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Transport of S-Adenosylmethionine in *Saccharomyces cerevisiae*

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The properties of a specific system for the transport of *S*-adenosylmethionine in yeast are described. The process was *p*H-, temperature-, and energy-dependent, and showed saturation kinetics. The K_m for the system was 3.3×10^{-6} M. Of the *S*-adenosylmethionine moieties tested, only *S*-adenosylhomocysteine competitively inhibited the uptake of the adenosylsulfonium compound. Adenine, adenosine, methionine, homocysteine, and the sulfonium compound *S*-methylmethionine were without effect. The analogue *S*-adenosylethionine showed competitive inhibition. Under conditions of inhibition of protein synthesis by cycloheximide or methionine starvation, permease activity was stable. The mutant *sam-p3* apparently was able to transport *S*-adenosylmethionine only by diffusion. Uptake by diploids containing this mutation was directly proportional to the gene dose.

Both general (6) and specific amino acid (2, 3, 7, 8, 10) permeases have been described in yeast. Mutations have also been isolated which affect several of these systems (5, 11, 14). An uptake system for *S*-adenosylmethionine, as well as a mutation affecting this system, has been reported (12). The general genetic character and physiological consequence of the mutation were described previously (12). The specific nature of the *S*-adenosylmethionine uptake system has since been under investigation and is the subject of this paper.

MATERIALS AND METHODS

Strains. Strain 80BM1 (*a ura met-2a*) was isolated from *Saccharomyces cerevisiae* strain 3701B (*a ura*) after ultraviolet irradiation. The derivation of strain 8038 (*a ura met-2a sam-p3*) was described previously (13). Strain 838J4-221 (*α ade met-2a sam-p3*) is a segregant of a cross between strains 8038 and JB4 (*α thr-2 ade-1*). Strains JB4 and ET48 (*α met-3*) were obtained from R. K. Mortimer, University of California, Berkeley. Diploid strains employed in gene dose studies included 838J4-221 × 8038 (*sam-p3/sam-p3*), 838J4-221 × 3701B (*sam-p3/+*), and ET48 × 3701B (+/+).

Medium and growth conditions. Medium B (12), a defined glucose-salts medium, was employed throughout. The cells were grown aerobically on a rotary shaker at 30 C. Growth was monitored with a Klett-Summerson photoelectric colorimeter (400 to 465 nm). At 32 Klett units, a culture contains 0.1 mg (dry weight) of cells per ml. The uptake of *S*-adenosylmethionine was studied during the exponential phase of growth to assure that conditions would be as physiologically significant and as reproducible as possible. Unless otherwise noted, methionine-requiring strains to be assayed for permease activity were grown with *S*-adenosylmethionine as a source of methionine for at least eight generations. Under these conditions, methionine-requiring strains show the same permease activity as wild-type strains. Strain 80BM1 was employed in all studies of the wild-type permease system unless otherwise indicated. Permease mutant strains (*sam-p3*) possessing a *met* marker were grown in the presence of methionine. Starvation of *sam-p3* strains for methionine does not increase their ability to take up *S*-adenosylmethionine.

Initial velocity of uptake of S-adenosylmethionine. Samples of 1.2 mg (dry weight) of cells were obtained by filtering an appropriate volume of cell culture on a membrane filter (0.45 μm pore size, 25 mm in diameter, Millipore Corp.) and quickly washing the filter with three 2-ml rinses of medium B at 30 C. The cells were then immediately resuspended in a 50-ml beaker containing 4 ml of medium B (*p*H 4.8) plus uracil (0.2 mM) and the desired concentration of *S*-adenosylmethionine-$^{14}CH_3$, with or without inhibitors, all of which had been previously equilibrated to 30 C in a water-bath shaker. Samples (1 ml) were removed at 30-sec intervals for 1.5 min (Clay Adams Selectapette), placed on membrane filters, and immediately washed with five 2-ml rinses of iced distilled water (there was no detectable leakage as a result of the ice water washes). The cells plus filter were then immediately placed in scintillation vials, each containing 10 ml of a mixture of toluene and Triton X-100 (1:2) plus 1 g of 2,5-diphenyloxazole and 0.06 g of 1,4-bis(5-phenyloxazolyl)-

benzene per liter. Radioactivity was determined on a Nuclear-Chicago Unilux III liquid scintillation counter.

