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Overproduction of Lysine by Mutant Strains of *Escherichia coli* with Defective Lysine Transport Systems

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Mutants selected on the basis of their resistance to S-(β-*aminoethyl)cysteine and overproduction of lysine were found to be defective in the lysine transport system. The overproduction of lysine was not due to mutation affecting either of the two regulatory enzymes aspartokinase and dihydrodipicolinic acid synthetase. Uptake of labeled lysine by the lysine-specific transport system was reduced to a negligible level, while uptake by the lysine, ornithine, arginine system was also affected. A hypothesis regarding the nature of these mutations and their effects on the regulation of lysine biosynthesis is discussed.*

KEY WORDS: *Escherichia coli*; lysine; mutants; defective transport.

INTRODUCTION

Regulation of the branched biosynthetic pathway for lysine, methionine, threonine, and isoleucine in *Escherichia coli* K12 has been studied in considerable detail by Patte *et al.* (1967), Cohen (1969), and Yugari and Gilvarg (1962, 1965). These workers have shown that the flow of intermediates from aspartate is regulated at three major points: the first enzyme of the common pathway, aspartokinase, and the branch-point enzymes homoserine dehydrogenase (HS dehydrogenase) and dihydrodipicolinic acid synthetase (DHDP synthetase) (Fig. 1).

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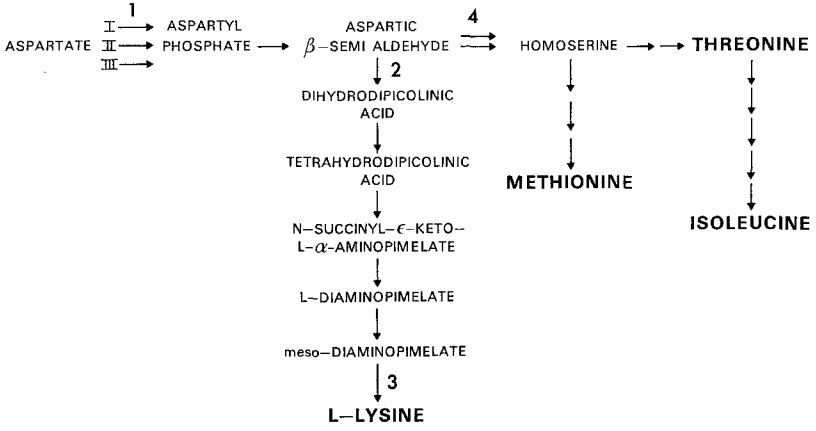

Fig. 1. Outline of the branched biosynthetic pathway to lysine, methionine, threonine, and isoleucine in *E. coli*. Enzymes depicted by arrows at the numbered positions are (1) aspartokinases I, II, and III, (2) dihydrodipicolinic acid synthetase, (3) diaminopimelate decarboxylase, and (4) homoserine dehydrogenases I and II. Enzymes 1, 2 and 3 are involved in the regulation of lysine biosynthesis.

Aspartokinase is comprised of three isoenzymes: aspartokinase I, which is repressed and feedback-inhibited by threonine; aspartokinase II, which is repressed by methionine; and aspartokinase III, which is repressed and feedback-inhibited by lysine (Patte *et al.*, 1967). As channeling does not occur, repression and feedback inhibition of aspartokinase III do not effectively prevent the flow of intermediates to lysine in the absence of threonine and methionine at concentrations required for repression. DHDP synthetase is the first enzyme of the lysine-specific pathway (Fig. 1); it is sensitive to feedback inhibition by lysine (Yugari and Gilvarg, 1962), but there is no information in the literature regarding its repressibility by lysine. The last enzyme of the lysine pathway, diaminopimelic acid decarboxylase (DAP decarboxylase), is repressed and inhibited by its product lysine (Patte *et al.*, 1962), so preventing the cells being deprived of diaminopimelic acid, which is a major component of the bacterial cell wall.

As a preliminary to the selection of deregulated lysine mutants in higher plants, the efficacy of a selection method based on resistance to lysine analogues was examined in *E. coli* by Brock *et al.* (1973). The authors selected a class of spontaneously occurring mutants which were resistant to the lysine analogue *S*-(β-aminoethyl)cysteine (AEC) incorporated into minimal medium at a concentration of 10 mM. These mutants were then further screened for strains which excreted sufficient lysine to sustain growth of a lysine-requiring auxotroph. Addition of methionine, threonine, and isoleucine (each 10 mM) to the medium was reported to restore the sensitivity of the mutants to AEC. It

was postulated that this first class of mutants would contain mutations affecting the lysine-specific pathway beyond the branch point, possibly at the first enzyme, DHDP synthetase, which is known to be a regulatory point.

