BIOCHEMICAL BASIS FOR THE ADENINE REQUIREMENT OF ad_3 MUTANTS OF SACCHAROMYCES.

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Received January 30, 1967

In Saccharomyces thirteen non-allelic adenine requiring mutants (ad_1-ad_{13}) have been reported (Microbial Genetics Bulletin, 1966). The metabolic defects associated with these mutants are well characterized only for ad_1 which accumulates 5-amino imidazole ribotide (AIR) (Friedman and Moat, 1958), and ad_{12} and ad_{13} which lack adenylosuccinate synthetase and adenylosuccinase activity respectively (Dorfman, 1966). The biosynthetic pathway for adenine in yeast seems to be identical with that described for other organisms (cf. Magasanik, 1962). Mutations at the ad_3 locus of Saccharomyces are of particular interest because they result in a requirement for histidine as well as adenine (Roman, 1956). Although it has been suggested by Levinthal et al (1962) that ad_3 mutants accumulate 5-amino 4-imidazole carboxamide ribotide (AICAR), no evidence supporting this contention has been offered. In the present paper experiments are described which establish the primary metabolic defect of ad_3 leading to the adenine requirement.

MATERIALS AND METHODS

The yeast strains were derived from stocks obtained from H. Roman. They were grown aerobically at 30°C to stationary phase in synthetic complete media (Roman, 1956).

(1) Aided by a traineeship from The National Aeronautics and Space Administration.

Cells were disrupted as described previously (Eaton, 1962). The disrupted cells were suspended in an equal volume of distilled water and centrifuged at 14,500xg for 10 minutes. The protein was precipitated with two volumes of 95% ethanol. After centrifuging, the supernatant was lyophylized, and the residue was dissolved in minimal amounts of distilled water for subsequent analysis.

The compound characteristically accumulated by ad_3 mutants was isolated by ion exchange chromatography and was detected by the Bratton-Marshall reaction for arylamines (Flaks et al, 1957). Additional analytical procedures used were the Pauly test for imidazoles (Koessler and Hanke, 1919), the orcinol test for pentose (Mejbaum, 1939), phosphate test (Fiske and Subbarow, 1925) and microbiuret for protein (Zamenhof, 1957).

Cellulose thin layer plates spread at a thickness of 0.5 mm. were used for chromatography.

AICAR transformylase activity was assayed by measuring the disappearance of AICAR according to the method of Flaks and Lukens (1963). The cells, disrupted as described above, were suspended in an equal volume of 0.1M phosphate buffer at pH7.6 and centrifuged at 22,000xg for 15 minutes. The supernatant was dialyzed 24 hours against the same buffer at 0.01M concentration.

RESULTS AND DISCUSSION

Characterization of the Bratton-Marshall positive material, isolated by ion exchange chromatography, from ad_3 mutants is given in Table I. The compound has a UV absorption maximum (268mu) and Bratton-Marshall chromatophore absorption maximum (540 mu) identical to that described by Flaks et al (1957) for AICAR. It gives a positive Pauly test for imidazoles and contains ribose and organic phosphate.

The alkaline phosphatase-treated compound was identical to the

commercially available AICARiboside. The two materials moved together in three solvent systems and had identical UV and Bratton-Marshall spectra. This clearly established that AICAR accumulates in ad_3 mutants.

TABLE 1

Characterization of Accumulation Product of ad_3 Mutants

	Isolated Compound		
	Untreated	Hydrolyzed[1]	AICARiboside[2]
Absorbance maximum	268mu	268mu	268mu
Bratton-Marshall chromatophore absorbance maximum	540mu	540mu	540mu
Imidazole	+	+	+
Pentose	+	+	+
Organic Phosphate	+	-	-
R_f (propanol: ammonia: water=7:1:2)	0.08	0.48	0.48
R_f (chloroform:methanol :10%formic acid=3:3:1)	0.67	0.53	0.53
R_f (n-butanol:5% acetic acid=1:1)	0.20	0.36	0.36

[1] Treated with alkaline phosphatase (Worthington).
[2] Purchased from California Biochemical Corporation.

