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AMINO ACID ACCUMULATION IN ETHIONINE-RESISTANT *SACCHAROMYCES CEREVISIAE*[1]

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ABSTRACT

SORSOLI, W. A. (Oregon State University, Corvallis), K. D. SPENCE, AND L. W. PARKS. Amino acid accumulation in ethionine-resistant *Saccharomyces cerevisiae*. J. Bacteriol. **88**:20-24. 1964.—Recessive ethionine-resistant strains of *Saccharomyces cerevisiae* possess a genetic lesion affecting the concentration of amino acids nonspecifically in the expandable pool of the organism. This area, which may be linked to a methionine gene, is highly mutable and accounts for resistance to certain amino acid analogues. Other mutations result in ethionine resistance by a mechanism unrelated to the amino acid uptake system.

During studies of methionine metabolism in *Saccharomyces cerevisiae*, mutants resistant to methionine analogues were isolated. Limited physiological and genetic analyses were made of a series of ethionine-resistant clones for comparison with the wild-type organism. Some genetically recessive mutants were found to have an impaired mechanism for the accumulation of exogenously supplied amino acids.

An energy-dependent accumulation process for amino acids has been known for yeast for many years (Taylor, 1947, 1949). In contrast with the highly specific amino acid permeases of *Escherichia coli* (Kepes, 1962), yeast possess a less specific concentrating mechanism (Halvorson and Cohen, 1958). This report supports the latter findings, and describes some experiments made with a yeast mutant found to lack the ability to concentrate amino acids as a result of a specific genetic lesion.

MATERIALS AND METHODS

Ethionine-resistant mutants were isolated from *S. cerevisiae* strain 3701B, a haploid, uracil-requiring clone, by exposure to ultraviolet light at approximately 12 ergs per mm^2 per sec for 80 sec. The irradiated organisms were spread onto the surface of a minimal synthetic medium (Hawthorne, 1955) supplemented with the normally inhibitory concentration of 25 mg per liter of DL-ethionine, and resistant clones were isolated. A large number of mutants were obtained in this way. A strain designated A3E90, resistant to ethionine in concentrations up to 75 mg per liter, was isolated in this manner and used in subsequent investigations. Cursory studies with other isolates indicate that the mutation in A3E90 is not rare among the ethionine-resistant organisms.

For genetic manipulation, the following method was employed for the procurement of zygotes. Organisms containing complementary nutritional requirements were grown separately in a yeast extract-supplemented medium (Starr and Parks, 1962) at 30 C for 24 hr, harvested, and inoculated in approximately equal numbers into fresh medium of the same composition. Prototrophic recovery of the mated cells was accomplished on the synthetic medium. Figure 1 is a schematic representation of the crosses made and the genotypes involved.

Diploids for sporulation were grown in yeast extract-supplemented medium at 30 C for 24 hr with agitation. The cells were harvested, washed three times in sterile-distilled water, and centrifuged; the resulting pellet was applied onto the surface of sporulation medium (Fowell, 1955). After 48 hr of incubation at 30 C, the cells were sufficiently sporulated for dissection. The dissection techniques of Johnston and Mortimer (1959) were used. A highly active digestive juice from *Helix aspersa* was prepared in our laboratory and sterilized by filtration. Segregated tetrads were allowed to grow until the colony reached a size convenient for transfer to a medium for storage.

Scoring for the nutritional and ethionine-resistant markers was accomplished by making a suspension of individual clones in a small

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amount of sterile-distilled water. These suspensions were streaked onto synthetic media containing selected deletions necessary for the analyses. Ethionine was added to other media at a concentration of 50 mg per liter. The methionine requirement of JB4 may be satisfied by homoserine. In all cases, homoserine was used in place of methionine to avoid direct competition between methionine and ethionine in those media where the analogue was used. Scoring was based on the growth or lack of growth by the clone after 72 hr of incubation at 30 C. The diploids and parental haploids were similarly scored at the same time.

Organisms to be used in the investigations of methionine and ethionine accumulation were grown in the yeast extract-supplemented medium at 30 C with agitation. The cells were harvested, washed three times in distilled water, and resuspended in synthetic medium containing the amino acid at a final concentration of 1 mM. The reaction mixture was incubated at 30 C on a rotary shaker; 20-ml samples were taken at 0, 0.5, 1, 2, and 3 hr. These samples were immediately cooled in an ice bath to retard further reaction. The cells were washed three times in ice-cold 0.67 M phosphate buffer (pH 6.6), and were resuspended in the same buffer to a volume of 6 ml. A 5-ml portion of the suspension was boiled for 20 min and centrifuged; the supernatant fluid was used for assay of the accumulated methionine in the cells. The microbiological assay used *Streptococcus faecalis* strain 9790 as the test organism, and was carried out in commercially available Methionine Assay Medium (Difco). The procedure suggested by the supplier was followed exactly.