For measurement of the effect of temperature on the initial velocity of uptake of *S*-adenosylmethionine, each reaction vessel and solution was brought to the indicated temperature in a water bath and maintained at that temperature during the sampling period. For the measurement of the *p*H effect, medium B was replaced by 0.1 M sodium phosphate, 0.05 M citrate-phosphate, 0.05 M sodium citrate, and 0.05 M HCl-KCl buffering systems, with *p*H values ranging from 1 to 9. To study the effects of inhibition of protein synthesis on permease activity, cells were prepared as usual, and uptake was monitored after transfer to either (i) medium B plus uracil (0.2 mM) without a methionine source, or (ii) medium B plus uracil (0.2 mM) plus *S*-adenosylmethionine (0.1 mM), to which cycloheximide (2 μg/ml) was added.

Extraction and identification of the accumulated radioactivity. After incubation for 5 min in *S*-adenosylmethionine-$^{14}CH_3$, cells were filtered on membrane filters, washed with ice water, and extracted for 5 min in ice-cold 100% ethanol. After the centrifugation and removal of the ethanol, the cells were extracted for 10 min in cold 0.2 N perchloric acid. The supernatant extract obtained was neutralized to *p*H 2 with $KHCO_3$ and chromatographed on paper with a solvent of butanol-acetic acid-water (60:15:25, v/v). In addition, a portion of the neutralized extract was heated in a boiling-water bath for 30 min and chromatographed in the same manner. Compounds were identified by simultaneous chromatography with known controls.

Chemicals. *S*-adenosyl-L-methionine and *S*-adenosyl-L-homocysteine were obtained from Boehringer Mannheim Corp., and *S*-adenosyl-L-methionine-$^{14}CH_3$, from New England Nuclear Corp. *S*-adenosyl-L-ethionine and additional *S*-adenosyl-L-methionine were prepared in this laboratory (9). All other compounds were in the L-form and were obtained from other commercial sources.

RESULTS

Properties of the S-adenosylmethionine transport system: extraction and identification of the accumulated radioactivity. In excess of 90% of the labeled compound taken up by the cells was found in the 0.2 N perchloric acid exract, and within the limits of detection all of the radioactivity was found on one spot, which corresponded to the control spot of *S*-adenosylmethionine. The identification of *S*-adenosylmethionine was verified by chromatography of the heated extract. After hydrolysis at *p*H 2, more than 70% of the radioactivity was found on a second spot which corresponded to methylthioadenosine (R_f 0.8), the hydrolytic product of *S*-adenosylmethionine under these conditions.

Effects of pH and temperature. The initial velocity of entry of *S*-adenosylmethionine was found to be dependent on both *p*H and temperature (Fig. 1 and 2), with optima at *p*H 4.8 and 35 C.

Kinetics of uptake. The *S*-adenosylmethionine transport system was saturable, with a K_m of 3.3×10^{-6} M and a V_{max} of 3 nmoles of *S*-adenosylmethionine per mg (dry weight) of cells per min (Fig. 3).

Addition of either 20 mM sodium azide (Fig. 4) or 10 mM sodium cyanide strongly inhibited the uptake of the adenosylsulfonium compound. This supports the conclusion that the uptake of *S*-adenosylmethionine is mediated by an energy-requiring mechanism.

Specificity of uptake. Of the compounds tested, only *S*-adenosylethionine and *S*-adenosylhomocysteine inhibited the uptake of *S*-adenosylmethionine. Adenine, adenosine, adenosine triphosphate, methionine, *S*-methylmethionine, and homocysteine had no effect. These results are detailed in Table 1.

The inhibition of *S*-adenosylmethionine transport by *S*-adenosylethionine and *S*-adenosylhomocysteine was competitive (Fig. 5 and 6). The apparent dissociation constants (K_i) were found to be 5×10^{-5} M for *S*-adenosylethionine and 2.5×10^{-4} M for *S*-adenosylhomocysteine.

Inhibition of uptake by cycloheximide and methionine starvation. Grenson (1, 4) monitored the effect of cycloheximide treatment and amino acid starvation on the uptake of several amino acids. It was clear from the results that in the cases studied the accumulation of intracellular compounds, and not the rapid turnover of the permease protein, was responsible for the rapid loss of uptake ability during the inhibition of protein synthesis. To determine the nature of the response of the *S*-adenosylmethionine permease to the inhibition of protein synthesis, uptake of the sulfonium compound was monitored both in the presence of cycloheximide (2 μg/ml) and during methionine starvation. For comparative purposes, the uptake of methionine, the immediate precursor of *S*-adenosylmethionine, was also followed in the same cultures. The results of these studies are shown in Fig. 7.

