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Amino Acid Transport and Metabolism in Nitrogen-Starved Cells of *Saccharomyces cerevisiae*

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Nitrogen-starved yeast derepress a general amino acid permease which transports basic and hydrophobic amino acids. Although both groups of amino acids are metabolized, the derivatives of the basic amino acids are retained by the cells, whereas those of the hydrophobic amino acids are released as acidic and neutral deaminated derivatives. The release of the deaminated derivatives of the hydrophobic amino acids only occurs in the presence of glucose, which presumably produces amino acceptors. The accumulation of intracellular amino acids results in transinhibition of the uptake of exogenous amino acids whether the intracellular amino acid is a basic amino acid or the product of intracellular transamination from a hydrophobic amino acid. Variation of permease and transaminase activity was measured during growth under repressed (ammonia-grown) and derepressed (proline-grown) conditions. Maximum levels for both activities occurs at the mid-exponential phase.

Grenson et al. (7) showed that nitrogen starvation in *Saccharomyces cerevisiae* results in the derepression of a general amino acid permease which shows a high capacity for the transport and accumulation of basic and hydrophobic amino acids. Confirmation of the derepression of amino acid permeases by nitrogen starvation of ammonia-grown cells has been reported by other laboratories (6, 10, 15) and for other yeasts (12).

In this paper we report that nitrogen starvation of *S. cerevisiae* results concomitantly in the derepression of the general amino acid permease and specific transaminases. As a result of the combined action of the permease and transaminases, deaminated derivatives are produced from the hydrophobic amino acids, which leave the cells. The metabolic derivatives of the basic amino acids, however, are retained by the cells. The release of deaminated derivatives of hydrophobic amino acid requires the presence of glucose. It is presumed that glucose provides the energy for transport and the amino acceptor for transamination. A similar phenomenon with almost identical substrate specificity was described by Hunter and Segel (8) for the mold *Penicillium chrysogenum*.

This paper describes the conditions required for derepression of the general amino acid permease and for the release of deaminated derivatives. Preliminary observations on the variation of these activities during growth of cells under repressed and derepressed culture conditions are also described.

MATERIALS AND METHODS

Growth of cultures. Cells were grown in filter-sterilized Wickerham synthetic medium (24) containing either glucose (to a final concentration of 5%) or maltose (final concentration of 2%) and succinate (final concentration of 0.2%). The nitrogen source was added aseptically either as ammonia [0.02 M as $(NH_4)_2SO_4$] or proline (1 mg/ml). Cultures were grown overnight at 30°C in a shaking water bath. Cells were harvested by centrifugation (in a Sorvall RC2B centrifuge [Newtown, Conn.]), washed with distilled water, and stored at 4°C. Samples were resuspended to a final concentration of 2% (vol/vol) before use. The yeast strains used were Y185, obtained from H. O. Halvorson of Brandeis University, and L14, used in previous studies in this laboratory.

Uptake of amino acids. Uptake of amino acids was carried out on 1-ml samples containing 0.5 ml of a 2% cell suspension, 0.2 ml of 0.05 M phthalic acid buffer (pH 5.5), 0.1 ml of cycloheximide (1 mg/ml), and 0.1 ml of 20% glucose. After 15 min of preincubation at 30°C, 0.1 ml of radioactive amino acid was added to a final concentration of 0.2 mM, with a specific activity of approximately 0.5 μCi/μmol. Samples (0.1 ml) were withdrawn from the uptake mixture, pipetted into 5 ml of ice-cold water over glass-fiber filter disks (Whatman, 934 AH), and washed with 10 ml of ice-cold water under suction. Filter disks were placed in vials with 5 ml of ACS scintillation fluid (Amersham/Searle, Arlington Heights, Ill.) and counted in a Beckman (Palo Alto, Calif.) or an Intertechnique (Plaisir, France) scintillation counter. Results are expressed as nanomoles of amino acid accumulated per milligram of protein. The protein content of a cell suspension was derived from a standard curve relating cell wet weight to protein. Protein was determined by the biuret proce-

dure (9). The figures depict individual experiments, which were shown to be consistently reproducible.

Determination of products of amino acid metabolism. The presence of deaminated neutral and acid derivatives in the medium of cell suspensions actively transporting amino acids was determined using a modification of the method of Hunter and Segel (8), by which the derivatives are partitioned between an amyl alcohol layer and neutral or acidified uptake medium, respectively. Cells were incubated in the usual uptake medium for 15 min at 30°C, after which ^{14}C-labeled amino acid was added (0.2 mM final concentration). After 30 min, cells were concentrated by centrifugation, washed, and collected by filtration for determination of ^{14}C label in the cells. The supernatant was adjusted to approximately pH 7.0, and an equal volume of amyl alcohol was added. The tubes were then mixed with a Vortex mixer (Scientific Industries, New York) for 10 s and then spun in a Sorvall SP/X centrifuge for 10 min at full speed (ca. 3,000 × *g*). Samples (0.1 ml) from the two immiscible layers were added to ACS scintillation fluid (Amersham/Searle) and counted in an Intertechnique scintillation counter. The ratio of counts in the amyl alcohol layer to those in the water layer is used to determine the amount of neutral derivatives produced. A second extraction was carried out after adjusting the supernatant to pH 1.2 to determine the amount of acid derivatives produced. Correction was made for the distribution of the unmodified amino acid.

