonaerobic to the aerobic enzyme, presumably by the loss of one subunit [6]. The main purpose of this study was to ascertain, through an immunological approach, whether there was a common subunit to both the enzymes.

Materials and Methods

Saccharomyces cerevisiae, strain LK2G12, was used in this study. Its cultivation, preparation of cell-free homogenates, isolation of subcellular fractions and methods of enzyme analysis have been described earlier [3].

Chemicals. Coenzyme A and ATP were obtained from Mannheim-Boehringer Corporation (Indianapolis, IN), reagents for acrylamide gel electrophoresis and sodium dodecyl sulfate (SDS) were purchased from Bio-Rad Laboratories, (Richmond, CA). Dithiothreitol was purchased from Calbiochem Corporation (San Diego, CA), guanidine hydrochloride and urea were purchased from Schwarz/Mann Chemical Co. (Orangeburg, NY). Immunodiffusion discs were purchased from Miles Laboratories (Elkhart, IN). All other reagents were of reagent grade.

Highly purified, crystalline, aerobic isozyme (spec. act., 390—468) was prepared as described earlier [5]. The nonaerobic isozyme was partially purified by conventional techniques involving differential centrifugation, solubilization of the bound enzyme from the microsomal fraction, $(NH_4)_2SO_4$ fractionation followed by affinity chromatography on agarose-organomercurial columns, and subsequent elution by ATP from agarose-AMP columns. Such preparations had a spec. act. of approx. 155 and resulted in an overall purification of 129-fold (Satyanarayana, T. and Klein, H.P., unpublished data).

Protein was determined by the method of Lowry et al. [7] using bovine serum albumin as standard. Polyacrylamide gel electrophoresis was carried out as described earlier [5].

Acetyl-CoA synthetase subunit preparation. The method used was essentially that described by Weber et al. [8], utilizing the vigorous conditions described in their 'Method 2' (i.e., denaturation with guanidine hydrochloride followed by alkylation, with reagents suitably varied for the amount of enzyme protein used).

Analytical ultracentrifugation. All centrifugations were carried out in a Beckman Model E analytical ultracentrifuge as described earlier [5]. Sedimentation equilibrium runs were carried out at 16 000 rev./min at 16°C, and sedimentation velocity runs were carried out at 60 000 rev./min at 20°C.

Preparation of antiserum. The antiserum for the aerobic isozyme subunit was prepared in a similar manner to that used for the native enzyme as described earlier [4]. Immunodiffusion and enzyme inhibition assays were carried out essentially as described in those studies.

Results

Since the aerobic acetyl-CoA synthetase isozyme subunit, when subjected to polyacrylamide gel electrophoresis in the presence of SDS, yielded a single

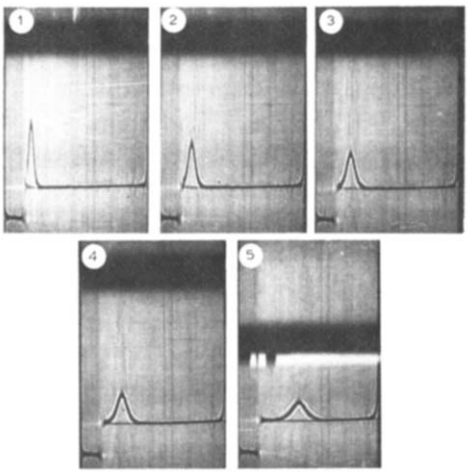

Fig. 1. Sedimentation pattern of the aerobic acetyl-CoA synthetase subunit. The picture represents (1) 8; (2) 16; (3) 24; (4) 32; and (5) 64 min after attaining the speed of 60 000 rev./min. (The last frame (5) contains the interference pattern at the same time.) Schlieren diaphragm angle, 70°. Protein concentration 3.5 mg/ml.

band with an apparent molecular weight of 82 500—83 000 [5] and since the molecular weight for the native enzyme was estimated to be 250 000 [5], the aerobic isozyme is assumed to be composed of three subunits having identical or nearly identical molecular weight.

Sedimentation velocity and sedimentation equilibrium experiments

In the present study, molecular weight estimations were performed on the subunit of the aerobic enzyme using ultracentrifugation techniques. Sedimentation velocity experiments were carried out on the aerobic subunit prepared in 0.01 M sodium phosphate buffer, containing 0.1% SDS. A value of 3.6 for $s_{20,w}$ was calculated from the single symmetrical peak observed (Fig. 1). The molecular weight of the subunit was determined by the low-speed sedimenta-

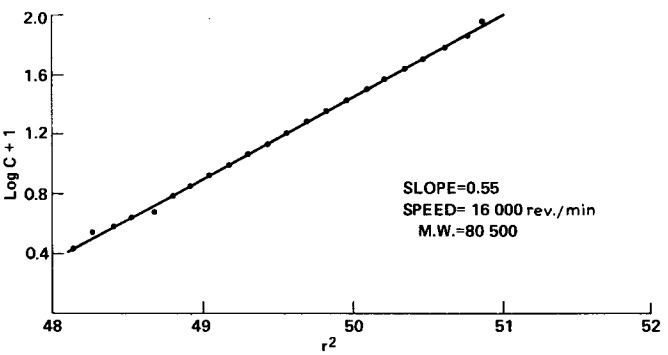

Fig. 2. Typical plot of log of interference fringe displacement vs. r^2 plot used for the analysis of meniscus depletion sedimentation equilibrium data at 16 000 rev./min (slope 0.55). The concentration range covered is approx. 0.07—2.10 mg/ml.

tion equilibrium technique [9]. Plots of the natural logarithm of fringe displacement vs. r^2 yielded a straight line as shown in Fig. 2. Using a value for the partial specific volume calculated from amino acid analysis of the native enzyme ($\bar{v} = 0.733$), the molecular weight of the subunit was calculated to be 80 500 ± 500.

