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Growth Inhibition by α-Aminoadipate and Reversal of the Effect by Specific Amino Acid Supplements in *Saccharomyces cerevisiae*

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The growth of *Saccharomyces cerevisiae* wild-type strain X2180 in minimal medium was inhibited by the addition of higher-than-supplementary levels of α-aminoadipate. This inhibitory effect was reversed by the addition of arginine, asparagine, aspartate, glutamine, homoserine, methionine, or serine as single amino acid supplements. Mutants belonging to the *lys2* and *lys14* loci were able to grow in lysine-supplemented α-aminoadipate medium, although not as well as when selected amino acids were added. Growth in α-aminoadipate medium by all strains was accompanied by an accumulation of α-ketoadipate. Glutamate:ketoadipate transaminase levels were derepressed two- to fivefold in *lys2* mutants using α-aminoadipate as a nitrogen source. Wild-type strain X2180 growing in amino acid-supplemented AA medium exhibited higher levels of α-aminoadipate reductase. Mutants unable to use α-aminoadipate without amino acid supplementation were obtained by treatment of *lys2* strain MW5-64 and were shown to have glutamate:ketoadipate transaminase activity and to lack α-aminoadipate reductase activity. Altered cell morphologies, including increased size, multiple buds, pseudohyphae, and germ tubes, evidenced by cells grown in α-aminoadipate medium suggest that higher-than-supplementary levels of α-aminoadipate result in an impairment of cell division.

The addition of certain amino acids to minimal media inhibits the growth of both procaryotes and eucaryotes (4, 15, 18). The growth inhibition in bacteria is a consequence of antagonism between related amino acids. For example, the growth inhibition of *Escherichia coli* K-12 by valine and the reversal of the inhibition by isoleucine is due to the total inhibition of the valine-sensitive acetohydroxy acid synthases I and III and a lack of expression of the valine-resistant acetohydroxy acid synthase II, which results in a limitation for isoleucine (5). In eucaryotic cells, the study of growth inhibition resulting from the addition of amino acids is complicated by the unequal distribution of amino acid pools between the cytoplasm and organelles such as mitochondria, the nucleus, and vacuoles (6, 12, 20). In addition, in yeasts the transport of amino acids into organelles is a dynamic process dependent upon metabolic conditions (2, 14). Basic amino acids, when added to media containing a poor nitrogen source, inhibit the growth of *Saccharomyces cerevisiae* (18).

The amino acid α-aminoadipate (AA), an intermediate of the lysine biosynthetic pathway (Fig. 1) in yeasts and other higher fungi (19), can support the growth of appropriate lysine auxotrophs when added as a supplement to minimal medium. Chattoo et al. (3) observed that wild-type *S. cerevisiae* does not use AA as a principal nitrogen source, whereas *lys2* and *lys5* strains with deficient levels of AA reductase (EC 1.2.1.26) do. They selected *lys2* and *lys5* mutants based on the ability of the mutants to use AA as the sole nitrogen source and concluded that the ability of these mutants to use AA as a nitrogen source is directly or indirectly related to the decreased levels of AA reductase activity.

In this report, we present evidence that AA, when used in concentrations sufficient to provide a nitrogen source, inhibited the growth of *S. cerevisiae* in ammonia-containing medium. The growth inhibition was reversed by the addition of supplemental levels of specific amino acids. Results from studies of wild-type strains and two classes of mutants demonstrate the pathway for the use of AA as a nitrogen source as well as the independence of the growth inhibition effect of AA from the levels of AA reductase activity. Cells grown in AA medium exhibited altered morphologies, similar to those of Hartwell's *cdc*

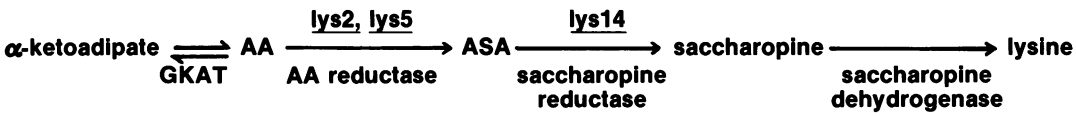

FIG. 1. Biochemical steps for the conversion of AA to α-ketoadipate or lysine and the position of mutants.

mutants (11), suggesting that the growth inhibition caused by AA is due to an impairment of normal cell division.