Chromatography of metabolic products. To determine whether basic amino acids were metabolized, cell extracts were chromatographed. Cells were allowed to accumulate and metabolize uniformly labeled ^{14}C-amino acids for 30 min at 30°C. They were extracted with hot water for 20 min (100°C), and cells were removed by centrifugation. The supernatant was lyophilized overnight and taken up in 100 μl of water. The reaction mixtures used for in vitro transaminase activity were also analyzed; after incubation they were heated to 100°C for 5 min and treated as above. Portions (10 ml) of each extract were applied to thin-layer cellulose chromaotgrams (Eastman 13255 cellulose) and developed with an *n*-propanol-NH_4OH (70%/30%) mixture. Chromatograms were air-dried and cut into 1-cm strips from base line to solvent front for each extract. The strips were placed in vials with ACS scintillation fluid and counted in a Beckman scintillation counter. The results were compared with amino acid standards and related to known R_f values for metabolic products, where available.

Preparation of cell extracts and assay for transaminase activity. Packed cells were mixed with cold tris(hydroxymethyl)aminomethane-hydrochloride buffer (0.05 M, pH 7.5) and glass beads (1 mm; Arthur H. Thomas, Philadelphia, Pa.) in a ratio of 1:2:4 and disrupted using a Braun homogenizer (type 2876, Melsungen, W. Germany) at 2,000 rpm for 2 min. The homogenates were centrifuged at 27,000 × *g* for 15 min in a Sorvall SS34 rotor (RC2-B), and the supernatant was used for determination of transaminase activity.

Transaminase activity was determined by incubating extracts with 2.0×10^{-4} M ^{14}C-labeled amino acid, 10^{-2} M α-ketoglutarate, oxaloacetate, or pyruvate, and 10^{-4} M pyridoxal phosphate in 0.02 or 0.1 M phosphate buffer (pH 7.0). Samples were taken at intervals and adjusted to pH 1.2, and the amount of α-keto acid formed was determined by the amyl alcohol partition method described above. Results are expressed as nanomoles per milligram of protein. Protein was determined by the method of Lowry et al. (14).

RESULTS

Amino acid transport in derepressed cells. Derepression of amino acid transport was observed in proline-grown (derepressed) cells as first described by Grenson et al. (7). Transport of amino acids in proline-grown cells is 4- to 360-fold higher than that in ammonia-grown (repressed) cells depending on the amino acid and yeast strain used (Table 1).

Derepression of amino acid transport was also observed when ammonia-grown cells were shaken for 1 to 2 h in distilled water containing glucose, as described by Kotyk and Rihova (10). Although Kotyk and Rihova showed that derepression under these nitrogen starvation conditions is inhibited by cycloheximide, they proposed that the glucose-stimulated amino acid transport by ammonia-grown cells is the result of the synthesis of special energy reserves for amino acid transport. They proposed that these special energy reserves are inorganic polyphosphates, since they observed a stoichiometric relationship between amino acid uptake and polyphosphate disappearance during uptake. To test this hypothesis, derepression of the general amino acid permease and amino acid transport activity was measured in phosphate-depleted, ammonia-grown, and proline-grown cells, respectively. Ammonia- and proline-grown cells were depleted of their phosphate and polyphosphate reserves by overnight incubation in complete growth medium minus inorganic phosphate (22).

The data in Table 2 show that derepression of the general amino acid permease (measured as arginine transport) in phosphate-depleted, ammonia-grown cells does not occur unless inorganic phosphate is present during the 2-h incubation with glucose (experiment 3 versus 1 and 2). This could mean that phosphate repletion is required for either derepression or polyphosphate synthesis. However, the data on the transport of identically phosphate-depleted, proline-grown cells show that phosphate repletion is not required for amino acid transport by previously derepressed cells. The high level of arginine uptake by phosphate-depleted, proline-grown cells without prior phosphate repletion (experiments 4 and 5 versus 1 and 2) shows that the phosphate repletion is required for de-

TABLE 1. *Rate of amino acid uptake by repressed (ammonia-grown) and derepressed (proline-grown) cells*[a]

