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Regulation of *argE-argH* Expression with Arginine Derivatives in *Escherichia coli*: Extreme Non-uniformity of Repression and Conditional Repressive Action

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In regulatory studies of the arginine biosynthetic system of *Escherichia coli*, α-*N*-acetyl-L-arginine (AcA) is a useful restrictive arginine source. In strain 39A-23R3 (*argA*$^-$), at 25 μg/ml, AcA gives suboptimal growth rates and is fully derepressive for acetylornithinase (specified by *argE*) and approximately 50% derepressive for argininosuccinase (specified by *argH*). At 10 μg/ml, the growth rate decreases, whereas the extent of derepression is unchanged; at 500 μg/ml, full repression results. In strain 3670 (*argB*$^-$*argG*$^-$), AcA (25 μg/ml) leads to partial derepression of acetylornithinase but full repression of argininosuccinase. Thus, the repression patterns for both strains, although not identical, are nonuniform. AcA utilization is antagonized by α-*N*-acetyl-L-ornithine (AcO). In strain 3670 (blocked before and after acetylornithinase), the growth rate on AcA (25 μg/ml) is lowered by AcO (500 μg/ml); acetylornithinase is completely derepressed, whereas argininosuccinase is fully repressed. This difference in regulatory behavior represents extreme nonuniform repression. Unexpectedly, the effect of AcO is attributable to the conversion of AcO to citrulline (Cit). In strain 3670, mixtures of AcA (25 μg/ml) and Cit (300 μg/ml) permit complete derepression of acetylornithinase; there is evidence that Cit enters the cell. In contrast, in the arginine-limited chemostat, Cit represses acetylornithinase. These opposite regulatory effects of Cit appear to stem from the difference in arginine restriction. AcA enters the cell via AcO permease and is deacylated by acetylornithinase (K_m, 5.0 mM). AcA competitively inhibits AcO cleavage (K_i, 2.4 mM), but Cit is not inhibitory. The antagonism of AcA utilization by AcO or Cit is thought to be exerted at the AcO permease.

In *argR*$^+$ strains of *Escherichia coli*, the formation of the enzymes of the arginine biosynthetic pathway (17, 32; Fig. 1 and Table 1) is derepressible by restricting the arginine supply (19; A. P. Bollon, T. Leisinger, and H. J. Vogel, Genetics **61**:s6, 1969). This paper describes a novel mode of arginine restriction, achieved in batch cultures with the aid of α-*N*-acetyl-L-arginine, either singly or in conjunction with the arginine precursers α-*N*-acetyl-L-ornithine or L-citrulline. In this manner, an extreme case of nonuniform repression and an unusual instance of contrasting regulatory effects of citrulline have come to light.

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MATERIALS AND METHODS

Organisms. The *E. coli* strains used are strain W (ATCC 9637) and the following mutants derived from it: strain 160-37 (*argE*$^-$) (17), strain 39A-23R3 (*argA*$^-$) (20), strain W2D (*argR*$^-$*pro*$^-$) (28), strain 3670 (*argB*$^-$*argG*$^-$*pro*$^-$), *strain 3616* (*argB*$^-$*argH*$^-$*pro*$^-$), strain 265 (*argC*$^-$*argH*$^-$*pro*$^-$), and strain 55P (*argB*$^-$*argD*$^-$*pro*$^-$, α-*N*-acetyl-L-ornithine permease$^-$) derived from strain 55 (22). An incidental *pro*$^-$ marker is present in some of these strains; growth media, therefore, are supplemented with L-proline (25 μg/ml) which does not effect repression-derepression in the arginine system. The nomenclature for arginine genes of *E. coli* has been described (14; also see Discussion).

Cultivation. The organisms are grown in stationary culture at 37 C in (minimal) glucose-salts medium (26), supplemented as indicated.