MATERIALS AND METHODS

Strains and media. Representatives of strains used for this study are listed in Table 1. Lysine auxotrophs were obtained by treatment of wild-type strain X2180-1A with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and characterized by complementation tests, accumulation of specific intermediates, and enzyme assays. Mutants unable to use AA as a nitrogen source without supplementation were derived from the *lys2* mutant MW5-64 by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and enrichment with nystatin. Media used for maintenance and growth studies included the following: nutrient medium (9); basal medium without a nitrogen source (NN) (glucose, 10 g; yeast nitrogen base without amino acids and ammonium sulfate, 1.7 g; and distilled water to 1 liter); minimal medium (MM) (NN plus ammonium sulfate, 1 mg/ml), and AA medium (NN plus DL-AA, 1 mg/ml, unless stated otherwise).

Growth studies. The growth response in various media was recorded turbidimetrically up to an optical density at 550 nm (OD_{550}) of 2.0 and microscopically as the increase in cell size and number. A standard inoculum (0.5 ml of a suspension with an OD_{550} of 1.0) of fresh cells was used to inoculate 25-ml portions of medium in culture flasks. The flasks were incubated in an air shaker at 30°C. Samples removed at intervals were vigorously shaken in a Vortex mixer before examination.

TABLE 1. *S. cerevisiae* strains

Strain[a]	Genotype	Phenotype	Source
X2180-1A	a *SUC2 mal gal2 CUP1*	Does not use AA as nitrogen source without amino acid supplement	Yeast Genetics Stock Center, Donner Laboratory, Berkeley, Calif.
MW5-64	a *lys2*	Uses AA as nitrogen source	This study[b]
MW7-62	a *lys2*	Does not use AA as nitrogen source without amino acid supplement	Derived from strain MW5-64
7305d	α *lys14*	Uses AA as nitrogen source	F. Messenguy

[a] Strains listed are representatives of phenotypic classes used in this study.

[b] Mutant strains obtained by treatment of strain X2180-1A with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

Phase-contrast photomicroscopy. Observations of changes in cell morphology were recorded on Kodak Panatomic X film with a Leitz microscope with a 40X phase objective.

Determination of α-ketoadipate. The accumulation of α-ketoadipate in culture supernatants was determined by the 2,4-dinitrophenylhydrazone assay, thin-layer chromatography, and tracer experiments (1, 17).

Preparation of cell extracts. Cells were disrupted in a Braun model MSK cell homogenizer (16), and crude extracts were dialyzed by passage through a Sephadex G-25-80 column. The protein concentration in the extracts was determined by the biuret method (8) with bovine serum albumin as a standard.

AA reductase assay. AA reductase activity was determined by a modified assay based on the conversion of AA to α-aminoadipic-δ-semialdehyde (16).

GKAT assay. Glutamate:α-ketoadipate transaminase (GKAT) (EC 2.6.1.39) activity was assayed by determining the counts per minute of α-ketoadipate formed from ^{14}C-AA in vitro. The reaction mixture consisted of the following: pyridoxal phosphate, 1.75 μmol; α-ketoglutarate, 7.75 μmol; DL-AA, 7.75 μmol (cold AA); 1-^{14}C-AA, 0.9 nmol (53 mCi/mmol); 0.1 M potassium phosphate buffer, pH 7.5; and dialyzed extract, 0.5 to 1 mg of protein in a final volume of 0.5 ml. After 2 h of incubation at 25°C, the reaction was stopped, and the mixture was fractionated with a Pasteur pipette column of Dowex-1 formate (200 to 400 mesh) resin. Unreacted ^{14}C-AA was washed with 3 ml each of sodium formate buffers at pH 5.0, 4.0, 3.5, and 3.25 (1 N NaOH was added to 4.5 N formic acid to achieve the desired pH). The α-[^{14}C]ketoadipate was eluted by 6 ml of 4.5 N formic acid and 3 ml of 88% formic acid. Fractions were collected in vials, dried, and counted. The control lacked α-ketoglutarate.

RESULTS

Growth inhibition effect of AA. Wild-type strain X2180 did not grow in AA medium, and growth in MM was inhibited by the addition of AA (Fig. 2). The degree of inhibition was dependent upon the concentration of AA; the addition of 100 μg of AA per ml caused little or no inhibition, and 1 mg of AA per ml caused the maximum inhibition. Low concentrations (30 to 100 μg/ml) of AA satisfy the growth requirements of lysine auxotrophs blocked before the AA step (7). Although DL-AA was used in most experiments, L-AA had the same inhibitory effect. The L isomer also seems to be the form that the cell uses as a nitrogen source since *lys2* mutants grew twice as well in L-AA medium as in DL-AA medium.