Class	Amino acid	Transport activity[b]					
		Strain Y185			Strain L-14		
		Repressed	Derepressed	Ratio	Repressed	Derepressed	Ratio
1. Basic	Arginine	4.4	17.84	4.1	0.18	12.94	72.0
	Lysine	3.0	17.32	5.8	0.40	12.53	31.3
	Histidine	0.88	19.09	21.7	0.16	12.25	76.6
2. Imino	Proline	0.05	0.90	18.0	0.006	0.8	133.3
3. Dicarboxylic	Glutamic acid	0.42	10.12	24.1	0.02	7.2	360
	Aspartic acid	0.4	3.44	8.6	0.03	2.7	90
4. Neutral	AIB[c]	0.3	3.0	10.0	0.02	1.98	99
	Glycine	0.55	17.03	31.0	0.10	9.69	96.9
	Alanine	2.4	15.08	6.3	0.10	4.24	42.4
5. Hydrophobic	Methionine	0.25	9.99	40.0	0.07	14.96	213
	Phenylalanine	1.8	5.22	2.9	0.08	4.72	59.0
	Tyrosine	3.04	15.8	5.2			
	Leucine	1.25	4.74	3.8	0.26	4.30	15.5
	Isoleucine	0.93	5.99	6.4	0.167	8.19	49.0
	Valine	1.08	8.76	8.1	0.11	7.13	64.8

[a] Conditions as described in Fig. 1.
[b] Transport rate expressed as nanomoles/milligram of cell protein per 5 min at 30°C.
[c] α-Amino isobutyric acid.

TABLE 2. *Rate of arginine transport by phosphate-depleted, ammonia- and proline-grown cells*[a]

Expt	Cells	Repletion conditions	Arginine transport activity	
			Edogenous	Glucose
1	Ammonia grown	Water	709	939
2	Ammonia grown	Glucose	962	957
3	Ammonia grown	Glucose + phosphate	3,564	10,231
4	Proline grown	Water	3,105	17,132
5	Proline grown	Glucose	8,181	19,240
6	Proline grown	Glucose + phosphate	12,294	23,984

[a] Phosphate-depleted, ammonia- and proline-grown cells were shaken for 2 h at 30°C in distilled water, 5% glucose, or 5% glucose plus 0.1% phosphate. Arginine uptake was measured after a wash in distilled water either in the absence or presence of 2% glucose. Arginine was present at a concentration of 0.2 mM (1.0 μCi/μmol). Uptake is expressed as counts per minute per 0.1 mg of cell protein in 15 min at 30°C.

repression of arginine uptake by ammonia-grown cells (experiment 3 versus 2) rather than for polyphosphate synthesis. Phosphate repletion may secondarily contribute to amino acid uptake as an energy source since repletion increases both the endogenous and glucose-stimulated rates of arginine uptake of proline cells (experiment 6 versus 5).

Release of amino acid derivatives. The derepression of the general amino acid permease whether by growth in proline or nitrogen starvation in the presence of glucose results, in addition to an increase in the rate of uptake of both basic and hydrophobic amino acids, in the release into the medium of deaminated derivatives of the hydrophobic amino acids (Fig. 1). The release phenomenom depends on the presence of a fermentable sugar (Fig. 2). Glucose and fructose stimulate uptake and efflux, whereas ethanol and acetate, although rapidly oxidized, have no effect (not shown). Galactose and maltose, which are not metabolized in non-induced cells, stimulate neither transport nor efflux (data not shown). Glucose added at any time during uptake stimulated both transport and release of the radioactive label (Fig. 3).

Competitive inhibition of amino acid transport. The measurement of reciprocal inhibition of uptake among the amino acids histidine, lysine, alanine, valine, and glutamic acid (Table 3) shows that the substrate specificity of the general amino acid permease is similar to that reported for other yeast strains (4, 6, 7). The affinity is highest for basic amino acids, followed by that for the neutral and hydrophobic amino acids, and is lowest for dicarboxylic amino acids.

The data of Fig. 4 show that in a mixture of

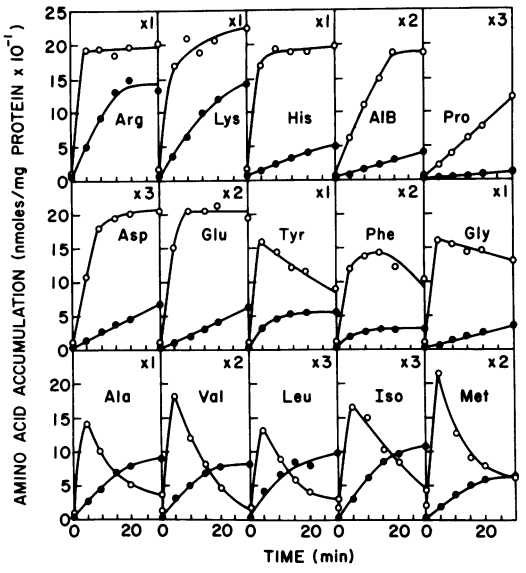


FIG. 1. *Amino acid accumulation in ammonia-grown (●) and proline-grown (○) cells. The uptake medium contained 0.2 mM amino acid, 2% glucose, 100 μg of cycloheximide per ml, and 1% (vol/vol) cell suspension in 0.01 M phthalate buffer (pH 5.5) at 30°C. Glucose was added 15 min before the amino acids. Ordinate scale was reduced (1 to 3×) as indicated for each amino acid. All amino acids were uniformly labeled with ^{14}C, except [methyl-^{14}C]methionine and [^{3}H]phenylalanine.*

basic and hydrophobic amino acids, at concentrations that do not significantly inhibit their respective uptakes, the time course of valine accumulation and the release of the carbon chain is unaffected by the presence of lysine. In data shown in Fig. 5, the time course of valine uptake and carbon chain release is considerably protracted by the presence of concentrations of a basic amino acid, in this case histidine, which inhibits valine uptake. However, the final result is unchanged; all the valine of the medium is cycled through the cell and deaminated.