By analogy to the pathway of adenine biosynthesis in other organisms, the accumulation of AICAR suggests that the enzyme AICAR transformylase, needed to convert AICAR to 5-formamido 4-imidazole carboxamide ribotide (FAICAR), is either lacking or non-functional in ad_3 mutants. AICAR transformylase is present in extracts of adenine-independent strains and adenine-requiring mutants other than ad_3 (Figure I). The AICAR was recovered in part by mild acid hydrolysis, indicating the formation of FAICAR as a reaction product (Flaks and Lukens, 1963). No AICAR

transformylase activity could be detected in extracts of ad_3.

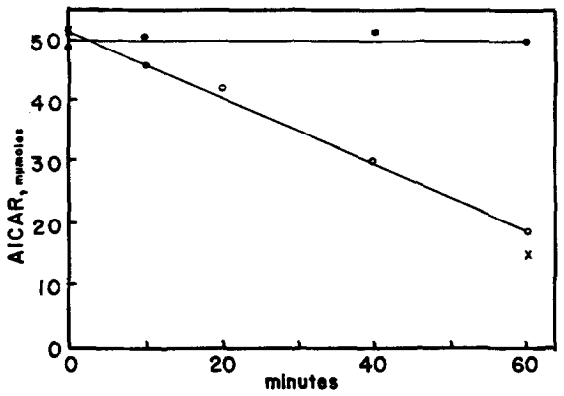

Figure 1. AICAR transformylase activity in wild type and adenine requiring mutants of Saccharomyces. The complete assay mixture contained per ml: 50muMoles AICAR, 100muMoles formyltetrahydrofolic acid (FTHFA), 10uMoles phosphate buffer (pH 7.6), 10uMoles $MgCl_2$, 8 mgm protein. Incubated at 30°C. AICAR was quantitated by Bratton-Marshall reaction using AICARiboside as standard. The control, without FTHFA (not shown), had no activity. o_____o adenine-independent extract, •_____• ad_3 extract, x_____x adenine-independent extract + ad3 extract.

In order to ascertain whether a lack of enzyme, or the presence of an inhibitor, accounted for the absence of activity in extracts of ad_3 mutants, the extract from adenine-independent strains was assayed in the presence of extract from ad_3. There was no inhibition of the AICAR transformylase from adenine-independent yeast by extracts of ad_3. These results are shown in Figure 1. The primary block in adenine biosynthesis in ad_3, therefore, appears to be caused by the inability of the mutant to produce a functional AICAR transformylase.

Mutation at the ad_3 locus also appears to interfere with earlier steps in adenine biosynthesis. It has been shown that AIR, which is formed before AICAR in adenine biosynthesis, accumulates in ad_1. However,

the expected accumulation of AIR in ad_1ad_3 double mutants is not observed (Dorfman, 1964). This effect on the earlier steps of adenine biosynthesis is supported by work now in progress, which indicates that, although a small amount of the AICAR accumulated by ad_3 mutants is derived from the adenine pathway, AICAR accumulates primarily as a byproduct of histidine biosynthesis. Calculations based on the amount of AICAR accumulating as a byproduct of histidine biosynthesis show that less histidine is synthesized in ad_3 mutants than is needed to allow detectable growth of this mutant in histidine-free media.

The basis for the histidine requirement of ad_3 is still not known. Inhibition of the first enzyme of the histidine biosynthetic pathway by AICAR has been reported to result in the histidine requirement of a similar adenine-histidine requiring mutant in Schizosaccharomyces pombe. The Schizosaccharomyces pombe mutant like the ad_3 mutant in Saccharomyces, lacks AICAR transformylase activity (Whitehead, 1966). However, in ad_3 mutants of Saccharomyces, the first enzyme of the histidine pathway measured in cell free extracts is not inhibited by concentrations of AICAR at ten times the concentration which accumulates *in vivo*.

Thus, while we have established the primary metabolic block in ad_3, the consequences of this block on the biosynthesis of histidine and on the earlier steps of the adenine pathway remain to be clarified.

ACKNOWLEDGMENT

We wish to thank Lederle Laboratories for their generous gift of formyltetrahydrofolic acid.

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