Effect of α-Acetylation on Utilization of Lysine Oligopeptides in *Escherichia coli**

Richard Losick and Charles Gilvarg

From the Frick Chemical Laboratory, Princeton University, Princeton, New Jersey 08540

SUMMARY

A method has been developed for separating α-acetyllysine oligopeptides from ε-acetylated contaminants by chromatography on carboxymethyl cellulose columns with buffered eluents.

An *Escherichia coli* lysine auxotroph will utilize α-acetyltrilysine as a source of lysine, but will not utilize α-acetyldi- or α-acetyltetralysine. It was concluded that the nutritional ineffectiveness of α-acetylated di- and tetralysine is due to an inability to penetrate the cell envelope.

Growth on α-acetyltrilysine was a linear function of time, and growth rate was dependent on the concentration of the acetylated tripeptide. It was therefore concluded that α-acetyltrilysine limits the availability of lysine to the *E. coli* auxotroph. This property was used to study derepression in the lysine pathway. The pyruvate-aspartic semialdehyde-condensing enzyme and the diaminopimelate epimerase concentrations in cells grown on α-acetyltrilysine increased to a considerably lesser extent than dihydrodipicolinic acid reductase, indicating a lack of coordinate repression in the lysine pathway.

During the course of an investigation of the ability of lysine oligopeptides to support growth of an *Escherichia coli* lysine auxotroph, it was found that di-, tri-, and tetralysine were nutritionally effective (1). However, pentalysine and higher homologues were unable to support growth. The acetylation of an ε-amino group in the peptides that could be utilized, although it eliminated the growth activity of the lysine residue to which the acetyl group was attached, did not affect the nutritional activity of the associated lysine residues in the peptide. On the other hand, acetylation of the α-amino group markedly reduced the nutritional effectiveness of the oligopeptides. While the inertness of α-acetyldilysine and α-acetyltetralysine seemed to be complete, some growth was obtained on α-acetyltrilysine. The synthesis of the α-acetylated peptides had been carried out at a pH that would lead to preferential acetylation in the α position, but enough ε-acetylated peptide might have been present to give

a weak growth response. The preliminary observation that only the α-acetylated tripeptide, which is intermediate in both size and net charge to the di and tetra homologues, was active appeared sufficiently striking to merit confirmation. Accordingly, a procedure was devised that was capable of resolving α- and ε-acetylated lysine oligopeptides. The purified α-acetylated lysine oligopeptides maintained the discontinuity in response, only the α-acetyltrilysine being active. Moreover, with the purified material, it was apparent that the growth response was unusual in that the bacterial mass increased linearly with time.

Ames and Garry (2) had shown that the slow growth of histidine auxotrophs on α-formylhistidine resulted in a derepression of the enzymes involved in histidine biosynthesis. The poor growth on the α-acetyltrilysine suggested that this compound might similarly find utility as a means of selectively derepressing the enzymes involved in lysine biosynthesis. This was shown to be so for the dihydrodipicolinic acid reductase, one of the enzymes in the central part of the pathway.

EXPERIMENTAL PROCEDURE

Materials

Lysine Oligopeptides—The lysine oligopeptides were prepared by trypsin hydrolysis of poly-L-lysine hydrobromide (Pilot Chemicals) according to a modification of the procedure of Waley and Watson (3). Polylysine-HBr (21 mg per ml) was incubated with trypsin (2.1×10^{-3} mg of nitrogen per ml) in 0.083 M KCl at room temperature. The pH was maintained at 7.6 by the periodic addition of 0.3 M NaOH. The reaction was essentially complete in 3 hours. At these low concentrations of trypsin, the hydrolysate consists of a mixture of dilysine, trilysine, and some tetralysine. Higher concentrations of the enzyme are to be avoided because trypsin can catalyze a transpeptidation reaction which converts trilysine and dilysine to free lysine (4).

The oligopeptides were freed of trypsin by passage through a Sephadex G-25 column. In order to separate the lysine peptides from each other, the enzyme-free hydrolysate was placed on a carboxymethyl cellulose column (2.2×40 cm). Up to 100 mg of material can be resolved by such a column. Selective elution based on net charge was achieved with a gradient of logarithmically increasing salt concentration according to the procedure of Stewart and Stahlmann (5). The composition of the hydrolysate and the separation obtained are shown in Fig. 1. The first band of ultraviolet light-absorbing material in the effluent represents material not adsorbed by the column and is primarily bro-

mide ion. Sodium chloride was removed from the peptide fractions by placing them on a Dowex 50 (H$^+$) column (0.8 × 3 cm). The column was of minimal size capable of retaining all the peptide (the more highly charged peptide adheres preferentially to Dowex 50) and still allowing most of the salt to pass through. The peptides were then eluted with 1 M NH$_3$ and taken to dryness under reduced pressure at 50°. They were then dissolved in water, and the pH of the solution was adjusted to 7 with 0.1 M HCl.

