ENZYMOLOGY OF THE PIGMENTED ADENINE-REQUIRING MUTANTS OF SACCHAROMYCES AND SCHIZOSACCHAROMYCES

C. R. Fisher

Biology Division, Oak Ridge National Laboratory,
Oak Ridge, Tennessee

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The red or purple adenine-requiring mutants of Saccharomyces, Schizosaccharomyces, and Neurospora have been categorized genetically as resulting from the mutation of two separate loci. In Neurospora crassa these are the ad-3A and ad-3B loci (de Serres, 1956); in Saccharomyces cerevisiae, the ad-1 and ad-2 loci (Roman, 1956); and in Schizosaccharomyces pombe, the ad-6 and ad-7 loci (Leupold & Gutz, 1965). Attempts to arrange these mutants as genetic blocks along the purine biosynthetic pathway have been limited to the identification of the accumulated biochemical intermediates (Bernstein, 1961; Levinthal, Fogel and Hurst, 1962; French, personal communication; Silver and Eaton, 1968). These studies have indicated that the two loci collectively encode the two enzymes phosphoribosyl-amino-imidazole-succinocarboxamide synthetase (5'-phosphoribosyl-4-carboxy-5-amino-imidazole: L-Aspartate ligase (ADP), EC 6.3.2.6) and phosphoribosyl-amino-imidazole carboxylase (5'-phosphoribosyl-5-amino-4-imidazolecarboxylate carboxy-lyase, EC 4.1.1.21). However, the results of the Neurospora studies have not allowed a conclusive locus-enzyme correlation to be made (Bernstein, 1961; French, personal communication). Levinthal, Fogel and Hurst (1962) reported that ad-1 strains of Saccharomyces accumulate amino-imidazole ribonucleotide (AIR) and that ad-2 strains accumulate 5-amino-4-imidazolecarboxylate ribonucleotide (CAIR). Dorfman (1963) reported that both ad-1 and ad-2 strains accumulate AIR, and Silver and Eaton (1968, 1969) and Dorfman (1964) found that ad-1 strains accumulate CAIR and

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ad-2 strains accumulate AIR. The data on Saccharomyces presented in this report are in agreement with the latter conclusions.

This report describes a method of isolating and assaying for phosphoribosyl-amino-imidazole-succinocarboxamide synthetase in soluble protein extracts from Saccharomyces and Schizosaccharomyces and shows that this enzyme is encoded by the ad-1 locus of Saccharomyces and by the ad-7 locus of Schizosaccharomyces. Data showing that this enzyme is encoded by the ad-3A locus of Neurospora will be published elsewhere (Fisher, 1969).

MATERIALS AND METHODS

Schizosaccharomyces pombe strains L 250 (ad-6), L 409 (ad-7), M 210 (ad-6), and M 217 (ad-7) were obtained from Dr. H. Gutz, Southwest Center for Advanced Studies. Saccharomyces cerevisiae strains S733A (ad-1) and X1687-101B (ad-2) were obtained from Dr. R. C. von Borstel, Oak Ridge National Laboratory.

The isolation and assay of phosphoribosyl-amino-imidazole-succinocarboxamide synthetase were performed according to the method developed for the study of this enzyme in N. crassa (Fisher, 1969). The organisms were grown in 12-liter fermentors containing 10 liters of medium composed of 1% yeast extract, 2% peptone, 2% dextrose, and 2 ml polyglycol antifoam. Incubation at 25° with aeration and stirring was continued for 40 hours. The cells were centrifuged, washed, suspended in buffer, and broken in a Gaulin Press. Cell debris and ribosomes were removed by centrifugation, and the proteins were precipitated with saturated $(NH_4)_2SO_4$. Proteins soluble in 2.0 M $(NH_4)_2SO_4$ but not in 2.5 M $(NH_4)_2SO_4$ were desalted on Sephadex G-25, concentrated, and subjected to hydroxylapatite chromatography utilizing a 1-liter 0.005 to 0.2 M linear phosphate gradient for elution. Ten-ml fractions were collected and assayed for synthetase activity.

Synthetase activity was determined by assaying for the conversion of 5-amino-4-imidazole-N-succinocarboxamide ribonucleotide (SAICAR) to 5-amino-4-imidazolecarboxylate ribonucleotide (CAIR). The conversion of SAICAR to CAIR was measured by converting CAIR to amino-imidazole ribonucleotide (AIR) and assaying for the amount of AIR produced (Fisher, 1969).

