Ureidosuccinate Is Transported by the Allantoate Transport System in *Saccharomyces cerevisiae*

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The regulatory characteristics exhibited by ureidosuccinate transport in *Saccharomyces cerevisiae* led us to hypothesize that this biosynthetic intermediate was transported via the degradative allantoate transport system. The hypothesis was verified by the finding that neither *dal5* nor *urep1* mutant strains could transport allantoate or ureidosuccinate. Mutations in the two loci were tightly linked and failed to complement one another, suggesting that they were allelic. The use of a common transport system for accumulation of both biosynthetic and degradative metabolites explains the paradoxical characteristics observed for control of ureidosuccinate and allantoate transport.

Ureidosuccinate (USA) is the first specific intermediate in pyrimidine biosynthesis (6). Lacroute and his colleagues (4, 5) characterized transport of this precursor and found it to be mediated by a low K_m (27 μ M) active transport system that was controlled in a quite puzzling manner. Production of most biosynthetic transport systems is repressed by the accumulated metabolite or end product of the pathway (2, 4); this one was not. No biosynthetic systems have been shown to be sensitive to nitrogen catabolite repression or trans-inhibition (3); USA transport appeared to be sensitive to both types of regulation (5, 6). In other words, the USA transport system was not regulated like a biosynthetic permease, but rather like a catabolic nitrogen accumulation system. Greth et al. (5), in their description of the USA transport system, perceptively noted that "ureidosuccinic acid may not be the most important natural substrate of the ureidosuccinate permease."

We recognized this paradox in regulation while preparing a review of the literature (3) and searched for a degradative system whose substrate was structurally similar to USA. Allantoate is structurally similar both to USA and to compounds that competitively inhibit its uptake (2, 4-6) (Fig. 1). These correlations raised the possibility that USA was transported by way of the nitrogen-repressible allantoate transport system we described earlier (7, 8). Experiments testing this hypothesis are described in this report.

(A preliminary description of this work has appeared already [V. Turoscy and T. G. Cooper, Tenth Int. Conf. Yeast Genetics and Molecular Biology, abstr. 237, 95, 1980].)

MATERIALS AND METHODS

Strains. The strains used in this work are listed in Table 1.

Culture conditions. Wickerham medium (buffered to the desired pH [pH 6.0, unless specified otherwise] by the addition of 1% sodium citrate [pH was adjusted with HCl]) was used throughout these experiments (9). Glucose (0.6%) and ammonium sulfate (0.1%) were added as the sole carbon and nitrogen sources, respectively. Cell density was mea-

sured with a Klett-Summerson colorimeter (500- to 570-nm band-pass filter). One hundred Klett units are equivalent to approximately 3×10^7 cells per ml of culture.

Resting cell cultures were prepared as described below. Cultures were grown overnight in buffered glucose-ammonia medium to a cell density of 40 to 60 Klett units. At this point, they were harvested by filtration, washed with several volumes of nitrogen-free medium, and suspended in the original volume of prewarmed, preaerated buffered medium devoid of any nitrogen source. After incubation at 30°C for 24 to 28 h, the cultures were either (i) used to assay allantoate transport or (ii) transferred to prewarmed pH 5.0 medium devoid of a nitrogen source and preincubated for 1 h, followed by assay of USA uptake. The resting cell density at the time of assay was approximately 120 to 190 Klett units (7).

Purification of [14 C]USA. [14 C]USA was a gift from F. Lacroute. The radioactive material was purified on preparative thin-layer silica gel plates developed for 2 to 2.5 h in a butanol-formic acid-water (75:15:10, vol/vol/vol) solvent system. This resolved several major radioactive species

FIG. 1. Chemical structures of allantoate, USA, and analogs demonstrated by Greth et al. (5) to be effective competitive inhibitors of USA transport.

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TABLE 1. Strains of *S. cerevisiae* used in this work

Strain	Genotype
M927	<i>MATa his6 ura1 dal1-N16 dal2-N18</i> <i>MATa ade6 leu1 dal1-N16 dal2-N18</i>
M938	<i>MATa inol-13 met8 dal1-N16 dal2-N18 dal5-N1262</i> <i>MATa his6 ura1 dal1-N16 dal2-N18 dal5-N1262</i>
M1068	<i>MATa ura1 dal1-N16 dal2-N18 urep1^a</i> <i>MATa his6 dal1-N16 dal2-N18 urep1</i>

^a *urep1* allele was derived from strain FL475-1C (*MATa urep1*), generously provided by M. R. Chevallier.

contained in the original sample (1). These species were then visualized by autoradiography. Nonradioactive USA visualized with Ehrlich reagent was used as a standard. The radioactive species, which migrated with an *R_f* value identical to that of the pure USA standard, was identified and scraped from the plate into 2 ml of cold distilled water. Silica gel was removed from the solution by straining it through glass wool followed by centrifugation for 20 min at 7,700 × *g*. The supernatant solution was lyophilized to dryness, suspended in sterile distilled water, and stored at -20°C. Chromatographic homogeneity of the purified material was verified by thin-layer chromatography.

