Biochimica et Biophysica Acta, 465 (1977) 138—151

BBA 77604

UREIDOSUCCINIC ACID PERMEATION IN *SACCHAROMYCES CEREVISIAE*

M.L. GRETH, M.R. CHEVALLIER and F. LACROUTE

Laboratoire de Génétique Physiologique, I.B.M.C., 15, rue R. Descartes, 67084 Strasbourg-Cedex (France)

(Received October 18th, 1976)

Summary

Some strains of *Saccharomyces cerevisiae* exhibit a specific transport system for ureidosuccinic acid, which is regulated by nitrogen metabolism. Ureidosuccinic acid uptake occurs with proline but with ammonium sulfate as nitrogen source it is inhibited. The V for transport is 20—25 μmol/ml cell water per min. The apparent K_m is $3 \cdot 10^{-5}$ M. For the *urep1* mutant (ureidosuccinic acid permease less) the internal concentration never exceeds the external one.

In the permease plus strain ureidosuccinic acid can be concentrated up to 10 000 fold and the accumulated compound remains unchanged in the cells. Energy poisons such as dinitrophenol, carbonyl cyanide-*m*-chlorophenyldrazone (CCCP) or NaN_3 inhibit the uptake. No significant efflux of the accumulated compound occurs even in the presence of these drugs.

The specificity of the permease is very strict, only amino acids carrying an α-*N*-carbamyl group are strongly competitive inhibitors.

The high concentration capacity of the cells and the lack of active exit of the accumulated compound support the hypothesis of a carrier mediated active transport system.

Introduction

Ureidosuccinic acid uptake and its regulation in pyrimidine synthesis in *Saccharomyces cerevisiae* have been studied previously by Lacroute [1,2]. The importance of the source of nitrogen as well as the presence of a specific permease for its uptake has already been described by Drillien and Lacroute [3]. As it is possible to obtain mutants blocked in the utilization of ureidosuccinic acid, we studied in more detail this uptake process and the main properties of the permease. Our results are similar to the results obtained by Grenson et al.

Abbreviation: CCCP, cyanide-*m*-chlorophenyldrazone

[4] with the different amino-acid permeases in *S. cerevisiae* (one-sided transport) but very different from the results obtained on cytosine permeation and on uracil permeation in *S. cerevisiae* [5].

Materials and Methods

Yeast strains

The strains used were *S. cerevisiae* FL 100 (haploid, *a* mating type) and the derived strains *ura 2-60 ura 4-3* and *ura 2-60 ura 4-3 urep1* (haploid, *a* mating type), isolated in our laboratory. *Ura 2-60 ura 4-3* strain is defective for the aspartic transcarbamylase structural gene *ura 2* and for the dihydroorotase structural gene *ura 4*. *Urep1* strains lack the ureidosuccinic acid permease [3].

Growth conditions

The standard minimal medium contained per liter: 6.7 g of yeast nitrogen base without amino acids (Difco) and 20 g of D-glucose. Proline medium, used for permease derepression assays was identical except that ammonium sulfate was replaced by L-proline (3 mg/ml) as nitrogen source. When *ura*$^-$ strains were used, 30 μg/ml of uracil were added. Yeast cultures were grown aerobically at 28° C with reciprocal shaking. Growth was monitored on a Klett Summerson photoelectric colorimeter (blue filter). All experiments were conducted on exponentially growing cultures (about $1 \cdot 10^7$ cells/ml) between 60—80 Klett units.

Uptake measurements

For the measurement of the initial velocity of uptake, 1 ml of an exponentially growing culture was pipetted into a test tube containing [^{14}C]ureidosuccinic acid (usually $2.86 \cdot 10^{-3}$ μmol) shaken manually for 30 sec., filtered through a membrane filter LMR 4, 0.8 μm pore size (Labo-Moderne, Paris). The filters were then washed twice with 10 ml of cold water, dried, and the retained radioactivity counted in 8 ml of toluene plus 2,5-diphenyl-oxazole (5 g/l) in a scintillation spectrometer SL 30 Intertechnique-France. Blanks with portions of the radioactive solution were also filtered to determine the radioactivity retained by the filters alone. To calculate the internal concentration of ureidosuccinic acid an intracellular volume of $8 \cdot 10^{-4}$ ml/100 Klett units (blue filter) was taken, according to Jund [6] and Lacroute (unpublished results). [^{14}C]ureidosuccinic acid was usually diluted to obtain a specific radioactivity of 10 Ci/M.

Chase experiments

Usually 40 ml of a culture were first equilibrated with [^{14}C]ureidosuccinic acid for 30 min. Then 30 ml of the culture were filtered and the membrane carefully rinsed with medium prewarmed to 28° C. The adsorbed cells were then resuspended in 30 ml of medium and separated into two fractions. One fraction was supplemented either with non radioactive ureidosuccinic acid or with the inhibitor under study. The other fraction was used as control sample. Intracellular radioactivity was followed as a function of time of incubation by filtration and washing of 1 ml samples as described above.

Inhibition of ureidosuccinic acid permease

The possible inhibitors were generally tested at following final concentrations: 50 μg/ml, 5 μg/ml and 0.5 μg/ml. 1 ml of the *ura 2-60 ura 4-3* strain was pipetted into test tubes containing [^{14}C]ureidosuccinic acid ($2.86 \cdot 10^{-3}$ mM, $2.68 \cdot 10^{-2}$ mM and $2.86 \cdot 10^{-1}$ mM), with and without the possible inhibitor. Uptake of radioactive material was measured in initial velocities. For the study of competitive inhibition, six concentrations of ureidosuccinic acid were chosen and four concentrations of inhibitor.