A second class of mutants was obtained from the first class by selecting for spontaneous mutants which could grow in the presence of methionine, threonine, isoleucine, and AEC (each 10 mM). It was expected that the second class of mutants would be affected at aspartokinase III, rendering it insensitive to feedback inhibition and/or repression.

Representatives of both classes of mutants were kindly supplied by Dr. R. D. Brock to facilitate a study designed to determine which genes were affected by these mutations. When the results obtained with the two regulatory enzymes, aspartokinase III and DHDP synthetase, did not support the hypothesis, another site for the primary mutation was sought. The most probable site appears to be the lysine transport system, which is extensively modified in all strains examined.

MATERIALS AND METHODS

Chemicals

S-(β-Aminoethyl)cysteine was synthesized by Dr. J. Phillips, Plant Chemotherapy Section, Division of Plant Industry, CSIRO, and tested for purity by mass spectrography. Aspartic-β-semialdehyde was synthesized from DL-allylglycine by the method of Black and Wright (1955). High specific activity lysine-C^{14} was obtained from the Radiochemical Centre, Amersham. Other chemicals were obtained from Sigma and Calbiochem.

Media

The minimal medium used was medium 56 (Monod *et al.*, 1951). This consists of 13.6 g KH_2PO_4, 2 g $(NH_4)_2SO_4$, 0.2 g $MgSO_4.7H_2O$, 0.01 g $CaCl_2$, 0.5 mg $FeSO_4.7H_2O$ per liter adjusted to *p*H 7.4. After autoclaving, sterile glucose was added to a final concentration of 0.2% (w/v). Amino acids were added as sterile solutions after autoclaving to the final concentrations indicated elsewhere in the text.

Strains

Wild-type *E. coli* K12 strain 3000–3 was used to derive the two classes of mutants studied. Spontaneous mutants supplied by Dr. R. D. Brock were purified and maintained on minimal medium slopes and as silica gel dehydrated cultures.

Resistance to AEC and Overproduction of Lysine

Resistance to AEC was tested initially in plate tests and subsequently by measuring growth rates in liquid culture as increase in absorbance at 650 nm at 37 C following the addition of known concentrations of AEC.

Cross-feeding tests to detect the excretion of lysine into the medium were carried out in minimal medium plates to which a lysine-requiring strain of *E. coli* AT992 or 352 had been added before pouring. These plates were inoculated with the strain to be tested and examined for cross-feeding effects after 48 hr at 37 C.

Preparation of Extracts for Enzymatic Analysis

Cells grown overnight were diluted 1:10 with fresh medium and grown in shaken culture at 37 C to a density of 0.5 $A_{650\ mm}^{1cm}$. Cells were harvested by centrifugation at 8000 *g* for 20 min at 0 C, washed twice with ice-cold 0.02 M KH_2PO_4–NaOH buffer, *p*H 6.75, and then dispersed in 0.02 M KH_2PO_4–NaOH buffer, *p*H 6.75, with β-mercaptoethanol (0.03 M) prior to disruption by a French press at a pressure of 12,000 lb/inch^2. The opalescent product was then centrifuged at 20,000 *g* for 15 min at 0 C to remove cell debris. The supernatant was dialyzed 3 × 1 hr against 0.02 M KH_2PO_4–NaOH, *p*H 6.75, with β-mercaptoethanol (0.03 M) at 4 C. The dialyzed extract was used immediately in assay work. When necessary, the extract was stored overnight at 0 C but not frozen.

Protein estimation was by the method of Lowry *et al.* (1951).

Enzyme Assay

Aspartokinase activity was determined by the method of Truffa-Bachi and Cohen (1970) and recorded at the absorption maximum of $A_{505\ nm}$. All reagents were freshly prepared on the day of use. Linearity of chromophore production with concentration was confirmed using succinhydroxamate as standard.

DHDP synthetase activity was assayed by the method of Yugari and Gilvarg (1965), using imidazole buffer and following the rate of increase in absorbance at 270 nm. Purified aspartic β-semialdehyde was used in the reaction mixture at concentrations which were not rate limiting.

Uptake of L-Lysine-C^{14}

L-Lysine-C^{14} with a specific activity of 342 mCi/mmole was used to prepare a stock solution of 1 mM L-lysine containing 20 μCi/ml.