RESULTS AND DISCUSSION

The elution patterns of protein and synthetase activity from hydroxylapatite were similar for Saccharomyces and Schizosaccharomyces. Synthetase activity elutes early in the gradient as a single peak (Fig. 1). The ad-1 strain of Saccharomyces and the ad-7 strains of Schizosaccharomyces lacked synthetase activity, whereas the ad-2 strain of Saccharomyces and the ad-6 strains of Schizosaccharomyces possessed synthetase activity. These data indicate that phosphoribosyl-amino-imidazole-succinocarboxamide synthetase is encoded by the ad-1 locus in Saccharomyces and by the ad-7 locus in Schizosaccharomyces.

No attempt was made to characterize the enzyme obtained from yeast. Its optimum pH, temperature, ionic concentrations, etc. may be different from those determined for the Neurospora enzyme, but are apparently not sufficiently different to cause difficulty with the assay.

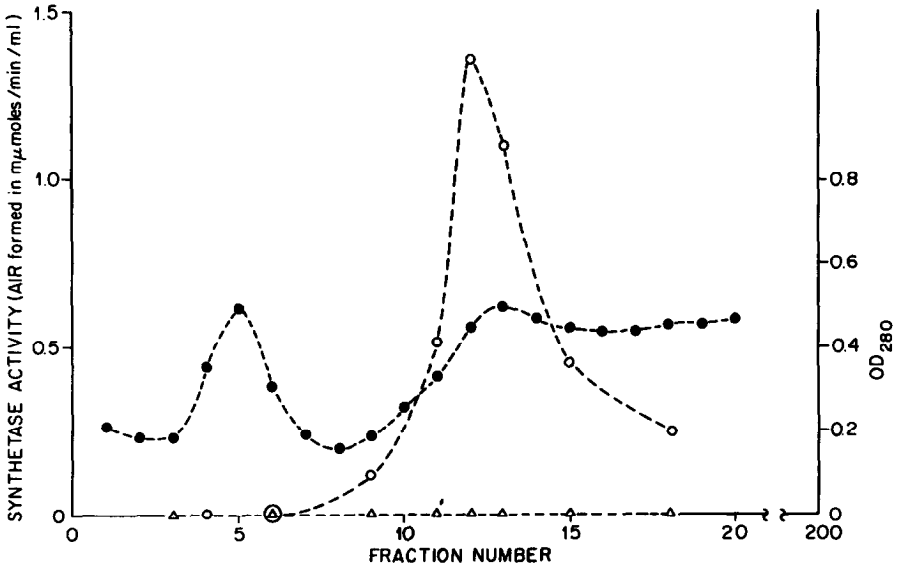

Figure 1. Typical elution of synthetase activity from hydroxylapatite. The reaction mixture contained in μmoles/ml: $MgCl_2$ 42; ADP, 10; potassium phosphate (pH 6.0), 95; 2-(N-morpholino) ethanesulfonic acid (MES) buffer (pH 6.0), 20; SAICAR, 0.4; and 0.1 ml of the appropriate hydroxylapatite fraction. The total volume was 0.525 ml and incubation was at 37°. ●-----●, OD 280 of eluate; 0-----0, synthetase activity of ad-2 strain of Saccharomyces and ad-6 strains of Schizosaccharomyces; Δ-----Δ, synthetase activity of ad-1 strain of Saccharomyces and ad-7 strains of Schizosaccharomyces. The data plotted are from S. pombe strains M210 and M217 and are representative of the data obtained with the other strains.

It is interesting to note that, in addition to the enzyme responding to similar isolation and assay conditions, there are also genetic similarities between the loci of the three genera. The locus specifying synthetase activity is noncomplementing in each organism (Woods and Bevan, 1966). The other locus, which can be assumed to specify the carboxylase activity, shows intragenic complementation (Table I).

If the genes and gene products are as similar as might be suggested, it is likely that CO_2 stimulation of growth, analogous to that reported by de Serres (1967) for Neurospora, should be observed in some ad-2 strains of Saccharomyces and ad-6 strains of Schizosaccharomyces. This would serve as a further confirmation of the carboxylase nature of that enzyme in the absence of an in vitro assay system.

TABLE I

Summary of locus-enzyme relationships and intragenic complementation

Organisms and loci	Locus-enzyme relationship* AIR⇐⇒CAIR⇐⇒SAICAR		Intragenic complementation
Neurospora crassa			
ad-3A	-	+	negative
ad-3B	+	-	positive
Saccharomyces cerevisiae			
ad-1	-	+	negative
ad-2	+	-	positive
Schizosaccharomyces pombe			
ad-6	+	-	positive
ad-7	-	+	negative

*+ indicates that the enzyme is encoded by this locus; - indicates that the enzyme is not encoded by this locus.

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Note added in proof: Dr. Robin Woods (personal communication) has found that the growth of certain ad-2 strains of Saccharomyces is stimulated by CO_2.