Use of AA and reversal of inhibition of AA in MM by amino acid supplementation. In prelimi-

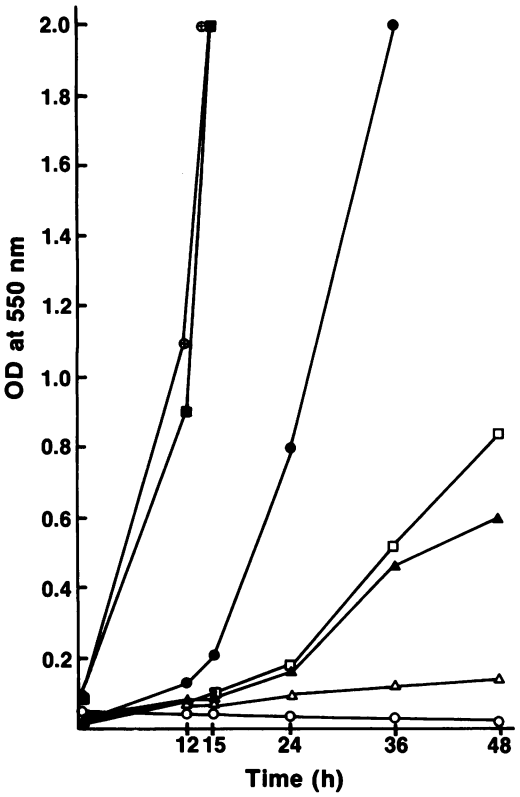

FIG. 2. Growth response of wild-type strain X2180 in the following: AA medium with (▲) or without (○) methionine (100 μg/ml) supplement; NN containing ammonia at 500 μg/ml alone (⊕) or with AA at 1 mg/ml (△), 500 μg/ml (□), or 250 μg/ml (●); or NN containing ammonia at 500 μg/ml, AA at 500 μg/ml, and methionine at 100 μg/ml (■).

nary experiments, we observed that AA at 1 mg/ml did not inhibit the growth of strain X2180 in nutrient medium or MM supplemented with casein hydrolysate. Subsequently, we demonstrated that supplementation of AA medium with any group I amino acids at 50 to 100 μg/ml resulted in good growth (asparagine, aspartic acid, and glutamine resulted in an OD_{550} of 2.0 within 24 h) and a significant accumulation of α-ketoadipate in the culture supernatant (Table 2). The accumulation of α-ketoadipate indicated the use of the amino group from AA. Group II amino acids provided slight growth but no accumulation of α-ketoadipate by 72 h, and group III amino acids were ineffective. Only aspartate supplementation resulted in good growth but no accumulation of α-ketoadipate, suggesting that cells grew using only the aspartate as a nitrogen source. Methionine and other group I amino acids also reversed the inhibition of strain X2180 by AA in MM (Fig. 2).

Ability of specific lysine auxotrophs to grow in AA medium. So far only *lys2* and *lys5* mutants are known to use AA as nitrogen source (3). Mutant strains which belong to the *lys14* locus (L. A. Urrestarazu, C. W. Borell, and J. K. Bhattacharjee, unpublished data) were also able to grow, but with a longer lag phase, in AA medium without amino acid supplementation, and they accumulated α-ketoadipate in the culture supernatant (Table 3). Mutant strains, including MW7-62 derived from a *lys2* mutant, lost their ability to grow in AA medium, and like the wild-type strain, they used AA as a nitrogen source when supplemented with methionine and accumulated α-ketoadipate.

AA reductase and GKAT activities of cells grown in AA medium. To elucidate the biochemical basis for the use of AA as a nitrogen source and to study the possible relationship between the lack of AA reductase and the ability to use AA as the principal nitrogen source, the levels of AA reductase and GKAT activities were determined in representative strains (Table 4). Wild-type strains and all mutant strains exhibited GKAT activity whether grown in MM or AA medium. The *lys2* mutant MW5-64 exhibited a fivefold higher GKAT activity when grown in AA medium as compared with MM. Wild-type cells grown in homoserine- or asparagine-supplemented AA medium had two- to threefold

TABLE 2. Effects of amino acid supplementation on growth of wild-type strain X2180 in AA medium and accumulation of α-ketoadipate in growing cultures

Nitrogen source[a]	Amino acid supplement[b]	OD_{550} at 72 h	α-Ketoadipate accumulation[c]
None	None	0.0	0.0
NH_4	None	2.0	0.0
AA	None	0.0	0.0
AA	Arginine, asparagine, glutamine, homoserine, methionine, or serine (group I)	0.7 to 2.0	0.4 to 4.1
AA	Glutamate, glycine, isoleucine, phenylalanine, proline, or threonine (group II)	0.1 to 0.2	0.0
AA	Alanine, cysteine, leucine, lysine, tryptophan, tyrosine, or valine (group III)	0.0	0.0
AA	Aspartate	2.0	0.0

[a] NN contained the nitrogen source at 1 mg/ml.
[b] Supplements were added at a concentration of 100 μg/ml.
[c] Accumulation is expressed as micromoles of α-ketoadipate formed per milliliter of culture supernatant after 72 h of growth.