Transinhibition of amino acid transport. Transinhibition by preloading nitrogen-starved cells with either arginine or valine is shown in Fig. 6. In this experiment, the cells are allowed to accumulate unlabeled amino acid for the times shown before ^{14}C-labeled amino acid is added. As can be seen, between 5 and 10 min after preloading, the initial rate of uptake is reduced by about 90%. It is significant that the time course for the establishment of the transinhibition is the same for the two amino acids although the valine carbon chains are not retained by the cells. Transinhibition has also been demonstrated with lysine and α-amino isobutyric acid (AIB) (results not shown).

Metabolism of amino acids by whole cells. Although the cycloheximide present in the medium during uptake was sufficient to prevent incorporation of amino acids into proteins, metabolic alteration of the amino acids such as transamination could still occur. To investigate this possibility, the medium was examined for deaminated derivatives as described in Materials and Methods.

The release of metabolic products of valine and lysine was determined in repressed and derepressed cells (Table 4). Valine was found to be extensively metabolized by derepressed

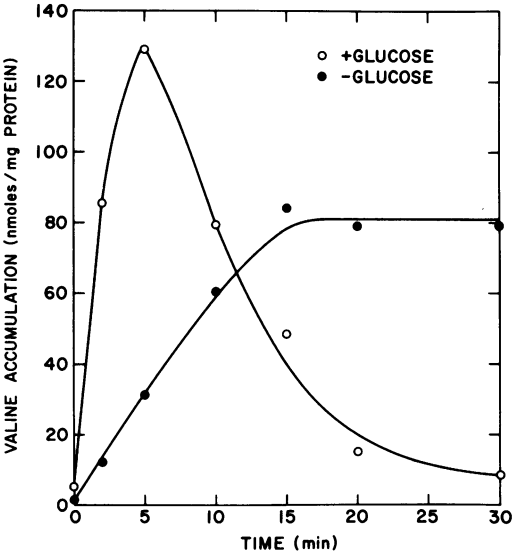


FIG. 2. *Effect of glucose on the accumulation and release of ^{14}C radioactivity from L-valine. Conditions as in Fig. 1.*

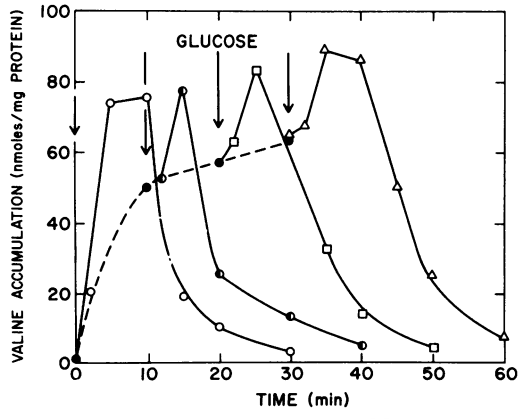


FIG. 3. *Effect of time of glucose addition on accumulation and release of ^{14}C radioactivity from L-valine. Arrows indicate time of glucose addition. No glucose control (●). (Conditions as in Fig. 1.)*

TABLE 3. *Competition among amino acids for transport*[a]

Inhibitor (mM)	Inhibition (%) with substrate (0.2 mM):				
	Glutamate	Valine	Alanine	Lysine	Histidine
Glutamate					
0.2		15	12	0	0
2.0		2	−6	0	0
20.0		88	100	0	0
Valine					
0.2	77		17	18	−[b]
2.0	96		88	35	33
20.0	100		100	90	69
Alanine					
0.2	44	46		20	−
2.0	97	96		31	31
20.0	100	100		90	92
Lysine					
0.2	72	44	62		−
2.0	97	97	92		70
20.0	100	100	100		91
Histidine					
0.2	72	71	87	−	
2.0	98	99	97	79	
20.0	100	100	100	96	

[a] Each amino acid was used as a labeled substrate for transport at a concentration of 0.2 mM as described in the legend of Fig. 1. Competing unlabeled amino acids were added at the indicated concentrations, and percent inhibition of transport was calculated from 5-min uptakes.

[b] −, Not done.

