PHOSPHORIBOSYL-5-AMINO-4-IMIDAZOLECARBOXAMIDE FORMYLTRANSFERASE ACTIVITY IN THE ADENINE-HISTIDINE AUXOTROPH AD-3 OF S. CEREVISIAE

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Point mutations of the ad-3 locus in the yeast Saccharomyces cerevisiae cause a simultaneous requirement for adenine and histidine. The ad-3 mutants have been shown to be deficient in three enzymes involved in synthesis and interconversion of one-carbon adducts of tetrahydrofolic acid (Jones and Magasanik, 1967). As a result of this lesion the mutants have no single carbon derivatives of tetrahydrofolate at the formate level of oxidation.

Mazlen and Eaton (1967) have recently reported that ad-3 mutants lack phosphoribosyl-5-amino-4-imidazolecarboxamide (AICAR) formyltransferase. It is the purpose of this report to show that activity for AICAR formyltransferase is present in extracts of ad-3 mutants. Activity can only be seen, however, if 10-formyltetrahydrofolate (the requisite coenzyme for this reaction) is provided in the reaction mixture. Failure to demonstrate activity for this reaction if other one-carbon donors are provided supports our previous finding that the ad-3 mutant is unable to convert these derivatives to 10-formyltetrahydrofolate.

MATERIALS AND METHODS

Yeast were grown aerobically at 30° C to late exponential

phase in a synthetic minimal medium (Manney, 1964) supplemented with adenine, histidine, tryptophan, and uracil at 20 mg/liter and leucine at 120 mg/liter. The cells (1.4 gm. wet weight) were harvested, washed, and frozen. The frozen cells were disrupted in a Hughes press, suspended in an equal volume of 0.1M potassium phosphate buffer, pH 7.5, and the mixture was centrifuged at 25,000 x g for twenty minutes. The supernatant was dialyzed for 18 hours against 0.01M phosphate buffer, pH 7.5.

AICAR formyltransferase and inosinic acid cyclohydrolase were assayed as described by Flaks and Lukens (1963).

Chemicals were obtained from the following sources: d,l-L-tetrahydrofolic acid (FH_4) from Sigma Chemical Company and calcium 5-formyl-tetrahydrofolate from American Cyanamid. 5, 10-Methenyltetrahydrofolic acid was prepared from calcium 5-formyltetrahydrofolate according to Rabinowitz and Pricer (1956). 10-Formyltetrahydrofolate was prepared from 5, 10-methenyltetrahydrofolic acid by incubating a solution of the latter compound in 2 x 10^{-2}M Tris buffer, pH 8.5, for two hours at room temperature. The stock solution of tetrahydrofolate was at a concentration of 10 mg./ml. in 0.04M mercaptoethanol, pH 6.8. AICAR was prepared according to Levin (1959) and was converted to the potassium salt immediately before use; 5'-phosphoribosyl-5-formamido-4-imidazolecarboxamide (FAICAR) was synthesized according to Flaks, Erwin and Buchanan (1957).

RESULTS AND DISCUSSION

The specific activity for AICAR formyltransferase in the presence and absence of tetrahydrofolate and its derivatives is given for the wild type yeast in Table I. There is activity for this enzyme in the absence of added one-carbon derivatives. Tetrahydrofolate itself does not stimulate the reaction. Rather it causes a diminution in activity.

The addition to the incubation mixture of a single carbon adduct of FH_4, be it 5-formyl, 10-formyl or 5, 10-methenyl FH_4, or of FH_4, formate and ATP, the substrates of 10-formyltetrahydrofolate synthetase, stimulates the reaction. If the extracts are pretreated with charcoal to remove tetrahydrofolate derivatives, the endogenous rate is reduced and the reaction shows a greater dependence on added single carbon donors.

Table I

Dependence of AICAR formyltransferase activity on added single carbon donors in wild type and ad-3 mutant yeast.