ε-N-Monoacetylated Lysine Oligopeptides—These peptides were prepared from di-, tri-, and tetralysine by reaction with acetic anhydride at pH 10 to 11 (1).

α-N-Acetylated Lysine Oligopeptides—Di-, tri-, and tetralysine were allowed to react with acetic anhydride at pH 7 (1). Because of the large difference in dissociation constants between the α-amino groups (pK 7.1 to 7.6) and the ε-amino groups (pK 10.2 to 10.8) at neutrality, the ε-amino groups will be almost fully protonated while the α-amino groups will be partially protonated. Therefore, acetylation will proceed preferentially on the α-amino groups. There is, however, the possibility of some ε-acetylation particularly because of the greater number and greater nucleophilicity of the ε-amino groups.

The carboxymethyl cellulose column, which resolves compounds on the basis of net charge, can be used to separate the monoacetylated peptides from diacetylated and unacetylated peptides in the reaction mixture. However, at pH 6.5 ε-monoacetyl peptides have about the same net charge as the corresponding α-acetyl peptides and are not separated by a carboxymethyl cellulose column under the usual conditions. Therefore, in order to effect their separation, advantage was again taken of the large difference in dissociation constants of the α- and ε-amino groups. By buffering the eluent in the carboxymethyl cellulose column to a pH intermediate to the pK values of the α- and ε-amino groups, the free α-amino group of a ε-monoacetylated peptide becomes unprotonated and lowers the net charge on the molecule. The α-acetyl peptides, not having free α-amino groups, are relatively unaffected by the higher pH. Therefore, buffering the eluent in the carboxymethyl cellulose column effects a difference in net charge between α-acetyl peptides and ε-monoacetylated peptides and permits their separation on a carboxymethyl cellulose column.

For purification of monoacetylated di-, tri-, and tetralysine, a carboxymethyl cellulose column (0.9 × 20 cm) was used. The elution gradient was established with a mixing chamber containing 1 liter of 0.01 M glycine buffer into which a solution of 0.82 M NaCl and 0.01 M glycine buffer was allowed to flow. The column was previously equilibrated with 0.01 M glycine buffer. The peptides were also adjusted to the appropriate pH before being placed on the column.

Fig. 2 illustrates the results obtained in the case of α-acetyltetralysine. An unbuffered NaCl solution (pH 6.5) elutes both α-acetyltetralysine and ε-monoacetyltetralysine in the same position. It was found, however, that buffering with glycine at pH 9.3 would cause ε-monoacetyltetralysine to elute at an earlier position (Fig. 2a). Fig. 2b shows that an artificial mixture of α-acetyltetralysine and ε-monoacetyltetralysine was resolved into two distinct peaks at pH 9.3. Finally, Fig. 2c shows that unfractionated acetyltetralysine is resolved into two distinct peaks when eluted at pH 9.3. The first peak corresponds in position to ε-monoacetyltetralysine and supports rapid growth, while the second, which corresponds to α-acetyltetralysine, does not sup-
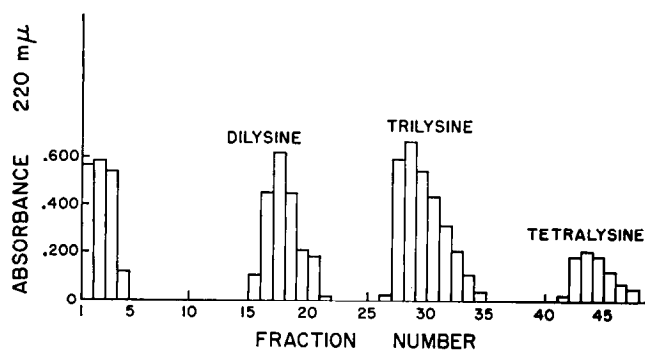

FIG. 1. Chromatographic separation of lysine oligopeptides obtained from tryptic hydrolysis of polylysine. See "Materials" for details. Fraction volume was 30 ml; a carboxymethyl cellulose column, Na$^+$ form (2.2 × 40 cm), was used; 2 liters of 0.82 M NaCl were in the reservoir, and 4 liters of H$_2$O were in the mixing chamber.
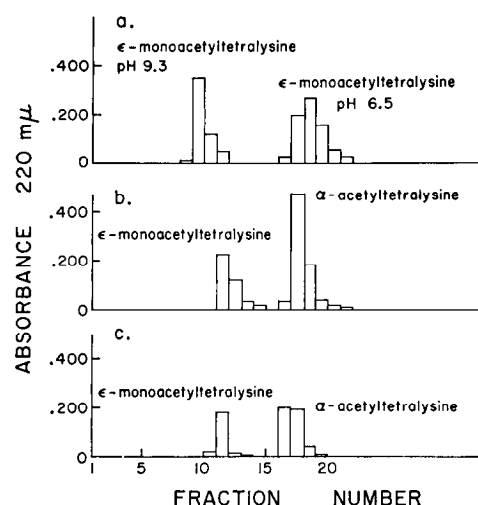