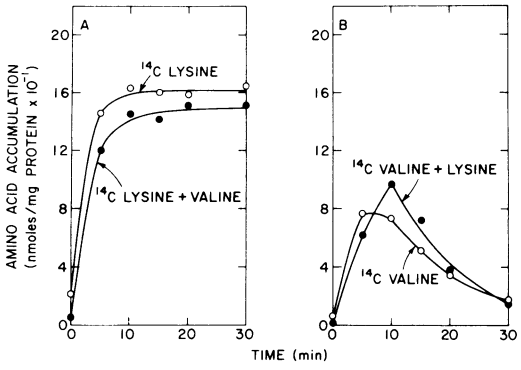


FIG. 4. *Accumulation of lysine and valine when present alone or in a mixture. (A) Lysine alone (○) or in the presence (●) of valine. (B) Valine alone (○) or in the presence (●) of lysine. Both amino acids were used at a 0.2 mM final concentration.*

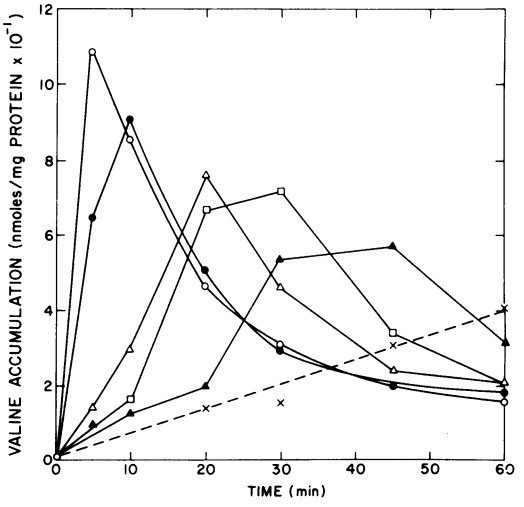


FIG. 5. *Time course of valine accumulation in the absence (○) or presence of increasing amounts of histidine: 0.1 mM (●), 0.2 mM (△), 0.25 mM (□), 0.3 mM (▲), and 0.35 mM (×). Valine was present at 0.2 mM.*

cells. Most of the valine from the medium was taken into the cell, metabolized, and released into the medium; 84% of the label in the medium was recovered as deaminated products (i.e., acid plus neutral amyl alcohol-soluble products). In repressed cells there was considerably less transamination: more amino acid remaining in the cell and less appearing in the medium. Metabolism (i.e., transamination) was glucose dependent. By contrast, lysine was almost completely transported into the cells and there was no significant release of metabolic products into the medium. Glucose stimulated basic amino acid transport but did not cause efflux of label.

The data in Table 5 compare amino acid metabolism in derepressed and repressed cells. In

derepressed cells significant metabolism of a number of acids is seen. The extent of metabolism of the hydrophobic amino acids varies from 54% for phenylalanine to 90% for leucine. Most of the metabolic products are accounted for as acid and neutral amyl alcohol-soluble derivatives, presumably the α-keto acid and fusel oil derivatives, respectively, of the amino acid concerned. The hydrophobic amino acids, beginning with phenylalanine, are arranged in the table in the order of increasing neutral product formation. The "volatile" fraction was calculated from the difference between the initial counts added to the incubation mixture and the total counts recovered from the medium plus cells; it is presumably CO_2. From 10 to 25% of the hydrophobic amino acids are converted to volatile products. Glutamic acid is also significantly metabolized in derepressed cells, most of the metabolic products being converted to volatile and acid derivatives.

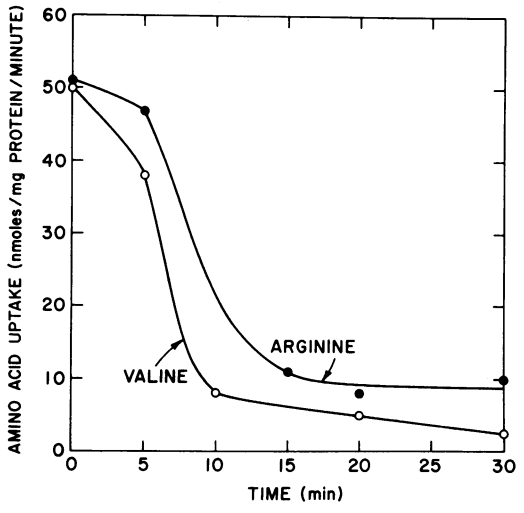


FIG. 6. *Transinhibition of valine (○) and arginine (●) transport in derepressed cells. Cells were allowed to accumulate unlabeled valine or arginine (0.2 mM final concentration) for the times indicated, at which times [^{14}C]valine or [^{14}C]arginine was added at a final specific activity of 0.5 μCi/μmol. Transport was then followed for 20 min, and the rate of uptake was calculated for each time point. Correction was made for changes of amino acid concentration due to transport and metabolism.*

The results from repressed cells (Table 5) show that although the extent of metabolism of the metabolized amino acids is only about 10% of that of derepressed cells, the products are qualitatively the same and are produced in similar proportions. Analysis of the medium for deaminated products shows that they begin to appear from zero time of uptake. There is no dependence on accumulation to a certain internal threshhold concentration.

The loss of label from the amino acids alanine and glycine seen in Fig. 1 involves metabolic reactions in addition to those mentioned above for the hydrophobic amino acids, but this was not studied further.