The reaction mixture contained in a volume of 0.6 ml. : potassium phosphate buffer, pH 7.4, 30 μmoles; AICAR, 0.13 μmoles; approximately 3 mg of protein; and where indicated 10-formyl FH_4, 0.25 μmoles; 5, 10-methenyl FH_4, 0.25 μmoles; 5-formyl FH_4, 0.24 μmoles; FH_4, 0.18 μmoles; sodium formate, 30 μmoles; ATP, 1.5 μmoles. Where ATP was present the reaction mixture contained 6 μmoles of $MgCl_2$. The specific enzyme activity is expressed as mμmoles AICAR utilized/ hr. per mg protein.

Additions	Wild type	Wild type charcoal treated	ad-3 mutant
none	4.5	3.3	0
FH_4	2.7	0	0
FH_4 + Na formate + ATP	13.5	11.1	0
5-formyl FH_4 + ATP*	16.7	8.4	0
5, 10-methenyl FH_4	10.7	9.3	2.2
10-formyl FH_4	6.0	14.7	11.1

*ATP is required for the conversion of 5-formyl FH_4 to 5, 10-methenyl FH_4 (Peters and Greenberg, 1958).

The ad-3 mutant has no activity in the absence of added single carbon donors, a result in agreement with the postulate that these organisms possess no derivatives of tetrahydrofolate at the formate level of oxidation. Tetrahydrofolate has no effect on the reaction, whether or not a mixture of sodium formate and ATP is present. It is also clear that 5-formyl FH_4 will not serve as a one-carbon donor for the formyltransferase reaction in the ad-3 mutant, though it will serve as one-carbon donor in the wild type.

The mutant exhibits apparently full activity for the enzyme when 10-formyl FH_4, the actual cofactor for the reaction (Hartman and Buchanan, 1959), is included in the reaction mixture. About 20% of normal activity is seen when 5, 10-methenyl FH_4 is the donor. At neutral pH and higher, 5, 10-methenyl FH_4 is non-enzymatically cleaved to give 10-formyl FH_4. As the pH of these assays was 7.4, the activity seen with the 5, 10-methenyl FH_4 may be attributable to the 10-formyl FH_4 produced non-enzymatically.

These results indicate that ad-3 mutants do indeed possess activity for AICAR formyltransferase. They also support our previous work indicating that the mutants lack 10-formyltetrahydrofolate synthetase, 5, 10-methenyl-tetrahydrofolate cyclohydrolase, and have greatly reduced levels of 5, 10-methylenetetrahydrofolate dehydrogenase, three enzymes responsible for synthesis and interconversion of formyltetrahydrofolate derivatives (Jones and Magasanik, 1967).

The discrepancy between our results and those of Mazlen and Eaton, who failed to observe activity for AICAR formyltransferase in ad-3 mutants, may reside in their choice of single carbon donor. The position of the formyl moiety in their formyltetrahydrofolate is never specified in their paper.*

Once the formyl group is introduced into the AICAR molecule chemically to yield FAICAR, the wild type and the ad-3 mutants act on this compound at similar rates to convert it to inosinic acid. The reaction mixture contained in a volume of 0.6 ml.; Tris buffer, pH 7.4, 30 μmoles; FAICAR, 0.13 μmoles and 0.3 to 0.4 mg protein. Specific activities for the IMP cyclohydrolase reaction, expressed in mμmoles FAICAR utilized per hr. per mg. protein were 948 and 1122 for the wild type and mutant respectively.

*Calcium 5-formyltetrahydrofolate is the commercially available product.

In summary, activity for AICAR formyltransferase can be demonstrated in an ad-3 mutant of S. cerevisiae. This activity is dependent on the presence of the requisite cofactor, 10-formyl FH_4. The wild type yeast, possessing as it does the enzymes for interconverting various derivatives of FH_4, is able to utilize a variety of single carbon donors for this reaction.

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