Methionine uptake decreased much more rapidly with cycloheximide treatment than with methionine starvation (Fig. 7a). This is consistent with the conclusions of Grenson (4) mentioned above, since only the cycloheximide treatment would allow the accumulation of an intracellular pool of either methionine or possibly a methionine derivative. The uptake of *S*-adenosylmethionine, however, showed a very similar response to both cycloheximide and methionine starvation (Fig. 7b). This response

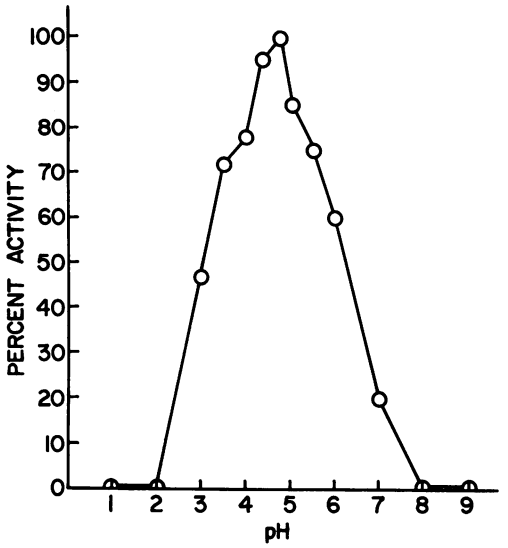

FIG. 1. *Effect of pH on uptake of S-adenosylmethionine (0.005 mM). Activity is initial velocity of entry in nanomoles of S-adenosylmethionine per milligram (dry weight) of cells per minute.*

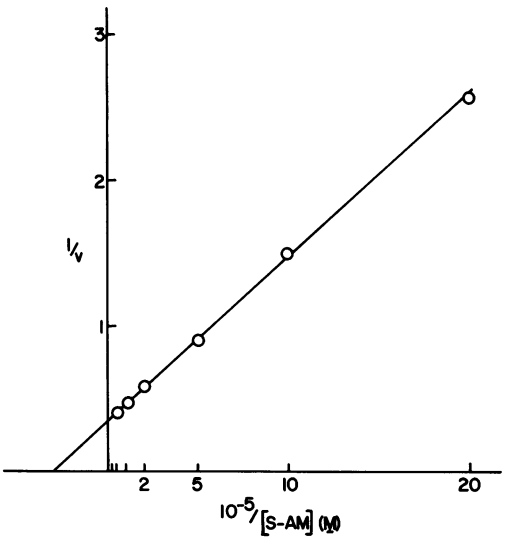

FIG. 3. *Lineweaver-Burk plot of uptake of S-adenosylmethionine. Velocity is in nanomoles of S-adenosylmethionine per milligram (dry weight) of cells per minute.*

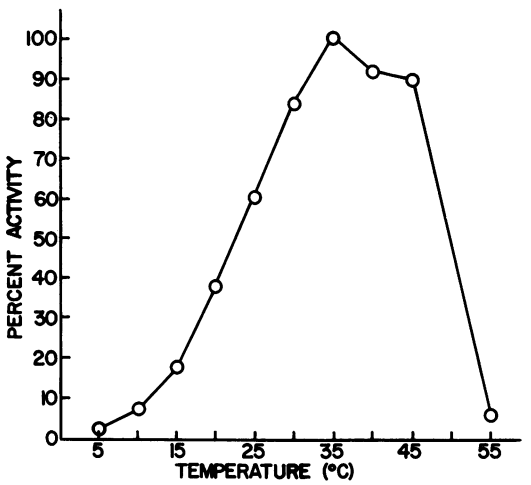

FIG. 2. *Effect of temperature on uptake of S-adenosylmethionine (0.005 mM). Activity is initial velocity of entry in nanomoles of S-adenosylmethionine per milligram (dry weight) of cells per minute.*

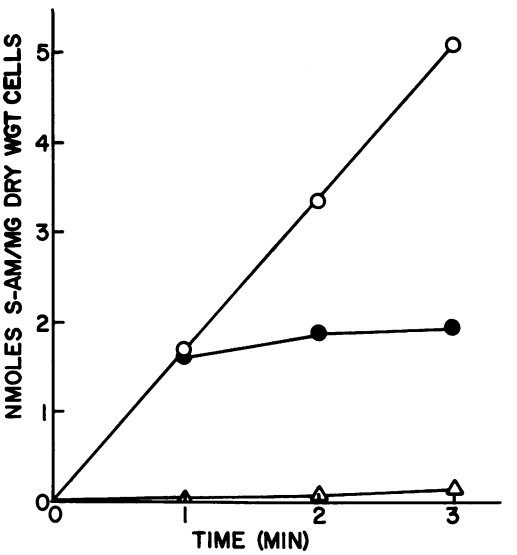

FIG. 4. *Effect of 20 mM sodium azide on the uptake of 0.005 mM S-adenosylmethionine. Symbols: ○, control with no azide; ●, azide added at 1 min; △, azide added at zero time.*

approximated the response of the methionine permease to methionine starvation, suggesting that under these conditions there is neither rapid turnover of the permease protein, nor any significant accumulation of compounds which might feed back on the system.