Whereas no radioactive products of lysine, histidine, or arginine appeared in the medium, chromatography of hot-water extracts of cells that had accumulated these amino acids showed that significant metabolism had occurred. Lysine was shown to be converted primarily to α-amino adipic semialdehyde and saccharopine, as described by Fjellstedt and Ogur (5). No effort was made to identify the products from histidine or arginine.

TABLE 4. *Amino acid metabolism by repressed and derepressed cells*[a]

Amino acid	Cells	Uptake conditions	Distribution of amino acid radioactivity (nmol/mg of cell protein)		
			Total amt of amino acid and derivatives		Amyl alcohol-soluble derivatives
			Cells	Medium	
Valine	Repressed	+ Glucose	153.1	46.9	19.6
		− Glucose	30.2	169.8	0.0
	Derepressed	+ Glucose	32.2	166.8	140.1
		− Glucose	8.9	191.1	2.4
Lysine	Repressed	+ Glucose	180.1	19.9	0.0
		− Glucose	31.3	168.7	0.0
	Derepressed	+ Glucose	194.6	5.4	0.0
		− Glucose	21.6	178.0	0.0

[a] Repressed and derepressed cells (1%, vol/vol) were incubated with ^{14}C-labeled L-valine or L-lysine for 30 min at 30°C in the presence or absence of 2% glucose. The distribution of radioactivity between cells and medium was determined as usual. The total amount of amyl alcohol-soluble derivatives in the medium was determined by partition between acidified medium (pH 1.2) and amyl alcohol.

TABLE 5. *Amino acid metabolism by repressed and derepressed cells*[a]

Amino acid	Distribution of amino acid radioactivity											
	Derepressed cells						Repressed cells[b]					
	Cells	Medium			Volatile	% Released from cells	Cells	Medium			Volatile	% Released from cells
		Acid	Neutral	Water soluble				Acid	Neutral	Water soluble		
Lysine	200	0	0	0	0	Nil	12.0	0	0	188.0	0	Nil
Histidine	195	0	0	5	0	Nil	10.0	0	0	190.0	0	Nil
Glutamic acid	61	34	8	41	56	59	15.6	0.6	0.2	183.6	0.5	0.7
Phenylalanine	2	58	30	90	20	54	5.8	4.1	4.0	184.1	0.0	4.1
Tyrosine	47	49	37	36	31	58	2.0	2.8	2.3	180.5	12.4	8.8
Methionine	10	45	61	29	55	80	2.2	1.7	6.5	179.3	10.3	9.3
Valine	6	42	93	23	36	85	5.4	2.0	5.0	180.1	7.5	7.3
Isoleucine	21	39	108	32	0	74	5.0	2.5	8.5	179.9	4.1	7.6
Leucine	5	29	151	15	0	90	4.1	2.0	16.5	177.4	0.0	9.3

[a] The distribution of label between cells and medium after 30 min of incubation at 30°C was determined for each amino acid as described for Table 4. The radioactive products in the medium were fractionated successively by partition into amyl alcohol from neutralized (pH 7.0) and acidified (pH 1.2) medium. The radioactivity remaining in the acidified medium is reported as "water soluble." The volatile product is calculated from the difference between total radioactivity added and the radioactivity recovered from the cells plus medium. The results are expressed as nanomoles per milligram of cell protein based on the original specific activity of each amino acid.

[b] Highly repressed.

Transaminase activity in cell extracts. Since the derivative released by derepressed cells is the result of intracellular transamination, the transaminase activity of extracts of repressed and derepressed cells was compared. Transaminase activity was tested with α-ketoglutarate, oxaloacetate, and pyruvate as amino acceptors. The results with α-ketoglutarate are shown in Table 6. High transaminase activity is observed with tyrosine, phenylalanine, leucine, and isoleucine as substrates, whereas aspartic acid, valine, and methionine are only slowly transaminated. No transamination of glycine was detected. These substrate specificities are similar to those reported in the literature for other yeast strains and represent the activities of more than one transaminase (1, 8, 16, 17). Transaminase activity with oxaloacetate as amino acceptor is somewhat less for most amino acids, whereas that with pyruvate is extremely low. In the case of alanine, however, significant transaminase activity was observed with pyruvate as an acceptor, but only negligible activity was observed with α-ketoglutarate and oxaloacetate as acceptors. Although neither lysine nor histidine produced derviatives that entered amyl alcohol, chromatography of the incubation medium revealed that derivatives similar to those in whole cells were produced.

There were significant differences between the levels of transaminase activity in extracts from repressed and derepressed cells. Using α-ketoglutarate as amino acceptor, transaminase activity was two to three times higher in derepressed cells (Table 6).