RESULTS AND DISCUSSION

Our objective in this work was to determine whether or not allantoin and USA enter the cell by way of a common transport system. A corollary of this hypothesis is that mutations in the *DAL5* and *UREP1* loci, which were independently isolated on the basis of failing to transport allantoin or USA, respectively (4-6, 8), are allelic.

Therefore, for the first test of our hypothesis, we constructed homozygous and heterozygous *dal5* and *urep1* mutant strains and measured their ability to use allantoin as the sole nitrogen source. Wild-type cells grew with a 265-min doubling time when provided with allantoin as the sole nitrogen source (Table 2). In contrast, *dal5/dal5* or *urep1/urep1* homozygous mutant strains failed to grow. The inability of a *dal5/urep1* heterozygote to grow on allantoin indicated that the mutations failed to complement one another for this function, a characteristic expected of allelism.

TABLE 2. Doubling times of wild-type and mutant strains of *S. cerevisiae*^a

Nitrogen source	Doubling time (min)			
	Wild type	<i>dal5/dal5</i>	<i>dal5/urep1</i>	<i>urep1/urep1</i>
Ammonia	125	160	160	130
Proline	302	285	295	320
Proline + USA	800	300	330	353
Allantoate	265	NG ^b	NG	NG

^a Cell cultures of each genotype above were grown overnight to cell densities between 30 and 50 Klett units in glucose-ammonia medium. At this point, cultures were filtered and diluted into fresh medium containing the appropriate nitrogen source for doubling time determination. Ammonia and allantoin were provided at a concentration of 0.1%, proline was at 0.2%, and USA was at 100 µg/ml of culture.

^b NG, No detectable growth.

The same result was obtained with growth conditions which defined the *urep1* mutation, i.e., loss of growth inhibition of USA (4-6). Growth of the wild-type strain was markedly inhibited by USA (Table 2, compare proline versus proline plus USA). Such inhibition was not observed, however, when either a homozygous mutant or the *dal5/urep1* heterozygote was used in the experiment. As before, the mutations appeared to be allelic.

This conclusion was tested further by analyzing a cross containing the *dal5* and *urep1* alleles in repulsion. If the mutations were allelic, one would expect all the asci to assort in a parental, 4-:0+, configuration for growth on allantoin. This was the result observed for 15 of 15 asci analyzed; the mutations were tightly linked.

Determination of allantoin and USA transport activities confirmed conclusions derived from the growth experiments. A wild-type strain (M927) was able to accumulate either allantoin or USA (Fig. 2A and B). However, transport of both compounds was lost in *dal5* and *urep1* mutant strains. Finally, USA was found to be a competitive inhibitor of allantoin transport (Fig. 3).

In sum, these data indicate that USA and allantoin are transported into the cell by a common transport system. Mutations in strains isolated by Lacroute and his colleagues (4-6) and in this laboratory (8) are allelic even though the

FIG. 2. Allantoin and USA accumulation in wild-type, *dal5*, and *urep1* mutant strains of *S. cerevisiae*. (A) Resting cell cultures of strains M927 (*dal1 dal2*), M938 (*dal1 dal2 dal5*), and M1068 (*dal1 dal2 urep1*) were prepared as described in the text (final cell densities of 226, 182, and 214 Klett units, respectively). At zero time, a sample of each culture was placed in a small flask containing [¹⁴C]allantoin at a final concentration of 0.1 mM. After incubation as indicated in the figure, 1-ml samples were removed and assayed for the amount of allantoin accumulated. (B) Resting cell cultures of the same strains used in panel A were prepared after growth in citrate-buffered (pH 4.5) medium (final cell densities of 188, 120, and 166 Klett units, respectively). This pH has been shown to be optimal for USA transport (4). Radioactive USA accumulation was measured as described in panel A for allantoin.

FIG. 3. Effect of USA on the rate of allantoin transport at pH 5.0. A resting cell culture of strain M927 was prepared as described in the text (final cell density, 146 Klett units). The nitrogen-starved cells were transferred to nitrogen-free medium buffered at pH 5.0 and allowed to equilibrate for 1 h before assay. After equilibration, samples of the culture were added to vessels containing 0.5 mM USA (final concentration) and increasing concentrations of [^{14}C]allantoin. After 20 min of allantoin accumulation, the cells were treated as described earlier (7). The same procedure was performed in the absence of added USA.

methods of isolation were completely different. We and M. R. Chevallier and F. Lacroute (personal communication) propose to designate the locus responsible for production of the common transport system as *DAL5*. This decision derives from the fact that *DAL5* is the designation of a degradative transport system which is most consistent with the regulatory characteristics it possesses. The physiological identity of the USA transport system with that mediating

transport of the catabolic substrate allantoin explains its paradoxical regulatory characteristics.

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