FIG. 2. *a*, chromatography of ε-monoacetyltetralysine at pH 6.5 and pH 9.3. The first peak represents the results of elution at pH 9.3; the second peak is for results obtained at pH 6.5. *b*, chromatography of an artificial mixture of α-acetyltetralysine and ε-monoacetyltetralysine at pH 9.3. *c*, chromatography at pH 9.3 of synthetic unfractionated monoacetyltetralysine not previously purified by chromatography at pH 9.3. The fraction volume was 11 ml. A carboxymethyl cellulose column, Na$^+$ form (0.9 × 20 cm), was used; 500 ml of 0.82 M NaCl were in the reservoir, and 1 liter of H$_2$O was in the mixing chamber. For pH 9.3 chromatograms, 0.01 M glycine buffer was used in both mixing chamber and reservoir. Ultraviolet absorption due to glycine was subtracted from the curves.

port growth. As a check, the material corresponding to the first peaks in Fig. 2 (*a*, *b*, and *c*) was recovered and subjected to chromatography once again with unbuffered eluent. As expected, they were eluted in the same position as α-acetyltetralysine. Therefore, buffering permits separation of α-acetyltetralysine from ε-monoacetyltetralysine.

This same procedure was also used to purify α-acetyltrilysine and α-acetyldilysine. The corresponding ε-monoacetylated peptides were again used to confirm the observation that the buffered system would separate a mixture of the ε-monoacetylated and α-acetylated peptides. In the case of α-acetylated dilysine, optimal separation was achieved at pH 8.8.

As in the case of the unacetylated peptides, a Dowex 50 column (H$^+$ form) was used to free the peptides of the salt and glycine.

TABLE I

Comparison of peptide assays

Peptide	Spectrophoto-metric assay	Reaction with ninhydrin	Titration
	mg/ml	*mg/ml*	*mg/ml*
Trilysine.............	9.90		9.86
α-Acetyltrilysine.....	4.12	4.25	4.10

Characterization of Peptides—The oligopeptides and acetylated derivatives were identified by the salt concentration at which they came off the carboxymethyl cellulose column. To confirm these identifications independently, titration curves were determined and paper chromatography with the Waley-Watson solvent (3) was employed. The Radiometer titrator, model TTT1, and recorder, model SBR-2 (Radiometer, Denmark), were used for performing the titrations. Samples were brought to pH 2 with 2 N HCl and titrated with 0.1 N carbonate-free NaOH. Nitrogen gas was passed over the titration mixture to provide an atmosphere free from CO_2. A titration curve was also run with water as a control.

The titration curve for trilysine, for example, showed titrating groups with dissociation constants at pK 10.2 and 7.1. These titrating groups were concluded to be ε- and α-amino groups, respectively. At pK 10.2, 3 times as many equivalents were required for the titrating groups as at pK 7.1. This is in agreement with the fact that trilysine has three ε-amino groups and one α-amino group. The titration curves for α-acetylated di-, tri-, and tetralysine showed no measurable titrating groups near neutrality. Therefore, the α-amino groups were fully acetylated.

Methods

Peptide Assays—The concentrations of oligopeptides and acetylated derivatives were determined spectrophotometrically, by reaction with ninhydrin, and by titration. The absorbance at 220 mμ of a solution of trilysine of known concentration was measured on a Beckman model DU spectrophotometer. From this value the absorbance for an equimolar amount of the acetylated and unacetylated peptides was empirically predicted on the basis of the number of amide bonds. Tetralysine (3 peptide bonds) should have 3/2 the absorbance and dilysine (1 peptide bond) 1/2 the absorbance of an equimolar solution of trilysine (2 peptide bonds). Assuming that the acetyl bond has the same absorbance as a peptide bond, α-acetyltrilysine should have 3/2 the absorbance of an equimolar solution of trilysine. Trilysine was found to have a molar extinction coefficient at 220 mμ of 1.2×10^3 M^{-1} cm^{-1}, while the corresponding value for α-acetyltrilysine was 1.8×10^3 M^{-1} cm^{-1}. Peptide absorption was much greater at lower wave lengths, but these were avoided in order to reduce the possibility of spectrophotometric artifact. The spectrophotometric assay was used to measure peptide concentrations most frequently because of its convenience.