Chemicals and reagents. All chemicals used,

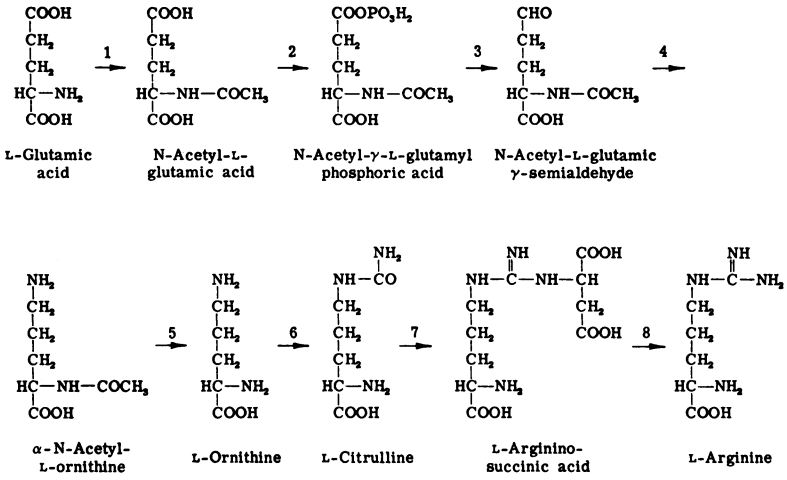

FIG. 1. *Path of arginine synthesis in E. coli. See Table 1 for the names of the enzymes catalyzing steps 1 through 8, respectively.*

TABLE 1. *Enzymes of arginine synthesis in Escherichia coli*

Step	Enzyme	
	Common name	Systematic name[a]
1	Acetylglutamate synthetase	Acetyl-CoA: L-glutamate *N*-acetyltransferase (EC 2.3.1.1)
2	Acetylglutamate kinase	ATP: *N*-acetyl-L-glutamate 5-phosphotransferase (EC 2.7.2.8)
3	Acetylglutamic γ-semialdehyde dehydrogenase	*N*-Acetyl-L-glutamate-5-semialdehyde: $NADP^+$ oxidoreductase (phosphorylating) (EC 1.2.1.38)
4	Acetylornithine δ-transaminase	N^2-Acetyl-L-ornithine: 2-oxoglutarate aminotransferase (EC 2.6.1.11)
5	Acetylornithinase	N^2-Acetyl-L-ornithine amidohydrolase (EC 3.5.1.16)
6	Ornithine transcarbamylase	Carbamoylphosphate: L-ornithine carbamoyltransferase (EC 2.1.3.3)
7	Argininosuccinate synthetase	L-Citrulline: L-aspartate ligase (AMP-forming) (EC 6.3.4.5)
8	Argininosuccinase	L-Argininosuccinate arginine-lyase (EC 4.3.2.1)

[a] Abbreviations: CoA, coenzyme A; ATP, adenosine triphosphate; $NADP^+$, nicotinamide-adenine dinucleotide phosphate; AMP, adenosine monophosphate.

including α-*N*-acetyl-L-arginine dihydrate (Cyclo Chemical Corp.), α-*N*-acetyl-L-ornithine (Calbiochem), and L-citrulline (Calbiochem), are commercially available. The following reagents are employed in assay procedures: *o*-aminobenzaldehyde (K & K Laboratories); argininosuccinate, glutathione, ninhydrin monohydrate, and pyridoxal 5-phosphate (Calbiochem); α-ketoglutaric acid and bovine albumin, fraction V (Schwarz/Mann); 2-methoxyethanol (Pierce Chemical Co.); and arginase (Worthington Biochemical Corp.).

Preparation of crude cell-free extracts. For the preparation of crude extracts, cells were cultivated in side-arm flasks containing 25 ml of minimal medium supplemented as indicated, allowed to attain a density corresponding to a reading of 35 in the Klett-Summerson colorimeter with no. 66 filter (3.5×10^8 cells/ml), collected at 3 C by centrifugation at 15,000 × *g* for 15 min, suspended in a mixture of 2 ml of 0.1 M potassium phosphate at pH 7.0 and 0.02 ml of 0.1 M glutathione, and disrupted, with chilling, in a 100-W MSE ultrasonic disintegrator.

Enzyme assays and protein determination. The assays and units for acetylornithine δ-transaminase (2, 27), acetylornithinase (26, 29), and argininosuccinase (3) have been described. Acetylarginine splitting activity is determined by a modification of the acetylornithinase assay (26), in which α-*N*-acetyl-L-arginine is substituted for α-*N*-acetyl-L-ornithine as substrate, and arginase (1.0 mg/ml) is incorporated in the enzymatic reaction mixtures to convert the initially produced L-arginine to L-ornithine.