TABLE 6. *In vitro transaminase activity of extracts of respressed and derepressed cells*[a]

Amino acid	Transaminase activity (nmol of deaminated product/mg of protein per 5 min)		
	Repressed cells	Derepressed cells	Ratio derepressed/repressed
Leucine	20.8	64.6	3.1
Isoleucine	17.7	53.3	3.0
Valine	4.6	7.2	1.6
Phenylalanine	19.2	71.8	3.7
Alanine	0.0	0.0	–[b]
Tyrosine	75.4	108.7	1.4
Methionine	0.0	5.1	∞
Glycine	0.0	0.0	–
Glutamic acid	7.7	10.8	1.4
Asparatic acid	8.9	13.6	1.5

[a] Cell extracts were prepared as described in Materials and Methods. Samples of the extracts were incubated with 2×10^{-4} M ^{14}C-labeled amino acid, 10^{-2} M α-ketoglutarate, and 10^{-4} M pyridoxal phosphate in 0.02 M phosphate buffer (pH 7.0) at 30°C for 15 min. Samples were removed at 2, 5, 10, and 15 min and acidified with concentrated HCl to pH 1.2, and the deaminated products present in solution were estimated by partition with amyl alcohol (see text).

[b] –, No activity with this substrate.

It is worth noting, with respect to methionine transport, that whereas efflux of ^{14}C label from cells was observed when methyl-labeled methionine was transported into cells, no efflux was observed with carboxyl-labeled methionine (Fig. 7).

Culture age dependence of amino acid

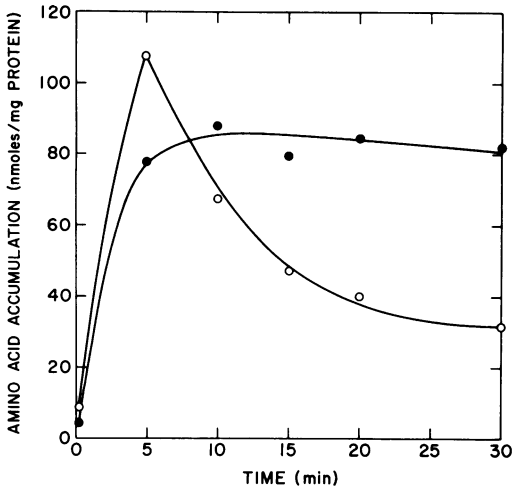


FIG. 7. *Transport of methionine labeled in different carbon atoms in derepressed cells. 1-[methyl-^{14}C]methionine (○) and carboxyl 1-[^{14}C]methionine (●) uptakes were followed as described in the legend of Fig. 1.*

transport and metabolism. Valine transport in repressed and derepressed cultures of strain Y185 was culture age dependent. In repressed cells (i. e., ammonia grown), valine transport remains essentially low throughout the growth curve except for a transient increase at the beginning of exponential growth, rapidly falling to a low level by the middle of the log phase (Fig. 8B). Transport in derepressed (proline-grown) cells also reached a maximum in early log phase. In vivo transaminase activity, indicated by the rapid loss of previously accumulated label, paralleled the development of transport capacity (note asterisks in Fig. 8A). Both transport and amino acid metabolism fell after mid-log phase in a manner similar to that of repressed cells.

DISCUSSION

Nitrogen limitation or starvation in yeast is known to result in a number of adaptations which allow the cells to use alternate nitrogen sources. Thomulka and Moat (18) showed that the transfer of cells from a rich medium containing amino acids to one containing only ammonia as a nitrogen source results in the derepression of the anabolic nicotinamide adenine dinucleotide phosphate (NADP)-linked glutamic dehydrogenase and glutamic-oxaloacetate transaminase, which together increase the ability of the cells to assimilate ammonia. The presence of ammonia in limiting amounts or its total absence further derepresses these enzymes for ammonia assimilation and derepresses a number of other enzymes, including the enzymes for the catabolism of asparagine (23), arginine (21), allantoin (2), and urea (3, 20) plus the general amino acid permease, which allow the cells to utilize alternate nitrogen sources.

The present study shows the effect of nitrogen limitation on the pattern of transport and metabolism of different amino acids (Fig. 1). On the one hand, the basic amino acids are accumulated and their carbon chains are retained against large concentration gradients. On the other, the hydrophobic amino acids are accumulated and undergo transamination with a release of their deaminated derivatives. The difference in pattern is the result of the difference in substrate specificity of the general amino acid permease and that of specific transaminases and their relative activities.

The release of carbon chains from certain amino acids when they serve as the sole nitrogen source has long been known. Felix Ehrlich first showed in 1904 that in yeast fermentations in which amino acids served as nitrogen