Reaction with ninhydrin and titration were used to verify the spectrophotometric assay independently. The molar color yield in the ninhydrin reaction of Moore and Stein (6) depends in part on the number of free amino groups. However, the color yield for α- and ε-amino groups is not the same (3). Hence this assay was used only for α-acetyl peptides for which polylysine can be used as a standard, since in polylysine the contribution of α-

amino groups is negligible compared to the number of ε-amino groups.

The titration curves obtained in the chemical characterization of the peptides were also used to determine the quantity of peptide.

The agreement among the three assays is illustrated in Table I.

Test System—Cultures were inoculated with M-26-26, a lysine auxotroph of *E. coli*. First isolated in the laboratory of B. D. Davis, M-26-26 lacks the decarboxylase which converts meso-diaminopimelic acid to L-lysine (7, 8). The bacterial cultures were grown in the medium of Davis and Mingioli (9).

Peptides and media were sterilized by autoclaving at 15 pounds of pressure for 15 min directly in Klett tubes. Sterile glucose solution (25%, 0.1 ml) was added aseptically after cooling. Growth was measured in a Klett-Summerson photoelectric colorimeter with a 660-mμ filter. Control tubes were prepared without added amino acid or peptide.

For the derepression experiments, cultures were grown in half-filled 1-liter flasks fitted with cotton plugs. Growth was determined by aseptically removing 1.0-ml samples and measuring the absorbance in a Beckman DU spectrophotometer at 660 mμ. Optical density units were converted to Klett units with the following empirical relationship.

$$O.D._{660} \times 280 = \text{Klett units}$$

A 500-ml culture grown to a density of 50 Klett units on 30 μg of lysine per ml was used for inoculation. The inoculum consisted of 0.1 ml for the Klett tube cultures and 10.0 ml for the 500-ml cultures.

Klett tube cultures were grown without agitation. Derepression cultures were grown aerobically on a New Brunswick shaker.

Diaminopimelic Acid Assay—This material was determined colorimetrically by its reaction with acidic ninhydrin (10).

Enzyme Assays—Extracts were prepared by rapidly cooling the culture to 5°. The cells were collected by centrifugation. The bacterial pellet was then resuspended with 0.02 M phosphate buffer, pH 7, and recentrifuged. (For control cultures, which were grown on lysine, the phosphate buffer used in this first resuspension also contained 100 μg per ml of lysine to maintain the same high concentration as the growth media.) The supernatant solution was discarded, and the pellet was resuspended in 0.1 ml of phosphate buffer for each 0.1 g of bacteria and then transferred to a Beckmann cellulose nitrate tube ($\frac{5}{8} \times 2\frac{1}{2}$ inches). The tube was floated in 75 ml of water in the "sonication" chamber of a Raytheon 10-kc sonic oscillator and treated for 13 min. Cell debris was removed by centrifugation at $18,000 \times g$ for 20 min.

The protein concentration of the crude extract was determined by the spectrophotometric method of Warburg and Christian (11).

RESULTS

Growth on Acetylated and Unacetylated Oligopeptides—Growth of M-26-26 on lysine and trilysine was exponential with time until the substrate was almost exhausted. The growth rate was the same for both compounds, and under the conditions employed, the generation time was 70 min (see Fig. 3). It can also be seen that although the yield of bacteria depended on the concentration of trilysine, the growth rate did not. When, however, the α-acetylated lysine oligopeptides were used as substrates,
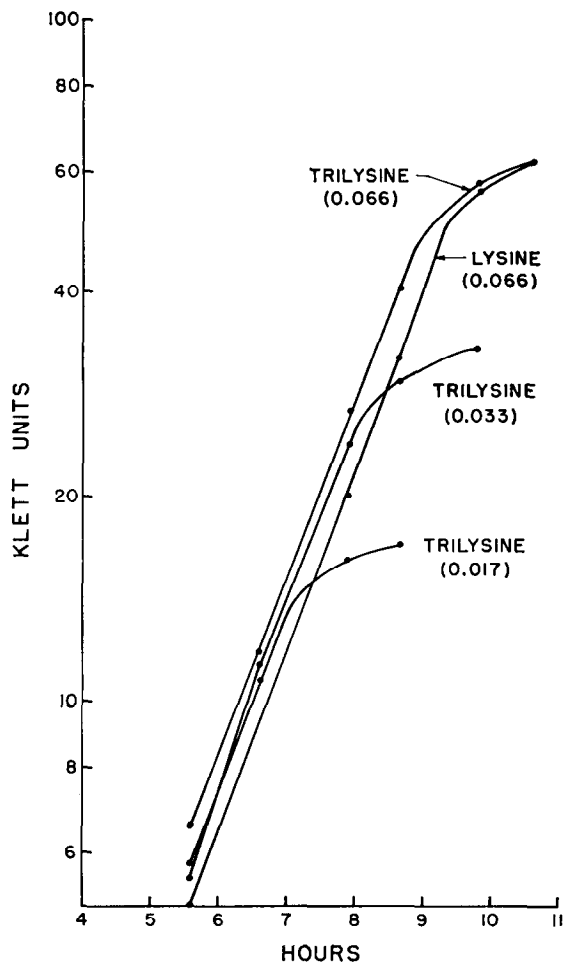
FIG. 3. Growth response of M-26-26 to lysine and trilysine. *Numbers* refer to concentration of substrate in microequivalents of lysine residues per ml. Incubation was performed at 33°.