Total protein is determined (11) with bovine albumin as protein standard.

RESULTS

Variable arginine restriction. In a search for restrictive arginine sources, we tested a series of compounds for their ability to support

the growth of arginine auxotrophs in glucose-salts medium (see Materials and Methods). The compounds (examined singly) included α-*N*-acetyl-L-arginine, L-arginylglycylglycine, α-*N*-benzoyl-L-arginine, α-*N*-carbobenzoxy-L-arginine, *N*-carbobenzoxyglycylglycyl-L-arginine, α-*N*-carbobenzoxy-*p*-tosyl-L-arginine, glycylglycyl-L-arginine acetate, α-*N*-*p*-tosyl-L-arginine, α-*N*-*p*-tosyl-L-arginine amide hydrochloride, and *p*-tosyl-L-arginine methyl ester hydrochloride. Of these, only α-*N*-acetyl-L-arginine, *N*-carbobenzoxyglycylglycyl-L-arginine, and glycylglycyl-L-arginine acetate gave a growth response at a concentration of 50 μg/ml. Further tests indicated that only α-*N*-acetyl-L-arginine is a potentially useful, limiting source of arginine. Figure 2 illustrates the growth of strain 39A-23R3 (*argA*⁻) on L-arginine hydrochloride or on different concentrations of α-*N*-acetyl-L-arginine dihydrate. At 30 μg/ml, acetylarginine gives a growth rate comparable to that obtained on an excess of arginine. At 5, 10, or 20 μg/ml (or at 25 μg/ml, not shown), the growth rates for acetylarginine are lowered. The utilization of acetylarginine thus qualitatively resembles that of α-*N*-acetyl-L-ornithine (16, 19, 21) but differs in that acetylarginine delivers arginine without dependence on several steps of the arginine biosynthetic path.

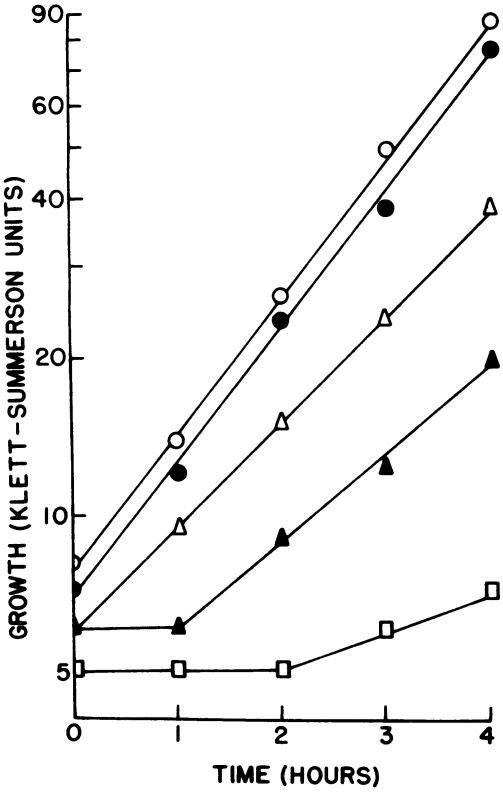

FIG. 2. *Growth of strain 39A-23R3 on arginine and various concentrations of acetylarginine. Arginine-grown inocula were cultivated in glucose-salts medium with supplements, as follows:* L-*arginine hydrochloride (30 μg/ml, ○); α-N-acetyl-*L-*arginine dihydrate (30 μg/ml, ●), (20 μg/ml, △), (10 μg/ml, ▲), (5 μg/ml, □).*

Since the suboptimal growth rates on suitable concentrations of acetylarginine probably reflect intracellular arginine restriction, experiments on the possible derepression of arginine enzymes were performed. Strain 39A-23R3 was cultivated in the presence of acetylarginine at various concentrations, and extracts were prepared and assayed for enzyme activities in the general manner described. For comparison, similar experiments were done with the *argR*⁻ strain W2D as well as with strain 39A-23R3 grown on an excess of arginine. Acetylarginine, at 500 μg/ml, is fully repressive for both acetylornithinase and argininosuccinase (Table 2). At the restrictive concentrations of 10 or 25 μg/ml, acetylarginine gives approximately full derepression for acetylornithinase, but only approximately 50% derepression for argininosuccinase. (Full derepression, in this context, is taken to correspond to the enzyme levels of the *argR*⁻ strain.) The different degrees of derepression of the two enzymes are noteworthy and indicate nonuniform repressor effectiveness.