Uptake of L-lysine-C^{14} by cells in midexponential phase was estimated by

the method of Rosen (1971) using chloramphenicol to prevent incorporation of the lysine into protein. The final concentration of lysine-C^{14} in the assay tube was 10 μM (0.2 μCi/ml). Arginine and AEC when used as competitors were 5 mM. Label retained in the cells following washing procedures was estimated by liquid scintillation in a toluene–PPO–POPOP scintillation fluid.

Retention of Lysine-C^{14} Against a Concentration Gradient

Retention of lysine-C^{14} against a concentration gradient was estimated by the method of Kessel and Lubin (1962) outlined below. Cells were harvested at a density equivalent to 0.2 $A^{1\ cm}_{650\ nm}$ and washed in ice-cold minimal medium. Cold shock has been shown to cause loss of the cell's free amino acid pool into the supernatant (Britten and McClure, 1962). The cells were suspended in minimal medium containing lysine-C^{14} (4 μCi/ml, 200 μM) at 0 C for 3 hr. The long incubation period was designed to charge the cells with lysine-C^{14} by diffusion to minimize the difference in lysine accumulation between wild-type and mutant strains. Subsequent to 1:40 dilution into minimal medium at 37 C, changes in lysine concentration within the cell were followed over 30 min.

Amino Acid Analysis of Cell Amino Acid Pools and Cell-Free Supernatant

Cells (800 ml) were grown to midexponential phase (0.5 $A_{650\ nm}$) and harvested by centrifugation at 8000 *g* for 15 min at 25 C to prevent loss of the amino acid pool from the cell. A sample of the supernatant was passed through a Millipore membrane (0.45 μ) to remove remaining cells before analysis. The cell pellet was washed gently with warmed minimal medium (37 C) and then pelleted in tapered tubes to facilitate estimation of the volume of cells. The pellet was treated with cold trichloracetic acid (TCA) (5%, 10 ml) to extract the free amino acid pool; the TCA was then removed by extraction with diethyl ether, and the water layer was retained. All preparations were stored at -10 C until analyzed.

A density of 0.5 $A_{650\ nm}$ was estimated to represent 10^9 cells/ml, i.e., 8×10^{11} cells in the total sample, which gave a packed cell volume of 1.3 ml. In calculating the TCA-soluble amino acid pool of the cells, the value obtained per milliliter of extract was converted to the value for the original cell density allowing that 75% of the cell volume was freely permeable to the cell's amino acid pool (Roberts *et al.*, 1955).

RESULTS AND DISCUSSION

Growth Characteristics in the Presence of AEC

Growth characteristics on agar plates of class I and class II mutants were

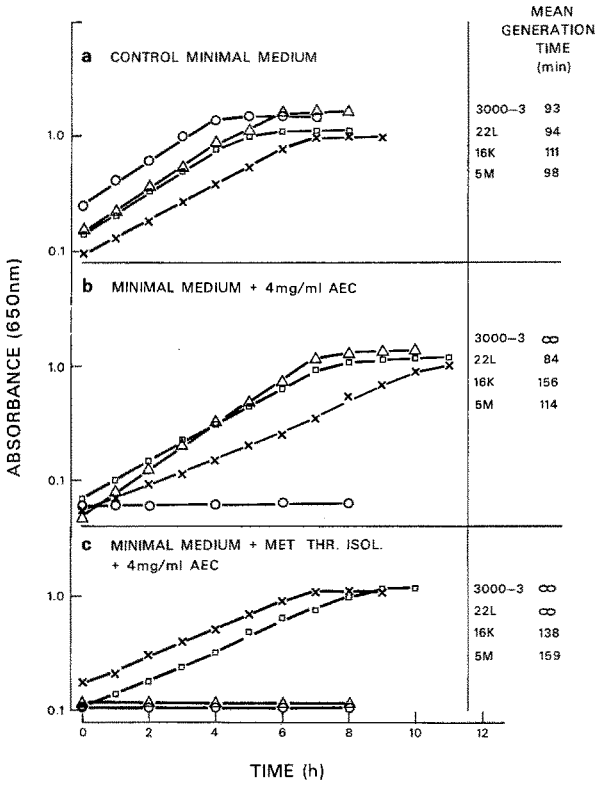

Fig. 2. Growth estimated as absorbance at 650 nm for wild-type and AEC-resistant mutants of *E. coli* in shaken liquid culture at 37 C. ○, Wild-type 3000–3; △, class I mutant 22L; ×, class II mutant 16K; □, class II mutant 5M. Zero hours corresponds to the time of adding AEC.

tested to confirm the results of Brock *et al.* (1973). Not all strains exhibited the responses reported as characteristic of the two classes of mutants (see Introduction). Strains giving the expected responses were chosen for quantitative study in liquid culture. These strains were grown in shaken culture at 37 C in the presence and absence of AEC (Fig. 2). No lag period is evident after the addition of AEC to early exponential phase cultures; the log $A_{650\ nm}$ is linear with time until stationary phase. The mean generation time (MGT) of the class I mutant was unaffected by the analogue in the absence of methionine, threonine, and isoleucine, but in the presence of these amino acids growth ceased. The MGT of the class II mutants was increased in the presence of AEC regardless of the presence or absence of methionine, threonine, and isoleucine.