FIG. 5. Growth response of M-26-26 to different concentrations of α-acetyltrilysine. *Numbers* refer to concentration in microequivalents of lysine residues per ml. Incubation was performed at 36°.

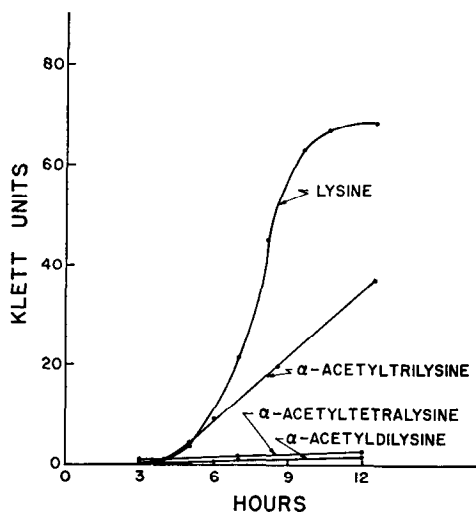FIG. 6. Dependence of growth rate on concentration of α-acetyltrilysine. Data were obtained from Fig. 5.

FIG. 4. Growth response of M-26-26 to α-acetyldi-, -tri-, and -tetralysine. The level of α-acetylated peptide in the growth tubes was set at 0.300 μeq of lysine residues per ml. The tube containing lysine had 0.066 μeq of lysine per ml. Incubation was performed at 36°.
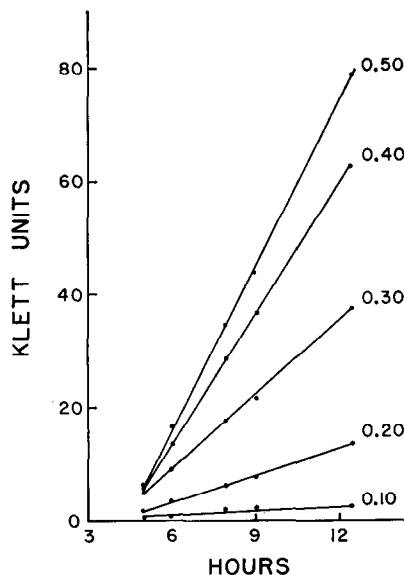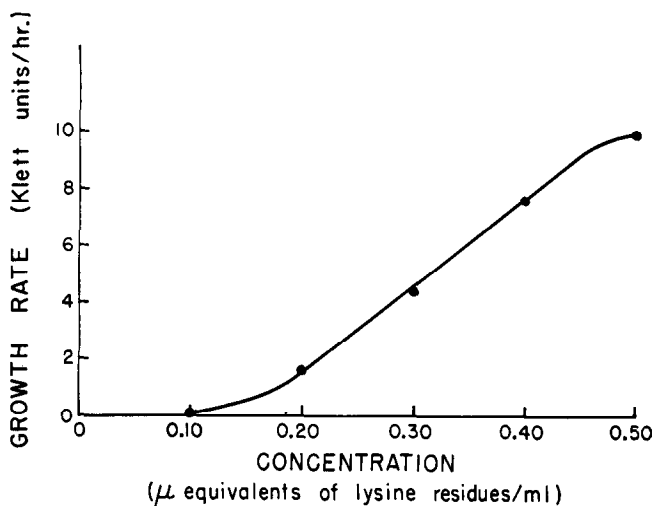
growth was either absent or severely limited: α-acetyldilysine and α-acetyltetralysine were completely inactive, but α-acetyltrilysine did support very slow growth (Fig. 4). Moreover, the slow growth on α-acetyltrilysine was a linear function of time rather than logarithmic, as is usually the case with bacterial cultures (Fig. 4).