Antagonism of acetylarginine utilization. Since acetylarginine permitted only partial derepression of argininosuccinase, we explored the possibility of achieving enhanced arginine

TABLE 2. *Relative specific activity of acetylornithinase and argininosuccinase in crude extracts of strain W2D (argR⁻) and strain 39A-23R3 (argA⁻argR⁺) grown on arginine or acetylarginine*

Enzyme	Specific activity[a]				
	Strain W2D	Strain 39A-23R3			
	Arg[b] (40)	Arg (40)	AcA[b] (10)	AcA (25)	AcA (500)
Acetylornithinase	100	21	99	95	20
Argininosuccinase	100	4	51	51	3

[a] Relative specific activities of 100 correspond to absolute specific activities (units per milligram of protein) of 75.0 and 16.8 for acetylornithinase and argininosuccinase, respectively.

[b] Abbreviations Arg, L-arginine hydrochloride; AcA, α-*N*-acetyl-L-arginine dihydrate. Concentrations (micrograms per milliliter) are given in parentheses under the respective supplements.

restriction by the use of an analogue of acetylarginine in conjunction with acetylarginine. Accordingly, growth tests were carried out, in the same general manner as before, with mixed supplements of acetylarginine (at 25 μg/ml) and an acylamino acid (at concentrations ranging from 5 to 625 μg/ml). The acylamino acids tested included *N*-acetyl-L-glutamate, *N*-acetyl-L-histidine, α-*N*-acetyl-L-lysine, *N*-acetyl-L-methionine, α-*N*-acetyl-L-ornithine, *N*-acetyl-DL-valine, α-*N*-benzoyl-L-arginine, *N*-benzoylglycine, and *N*-benzoyl-DL-methionine. Since some of these compounds are known to to be substrates of acetylornithinase, the experiments were carried out with the *argB*⁻-*argG*⁻ mutant strain 3670 which has blocks in the arginine path before and after acetylornithinase. Of the acylamino acids examined, only acetylornithine was found to antagonize the utilization of acetylarginine. The growth rate on acetylarginine, at 25 μg/ml, is increasingly lowered by acetylornithine at 500 and 1000 μg/ml. At 250 μg/ml, acetylornithine has no appreciable effect on the growth rate (Fig. 3).

Repression-derepression experiments were then performed with strain 3670 grown on acetylarginine or on mixed supplements of acetylarginine and acetylornithine (Table 3). At 25 μg/ml, acetylarginine leads to partial derepression of acetylornithine δ-transaminase and acetylornithinase, but to full repression of argininosuccinase. With the mixture of acetylarginine and acetylornithine used, the transaminase is largely derepressed and acetylornithinase is completely derepressed; argininosuccinase, however, is fully repressed within the accuracy of the assay. These results represent an

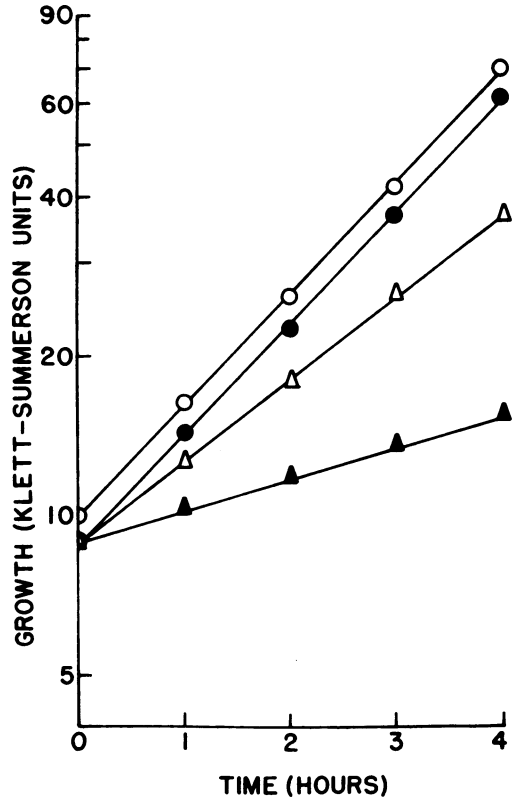

FIG. 3. *Growth of strain 3670 on acetylarginine and on acetylarginine-acetylornithine mixtures. Arginine-grown inocula were cultivated in glucose-salts medium with supplements, as follows: α-N-acetyl-L-arginine dihydrate (25 μg/ml, ○); mixtures of α-N-acetyl-L-arginine dihydrate (25 μg/ml, each) and α-N-acetyl-L-ornithine (250 μg/ml, ●), (500 μg/ml, △), (1000 μg/ml, ▲).*