Table I. Excretion of Lysine by Late Log Phase *E. coli* Cells Grown in Liquid Minimal Medium

	E. coli cells			
	Wild-type	Class I mutant	Class II mutants	
	3000–3	22L	16K	5M
Concentration of lysine excreted into the medium (μM)	0.1	65	130	170

Excretion of Lysine into the Medium

Class I and class II mutants were confirmed to be capable of cross-feeding lysine-requiring auxotrophs of *E. coli* (Brock *et al.*, 1973). It was determined (Table I) that the supernatant from late log phase cells of mutant strains contained about 650 (class I) and 1500 (class II) times the amount of lysine present in wild-type supernatant.

The results so far reported for the three mutants examined in detail are consistent with the model proposed by Brock *et al.* (1973) (see Introduction). Further classes of mutants may exist in that, although all mutants cross-fed lysine auxotrophs, not all behaved as class I or class II mutants in the plate tests. These strains have not been examined further.

Enzyme Assays: Repression and Inhibition of Regulatory Enzymes

Aspartokinase: Inhibition and Repression by Lysine

Aspartokinase activity was estimated in cells grown on minimal medium, lysine-free medium, and high-lysine medium (Table II). In wild-type cells grown on minimal medium, approximately 40% of the total aspartokinase activity was lysine sensitive (aspartokinase III); the remaining activity was threonine sensitive (aspartokinase I). When cells were grown in a medium supplemented with methionine (1 mM), threonine (1 mM), and isoleucine (0.5 mM) (lysine-free medium), derepression of aspartokinase III occurred concurrent with repression of aspartokinases I and II. In cells grown on medium containing lysine (1 mM), methionine (1 mM), threonine (1 mM), and isoleucine (0.5 mM) (high-lysine medium), all aspartokinase activity was strongly repressed.

Results obtained with the three mutant strains showed the same strong repression as wild type when grown on high-lysine medium but, in contrast, showed some degree of repression when grown on minimal medium (Table II). This phenomenon is probably due to the accumulation of lysine in the medium

Table II. Specific Activity and Lysine Inhibition of Aspartokinase in Extracts of Wild-Type and AEC-Resistant Mutants Grown Under Conditions of Repression and Derepression[a]

Strain	Minimal medium		Lysine-free medium		High-lysine medium[c]
	Specific activity	Percent lysine[b] inhibition	Specific activity	Percent lysine inhibition	Specific activity
Wild-type 3000–3	0.51	41	0.54	81	0.08
Class I mutant 22L	0.48	32	0.86	70	0.10
Class II mutants					
16K	0.33	34	0.74	100	0.10
5M	0.29	37	0.67	93	0.08

[a] Specific activity is expressed as $A_{505\ nm}$/mg protein/hr.
[b] Final concentration of L-lysine was 5 mM.
[c] Activity was too low to estimate lysine inhibition.

Table IIIA. Titration of Aspartokinase III Inhibition by L-Lysine[a]

Strain	Percent inhibition at different concentrations of L-lysine					
	13 mM	4.3 mM	1.4 mM	0.48 mM	0.16 mM	0.053 mM
Wild-type 3000–3	—[b]	81	75	44	8	0
Class I mutant 22L	71	78	70	60	17	—
Class II mutants						
16K	90	90	84	57	18	—
5M	88	85	73	56	15	—

[a] Cells were grown on lysine-free medium to maximize the proportion of aspartokinase III in the extract.
[b] Not tested.

Table IIIB. Titration of Aspartokinase III Inhibition by AEC[a]

Strain	Percentage inhibition at different concentrations of AEC				
	39 mM	13 mM	4.3 mM	1.4 mM	0.48 mM
Wild-type 3000–3	86	78	49	22	18
Class I mutant 22L	56	50	28	0	0
Class II mutants					
16K	88	73	28	3	3
5M	75	71	29	5	2

[a] Cells were grown on lysine-free medium to maximize the proportion of aspartokinase III in the extract.

during exponential phase (Tables I, VIA, and VIB). Repression below wild-type level on minimal medium was 6% (22L), 35% (16K), and 43% (5M). Some degree of derepression of aspartokinase III above wild-type level was obtained on lysine-free medium. Derepression was 59% (22L), 37% (16K), and 24% (5M).