In contrast to the exponential growth on the unacetylated peptides, which was independent of concentration, the linear growth rate on α-acetyltrilysine was dependent on the concentration of the acetylated tripeptide. Fig. 5 shows that as the concentration of α-acetyltrilysine was increased, the rate of growth increased with retention of its linearity. The dependence of this linear growth rate on concentration is shown in Fig. 6. The curve does not extrapolate back to zero concentration of α-acetyltrilysine. Therefore, a minimum concentration of about 0.10 μeq of lysine residues per ml is required for growth activity.
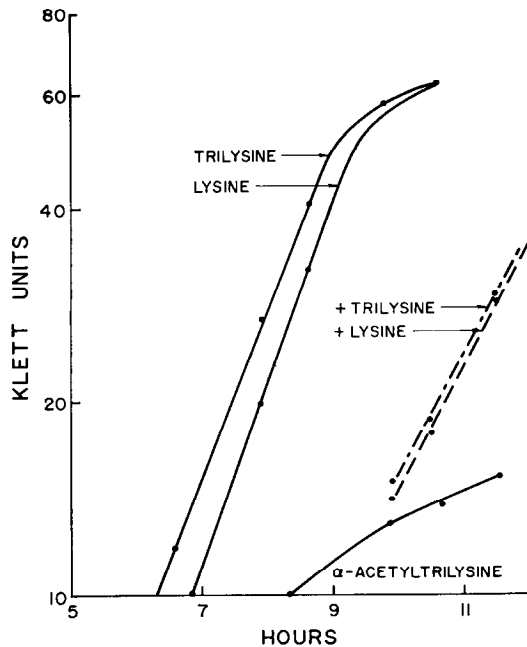
FIG. 7. Effect of α-acetyltrilysine on growth response of M-26-26 to lysine and trilysine. Three cultures were grown on α-acetyltrilysine (0.200 μeq of lysine residues per ml). After 9 hours, trilysine (0.050 μeq of lysine residues per ml) was added to one (·–··–), lysine (0.055 μeq per ml) to another (– – –), and nothing to the third. As controls, a culture was grown on lysine alone (0.066 μeq per ml), and another on trilysine alone (0.066 μeq of lysine residues per ml). Incubation was performed at 36°.
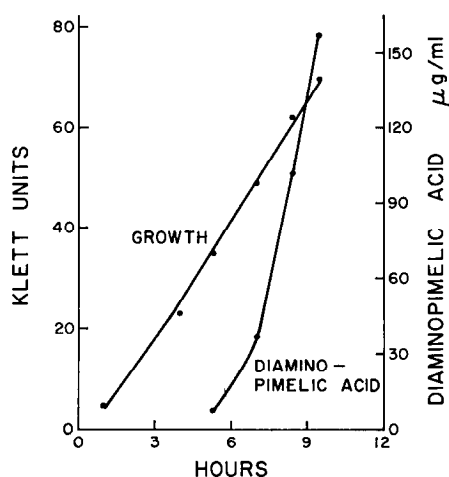

FIG. 8. Accumulation of diaminopimelic acid in a culture grown on α-acetyltrilysine (0.241 μeq of lysine residues per ml).

Since growth on the α-acetyltrilysine was very slow, it was important to test whether this peptide was inhibitory. Lysine and trilysine were added to cultures of M-26-26 growing on α-acetyltrilysine. Rate of growth after the addition of lysine and trilysine was not significantly different from what it would have been on the unacetylated materials in the absence of α-acetyltrilysine (Fig. 7).

M-26-26, the auxotroph used in all these studies, can revert to the wild type. In a culture growing continuously on α-acetyltrilysine, the number of wild-type bacteria should eventually

surpass the number of slowly growing M-26-26. In many experiments, cultures were grown on α-acetyltrilysine for as long as 13 hours. Therefore, it was necessary to determine how long a culture would retain its auxotrophic character. Samples of a culture growing on α-acetyltrilysine (0.300 μeq per ml) were plated out periodically onto agar plates with and without lysine. Both mutant and wild types grow on the lysine agar plate, but only wild type grows on the lysine-free plates. The relative number of bacterial colonies on lysine and lysine-free plates is a measure of the relative number of wild-type bacteria in the culture of M-26-26. Wild type was below 1% for at least 24 hours of growth.

The preceding experiments were all conducted at 36° without agitation. In order to see if the α-acetylated peptides would have the same properties under conditions of aerobic growth, 10-ml cultures were grown in 50-ml flasks on a shaker. Here again, only α-acetyltrilysine supported growth. The growth pattern on the tripeptide was still linear with respect to time but slightly faster under aeration. Increasing temperature from 32° to 36° to 38° increased the linear rate of growth on α-acetyltrilysine from 0.7 to 1.7 to 2.1 Klett units per hour, respectively. The α-acetylated di- and tetrapeptides were still unable to support growth. Therefore, the linearity of growth on α-acetyltrilysine and the lack of growth on α-acetylated di- and tetralysine is maintained under different conditions of aeration and temperature.