TABLE 3. Growth and accumulation of α-ketoadipate by mutant strains in AA medium

Mutant strain	OD_{550} at 24 h when grown in:			α-Ketoadipate accumulation[a]
	MM	AA medium	AA medium + methionine (50 μg/ml)	
MW5-64 (*lys2*)[b]	2.0	0.4	2.0	2.5
7305d (*lys14*)	2.0	0.2	0.3	2.2
MW7-62 (derived from *lys2*)[b]	2.0	0.0	0.2	2.2

[a] Accumulation is expressed as micromoles of α-ketoadipate formed per milliliter of culture supernatant (AA medium for strains MW5-64 and 7305d and AA medium plus methionine for strain MW7-62) after 72 h of growth.

[b] Media for these strains contained lysine (20 μg/ml).

higher levels of AA reductase activity than MM-grown cells. Strain MW7-62 and the parent strain lacked AA reductase, whereas the *lys14* strain grown in MM or AA medium had significant levels of activity.

Alteration of cell morphology in AA-grown cells. The growth of wild-type cells in AA medium was arrested as unbudded or singly budded cells which were somewhat larger than cells (3 to 4 μm) grown in MM. The mutant MW5-64 grown in lysine-supplemented (300 μg/ml) AA medium exhibited increased cell diameters averaging 9 to 11 μm, a single large vacuole, multiple buds, pseudohyphae, and germ tubes (Fig. 3). Cells of *lys2* mutants grown in MM with lysine (300 μg/ml) showed slightly increased size but did not show altered morphology. Furthermore, the unusual structures were not due to basic amino acid toxicity since the leaky mutant 7305d grown in unsupplemented AA medium also exhibited multiple buds, pseudohyphae, and germ tubes. The addition of group I amino acid supplements to the AA medium resulted in an increased percentage of normal, budding cells in wild-type and mutant strains.

DISCUSSION

The results presented here demonstrate that AA, when present in high concentrations in MM with ammonia as a nitrogen source, produces a toxic effect on wild-type *S. cerevisiae*. The effect is evidenced by an increased lag phase and the impairment of normal cell division. Supplementation of MM with selected amino acids reverses the growth inhibition effect of AA. Supplementation with group I amino acids also enables wild-type strains and mutant MW7-62 to use AA as a nitrogen source and enhances the growth of *lys2* and *lys14* mutants in AA medium.

The presence of AA reductase activity in *lys14* mutants and the accumulation of α-aminoadipic-δ-semialdehyde by these strains suggest that a deficiency of AA reductase or a lack of accumulation of intermediates resulting from this deficiency is not responsible for the ability of *lys2* and *lys5* mutants to use AA as a nitrogen source, as has been proposed previously (3). The ability of wild-type cells to use AA as a nitrogen source in selected amino acid-supplemented medium while exhibiting higher levels of AA reductase activity also demonstrates the independence of the toxic effect of AA from the levels of AA reductase. Furthermore, the loss by the MW7-62 mutant of the ability to use AA, while it still retains the *lys2* lesion, suggests that the mutation occurs at a distinct site.

The accumulation of α-ketoadipate by mutant and wild-type strains grown in AA medium and the derepression of GKAT in *lys2* mutants grown in AA medium suggest that the pathway for the use of AA as a nitrogen source involves a transamination reaction catalyzed by GKAT (Fig. 1). The absence of activity in controls which lack α-ketoglutarate indicates that the enzyme is a transaminase and not a deaminase. The GKAT catalyzes a reversible reaction and functions in the biosynthesis of lysine (13) (Fig. 1). The derepression of this enzyme in *lys2* mutants grown in AA medium and a derepression of AA reductase in strain X2180 grown in amino acid-supplemented AA medium suggest that the ability to reduce the toxic levels of AA via these enzymes may explain the ability to grow in AA medium.