As a check on this procedure, radioactively labeled methionine was assayed in similar cell suspensions. The same procedure was followed for the preparation of the reaction mixture described above, with the exception that 5 μc of C^{14}-labeled methionine were added. Samples were taken at the previously mentioned times and, after boiling, were assayed for the accumulation of methionine in the cells by use of a Tri-Carb liquid scintillation spectrometer. Samples (0.5 ml) in a 52% toluene-48% ethanol system were assayed with the use of a PPO-POPOP fluor combination (Pigg, Spence, and Parks, 1962).

C^{14}-ethionine was used to measure its accumulation by the resistant and sensitive strains. The procedure followed was identical to the measurement of methionine, with the exception that C^{14}-methionine was replaced with 1 μc of C^{14}-ethionine.

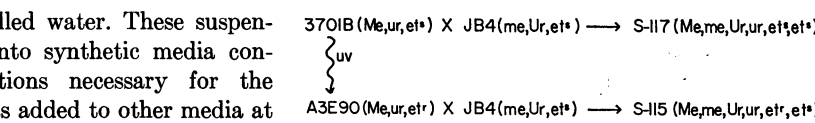

FIG. 1. *Schematic representation of genetic crosses indicating genotypes.*

Randomly C^{14}-labeled algal hydrolysate was used to measure the accumulation of the amino acids contained therein. The same procedure as previously described was used, with the exception that 149 mg per liter of Vitamin Free Casamino Acids (Difco) were used as carrier with 5 μc of C^{14}-algal hydrolysate.

Accumulation of amino acids at 0 C and in the presence of 0.5% sodium azide was assayed with the use of suspensions of the wild-type organism and the procedure for methionine concentration.

Cell quantities were measured nephelometrically with a Coleman model 9 Nepho-colorimeter. Direct microscopic cell counts and samples of aqueous cell suspensions dried to constant weight were used for preparing calibration curves.

Labeled amino acids and algal hydrolysate were obtained from the New England Nuclear Corp., Boston, Mass. Unlabeled amino acids were of highest available purity, and were obtained from commercial sources.

RESULTS AND DISCUSSION

The accumulation of methionine by the resistant and sensitive strains of yeast, as assayed microbiologically in a typical experiment, is shown in Fig. 2. Methionine is accumulated in the sensitive strain. The measurement of accumulated methionine is complicated by the cellular conversion of the amino acid to *S*-adenosyl-methionine (Schlenk and De Palma, 1957; Pigg, Sorsoli, and Parks, 1964). The sulfonium derivative would not be assayed by the microbiological technique (Pigg, Spence, and Parks, 1962). As a check on this procedure, the accumulation of C^{14}-methionine was determined. The accumulation of the label by the sensitive strains of yeast greatly exceeds that of the

resistant strain over a 3-hr period (Fig. 3). These results suggest that the concentrating mechanism for methionine and, therefore, theoretically for ethionine of the ethionine-resistant strain has been altered.

Because the convenience of a microbiological assay for ethionine was not available, use was made of C^{14}-labeled ethionine to measure its accumulation. The accumulation of the labeled ethionine was similar to that of methionine; a rapid accumulation by the sensitive strains and a very slow diffusion-like accumulation by the resistant strain was obvious (Fig. 4). Similar experiments employing C^{14}-labeled leucine and serine gave the same results. In addition, when randomly labeled algal protein hydrolysate was used as the amino acid source (Fig. 5), slow accumulation of labeled material was effected by the ethionine-resistant organism, in contrast with the wild-type organism. It seems evident that the mechanism of amino acid uptake in yeast is greatly altered by the mutation being studied. Relative nonspecificity in the mechanism is apparent. That the mutation is recessive in nature is indicated by the accumulation of both methionine and ethionine by the heterozygote. Another class of ethionine-resistant mutants has been isolated and shown to be dominant over the wild-type allele.