The possibility that changes had occurred in the affinity of the enzyme for the feedback inhibitor, lysine, was examined over a concentration range from 53 μM to 13 μM (Table IIIA). Only minor variations were observed between wild type and the mutant strains. AEC was found to be less effective than lysine in inhibiting aspartokinase activity from both wild type and mutant strains (Table IIIB).

Dihydrodipicolinic Acid Synthetase

DHDP synthetase was examined in wild type and in the three mutant strains (Table IV). The specific activity of this enzyme from wild-type cells did not vary with changes in the lysine concentration of the medium, indicating that the enzyme is not subject to repression. The enzyme is sensitive to feedback inhibition by lysine (Tables IV and VA). In wild type, more than 80% of the activity can be inhibited by lysine (1.4 mM). AEC is not as effective an inhibitor, the same concentration inhibiting only 58–62% of activity (Table VB). All the mutant strains were slightly less sensitive to lysine.

Apart from the differences already noted, the inhibition and repression characteristics of the mutants closely resemble those of wild type. This was not expected on the basis of the hypothesis of Brock *et al.* (1973), and therefore

Table IV. Specific Activity and Lysine Inhibition of Dihydrodipicolinic Acid Synthetase in Extracts of Wild-Type and AEC-Resistant Mutants Grown Under Conditions of Repression and Derepression[a]

Strain	Minimal medium		Lysine-free medium (1 mM Met Thr, 0.5 mM Ile)		High-lysine medium (1 mM Met Thr Lys, 0.5 mM Ile)
	Specific activity	Percent by L-lysine[b]	Specific activity	Percent by L-lysine	Specific activity
Wild-type 3000–3	0.018	62	0.019	84	0.021
Class I mutant 22L	0.021	90	0.029	57	—[c]
Class II mutants					
16K	0.015	84	0.019	58	—
5M	0.019	70	0.021	60	0.015

[a] Specific activity is expressed as $\Delta A_{290\ nm}$/mg protein/min.
[b] Final concentration of L-lysine was 4.3 mM.
[c] Not tested.

Table VA. Titration of Inhibition by L-Lysine of Dihydrodipicolinic Acid Synthetase in *E. coli*

Strain	Percent inhibition at different concentrations of L-lysine					
	4.3 mM	1.4 mM	0.48 mM	0.16 mM	0.053 mM	0.018 mM
Wild-type 3000–3	84	80	68	38	30	0
Class I mutant 22L	—[a]	50	50	20	—	—
Class II mutants						
16K	—	58	56	25	—	—
5M	—	60	60	25	—	—

[a] Not tested.

Table VB. Titration of Inhibition by AEC of Dihydrodipicolinic Acid Synthetase in *E. coli*

Strain	Percent inhibition at different concentrations of AEC				
	13 mM	4.3 mM	1.4 mM	0.48 mM	0.16 mM
Wild type 3000–3	73	62	58	41	19
Class I mutant 22L	—[a]	57	56	—	—
Class II mutants					
16K	—	58	56	—	—
5M	—	60	58	—	—

[a] Not tested.

another explanation for AEC resistance and overproduction (excretion) of lysine must be found. A degree of difference exists between class I and class II mutants in that the latter show more repression of aspartokinase on minimal medium.

Uptake of Labeled Lysine

In *E. coli*, lysine is accumulated within the cell against a concentration gradient by means of two transport systems (Schwartz *et al.*, 1959; Rosen, 1971). One of these is a nonspecific system capable of transporting lysine (K_m 0.5 μM), ornithine (K_m 1.4 μM), and arginine (LAO transport system). The second system is lysine specific, with a lower affinity for lysine (K_m 10 μM). To examine the lysine-specific transport system, the nonspecific system was overloaded with a high concentration of arginine (5 mM).