TABLE 3. *Relative specific activity of acetylornithine δ-transaminase, acetylornithinase, and argininosuccinase in crude extracts of strain W2D (argR⁻), strain 3670 (argB⁻ argG⁻), and strain 3616 (argB⁻ argH⁻) grown on various supplements*

Enzyme	Specific activity[a]						
	Strain W2D	Strain 3670			Strain 3616		
	Arg[b] (40)	Arg (40)	AcA[b] (25)	AcA + AcO[b] (25) (500)	Arg (40)	AcA (25)	AcA + AcO (25 (500)
Acetylornithine δ-transaminase	100	3	26	76	4	41	40
Acetylornithinase	100	22	47	110	23	49	50
Argininosuccinase	100	3	2	4	—[c]	—[c]	—[c]

[a] Relative specific activities of 100 correspond to absolute specific activities (units per milligram of protein) of 15.2, 72.0, and 12.5 for acetylornithine δ-transaminase, acetylornithinase, and argininosuccinase, respectively.

[b] Abbreviations: Arg, L-arginine hydrochloride; AcA, α-*N*-acetyl-L-arginine dihydrate; AcO, α-*N*-acetyl-L-ornithine. Concentrations (micrograms per milliliter) are given in parentheses under the respective supplements. Acetylarginine at 500 μg/ml is fully repressive for strains 3670 and 3616.

[c] The *argH*⁻ strain has no detectable argininosuccinase activity.

extreme case of nonuniform repression. Analogous experiments with the *argB*⁻*argH*⁻ strain 3616 (Table 3) revealed a significant difference: although acetylarginine (25 μg/ml) gives partial derepression, the addition of acetylornithine (500 μg/ml) gives no appreciable further derepression. The *argC*⁻*argH*⁻ strain 265 behaves like strain 3616. It was thought that the difference between the *argG*⁻ strain 3670 on the one hand and the *argH*⁻ strains 3616 and 265 on the other may be attributable to citrulline, which strain 3670, in contrast to the other two strains, can accumulate in substantial amounts when acetylornithine is provided in the medium.

Contrasting regulatory effects of citrulline. In view of the possible involvement of citrulline, this amino acid was tested as a potential antagonist of acetylarginine utilization. In growth tests with strain 3670, it could indeed be demonstrated that, in the presence of acetylarginine (25 μg/ml), L-citrulline (at 300 μg/ml) gives approximately the same growth rate as does acetylornithine (at 500 μg/ml); also, L-citrulline (at 300 μg/ml), in conjunction with acetylarginine (25 μg/ml), is as derepressive for acetylornithinase as is acetylornithine at 500 μg/ml (Table 4). Moreover, when mixed with acetylarginine (25 μg/ml), L-citrulline (300 μg/ml) proved as derepressive for acetylornithine δ-transaminase and acetylornithinase in strains 3616 and 265 as was acetylornithine (500 μg/ml) in strain 3670.

The indicated derepressive effect of citrulline through an antagonism of arginine utilization is noteworthy since it was shown earlier that citrulline can be repressive (D. F. Bacon and H. J. Vogel, Genetics **54**:319, 1966; T. Leisinger and H. J. Vogel, Genetics **60**:197, 1968). Thus, superimposed on the antagonism of arginine utilization, there might have been a repression by citrulline which, however, was not observed. The contrasting regulatory actions of citrulline are summarized in Table 5. It is inferred that the effect of citrulline depends on the nature of the arginine restriction used. In the arginine-limited chemostat, citrulline is repressive and, in arginine limitation with acetylarginine-citrulline mixtures, citrulline is not repressive.