Chemicals and abbreviations

L-ureido [^{14}C]succinic acid was synthesized by NEN Corp., Boston, but a further purification of this product was needed and performed as follows: a spot of L-ureido [^{14}C]ureidosuccinic acid (0.9 Ci/mol) was layered on a precoated thin-layer chromatography silica gel plate (0.25 mm, 20 × 20 cm). Solvent system was butanol/formic acid/water (75 : 15 : 10). Migration took place in a saturated chamber at 22° C. Visualization was effected with scanning on a Berthold-France pulse counter, at appropriate sensibility and scan frequency. Six peaks were always found, the greatest (40—50% of the radioactivity) corresponds to L-[^{14}C]ureidosuccinic acid. The other peaks were contaminating unidentified material. The ureidosuccinic acid peak was scraped off from the gel and extracted with cold distilled water, lyophilized and resuspended in an appropriate volume of water. For the colorimetric determination of ureidosuccinic acid the method of Gerhart and Pardee was applied [7].

DL-ureidosuccinic acid: (*N*-carbamyl-DL-aspartic acid) as well as the *N*-carbamyl analogs, the amino acids, and the amines were the purest grades available from Sigma Chemicals Co., St. Louis (U.S.A.). Allantoin was purchased from Fluka (Switzerland). Chlorhexidine was from Serva (Heidelberg), and the energy uncoupling agents from Sigma Chemical Co., St. Louis (U.S.A.). Yeast Nitrogen Base, without amino acids and without ammonium sulfate was obtained from Difco (Detroit, Mich. U.S.A.). Thiolutin: 6-acetamido-4-methyl-1,2-dithiolo|4,3-b|pyrrol 5 (4H) one was from Pfizer Laboratories Inc., Groton, Conn., U.S.A. The other chemicals were purchased from Merck AG, Darmstadt (G.F.R.).

Results

Effect of pH on permease activity

Cells of strain *ura 2-60 ura 4-3* grown in proline medium were centrifuged and resuspended in appropriate buffer mixtures (0.2 M boric acid, 0.05 M citric acid, 0.1 M trisodium orthophosphate) [8]. Permease activity determinations were performed at the different pH values as described under Materials and Methods. The results given in Fig. 1 show that the pH range of maximal permease activity is narrow: from pH 4.2 to pH 4.7. It corresponds to the usual pH range of the minimal and the proline medium.

Kinetics of ureidosuccinic acid entry

The *ura 2-60 ura 4-3* strain and the *ura 2-60 ura 4-3 urep1* strain were grown in proline medium. [^{14}C]ureidosuccinic acid was added and the radioactivity

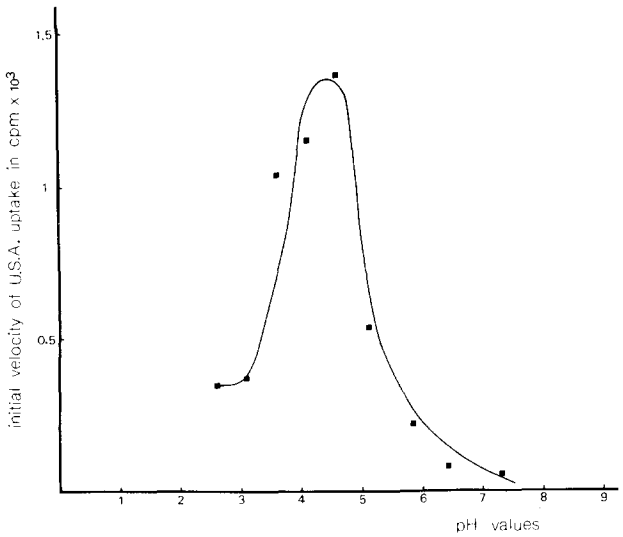


Fig. 1. pH profile for ureidosuccinic acid (U.S.A.) permease activity. Exponentially grown cells (about $1 \cdot 10^7$/ml) were harvested and the pellet resuspended in "universal buffer" containing 3% glucose at the appropriate pH values. For activity determination, $2.86 \cdot 10^{-3}$ µmol of [^{14}C]ureidosuccinic acid were added to 1 ml of cells and uptake measured as described under Materials and Methods. The maximal activity was found about pH 4.5.