Effect of Growth on α-Acetyltrilysine on Diaminopimelic Acid Accumulation—Samples were removed periodically from cultures of M-26-26 growing aerobically on α-acetyltrilysine (0.241 μeq of lysine residues per ml) and on lysine (0.228 μeq per ml) as a control. The samples were centrifuged to remove the bacteria, and the supernatant solution was assayed for diaminopimelic acid. The culture grown on the α-acetylated tripeptide produced large amounts of diaminopimelic acid. By the time the

TABLE II

Activities of some enzymes of lysine pathway after growth on lysine or α-acetyltrilysine

The pyruvate-aspartic semialdehyde-condensing enzyme was assayed according to the procedure of Yugari and Gilvarg (12). Diaminopimelate epimerase was assayed according to the procedure of Farkas and Gilvarg (13). Dihydrodipicolinic acid reductase was assayed by following the oxidation of TPNH spectrophotometrically. The reaction mixture contained 100 μmoles of Tris, pH 7.4; 1.5 μmoles of dihydrodipicolinic acid; 100 μg of TPNH; and 0.05 ml of diluted crude extract in a volume of 1.0 ml. Dihydrodipicolinic acid was synthesized according to the procedure of Farkas, Yugari, and Gilvarg (14). Crude extract from the control was diluted 1:10; crude extract from the α-acetyltrilysine culture was diluted 1:100. The diluted crude extracts were heated to 70° for 3 min to denature interfering enzymes before assaying.

Substrate	Condensing enzyme	Dihydrodipicolinic acid reductase	Epimerase
	O.D./min/mg protein	*μmoles TPNH/ min/mg protein*	*μmoles DPA[a]/ min/mg protein*
α-Acetyltrilysine............	7.7	0.151	0.0106
Lysine....................	2.8	0.010	0.0063

[a] α,ε-Diaminopimelic acid.

culture grown on α-acetyltrilysine had reached an opacity of 56 Klett units, 66 μg per ml of diaminopimelic acid had accumulated. The control culture after the same amount of growth had not accumulated any diaminopimelic acid (see Fig. 8).

Effect of α-Acetyltrilysine on Enzyme Derepression—The bacteria obtained from the cultures used to measure diaminopimelic acid accumulation were used as a source of crude extracts for enzyme assays. The bacteria of the control culture grown on lysine were harvested by centrifugation while the culture was still in the logarithmic phase of growth. In the crude extract, the contents of pyruvate-aspartic semialdehyde-condensing enzyme, dihydrodipicolinic acid reductase, and diaminopimelate racemase were determined (Table II). For the condensing enzyme and racemase, growth on α-acetyltrilysine caused about a 2-fold increase in specific activity over the control. For dihydrodipicolinic acid reductase, however, specific activity increased 15-fold.

DISCUSSION

The elaboration of a method capable of resolving α- and ε-acetylated lysine oligopeptides has made it possible to re-examine more confidently the effect of α-acetylation on the ability of the lysine oligopeptides to support the growth of a lysine auxotroph. In the lysine oligopeptide series, there was a sharp transition from the ability to be utilized to nutritional ineffectiveness in passing from tetralysine to pentalysine. It was possible to relegate the site at which this discrimination was effected to the cell envelope, since cell-free extracts rapidly hydrolyzed pentalysine to free lysine. This implied the existence, in the intact cell, of a permeability barrier that prevented access of the pentalysine to the hydrolytic enzyme, or enzymes, but permitted transport of the smaller peptides. The observation that acetylation of the α-amino group severely depressed the capacity to support growth represented additional evidence for such a site. It would be expected that an entry site capable of distinguishing between tetra- and pentalysine might have other stereochemical requirements for passage such as a free α-amino group. The finding that, of the lysine oligopeptides acetylated in the α position, only α-acetyltrilysine possesses some growth-supporting activity raises questions as to the mechanism that is able to distinguish it from α-acetyldilysine and α-acetyltetralysine. In view of the failure of α-acetyltrilysine to inhibit the response to trilysine, it seems unlikely that a common entry site is being used in the case of these two peptides. Therefore, it is possible that a site ordinarily involved in the transport of some other substance is responsible for the transport of the α-acetyltrilysine. The discrimination against α-acetyldilysine and α-acetyltetralysine would then be attributable to the specificity characteristics of this new site. It should be noted that *E. coli* contains the enzymatic machinery for breaking down α-acetyltetralysine to lysine (1), so that this new site would also be placed at the cell envelope.

Two aspects of the utilization of α-acetyltrilysine are somewhat unusual. The complete inability of low concentrations to support growth is surprising. However, such a threshhold for growth has been observed before in the case of bacterial cultures grown in the chemostat on extremely low concentrations of tryptophan (15). Presumably, at very low growth rates, the requirements for sufficiently stable essential molecules cannot be met, or possibly the excessive energy demands of turnover become too exorbitant.

The linear growth on the α-acetyltrilysine also merits comment. In a logarithmically growing culture the generation time is constant, and the number of cells doubles with each generation. In a linearly growing culture the generation time must double with each new generation.

