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Unstable *S*-Adenosylmethionine Synthetase in an Ethionine-Resistant Strain of *Neurospora crassa*

ERIC S. JACOBSON,†* GREGORY S. CHEN,†† AND ROBERT L. METZENBERG

Department of Physiological Chemistry, University of Wisconsin Medical School, Madison, Wisconsin 53706

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A pleiotropic ethionine-resistant mutant of *Neurospora* contains an *S*-adenosylmethionine synthetase that is labile to heat and dialysis but exhibits normal kinetics for methionine and ethionine.

Strain *eth-1*^{r} of *Neurospora* is a pleiotropic, ethionine-resistant, temperature-conditional-lethal, single-gene mutant. Unlike the wild type, it lacks the ability to repress normally the synthesis of methionine and the assimilation of sulfur (6, 8, 9). In this communication we present evidence that in this mutant the enzyme *S*-adenosylmethionine (SAM) synthetase is abnormally labile.

Enzyme was prepared and stored as an acetone powder of cells grown in Fries medium (2) containing methionine as sole sulfur source. At the start of an individual experiment, this acetone powder was extracted at 0°C for 20 min with 4 to 10 volumes of tris(hydroxymethyl)-aminomethane-hydrochloride (0.05 M, pH 7.4) containing methionine as the sole sulfur source. was removed by centrifugation at 35,000 × *g* for 20 min. Assay of the enzyme involved modification of the incubation mixture first used for yeast (10) and the assay method described by Shapiro and Ehninger (11). The assay gave results that were proportional to time and enzyme concentration, and the enzymatic product was characterized by co-chromatography in three solvent systems and by degradation to methylthioadenosine and homoserine (1) or, alternatively, to adenine, 5′-deoxy-5′-methylthioribose, and homoserine (3). Dialysis tubing was deionized before use by boiling successively in 2% Na_2CO_3 and 0.01 M ethylenediaminetetracetic acid and rinsing in glass distilled water.

We have confirmed Kerr and Flavin's observation (7) that the mutant, *eth-1*^{r}, contains less than 10% of the normal intracellular concentration of SAM. In addition, we found that the mutant contains from 2 to 20% of the normal amount of SAM synthetase. The activity of the wild-type enzyme was not diminished by mixing it with extract from the mutant.

It can be seen in Table 1 that SAM synthetase from *eth-1*^{r} was inactivated by dialysis against 100 volumes of tris(hydroxymethyl)aminomethane-hydrochloride (0.05 M; pH 7.4; 10^{-3} M in dithiothreitol), whereas that from the wild type was relatively stable. This has been observed repeatedly. Dialysis against ethylenediaminetetraacetic acid or 8-hydroxyquinoline, both chelators of heavy-metal ions, did not stabilize the mutant enzyme, and neither did dialysis against substrates or products. Often the wild-type enzyme was activated slightly by dialysis. We have no explanation for this finding.

SAM synthetase from *eth-1*^{r} is rapidly inactivated when incubated at 37°C. The wild-type enzyme is relatively stable under these conditions (Fig. 1). Michealis constants did not differ significantly between the strains. The values for methionine activation were about 8×10^{-5} M, whereas those for ethionine activation were about 1.0×10^{-3} M.

Kerr and Flavin (7) have suggested that *eth-1*^{r} is the locus of the structural gene for the SAM synthetase. Their arguments were based upon findings of low levels of both enzyme and product in the mutant. Our evidence for a qualitative alteration in the SAM synthetase of *eth-1*^{r} supports their position. However, our kinetic studies do not support the notion that the mutant enzyme selectively excludes ethionine from its catalytic site. Thus, the pleiotropic properties of the mutant must be explained on the basis of an enzyme that is unstable and of reduced activity. We suggest that the temperature-lethal property of *eth-1*^{r} may be due to failure of methyl-group activation above the critical temperature and that the lack of repression of methionine synthesis is due to the decreased end-product inhibition of cystathionine synthase, as shown by Kerr

† Present address: Division of Infectious Diseases, Department of Internal Medicine, Medical College of Virginia, Richmond, VA 23298.

†† Present address: University of Alabama School of Medicine, Birmingham, AL 35294.

TABLE 1. *Lability of SAM synthetase to dialysis*

Sample	Sp act (U/mg of protein)[a]		Activity remaining (%)
	Initial	After dialysis	
Wild type	0.076	0.120	158
eth-1^r	0.0165	0.0035	21

[a] Unit of activity is that defined by Mudd and Cantoni (10).

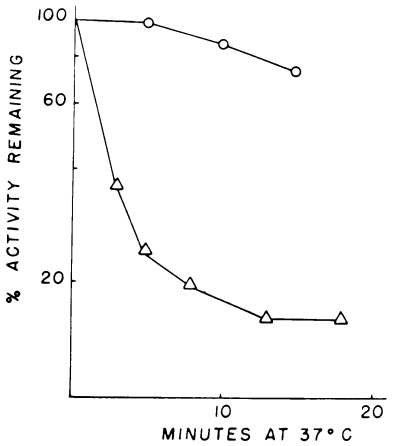

FIG. 1. *Inactivation of SAM synthetase at 37°C. Symbols: (○) Enzyme from wild type; (△) enzyme from eth-1*^{r}.

and Flavin (7). We suspect that, in the wild type, ethionine toxicity is amplified by the shut-off of methionine synthesis by a false end-product inhibitor, *S*-adenosylethionine. To support this idea, we note that ethionine can be activated by the methionine-activating enzyme (see above). We have found intracellular levels of *S*-adenosylethionine in the wild type to be 20-fold higher than those in the mutant (0.59 versus 0.028 mM). We suspect that, in the wild type, this concentration of *S*-adenosylethionine is able to reduce greatly the synthesis of methionine by "end-product inhibition" of cystathionine synthase, since Kerr and Flavin reported that only 0.1 mM SAM inhibited this enzyme by 97%. Therefore, we suggest that the mutant, with its 20-fold lower concentration of *S*-adenosylethionine, is able to continue to synthesize methionine and escapes "amplification" of the toxicity of ethionine. A somewhat similar suggestion has been made for a mutant of *Saccharomyces cerevisiae* (4). Finally, we propose that *eth-1*^{r} represents a class of fungal mutants that is analogous to the *metk* mutants of *Escherichia coli* (5).

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