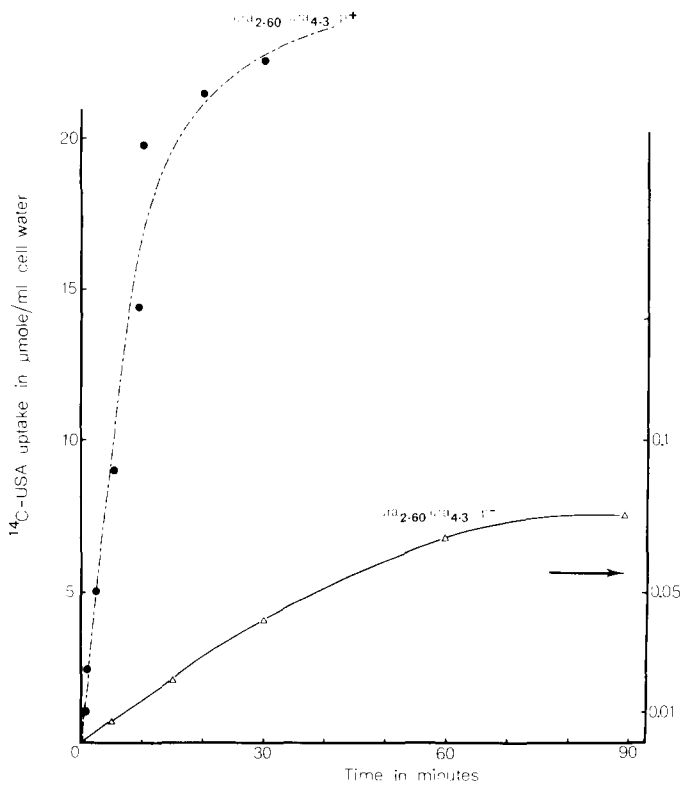


Fig. 2. Kinetics of ureidosuccinic acid (U.S.A.) entry. *S. cerevisiae* strain *ura 2-60 ura 4-3* ureidosuccinic acid permease plus and ureidosuccinic acid permease less were grown in proline medium and [^{14}C]ureidosuccinic acid added at time zero: $2.86 \cdot 10^{-2}$ mM for the permease plus strain and $5.6 \cdot 10^{-2}$ mM for the permease less strain. At the indicated times, samples were taken and the incorporated radioactivity determined. The upper curve corresponds to the permease plus strain. The lower profile corresponds to the permease less strain at a magnified scale (to the right hand). The internal ureidosuccinic acid concentration does not exceed the external one. The arrow indicates the external concentration of [^{14}C]ureidosuccinic acid.

retained by the cells was measured as a function of time of incubation. Fig. 2 shows the low entry into the permease less cells, in spite of the high external concentration: the internal concentration does not significantly exceed the external one. For the permease plus cells the figure shows the linearity of uptake during the first 5 min and the high level of internal ureidosuccinic acid concentrations reached after 20 min of uptake. We have calculated that the apparent equilibrium is due to partial exhaustion of the medium and that the uptake is linear as long as the external concentration is not significantly decreased.

Fate of accumulated ureidosuccinic acid

A culture of *ura 2-60 ura 4-3* cells in proline medium was loaded with $5.7 \cdot 10^{-2}$ mM [^{14}C]ureidosuccinic acid for two hours. Then, 20 ml of the culture were filtered, the cells resuspended in 1 ml of water and immersed in a hot water bath (100° C) for 10 min. After centrifugation, aliquots of the supernatant were layered on a silica gel 60 plate and further manipulations performed as described under L-[^{14}C]ureidosuccinic acid purification. Only one peak of radioactive material was found which had the same R_F value as the control applied on the same plate (R_F 0.32). Moreover, no significant radioactivity is found in the trichloroacetic acid insoluble precipitates of loaded cultures (see Table I). These results show that no transformation of the internally accumulated ureidosuccinic acid occurs.

Estimation of the apparent K_m

On strain *ura 2-60 ura 4-3* initial velocities of ureidosuccinic acid uptake were determined in the presence of various concentrations of [^{14}C]ureidosuccinic acid. The results, plotted according to Lineweaver and Burk, gave one straight line in the concentration range tested. The apparent K_m value was $2.73 \cdot 10^{-5}$ M, ±0.39 (see Fig. 3).

Ureidosuccinic acid permease derepression

An overnight culture of wild type strain FL 100, grown in minimal medium,

TABLE I

L-[^{14}C]UREIDOSUCCINIC ACID UPTAKE IN *ura 2-60 ura 4-3* STRAIN

External [^{14}C]ureidosuccinic acid concentration: $1.14 \cdot 10^{-2}$ mM; internal [^{14}C]ureidosuccinic acid concentration measurements in initial velocities (30 s). For trichloroacetic acid insoluble precipitates: 1 ml of the culture is mixed with 1 ml of cold trichloroacetic acid at 10%. After 20 min in an ice cold water bath, the mixture is filtered and radioactivity counted in PPO-toluene as usual.

Time of loading (min)	Incorporation of [^{14}C]ureidosuccinic acid (μmol/ml of cell water)	Incorporated radioactivity in trichloroacetic acid insoluble precipitates (μmol/ml of cell water)
15	26.36	0.007
30	52	⩽ to blank
60	58	⩽ to blank
90	61	0.0030
120	65	0.033
150	70	0.034

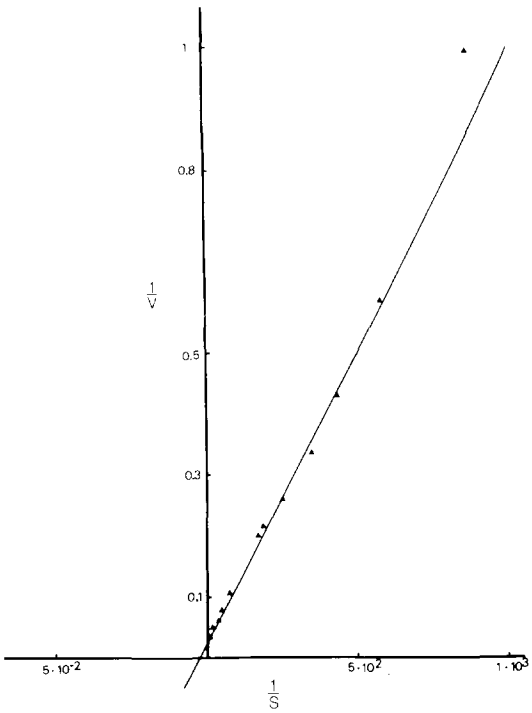


Fig. 3. Determination of the apparent K_m [^{14}C]ureidosuccinic acid uptake was performed on strain *ura 2-60 ura 4-3* ureidosuccinic acid permease plus in initial velocities measurements as function of external concentration, and under the usual experimental conditions. The extrapolated K_m is $2.73 \cdot 10^{-5}$ M ±0.39. Symbols: S, [^{14}C]ureidosuccinic acid external concentrations in mM; V, [^{14}C]ureidosuccinic acid incorporation in μmol/ml of cell water per 30 s incubation.