Among the hypotheses that might be constructed to explain such growth kinetics is one in which α-acetyltrilysine penetrates by means of a permease induced by lysine. In the α-acetyltrilysine-grown culture there is no exogenous lysine, and, therefore, new permease is not induced. Each daughter cell receives one-half as much permease as the parent cell. In each successive generation each bacterium is able to take in α-acetyltrilysine at one-half the rate of the previous generation. Since α-acetyltrilysine penetration limits growth, each new generation will grow one-half as fast as the previous generation. However, each new generation has twice as many cells, and therefore the over-all rate of growth is linear. Alternatively, α-acetyltrilysine could penetrate by means of a permease the synthesis of which, although not necessarily induced by lysine, is repressed by α-acetyltrilysine. Since α-acetyltrilysine represses synthesis of new permease, each daughter cell would, as in the previous model, receive only one-half as many permease molecules as the parent cell.

Novick and Weiner, studying the β-galactoside permease of *E. coli*, have shown that, in the absence of inducer, the permease content per cell falls (16). Apparently, the permease molecules are randomly divided among the daughter cells in each generation without synthesis of new permease. This model of β-galactoside permease dilution is analogous to the model proposed above to explain the linearity of growth on α-acetyltrilysine.

Advantage was taken of the slow growth on α-acetyltrilysine to investigate the regulatory control of some of the enzymes of the lysine pathway.[1] The observation that the dihydrodipicolinic acid reductase is capable of being derepressed adds it to the list of enzymes in this pathway that show extensive fluctuation in concentration in response to growth conditions. This list includes aspartyl kinase (17), aspartic semialdehyde dehydrogenase (17), and diaminopimelate decarboxylase (18). However, the limited responsiveness of the pyruvate-aspartic semialdehyde-condensing enzyme and the diaminopimelate epimerase, in contrast to these other enzymes, indicates that the enzymes of the lysine pathway, in the Waksman strain of *E. coli* at any rate, are not coordinately repressed.

The fact that large amounts of diaminopimelate accumulate when M-26-26 is growing on α-acetyltrilysine provides additional proof that the poor growth is due to lysine limitation rather than some other property of the peptide. It has been shown that there is an effective negative feedback regulation of the pyruvate aspartic semialdehyde-condensing enzyme by lysine (19). Under the usual conditions of growth on lysine, accumulation of diaminopimelate does not occur until growth has virtually ceased because of lysine depletion. The culture growing on α-acetyltrilysine behaves as if it too were in a state of lysine depletion.

REFERENCES

1. GILVARG, C., AND KATCHALSKI, E., *J. Biol. Chem.*, **240**, 3093 (1965).
2. AMES, B., AND GARRY, G., *Proc. Natl. Acad. Sci. U. S.*, **45**, 1453 (1959).
3. WALEY, S. G., AND WATSON, J., *Biochem. J.*, **55**, 328 (1953).

[1] ε-N-Formyllysine has been used to obtain slow growth with the Gif strain of *E. coli* (17). However, this compound was without activity for the Waksman strain used in the present studies.

4. KATCHALSKI, E., BERGER, A., AND LEVIN, Y., *Bull. Res. Counc. Israel, Sect. A*, **4**, 114 (1954).
5. STEWART, J., AND STAHMANN, M., *J. Chromatog.*, **9**, 233 (1962).
6. MOORE, S., AND STEIN, W. H., *J. Biol. Chem.*, **211**, 907 (1954).
7. DAVIS, B., *Nature*, **169**, 534 (1952).
8. DEWEY, D., AND WORK, E., *Nature*, **169**, 533 (1952).
9. DAVIS, B., AND MINGIOLI, E., *J. Bacteriol.*, **60**, 17 (1950).
10. GILVARG, C., *J. Biol. Chem.*, **233**, 1501 (1958).
11. WARBURG, O., AND CHRISTIAN, W., *Biochem. Z.*, **310**, 284 (1941).
12. YUGARI, Y., AND GILVARG, C., *J. Biol. Chem.*, **240**, 4710 (1965).
13. FARKAS, W., AND GILVARG, C., *J. Biol. Chem.*, **240**, 4717 (1965).
14. FARKAS, W., YUGARI, Y., AND GILVARG, C., *Federation Proc.*, **22**, 243 (1963).
15. NOVICK, A., *Ann. Rev. Microbiol.*, **9**, 101 (1955).
16. NOVICK, A., AND WEINER, M., *Proc. Natl. Acad. Sci. U. S.*, **43**, 553 (1957).
17. COHEN, G. N., PATTE, J. C., AND BOEZI, J., *Compt. Rend.*, **256**, 2939 (1963).
18. WHITE, P. J., KELLY, B., SUFFLING, A., AND WORK, E., *Biochem. J.*, **91**, 600 (1964).
19. YUGARI, Y., AND GILVARG, C., *Biochim. Biophys. Acta*, **62**, 612 (1962).