Properties of a mutant deficient in S-adenosylmethionine transport ability: kinetics of uptake. A mutant, designated *sam-p3*, has been described which has a greatly reduced ability to take up *S*-adenosylmethionine (12). The kinetic profile of uptake by the *sam-p3* mutant is shown in Fig. 8. Up to a concentration range 10-fold beyond that which saturates the wild type, uptake of *S*-adenosylmethionine remained linear with respect to the concentration of substrate. At a concentration of 10^{-5} M, a level leading to the maximal rate of uptake

TABLE 1. *Specificity of the S-adenosylmethionine permease*[a]

Unlabeled compound	Inhibition
S-adenosyl-L-ethionine	84
S-adenosyl-L-homocysteine	21
Adenine	0
Adenosine	0
Adenosine triphosphate	0
L-Methionine	0
S-methyl-L-methionine	0
L-Homocysteine	0

[a] The per cent inhibition of the initial velocity of uptake of 0.005 mM *S*-adenosyl-L-methionine-$^{14}CH_3$ by several unlabeled compounds (0.1 mM) is shown.

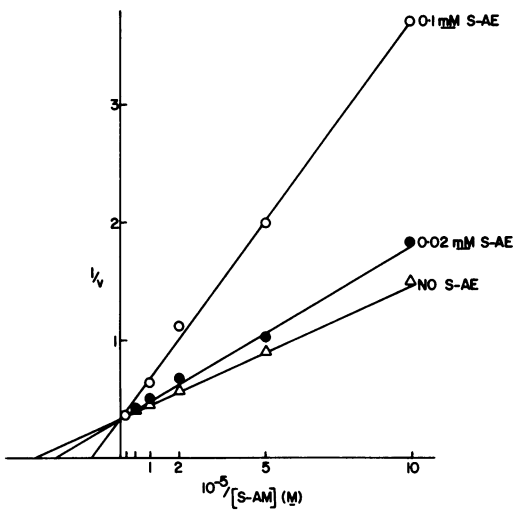

FIG. 5. *Competitive inhibition of S-adenosyl-L-methionine uptake by S-adenosyl-L-ethionine. Lineweaver-Burk plot. Velocity is in nanomoles of S-adenosylmethionine per milligram (dry weight) of cells per minute.*

in strains possessing the wild-type allele (3 nmoles per mg, dry weight, of cells per min), the uptake by the *sam-p3* strain was only 0.005 nmole per mg (dry weight) of cells per min. This is insufficient for the support of growth of *sam-p3* strains, in which methionine or adenine is required as a growth supplement (12). The results indicate that uptake of *S*-adenosylmethionine by the *sam-p3* mutant probably occurs only by a slow diffusion process.

Gene dose effect. The initial velocity of entrance of *S*-adenosylmethionine was assayed in diploids that were wild-type homozygotes (+/+), heterozygotes (+/*sam-p3*), and *sam-p3* homozygotes (*sam-p3*/*sam-p3*). The results of this test (Fig. 9) clearly demonstrate a gene dose effect.

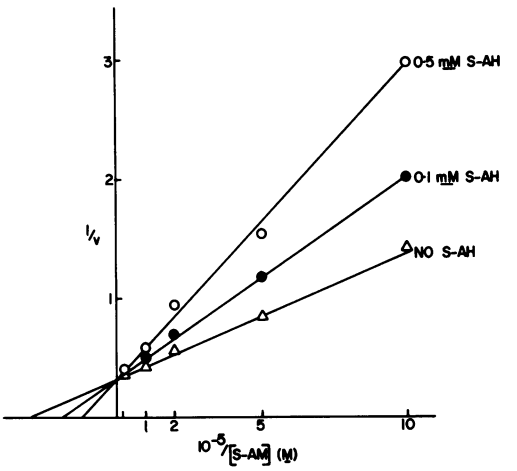

FIG. 6. *Competitive inhibition of S-adenosyl-L-methionine uptake by S-adenosyl-L-homocysteine. Lineweaver-Burk plot. Velocity is in nanomoles of S-adenosylmethionine per milligram (dry weight) of cells per minute.*

DISCUSSION

Experimental data indicate that the high-energy sulfonium compound *S*-adenosylmethionine is taken into the yeast cell by means of a very specific, high-affinity, active transport system.