was divided in three fractions and resuspended after filtration in proline medium, proline medium supplemented with cycloheximide (15 μg/ml) an inhibitor of protein synthesis [9] and proline medium supplemented with thiolutin (5 μg/ml) known to interfere with RNA synthesis [10]; [^{3}H]leucine was added to all cultures. Every 30 min, the initial velocity of uptake of ureidosuccinic acid was determined and protein synthesis was evaluated by measurement of the incorporation of [^{3}H]leucine into trichloroacetic acid insoluble precipitate (see Fig. 4A, 4B). After the shift into proline medium there is a rapid increase of the permease activity. The activity detected in minimal medium (Fig. 4A) corresponds mainly to the basal level of the permease in this medium. Protein synthesis was similar in both media and the absorbance was practically unchanged during the time of experimentation. In medium supplemented with cycloheximide only a slight increase in the permease activity was found, although protein synthesis was only little affected (Fig. 4B). In the medium supplemented with thiolutine, protein synthesis was more affected and the measured activity did not increase (data not shown). These results show that permease synthesis is more affected than total protein synthesis in the presence of either cycloheximide or thiolutine. This effect might be due to the fact that, in the presence of the inhibitors, even in proline medium, the amino acid composition of the intracellular pool does not change enough to suppress the inhibition or the repression due to some component of the pool.

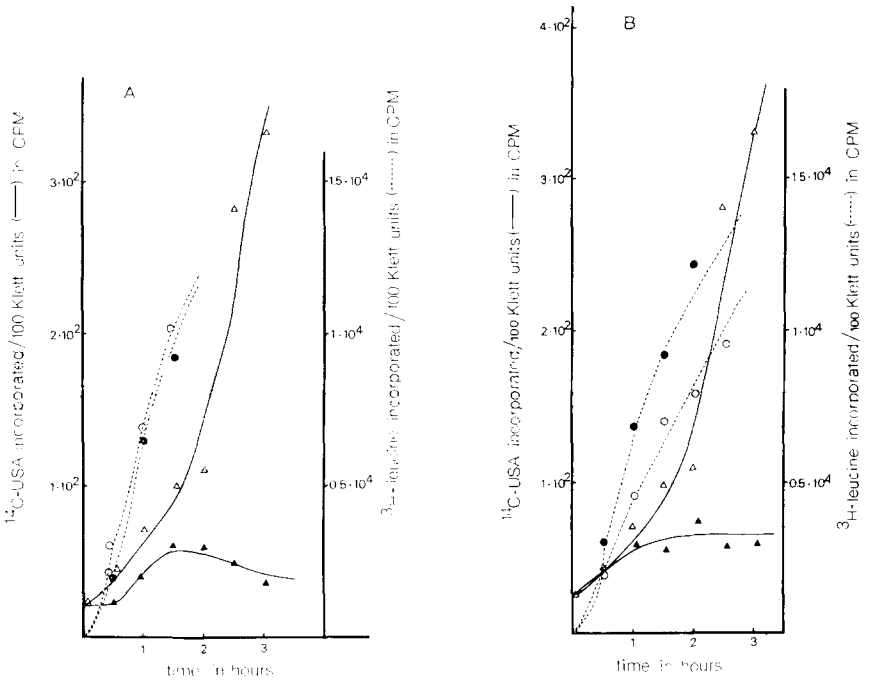


Fig. 4. (A) Ureidosuccinic acid (U.S.A.) permease derepression in proline medium. Wild type strain FL 100 is grown overnight in minimal medium, then diluted and resuspended either in proline medium or in minimal medium. In the first case, permease activity appears rapidly ([^{14}C]ureidosuccinic acid incorporation). In the second case the permease activity is strongly inhibited. In both media the protein synthesis is very active ([^{3}H]leucine incorporation). For assay conditions see Materials and Methods. Symbols: △———△, ureidosuccinic acid permease activity in proline medium; ●———●, protein synthesis in proline medium; ▲———▲, ureidosuccinic acid permease activity in minimal medium; ○———○, protein synthesis in minimal medium. (B) Ureidosuccinic acid (U.S.A.)-permease inhibition in proline medium supplemented with cycloheximide. Assay conditions are the same as in (A), except that one fraction of the culture is resuspended in proline medium supplemented with cycloheximide (15 μg/ml). Symbols: △———△, ureidosuccinic acid permease activity in proline medium; ●———●, protein synthesis in proline medium; ▲———▲, ureidosuccinic acid permease activity in proline medium plus cycloheximide; ○———○, protein synthesis in proline medium plus cycloheximide.

Ureidosuccinic acid inhibition after addition of ammonium ions to proline medium

To a culture of wild type strain FL 100, grown overnight in proline medium, 5 mg/ml of ammonium sulfate were added. Samples were taken as a function of time to determine initial velocities of [^{14}C]ureidosuccinic acid uptake (see Fig. 5). 10 min after the shift, about 50% of the initial permease activity was lost and after 30 min only 30% of the initial activity remained. The slight increase in permease activity after 25—30 min of incubation in the presence of ammonium ions has been observed in three independent experiments. The fast disappearance of permease specific activity during the first 20 min is strongly suggestive of an inhibition by ammonium ions or by a product accumulated in the cells in their presence. But the continuous decrease of the permease activity as a function of cellular growth is higher than expected assuming a complete repression of its synthesis. This implies either a late secondary inhibition or a degradation of the preexisting molecules.