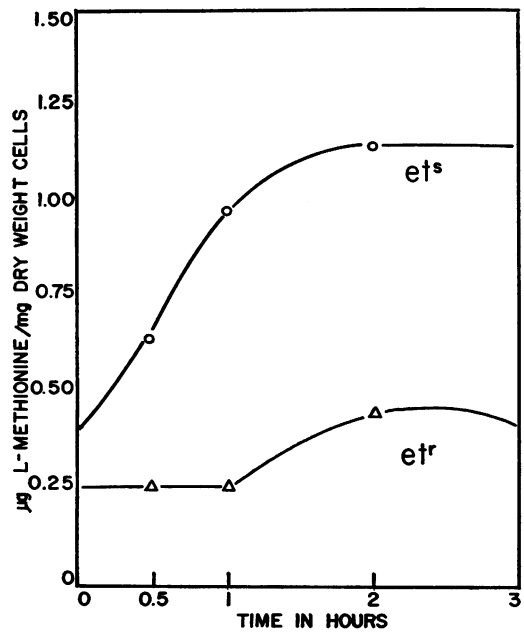

FIG. 2. *Microbiological assay of accumulated methionine.*

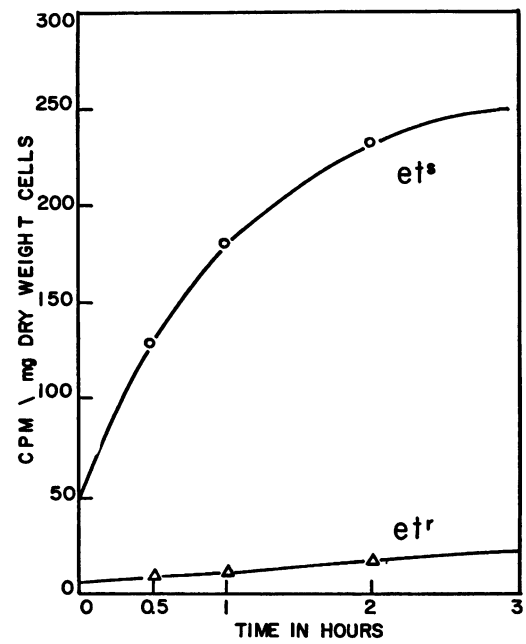

FIG. 3. *Accumulation of C^{14}-methionine.*

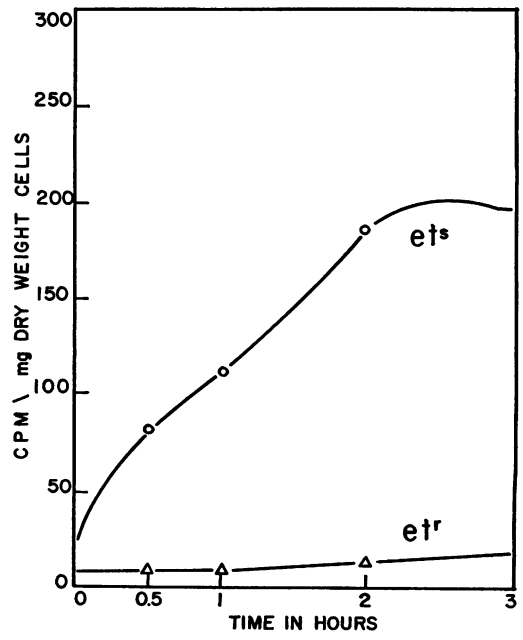

FIG. 4. *Accumulation of C^{14}-ethionine.*

If the ethionine resistance described here is due to a generally defective amino acid con-

centrating mechanism, these cells should also be resistant to other amino acid analogues. Such was the case; these organisms were able to grow in the presence of 56 mg per liter of *p*-fluorophenylalanine, even though this is one of the most toxic amino acid analogues (Halvorson and Spiegelman, 1952). The wild-type and dominant ethionine-resistant organisms do not grow under these conditions. The experiments were repeated with the addition of pyridoxine·HCl (4 mg per liter). This addition failed to stimulate amino acid uptake in the ethionine-resistant organisms, contrary to the findings in Ehrlich ascite cells (Riggs, Coyne, and Christensen, 1953). These results seem to eliminate the involvement of a pyridoxine deficiency in the accumulation process in the ethionine-resistant organism. The concentration of amino acids was shown to be temperature-dependent and energy-requiring (Fig. 6). These data suggest that ethionine resistance in these organisms is due to a mutation at some locus which in some manner alters amino acid transport of the cell. The concentrating mechanism apparently involves accumulation into the expandable pool of the organism (Cowie and McClure, 1959), because the process being studied is broadly specific.