The uptake of lysine-C^{14} (10 μM) was followed in *E. coli* wild-type 3000–3 in the presence and absence of arginine (Fig. 3a). In the presence of arginine, the initial rate of uptake of lysine was reduced to 35% of that

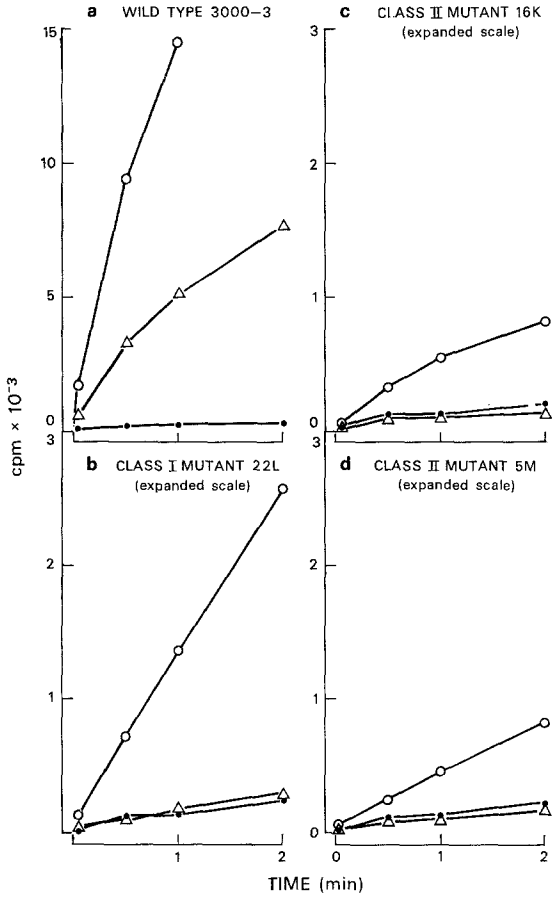

Fig. 3. Uptake of lysine-C^{14} (10 μM) into cells in the presence of chloramphenicol. ○, No competing amino acid present; ●, plus 5 mM arginine; △, plus 5 mM AEC. Note the expanded scale in (b), (c), and (d) relative to (a).

obtained in the absence of arginine. This residual amount represents the contribution of the lysine-specific transport system. In the presence of AEC (5 mM), lysine uptake was reduced to approximately 1.6% of the total uptake rate, indicating that AEC can compete with lysine for both transport systems.

When lysine uptake was examined in the two classes of mutants, there was a large reduction in the uptake rate of lysine-C^{14} both in the presence and in the absence of arginine. In class I mutants, the total uptake rate (i.e., in the absence of arginine) was reduced to 4–10% of wild-type rate in each of nine different strains treated. In class II mutants (Fig. 3c–d), the total uptake rate was reduced to 3–4% of wild-type rate. With added arginine to overload the

LAO transport system, uptake was reduced to the minimal rate observed in wild type in the presence of AEC. When AEC replaced arginine as a competitor, there was no further reduction in the uptake rate.

These data indicate that both classes of mutants are modified in the lysine-specific transport system, leaving only the nonspecific LAO system functioning. According to the data obtained from wild type, the LAO transport system is normally responsible for 60–65% of lysine uptake at this concentration and in the absence of arginine. In the mutants examined, although this is the only uptake system operating, the uptake rate is below that expected. It approximates 3–12% of the wild-type LAO uptake rate. This reduction in efficiency may be due to pleiotropic effects of the one mutation or to a mutation in the parent stock (E. A. Friederich, personal communication) of class I and class II mutants.

Retention of Lysine Within the Cell

Two different approaches were employed in an attempt to resolve whether the mutation(s) affected the active transport of lysine into the cell or its ability to

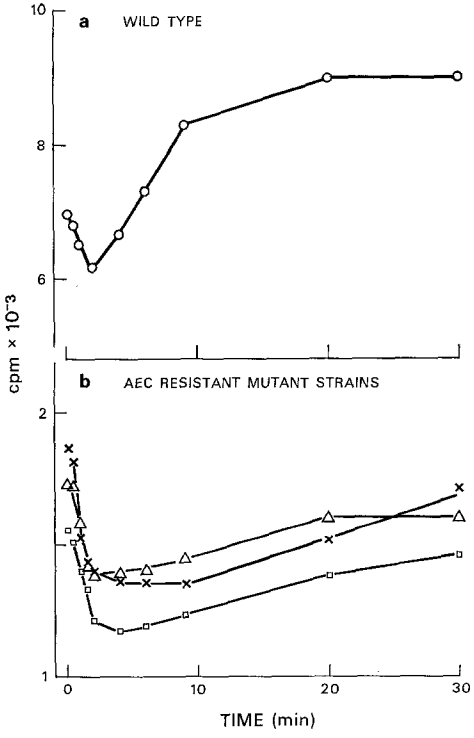

Fig. 4. Changes in lysine-C^{14} concentration within the cell following a 1:40 dilution of the extracellular lysine concentration at 37 C. ○, Wild-type 3000–3; △, class I mutant 22L; ×, class II mutant 16K; □, class II mutant 5M. Note the expanded scale in (b) compared to (a). Zero time represents the time of dilution.

retain lysine against a concentration gradient. First, the ability of the cell to retain lysine-C^{14} against a concentration gradient was examined (Fig. 4). Wild-type cells charged with lysine-C^{14} when diluted 1:40 into fresh medium lost lysine for approximately 1 min. After this period, the transport systems reaccumulated the lysine so effectively that the cells showed a net gain in lysine-C^{14} in less than 2 min. Neither class of mutants showed a net gain over the duration of the experiment. The difference in charging efficiency at 0 C between mutant and wild-type cells reflects either a degree of residual active uptake or a change in permeability by diffusion.