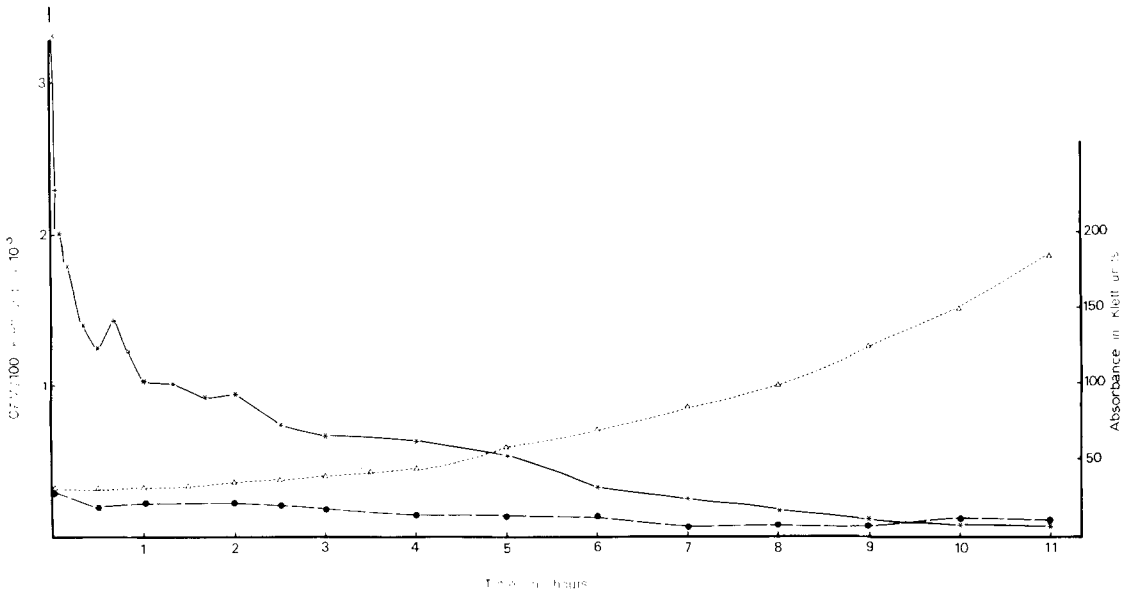


Fig. 5. Ureidosuccinic acid-permease inhibition after addition of ammonium ions to proline medium. Symbols: left hand scale: initial velocity of uptake of [^{14}C]ureidosuccinic acid in cpm/100 Klett units; [^{14}C]ureidosuccinic acid concentration: $5.7 \cdot 10^{-3}$ mM, ★———★. Initial velocity of uptake of [^{14}C]-ureidosuccinic acid of control culture keept in minimal medium in cpm/100 Klett units; ^{14}C external concentration: $5.7 \cdot 10^{-3}$ mM, ●———●. Right hand scale: absorbance in Klett units, △———△.

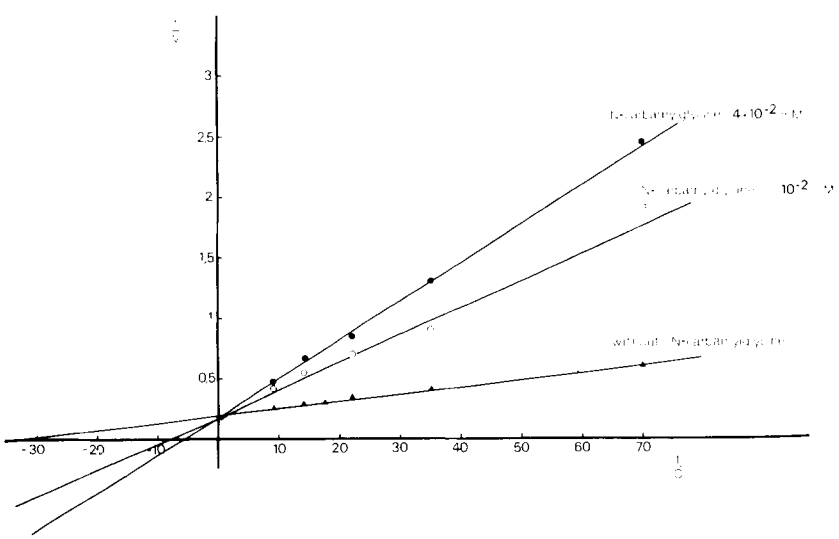


Fig. 6. Competitive inhibition of [^{14}C]ureidosuccinic acid uptake by *N*-carbamylglycine. Lineweaver-Burk plot showing competitive inhibition of [^{14}C]ureidosuccinic acid uptake by *N*-carbamylglycine 10^{-2} mM and $4 \cdot 10^{-2}$ mM in the presence of [^{14}C]ureidosuccinic acid concentrations ranging from $1.42 \cdot 10^{-2}$ mM to $1.14 \cdot 10^{-1}$ mM.

TABLE II

SPECIFICITY OF UREIDOSUCCINIC ACID PERMEASE. EFFECT OF POTENTIAL INHIBITORS ON [^{14}C]UREIDOSUCCINIC ACID UPTAKE

The external molarity of ^{14}C-ureidosuccinic acid was $3 \cdot 10^{-3}$ mM. The uptake was measured after 30 s of incubation and the potential inhibitor was added along with [^{14}C]ureidosuccinic acid before starting the incubation.