Immunodiffusion studies

Antiserum developed against the aerobic isoenzyme subunit reacted only with the homologous subunit isoenzyme. As is seen in Fig. 3, a single precipitin line was observed in the case of the aerobic isozyme subunit, and neither the subunit(s) of the nonaerobic acetyl-CoA synthetase isozyme nor the native aerobic or nonaerobic enzymes showed any reaction using immunodiffusion discs.

Antiserum reactive against native aerobic acetyl-CoA synthetase isozyme [4] also reacted with the subunit of the homologous isozyme (Fig. 4). No reaction, however, was observed with nonaerobic isozyme nor with the subunit(s) of the nonaerobic isozyme.

Effect of antisera on enzyme activity

The effect of antiserum prepared against the aerobic isozyme subunit was next tested on the enzyme activity of aerobic and nonaerobic acetyl-CoA synthetase. Fig. 5 shows that this antiserum inhibited the homologous enzyme activity but showed no effect on nonearobic acetyl-CoA synthetase activity. In both cases, nonimmune serum or serum isolated from rabbits injected only with SDS buffer showed no inhibition. In fact, a slight stimulation (10—15%)

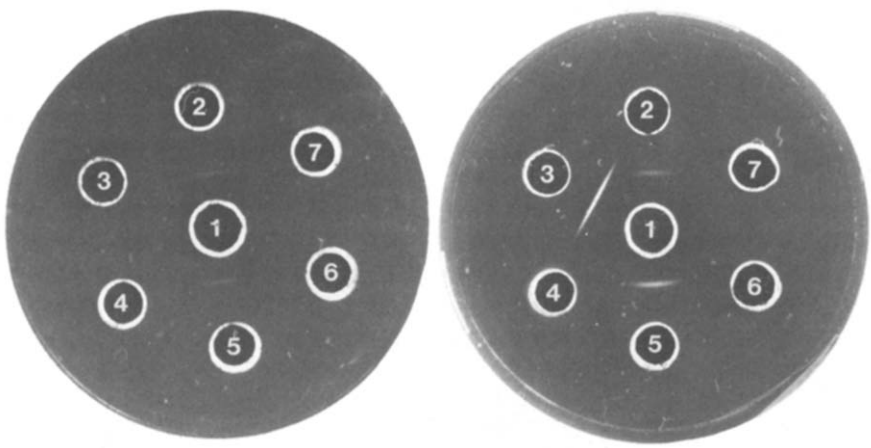

Fig. 3. Double diffusion study using antiserum of aerobic acetyl-CoA synthetase subunit in the center well (1); (2) and (5) aerobic isozyme subunit; (3) and (6) aerobic isozyme; (4) nonaerobic acetyl-CoA synthetase; (7) nonaerobic acetyl-CoA synthetase subunit(s).

Fig. 4. Double diffusion pattern using antibody of the native aerobic acetyl-CoA synthetase isoenzyme in the center well (1); (2) and (5) aerobic isozyme subunit; (3) aerobic isozyme; (4) nonaerobic isozyme; (6) nonimmune serum; (7) nonaerobic isozyme subunit(s).

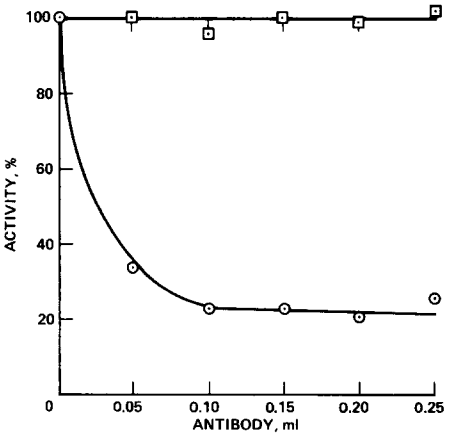

Fig. 5. Effect of antiserum to aerobic acetyl-CoA synthetase subunit on enzyme activity. ⊙———⊙, aerobic acetyl-CoA synthetase; ⊡———⊡, nonaerobic acetyl-CoA synthetase.

of both enzyme activities was observed and the values were corrected for this effect.

Discussion

Several lines of evidence indicate [1—6] the presence of two distinct acetyl-CoA synthetase isozymes in this strain of yeast. For the aerobic enzyme, a molecular weight estimate of 250 000 ± 500 was deduced from several different lines of evidence. The molecular weight of the nonaerobic isozyme was estimated to be 346 000 ± 500 based on the enzyme activity profile on Bio-Gel A 1.5 m columns (Satyanarayana, T. and Klein, H.P., unpublished data). Upon dissociation of the aerobic enzyme with SDS, earlier studies indicated a single polypeptide species of around 83 000 ± 500 on polyacrylamide gel electrophoresis (indicating a trimeric structure for the enzyme [5]). From the data presented here, this subunit was found to be homogeneous by sedimentation velocity studies as well as by sedimentation equilibrium studies. The straight line linearity observed during sedimentation equilibrium studies indicates the existence of a single species of around 80 500 ± 500.

Using preparations of this type, antibodies could be obtained against this subunit. If, in fact, the two isozymes were composed of identical subunits, it was reasonable to expect that an antibody preparation reactive to this subunit would react with both enzymes, although it is recognized that the quaternary structure of the two native proteins ultimately might determine immunologic reactivity. However, the data clearly indicate that only the aerobic enzyme is inhibited by antibody to the aerobic subunit, thus suggesting that the two proteins are not polymers of a common subunit. However, until pure preparations of the nonaerobic acetyl-CoA synthetase isozyme also become available, it will not be possible to obtain absolutely conclusive information concerning the possible chemical and structural similarities between the two enzymes.

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