The only natural product found to compete for the permease is *S*-adenosylhomocysteine; of the other compounds tested, only the analogue *S*-adenosylethionine showed any inhibition. These two compounds differ from *S*-adenosylmethionine by only one methyl or one methylene group; however, the apparent dissociation constants (K_i) are larger than the K_m of *S*-adenosylmethionine by factors of 15 for *S*-adenosylethionine and 76 for *S*-adenosylhomocysteine, demonstrating a greatly reduced affinity for the permease.

Like the specific amino acid permeases previously reported (4), the *S*-adenosylmethionine permease protein appears to be stable. In contrast to the apparent accumulation of methionine with the inhibition of protein synthesis by cycloheximide, however, there does not appear to be any increased conversion of methionine to *S*-adenosylmethionine, nor any accumulation of the sulfonium compound which might feed back and inhibit its own transport system.

The expression of diffusion kinetics by the *sam-p3* mutant demonstrates that a single mutation can result in the loss of the cell's ability to actively transport *S*-adenosylmethionine.

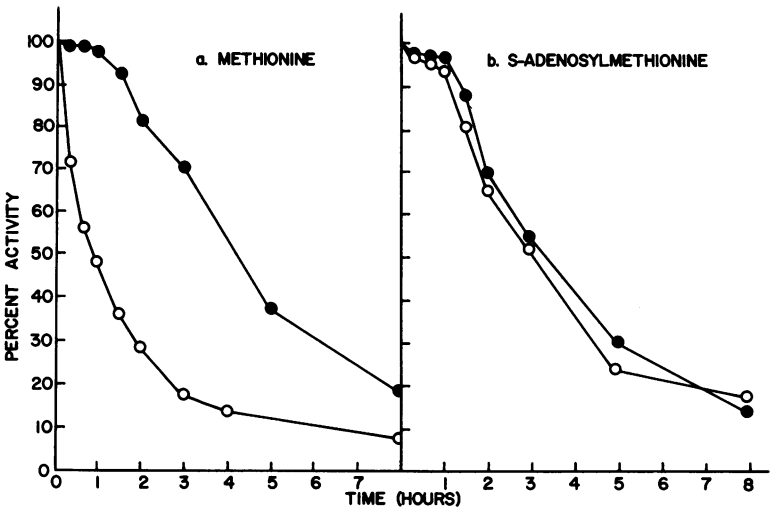

FIG. 7. *Effect of cycloheximide (2 μg/ml) and methionine starvation on the uptake of methionine (a) and S-adenosylmethionine (b). Symbols: O, cycloheximide; ●, methionine starvation. Activity is initial velocity of entrance in nanomoles per milligram (dry weight) of cells per minute.*

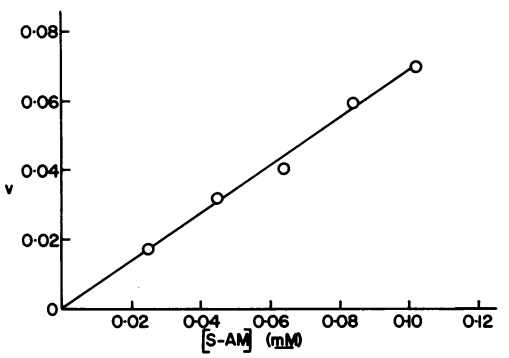

FIG. 8. *Uptake of S-adenosylmethionine by the sam-p3 mutant. Velocity is in nanomoles of S-adenosylmethionine per milligram (dry weight) of cells per minute. Strain 8038.*

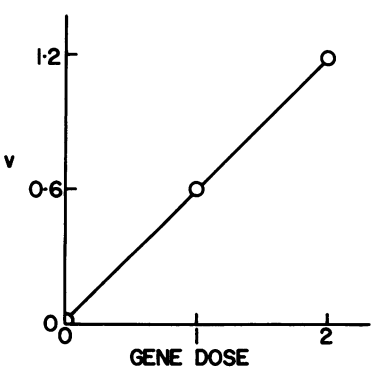

FIG. 9. *Gene dose effect on uptake of 0.005 mM S-adenosylmethionine by diploids. Gene dose is the number of wild-type alleles: sam-p3 homozygote (0), heterozygote (1), wild-type homozygote (2).*

ACKNOWLEDGMENTS

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