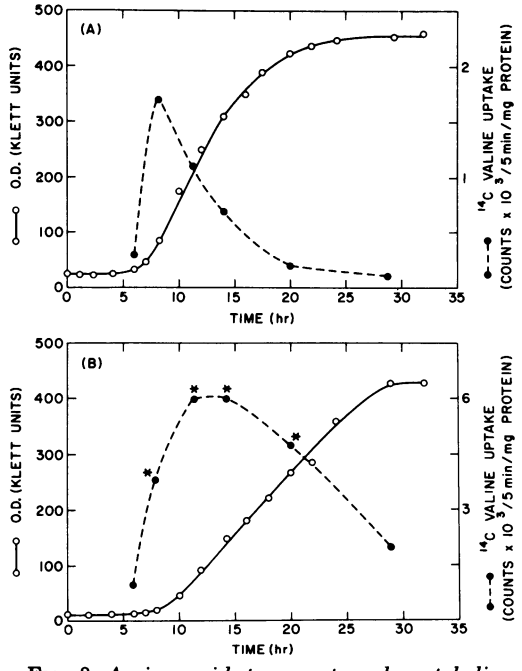
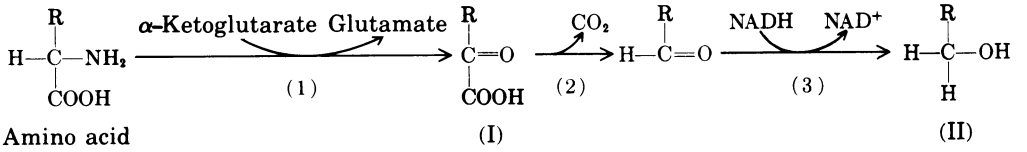


FIG. 8. *Amino acid transport and metabolism during the growth cycle of repressed and derepressed cells. Cells were grown in 2% maltose plus 0.2% succinate, with ammonia (A) or proline (B) as the nitrogen source. Growth was followed by increase in optical density (O.D.; ○). [^{14}C]valine transport was carried out on samples removed at the indicated times (●). The asterisks in (B) indicate that net loss of accumulated label was observed after 5 min of accumulation. (Identical results were obtained with cells grown on 5% glucose.)*

sources in the absence of ammonia, the carbon chains of certain amino acids are released as deaminated acids and "fusel oils" (see Webb and Ingraham [19] for a comprehensive and historical review). The deaminated acids (I) and fusel oils (II), which have been shown to be produced by all yeast strains studied, are produced by successive (1) transamination, (2) decarboxylation, and (3) reduction as shown below:



The acid and neutral deamination products released from the hydrophobic amino acids described in Table 5 are presumed to be the α-keto acid and fusel oil derivatives, respectively, of the specific amino acids.

The conditions for their release are similar to those described by Sentheshanmuganathan and Elsden for fusel oil formation from amino acids (16), namely, the presence of glucose. They attribute the requirement for glucose to its role as a source of: (i) energy for amino acid uptake, (ii) α-ketoglutarate as an amino acceptor, and (iii) NADH as a reductant of the aldehyde. A similar conclusion was reached by Hunter and Segel in their study of the nitrogen-starved cells of *P. chrysogenum* (8).

The manner in which nitrogen-starved yeast or *P. chrysogenum* utilizes hydrophobic amino acids in the presence and absence of glucose illustrates how the general amino acid permease together with specific transaminases accomplish the selective retention of the amino moiety of hydrophobic amino acids. In the absence of glucose, the amino acids are accumulated via the derepressed general amino acid permease activated by endogenous energy reserves. The accumulated amino acids are not, and indeed cannot be, metabolized in the absence of glucose. Upon the addition of glucose, however, the rate of amino acid uptake increases, but, more importantly, the amino moiety is transaminated to the α-ketoglutarate derived from glucose and the deaminated carbon chain released before or after further modification.

A comparison of the extent of derepression of the general amino acid permease versus the transaminases (Tables 1 and 6) shows that for most of the amino acids the increase is far greater for the permease (3 to 40 times) than for the transaminases (1.4 to 3.7 times). The basis of the derepression of the permease has been found to be essentially identical whether the cells are derepressed by growth in a proline medium, as described by Grenson et al. (7, 15), or by shaking in air with glucose in the absence of any exogenous nitrogen source, as described by Kotyk and Rihova (11) and Greasham and Moat (6). The relatively greater increase of permease activity does not necessarily mean a greater extent of permease synthesis over transaminase synthesis since permease activity is subject to inhibition by certain amino acids of the intracellular pool (i.e., transinhibition). Therefore, part of the increase of permease activity in derepressed cells could be due to a decrease in the intracellular amino acid pool.

Transinhibition has been demonstrated as a significant phenomenon in these studies. Transinhibition was found to increase during valine uptake (Fig. 6). The time course of the increase of transinhibition parallels the time course of the release of its deaminated derivatives to the medium. Thus, transinhibition appears paradoxically to increase with decrease of its intracellular concentration. The intracellular amino acid responsible for the transinhibition must be an amino acid derived from it by transamination. A similar phenomenon was reported by Hunter and Segel in *Penicillium* (8).

Transinhibition may account for the variation in amino acid transport activity during cell growth in repressed and derepressed cultures (Fig. 8). Both in ammonia and proline cultures there is a transient increase in permease activity during the early exponential phase of growth. In both cases this may represent variation in the level of transinhibition by changes in pool concentrations of specific amino acids. A similar variation with culture age has been described recently in *Streptomyces hydrogenans* (13). In the latter case, the authors present evidence that variation of transport activity during growth is due to changes of both negative and positive feedback effectors, namely, amino acids and specific, charged transfer ribonucleic acids (13).

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