The failure of citrulline to repress does not seem to stem from an impairment in citrulline uptake, as indicated by experiments with acetylarginine-citrulline mixtures in mutants blocked in the arginine path before citrulline (e.g., ornithine transcarbamylaseless mutants). In such experiments, the arginine biosynthetic enzymes are fully repressed, presumably through formation of arginine from citrulline which, therefore, appears capable of entering the cell.

Mode of acetylarginine utilization. Two diagnostic arginine auxotrophs failed to grow on α-*N*-acetyl-L-arginine—one of them (strain 160-37) is blocked at acetylornithinase, and the other (strain 55P) is deficient in the uptake of acetylornithine. It was thus indicated that acetylarginine is deacylated by acetylornithinase after entering the cell via acetylornithine

TABLE 4. *Relative specific activity of acetylornithinase in crude extracts of strains W2D (argR⁻) and 3670 (argB⁻ argG⁻) grown on various supplements*

Strain	Supplement[a]	Concn of supplement (μg/ml)	Relative specific activity
W2D	Arg	40	100[b]
3670	Arg	40	20
3670	AcA	25	40
3670	AcA + AcO	25 + 500	103
3670	AcA + Cit	25 + 100	57
3670	AcA + Cit	25 + 300	103

[a] Abbreviations: Arg, L-arginine hydrochloride; AcA, α-*N*-acetyl-L-arginine dihydrate; AcO, α-*N*-acetyl-L-ornithine; Cit, L-citrulline.

[b] A relative specific activity of 100 corresponds to an absolute specific activity of 70.7 U/mg of protein.

TABLE 5. *Contrasting regulatory effects of citrulline*[a]

Restrictive arginine source	Addition to medium	Doubling time of cells (min)	Relative specific activity
AcA	None	90	55
AcA	Arg[b]	60	22
AcA	Cit	120	110
Arg[c]	None	120	100
Arg[c]	Cit	120	20

[a] Strain 3670 (*argB*⁻ *argG*⁻) was grown in minimal medium with a restrictive arginine source and additions to the medium, as indicated, and the specific activity of acetylornithinase was determined in crude extracts (see Materials and Methods). A relative specific activity of 100 corresponds to an absolute specific activity of 72.5 U/mg of protein. Abbreviations: AcA, α-*N*-acetyl-L-arginine dihydrate; Arg, L-arginine hydrochloride; Cit, L-citrulline. Acetylarginine and citrulline were used at 25 μg/ml and 300 μg/ml, respectively.

[b] As an addition to the medium, arginine hydrochloride was employed at 40 μg/ml.

[c] Arginine-limited chemostat (D. F. Bacon and H. J. Vogel, Genetics **54**:319, 1966; T. Leisinger and H. J. Vogel, Genetics **60**:197, 1968), operated at the doubling time shown.

permease. Citrulline appears to be taken up via a different permease since strain 55P readily grows on L-citrulline.

The cleavage of acetylarginine by acetylornithinase could be exhibited in vitro. The assay used is similar to that for acetylornithinase (26), with the modification that the enzymatic incubation mixture contains arginase (see Materials and Methods). Experiments on the velocity of deacylation as a function of acetylarginine concentration yielded linear double-reciprocal plots from which a K_m value of 5.0 mM was calculated. Acetylarginine could be shown to be a competitive inhibitor of the deacylation of acetylornithine. Experiments on acetylornithine cleavage without added acetylarginine and with acetylarginine at concentrations of 2.1 mM and 4.0 mM led to linear double-reciprocal plots intersecting at a single point of the ordinate. An average K_i value of 2.4 mM was computed (Fig. 4). In this inhibition study, the standard acetylornithinase assay was used to follow the deacylation of acetylornithine to ornithine. Acetylarginine makes some contribution to the color obtained, which was corrected for by suitable blanks. In separate experiments, it was shown that the concentration of acetylarginine does not decrease appreciably during the incubation of the reaction mixtures. Any trace amounts of arginine formed do not interfere with the assay. In similar experiments, L-citrulline was found not to inhibit acetylornithinase activity.

Preliminary studies on the uptake of ^{14}C-labeled α-*N*-acetyl-L-arginine by suitably blocked mutants (A. P. Bollon, W. L. McLellan, and H. J. Vogel, unpublished data) gave results consistent with the above-mentioned negative growth tests. Strain 160-37 readily takes up acetylarginine, whereas strain 55P does not. α-*N*-Acetyl-L-ornithine and L-citrulline are indicated to antagonize acetylarginine uptake, although the antagonism by acetylornithine appears to be relatively very weak.