In a second series of experiments, the ability of exponentially growing

Table VIA. Concentrations of Certain Amino Acids Present in the Cell-Free Supernatant of *E. coli* Strains 3000–3, 22L, 16K, and 5M

	Amino acid concentration in supernatant (μM)			
	3000–3	22L	16K	5M
Lysine	0.6	31.3	41.8	70.6
Arginine	<0.5	<0.5	<0.5	<0.5
Aspartate	3.8	5.1	3.0	3.6
Glutamate	11.9	10.7	9.0	10.1
Glycine	5.0	5.0	6.1	6.1
Alanine	12.7	15.0	8.1	14.0

Table VIB. Concentration of Certain Amino Acids Present in the Cold TCA Soluble Amino Acids of *E. coli* Strains 3000–3, 22L, 16K, and 5M

	Concentration of cold TCA soluble amino acids present in 10^9 cells[a] (i.e., in 1 ml original cell suspension) (μM)			
	3000–3	22L	16K	5M
Lysine	8.55	18.1	39.3	45.4
Arginine	<0.25	0.9	1.8	2.4
Aspartate	2.9	5.4	4.3	4.0
Glutamate	74.3	51.4	86.8	65.4
Glycine	17.2	11.0	12.3	14.8
Alanine	37.9	26.5	35.4	40.5

[a] The free amino acid pool of the cell is estimated to occupy 75% of the total cell volume (Roberts *et al.*, 1955).

cells to retain endogenously synthesized lysine was examined by comparing the concentration of a number of different amino acids in cell extracts and supernatants (Tables VIA and VIB). In wild-type cells, the concentration of lysine in the cold TCA soluble amino acid pool of the cell exceeded that in the cell supernatant by fourteenfold. Of the other amino acids examined, with the exception of aspartate, the concentration of amino acid in the internal pool exceeded that in the supernatant, indicating that this is the normal balance between the internal and external environments of the cell.

In the mutant strains, normal balance was maintained for the amino acids other than lysine. The lysine concentration in the supernatant equaled or exceeded the concentration in the cell pool. As the cells were grown in minimal medium, this indicates that the mutant cells were not able to retain the lysine synthesized. The data show that the free lysine content of the mutant strains (Table VIB), exceeded that found in wild-type cells by two- to fivefold. Furthermore, the supernatant of the mutant strains exceeded by fifty- to a hundredfold the lysine concentration of the wild-type supernatant (Table VIA).

CONCLUSIONS

Examination of the mutant strains confirmed that they are resistant to AEC and excrete lysine into the medium. In the three mutants examined in detail, study of the regulatory enzymes aspartokinase III and DHDP synthetase showed that these enzymes are still sensitive to repression, derepression, and feedback inhibition. These experiments do not quantitate the degree of repression to the concentration of lysine.

The ability of these mutants to take up and retain lysine against a concentration gradient has been examined by three separate approaches: (1) by following the uptake of labeled lysine into the cell, (2) by following the loss of labeled lysine from the cell after dilution of the external lysine concentration, and (3) by analysis of the cold TCA soluble amino acid content of growing cells compared with their surrounding medium.

In the three mutant strains examined, there is an impaired ability to take up lysine from the medium at both 37 and at 4 C. It is clear that the supernatants from mutant strains contain about 100 times the lysine concentration found in the supernatant of wild-type cells. The change in the ratio of intracellular to extracellular lysine is not as great as this since the mutants also have a two- to fivefold increase in intracellular lysine. Even so, the intracellular lysine concentration (18–45 μM) is insufficient to effectively inhibit the enzymes concerned with lysine biosynthesis since these enzymes, aspartokinase III and DHDP synthetase, require approximately tenfold this concentration for 50% inhibition. Aspartokinase III is repressed to about 60% of the enzyme

level found in wild type under similar conditions. These data reflect the situation at the time of sampling, when lysine concentration would be maximal. Throughout the earlier stages of growth, repression and feedback inhibition of lysine synthesis and excretion would be even further reduced.