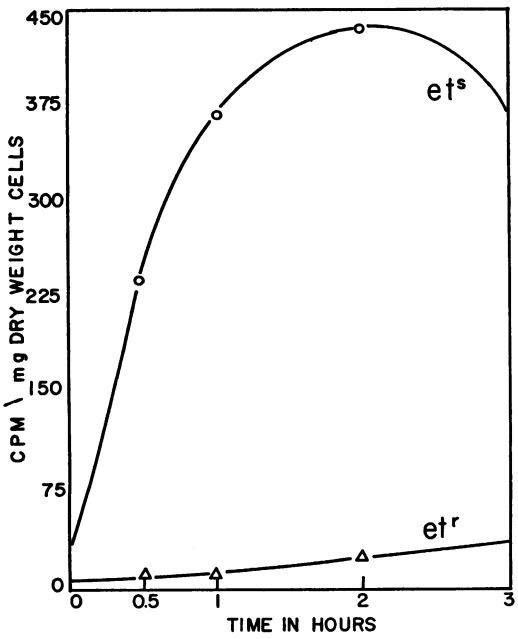

FIG. 5. *Accumulation of algal hydrolysate randomly labeled with* C^{14}.

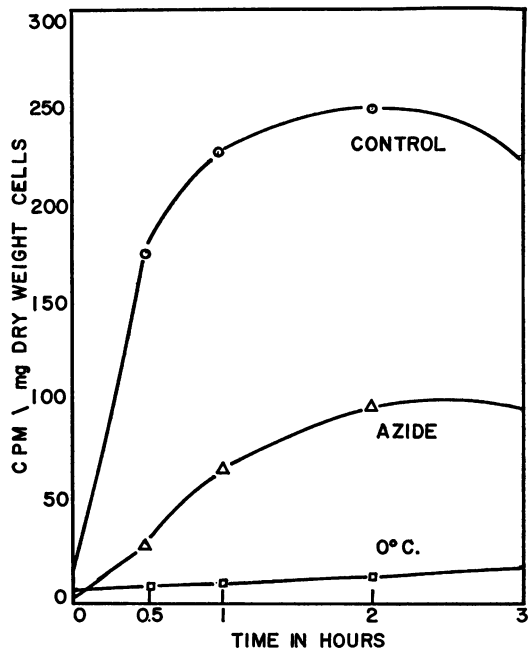

FIG. 6. *Energy requirement and temperature dependence of the accumulation process in the wild-type organism.*

TABLE 1. *Segregation patterns obtained from the genetic analyses of diploids S-115 and S-117*

Zygote no.	Segregation ratio	*Me:me*	*Ur:ur*	$et^r:et^s$
S-115	2:2	23	16	20
	3:1	1	4	3
	4:0	0	2	0
S-117	2:2	14	13	0
	3:1	1	1	0
	4:0	0	0	0

Amino acid analogue resistance would be due to the inability of the analogue to enter the amino acid pool of the cell. Analogue incorporation in protein synthesis is governed by the concentration of the analogue in the pool (Kempner and Cowie, 1960).

The possibility remained that a large number of mutations or deletions involving many binding sites or permeases had occurred. If resistance of the cell was due to a single gene mutation, then one would expect the character to segregate in a 2:2 ratio in the spores of the heterozygote. To test this possibility, the diploids S-115 (et^r et^s)

and the control S-117 (et^s et^s) were sporulated and dissected; the clones resulting from individual spores were analyzed for nutritional as well as the ethionine-resistance markers. The results of the analyses are shown in Table 1. Because the segregation ratio of all characters is predominantly 2:2, it can be postulated that the mutation to ethionine resistance is the result of mutation at a single locus. Of the eight expected segregational classes from the cross involving ethionine resistance, only four were generally recovered. Parental genotypes for the ethionine resistance and methionine markers were recovered with a greater than 20-fold frequency over the recombinant types. Although relatively close genetic linkage is suggested, larger numbers of asci must be dissected to gain definitive evidence for linkage.

Because accumulation in the expandable pool apparently is an enzymatic process (Halvorson and Cowie, 1961), the mutations being studied here could result in loss of this enzymatic mechanism. If retention of the accumulated amino acids involved their binding to sites in the expandable pool, a mutation affecting the structure of these would also give the observed results. It is necessary, however, to conclude that additional factors unrelated to the mutations described here may participate in amino acid uptake, because Maw (1963) demonstrated relative specificity in other experiments involving the accumulation of the sulfur-containing amino acids.

ACKNOWLEDGMENT

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