Compound	Molarity of inhibitor (% uptake)			Compound	Molarity of inhibitor (% uptake)		
	$3 \cdot 10^{-3}$ mM	$3 \cdot 10^{-2}$ mM	$3 \cdot 10^{-1}$ mM		$3 \cdot 10^{-3}$ mM	$3 \cdot 10^{-2}$ mM	$3 \cdot 10^{-1}$ mM
No addition	100	100	100	L-Cystine	106	109	108
Glycine	104	102	100	DL-Methionine	107	99	103
L-Alanine	103	94	104	L-Phenylalanine	100	105	99
L-Valine	98	96	91	L-Tyrosine	104	102	103
L-Leucine	106	94	112	L-Tryptophan	108	106	110
L-Aspartic acid	108	115	108	L-Histidine	118	121	121
L-Asparagine	106	114	80	L-Adenine	—	112	102
L-Glutamic acid	108	115	110	Succinic acid	109	107	107
L-Glutamine	110	95	100	Aspartic acid	108	115	108
L-Arginine	107	109	95	Fumaric acid	98	115	101
L-Ornithine	104	99	103	α-Keto-glutaric acid	111	108	112
L-Lysine	105	104	115	Orotic acid	89	113	—
L-Serine	101	103	102	Urea	97	121	113
L-Threonine	105	110	106	Ammonium sulfate	98	115	110
DL-Cysteine	104	106	104				

TABLE III

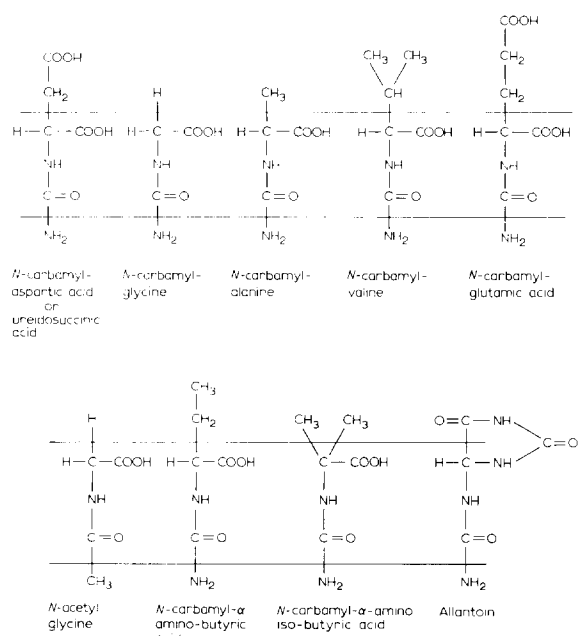
APPARENT AFFINITY CONSTANTS OF THE [^{14}C]UREIDOSUCCINIC ACID UPTAKE SYSTEM FOR STRUCTURAL ANALOGS

Ki constants were calculated from the experimental results obtained on Lineweaver-Burk plots from the values of the appropriate intercepts in the presence and absence of inhibitor. For more details see Results. The K_m value for [^{14}C]ureidosuccinic acid uptake is $2.73 \cdot 10^{-5}$ M ±0.39.

Structural analogs	$K_i \cdot 10^{-5}$ (M)
N-Acetyl glycine	2.90
N-Carbamyl glycine	1.62
N-Carbamyl alanine	0.90
N-Carbamyl valine	0.91
N-Carbamyl butyric acid	0.94
N-Carbamyl glutamic acid	0.80
N-Carbamyl isobutyric acid	No inhibition
Allantoin	No inhibition
Methylurea	No inhibition
Ethylurea	No inhibition
N-Phospho acetyl-1-aspartic acid	No inhibition

The specificity of the ureidosuccinic acid permease

A competitive inhibition of ureidosuccinic acid uptake by the permease was looked for with the naturally amino acids and with several compounds structurally analogous to the ureidosuccinic acid (see Tables II and III). Among the compounds tested, only some amino-acids carrying an α-*N*-carbamyl group and *N*-acetyl-glycine were found to be competitive (see Table III and Fig. 6). Several of the carbamyl compounds tested have a K_i smaller than the apparent K_m of ureidosuccinic acid for its uptake system; this is an indication that ureidosuccinic acid may not be the most important natural substrate of this permease.



Scheme I. Structures of the *N*-carbamyl analogs of ureidosuccinic acid assayed for competitive inhibition. Only allabtoin and *N*-carbamyl-α-amino iso-butyric acid are without effect on ureidosuccinic acid entry.

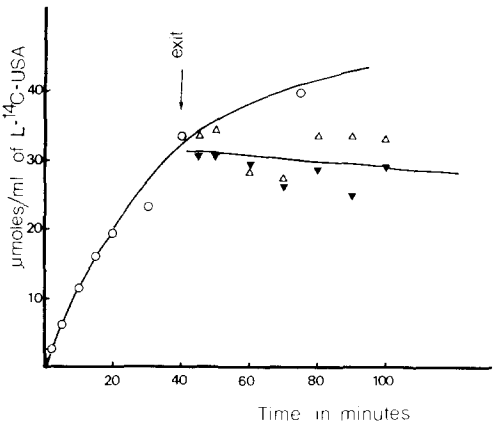


Fig. 7. Chase experiment with proline medium and with ureidosuccinic acid (U.S.A.). A culture of strain *ura 2-60 ura 4-3* was loaded in proline medium with $5.7 \cdot 10^{-3}$ mM [^{14}C]ureidosuccinic acid for 40 min, then filtered, washed and resuspended in proline medium and in proline medium supplemented with cold ureidosuccinic acid (200 μg/ml). Samples of 1 ml were withdrawn at time intervals and their radioactivity determined as described. Symbols: △———△, control in proline medium; ▲———▲, chase in proline medium supplemented with 200 μg/ml of cold ureidosuccinic acid; ○———○, [^{14}C]ureidosuccinic acid uptake in the loading phase.