Nutrient deprivation, certain *cdc* mutations, and α factor exert control over cellular reproduction, resulting in uniform arrest of cells at

TABLE 4. Levels of GKAT and AA reductase in wild-type and mutant strains of *S. cerevisiae* grown with different nitrogen sources

Strain	Nitrogen source[a]	Sp act of:	
		GKAT (pmol/min per mg)	AA reductase (μmol/min per mg)
X2180-1B	NH_4	1.7	0.48
X2180-1B	AA[b]	1.1	0.92 (1.6)
MW5-64	NH_4[c]	1.5	0.00
MW5-64	AA[c]	7.5	
MW7-62	NH_4[c]	1.1	0.00
7305d	NH_4	0.5	0.48
7305d	AA	0.8	0.39

[a] NH_4 was at 5 mg/ml; AA was at 1 mg/ml.

[b] Medium contained homoserine (or asparagine) (50 μg/ml) as a supplement.

[c] Medium contained lysine (20 μg/ml) as a supplement.

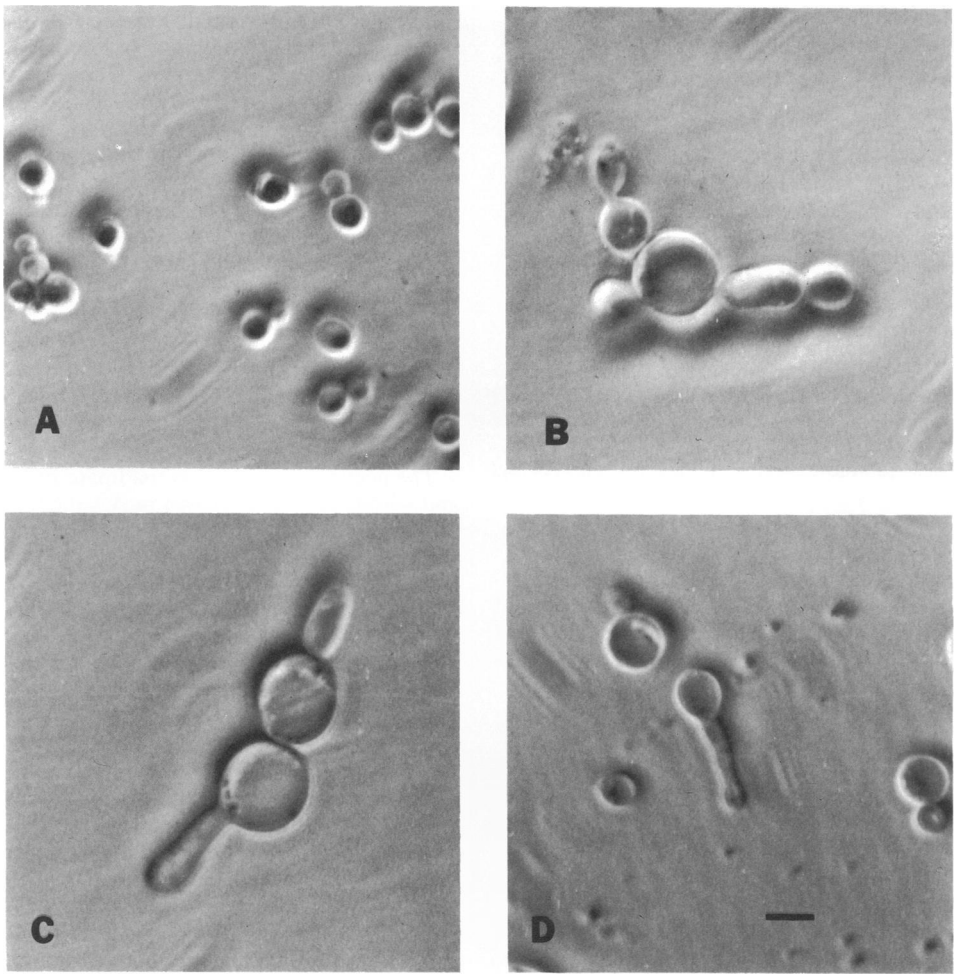

FIG. 3. Phase-contrast photomicrographs of MW5-64 cells after 48 h of growth in MM with lysine at 300 μg/ml (A) or in AA medium with lysine at 300 μg/ml (B, C, and D). Bar, 8 μm.

various diagnostic landmarks (10). Basic amino acid addition has a similar effect on cell morphology (18). The inhibitory effect of AA also appears to be the result of an impairment of cell division, as evidenced by the arrested growth and abnormal morphologies of wild-type and mutant strains grown in AA medium. The increased size of vacuoles in cells grown in AA medium suggests that the inhibition of growth and reversal of the toxic effect may be consequences of shifts in amino acid pools.

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