Britten and McClure (1962) have demonstrated the rapid exchange of molecules between the cell pool and the external environment. This is not dependent on the removal of free amino acid by incorporation into protein. Brown (1971) implicated the general aromatic permease in both the uptake and the loss of tyrosine from the cell. An essential feature of any hypothesis involving the same transport molecule in both the uptake and the efflux of an amino acid is the requirement that the uptake process be more efficient so that the cell accumulates the amino acid in a pool or pools. This may be achieved by a change in the affinity of the permease for the amino acid from a high affinity for a molecule in the external environment to a lower affinity for the same molecule in the internal environment (Brown, 1971).

This concept of differential affinity and continual movement of molecules in and out of the cell could explain the loss of lysine from a cell having a lysine permease with a lowered affinity for extracellular lysine. A lowered affinity of the permease for lysine would probably reduce the uptake of the lysine analogue AEC to levels which the cell could tolerate. Thus a single mutation could result in the pleiotropic effects of AEC resistance and the overproduction and excretion of lysine.

It has not been conclusively demonstrated that the strains examined carry a single mutation having pleiotropic effects. However, as nine class I and two class II mutants were examined, it is unlikely that the same double mutation would occur in all cases. The nature of the second mutation in the class II mutants conferring resistance to AEC in the presence of high concentrations of methionine, threonine, and isoleucine has not been investigated.

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REFERENCES

Black, S., and Wright, N. G. (1955). Aspartic β-semialdehyde dehydrogenase and aspartic β-semialdehyde. *J. Biol. Chem.* **213**:39.

Britten, R. J., and McClure, F. T. (1962). The amino acid pool in *Escherichia coli*. *Bacteriol. Rev.* **26**:292.

Brock, R. D., Friederich, E. A., and Langridge, J. (1973). The modification of amino acid composition of higher plants by mutation and selection. In *Nuclear Techniques for Seed Protein Improvement*, Proceedings of FAO/IAEA Meeting, Vienna, International Atomic Energy Agency, Vienna, pp. 329–338.

Brown, K. D. (1971). Maintenance and exchange of the aromatic amino acid pool in *Escherichia coli*. *J. Bacteriol.* **106**:70.

Cohen, G. N. (1969). The aspartokinases and homoserine dehydrogenases of *Escherichia coli*. *Current Topics Cell. Regul.* **1**:183.

Kessel, D., and Lubin, M. (1962). Transport of proline in *Escherichia coli*. *Biochim. Biophys. Acta* **57**:32.

Lowry, O. H., Rosebrough, N. J., Farr, A. R., and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265.

Monod, J., Cohen Bazire, J. P., and Cohn, M. (1951). Sur la biosynthèse de la β-galactosidase (lactase) chez *Escherichia coli*: La spécificité de l'induction. *Biochim. Biophys. Acta* **7**:585.

Patte, J. C., Loviny, T., and Cohen, G. N. (1962). Répression de la décarboxylase de l'acide méso-α, ε-diamino pimélique par la L-lysine, chez *Escherichia coli*. *Biochim. Biophys. Acta* **58**:359.

Patte, J. C., Le Bras, G., and Cohen, G. N. (1967). Regulation by methionine of the synthesis of a third aspartokinase and of a second homoserine dehydrogenase in *Escherichia coli* K12.

Roberts, R. B., Abelson, P. H., Cowrie, D. B., Boulton, E. T., and Britten, R. J. (1955). In *Studies in Biosynthesis in* Escherichia coli, Carnegie Institute of Washington Publication No. 607, Washington, D.C., pp. 58–59.

Rosen, B. P. (1971). Basic amino acid transport in *Escherichia coli*. *J. Biol. Chem.* **246**:3653.

Schwartz, J. H., Maas, W. K., and Simon, E. J. (1959). An impaired concentrating mechanism for amino acids in mutants of *Escherichia coli* resistant to L-canavanine and D-serine. *Biochim. Biophys. Acta* **32**:582.

Truffa-Bachi, P., and Cohen, G. N. (1970). Aspartokinase I and homoserine dehydrogenase I. In *Methods in Enzymology*, Vol. 17A, Academic Press, New York, pp. 694–699.

Yugari, Y., and Gilvarg, C. (1962). Co-ordinated end product inhibition in lysine synthesis in *Escherichia coli*. *Biochim. Biophys Acta* **62**:612.

Yugari, Y., and Gilvarg, C. (1965). The condensation step in diaminopimelate synthesis. *J. Biol. Chem.* **240**:4710.