Scheme I visualizes the requirement for competitive inhibition which is related to (a) the presence of a terminal carbamyl group or the presence of a peptide like bond and (b) on the configuration of the α carbon near the carbamyl group.

Reduction of ureidosuccinic acid entry by inhibitors of energy metabolism and by chlorhexidine

Inhibitors of energy metabolism have been tested on *ura 2-60 ura 4-3* strain.

TABLE IV

REDUCTION OF [^{14}C]UREIDOSUCCINIC ACID ENTRY BY ENERGY METABOLISM INHIBITORS, IN PERCENTAGES, RELATIVE TO CONTROL

Radioactivity determinations in initial velocities were performed as usual on samples of *ura 2-60 ura 4-3* cells, provided with [^{14}C]ureidosuccinic acid and with the inhibitor at appropriate concentrations.

Inhibitor	Molarity of inhibitor	Molarity of [^{14}C]ureidosuccinic acid	
		$2.86 \cdot 10^{-3}$ mM (% of inhibition)	$5.72 \cdot 10^{-3}$ mM (% of inhibition)
Dinitrophenol	$5 \cdot 10^{-4}$ M	70	88
CCCP	$5 \cdot 10^{-5}$ M	25	
	$1 \cdot 10^{-4}$ M	48	
	$1.5 \cdot 10^{-4}$ M	59	
	$2 \cdot 10^{-4}$ M	77	
Chlorhexidine	$2 \cdot 10^{-5}$ M	15	13
	$4 \cdot 10^{-5}$ M	13	16
	$6 \cdot 10^{-5}$ M	36	36
	$8 \cdot 10^{-5}$ M	56	54
	$1 \cdot 10^{-4}$ M	68	70
N_3Na	$7.5 \cdot 10^{-4}$ M	75	73

To 1 ml of culture, [^{14}C]ureidosuccinic acid and the inhibitor were added at the appropriate concentrations. The uptake of the labelled compound was estimated as usual (see Table IV).

Efflux of [^{14}C]ureidosuccinic acid from ura 2-60 ura 4-3 strain

(a) Chase by non radioactive ureidosuccinic acid. An exponentially growing culture was loaded with [^{14}C]ureidosuccinic acid. After 40 min of incubation, the culture was filtered, washed and resuspended either in proline or in proline medium supplemented with an excess of non labelled ureidosuccinic acid. Only a slight efflux of radioactive material, similar in both media, was detected (see Fig. 7).

(b) Effects of uncoupling agents and chlorhexidine on the level of internal ureidosuccinic acid. These assays were also performed on *ura 2-60 ura 4-3* strain. Loading was performed for 50 min with labelled ureidosuccinic acid. The inhibitors were then added. The concentrations were the same as those

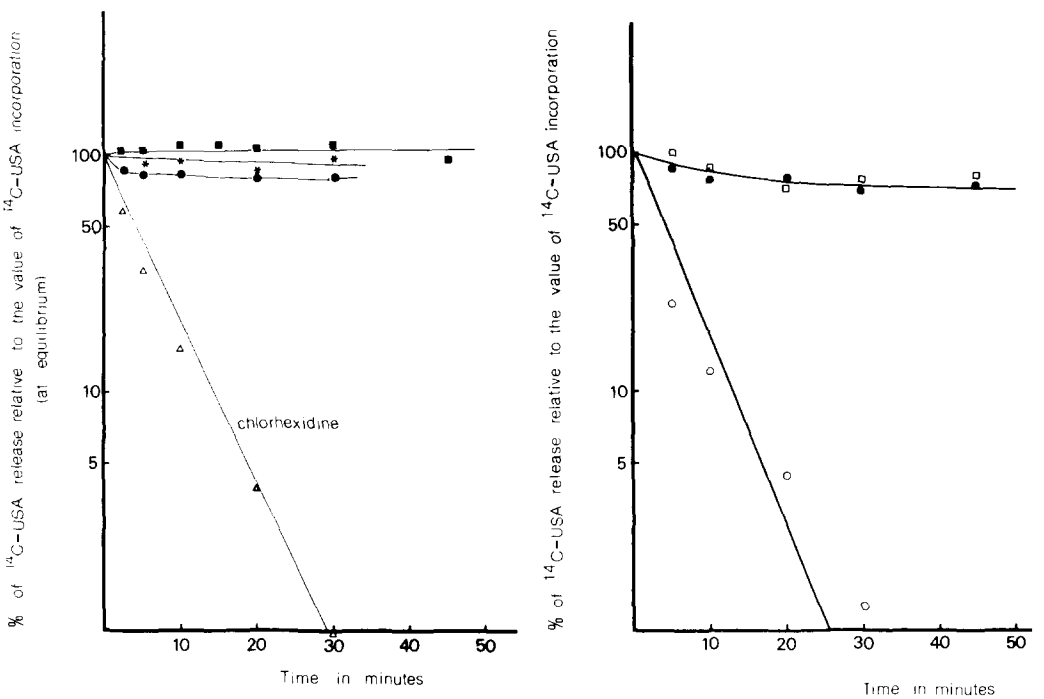


Fig. 8. Effect of dinitrophenol, of N_3Na, of ureidosuccinic acid (U.S.A.) and of chlorhexidine on [^{14}C]-ureidosuccinic acid in strain *ura 2-60 ura 4-3*. Symbols: ■———■, [^{14}C]ureidosuccinic acid exit in presence of dinitrophenol ($5 \cdot 10^{-4}$ M); ★——★, [^{14}C]ureidosuccinic acid exit in presence of N_3Na ($7.5 \cdot 10^{-4}$ M); ●———●, [^{14}C]ureidosuccinic acid exit in control (minimal medium); △———△, [^{14}C]ureidosuccinic acid exit in presence of chlorhexidine [10^{-4} M).