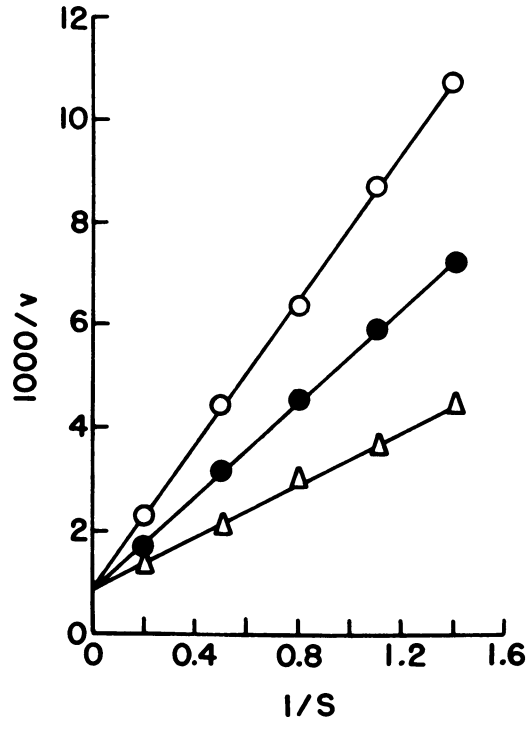

FIG. 4. *α-N-Acetyl-L-arginine as competitive inhibitor of the deacetylation of α-N-acetyl-L-ornithine by acetylornithinase. In the double-reciprocal plot, the reaction velocity (v) is expressed in Klett-Summerson readings per 15 min of incubation at 37 C; the acetylornithine concentration (S) is in micromoles per milliliter. Symbols: Δ, acetylornithine alone; ●, acetylornithine plus acetylarginine (2.1 mM); O, acetylornithine plus acetylarginine (4.0 mM).*

DISCUSSION

Extreme nonuniformity of repression. In strain W of *E. coli* (from which the strains used in this study are derived), eight structural genes, now termed *argA* through *argH*, are known, which respectively specify the first enzyme through the eighth enzyme of the arginine path; additionally, there is a regulatory gene, *argR*, which codes for the arginine repressor (23). In strain K-12 of *E. coli*, there is a ninth structural gene, *argI*, which, together with *argF*, determines the synthesis of species of ornithine transcarbamylase (8). Of the structural genes, four are clustered, and the remaining ones, as well as *argR*, are well dispersed in the genome, a gene distribution (5) that is of evolutionary interest (30). The sequence (7) of the clustered genes is *argECBH*. In *argR*$^+$ strains, the formation of the arginine biosynthetic enzymes is repressible by arginine; in *argR*$^-$ strains, the enzymes are formed at genetically derepressed rates. Repression by arginine has both transcriptional and translational components (12, 31; see these articles for further references).

The expression of the four clustered genes and its control present a number of intriguing features. The finding that the repression of acetylornithinase (specified by *argE*) and argininosuccinase (specified by *argH*) is not coordinate (3) and the isolation of a mutant with abnormal expression of *argCBH* (24) suggested the existence of at least two repressor recognition sites corresponding to the gene cluster; in

other words, *argE* and *argCBH* may constitute separate operons. Lack of coordination in repression by itself, of course, does not imply separate repressor recognition sites. For example, nonuniformity in the effectiveness of translational repression (R. H. Vogel and H. J. Vogel, Genetics **61**:s61, 1969; 31) may tend to bring about noncoordinate repression. Genetic studies (6, 9) have suggested that *argECBH* comprises two operons transcribed divergently, with a single operator site located between *argE* and *argC*, although the possibilities of two partially overlapping operators or two separate operators have not been excluded. Our present finding that acetylornithinase can be completely derepressed while argininosuccinase is completely repressed would, on the face of it, favor the presence of two operators. This all-or-none-type nonuniformity of repression may represent an extreme case of differential repressor effectiveness (10, 15). Also, we assume that extreme nonuniformity involves translational as well as transcriptional components of repression. Acetylornithine δ-transaminase (specified by *argD*, one of the dispersed genes) was examined for comparison and is seen to resemble acetylornithinase in repression behavior.

Conditional repressive action and channeling phenomena. Citrulline has now been shown to have contrasting regulatory effects, apparently as a function of the type of arginine restriction used. When arginine is limited in the chemostat, citrulline represses acetylornithinase; in contrast, when arginine is limited with the aid of acetylarginine-citrulline mixtures, citrulline does not repress acetylornithinase, although separate experiments indicate that citrulline enters the cell in the presence of acetylarginine, at the respective concentrations employed. The double mutant with which these experiments were carried out is blocked so as to be unable to convert citrulline to arginine; the sole source of arginine would be the acetylarginine provided. These results can be taken as suggesting that the conditional regulatory effects of citrulline reflect organizational phenomena associated with the channeling of citrulline and arginine depending on the circumstances under which these two amino acids are made available to the cell. However, possible alternative explanations have not been eliminated. Channeling of arginine-related compounds can be very marked in fungi (1, 4, 25), and appears to occur in *E. coli* (18).

There are at least two other aspects of the present study that are indicative of channeling phenomena. When strain 39A-23R3 is grown on acetylarginine at 10 or 25 μg/ml, closely similar repression patterns result, with approximately full derepression for acetylornithinase and approximately 50% derepression for argininosuccinase. The extent of derepression of the two enzymes thus remains constant. There is, however, a considerable difference in growth rate at the two concentrations of acetylarginine used. It appears, therefore, that there is a pronounced distinction between arginine as growth factor and arginine as small-molecule repressor. The implied compartmentalization seems interesting in that the compartments we would postulate are fed from the same source, namely endogenously liberated arginine resulting from the cleavage of acetylarginine. This finding is reminiscent of the reported difference between endogenous and exogenous arginine for the purposes of growth and repression (13). A channeling phenomenon may also underlie the behavior of strains 39A-23R3 and 3670, which, on acetylarginine at 25 μg/ml, give different repression patterns, although both patterns show nonuniform repression with respect to acetylornithinase and argininosuccinase.

An unexpected uptake antagonist. The enhanced derepression of acetylornithinase brought about by acetylornithine when admixed with acetylarginine in the *argB*$^-$*argG*$^-$ strain 3670, but not in the *argB*$^-$*argH*$^-$ strain 3616, implicated citrulline in the antagonism of acetylarginine utilization. Experiments with citrulline-acetylarginine mixtures proved exogenous citrulline to be a more potent antagonist of acetylarginine utilization than is exogenous acetylornithine, and to enhance the derepression of acetylornithinase even in strain 3616. Since the present study has provided evidence that acetylarginine enters the cell via acetylornithine permease and yields arginine upon deacetylation by acetylornithinase, the question arose whether the restrictive utilization of acetylarginine and its antagonism by acetylornithine or citrulline reflect events at the level of the deacetylating enzyme or of the permease (or of both).

The restrictive utilization of acetylarginine does not correlate with acetylornithinase concentration. During growth of arginine auxotrophs on acetylarginine, with or without added antagonist, the specific activity of acetylornithinase can rise several-fold as a result of derepression, and yet the growth rates remain constant. This behavior parallels precisely the case of acetylornithine (16, 19), whose utilization is limited by acetylornithine permease (21). Again, although both acetylornithine and acetyl-

arginine are substrates of acetylornithinase and acetylarginine is a competitive inhibitor of acetylornithine cleavage, citrulline does not inhibit the activity of acetylornithinase and, nevertheless, is the more potent of the two antagonists of acetylarginine utilization. Indeed, the results suggest that the antagonistic effect of acetylornithine essentially is exerted via citrulline. The antagonism thus does not seem to depend on the deacetylating enzyme but is indicated to occur at the level of acetylornithine permease. The preliminary uptake experiments with ^{14}C-labeled acetylarginine support this interpretation, particularly in view of the unnexpectedly small extent to which acetylornithine inhibits the uptake of acetylarginine. The inferred inhibition of acetylarginine uptake by citrulline appears to have a close analogy in the inhibition of acetylornithine uptake by arginine (16, 19), a mechanism that functions to counteract the entry of a biosynthetic precursor in the presence of the end product of a pathway. This mechanism, then, with acetylarginine in the place of acetylornithine and with citrulline in the place of arginine, could account for the antagonism observed.

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