Fig. 9. Effect of dinitrophenol, and of chlorhexidine on [^{14}C]ureidosuccinic acid (U.S.A.) exit in *ura 2-60 ura 4-3 urep1* strain. After a preloading with $5.7 \cdot 10^{-2}$ mM [^{14}C]ureidosuccinic acid for 50 min, in proline medium, the culture is filtered, divided in three parts and resuspended in minimal medium and in proline medium supplemented with dinitrophenol ($5 \cdot 10^{-4}$ M) or with chlorhexidine ($1 \cdot 10^{-4}$ M). The radioactivity is determined as usual. Symbols: □———□, control samples resuspended in minimal medium. ●———●, ureidosuccinic acid exit in presence of dinitrophenol; ●———●, ureidosuccinic acid exit in presence of chlorhexidine.

used for the assays of inhibition of uptake. The cells were sampled at appropriate times for measurements of retained radioactivity. Only a slight exit is observed in the presence of the uncoupling agents, but chlorhexidine, a surface active agent known to inhibit *Streptococcus faecalis* ATPase [11] induces a rapid efflux of the accumulated ureidosuccinic acid (see Fig. 8).

Efflux of [^{14}C]ureidosuccinic acid from ura 2-60 ura 4-3 urep1 strain

Experiments were conducted as described above except that loading was performed with high concentration of [^{14}C]ureidosuccinic acid. The release of the retained radioactivity was practically nil either in minimal medium or in proline medium supplemented with dinitrophenol. In proline medium supplemented with chlorhexidine, a rapid efflux was found. The fact that this occurs in the permease plus as well as in the permease less strain, suggests a general membrane alteration unrelated to the permease (see Fig. 9).

Discussion

Using a mutant strain *ura 2-60 ura 4-3* of *S. cerevisiae*, which can neither synthesize nor convert ureidosuccinic acid into dihydroorotic acid, we have seen that our strain possess a very specific ureidosuccinic acid permease. The specificity is well underlined by the fact that competitive inhibition was found only with *N*-carbamyl-amino acids or *N*-acetylglycine, amino acids and urea having no effect. The ureidosuccinic acid permease activity depends strongly on the nitrogen source in the growth medium. The transport system is very active in proline medium (a poor nitrogen source) and is much lower in ammonium sulfate medium. The kinetics of derepression (Fig. 4A) as well as the kinetics of repression (Fig. 5) do not allow discrimination between repression or inhibition of the permease on ammonium medium; most likely both phenomena contribute to the lowering of the specific permease activity. Cells having the permease can concentrate exogenous ureidosuccinic acid up to 10 000 fold. Its entry is inhibited by energy uncoupling agents. Analysis of the internally accumulated radioactivity indicates that it consists entirely of free ureidosuccinic acid. This cellular pool cannot be displaced by an excess of externally added ureidosuccinic acid or by energy uncoupling agents which inhibit the entry. The lack of exit from the loaded cells might be due to an inhibition of the carrier molecules on the internal side of the membrane. As this compound has only a very low diffusion constant this would ensure the high internal concentration. A passive diffusion constant of $k = 0.005$/min has been estimated using the data of Fig. 9 which show the efflux of ureidosuccinic acid from a *urep1* strain. This value is much lower than the values of 0.04/min and 0.48/min which have also been measured in yeast cells for cytosine and uracil respectively [5,12].

The ureidosuccinic acid transport system differs markedly from the cytosine transport system of *S. cerevisiae*, which has been previously described and where chasing of internal labelled cytosine by cold cytosine occurs, as well as efflux of internal cytosine in the presence of energy uncoupling inhibitors [5]. But it resembles to amino acid transport systems described in several cell types in its unidirectional aspects of transport [4,13—15].

Acknowledgements

This investigation was supported by grant no. 75.7.1337 from the Délégation Générale à la Recherche Scientifique et Technique. We thank H. Bussey for helpful comments and revision of the manuscript.

References

1 Lacroute, F. (1966) Doctoral Thesis, University of Paris
2 Lacroute, F. (1968) J. Bacteriol. 95, 824—832
3 Drillien, R. and Lacroute, F. (1971) J. Bacteriol. 109, 203—208
4 Crabeel, M. and Grenson, M. (1970) Eur. J. Biochem. 14, 197—204
5 Chevallier, M.R., Jund, R. and Lacroute, F. (1975) J. Bacteriol. 122, 629—641
6 Jund, R. (1974) Doctoral Thesis, University of Strasbourg
7 Gerhart, J.C. and Pardee, A.B. (1962) J. Biol. Chem. 237, 891—896
8 Perrin, D.D. and Boyd Dempsey □□ (1974) Buffers for pH and Metal Ion Control, pp. 48—49, Chapman and Hall Laboratory Manuals
8 Hartwell, L.H., Hutchinson, H.T. Holland, T.M. and McLaughlin, C.S. (1970) Molec. Gen. Genet. 106, 347—361
10 Tipper, D.J. (1973) J. Bacteriol. 116, 245—256
11 Harold, F.M. (1972) Bacteriol. Rev. 36, 172—230
12 Jund, R., Chevallier, M.R. and Lacroute, F. (1976) submitted to J. Membrane Biol.
13 Kotyk, A. and Rihova, L. (1972) Biochim. Biophys. Acta 288, 380—389
14 Ring, K. and Heinz, E. (1966) Biochem. Z. 344, 446
15 Pall, M.L. and Kelly, K.A. (1971) Biochem. Biophys. Res. Commun. 42, 940