N-ACETYL-γ-GLUTAMOKINASE AND N-ACETYLGLUTAMIC γ-SEMIALDEHYDE DEHYDROGENASE: REPRESSIBLE ENZYMES OF ARGININE SYNTHESIS IN ESCHERICHIA COLI

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In E. coli, ornithine is synthesized from glutamate via N-acetylated intermediates (Vogel, 1952, 1953), including N-acetylglutamate (Vogel, 1953; Vogel et al., 1953) and N-acetylglutamic γ-semialdehyde (Vogel, 1953). An in vitro enzymatic conversion of N-acetylglutamate to its γ-semialdehyde was first observed with extracts of Micrococcus glutamicus (Udaka and Kinoshita, 1958); a single enzyme was assumed to be involved. Evidence has now been obtained that, in E. coli, the reduction of N-acetylglutamate is mediated by two enzymes, which will be referred to as N-acetyl-γ-glutamokinase and N-acetylglutamic γ-semialdehyde dehydrogenase. The kinase catalyzes the formation of N-acetyl-γ-glutamyl phosphate:

$$\text{N-acetylglutamate} + \text{ATP} \overset{Mg^{++}}{\rightleftarrows} \text{N-acetyl-}\gamma\text{-glutamyl phosphate} + \text{ADP} \qquad (1)$$

The dehydrogenase (named for the reverse of the biosynthetic reaction) mediates the reduction of N-acetyl-γ-glutamyl phosphate to the γ-semialdehyde:

$$\text{N-acetyl-}\gamma\text{-glutamyl phosphate} + \text{TPNH} + H^{+} \rightleftarrows \text{N-acetylglutamic } \gamma\text{-semialdehyde} + \text{TPN}^{+} + P_i \qquad (2)$$

Accordingly, N-acetyl-γ-glutamyl phosphate (presumably as L isomer) is concluded to be an intermediate in the synthesis of ornithine, and hence of arginine, in E. coli.

Mixtures containing the kinase and the dehydrogenase can catalyze the conversion of N-acetylglutamate to N-acetylglutamic γ-semialdehyde, according to the sum of equations (1) and (2):

$$\text{N-acetylglutamate} + \text{ATP} + \text{TPNH} + \text{H}^{+} \overset{Mg^{++}}{\rightleftarrows} \text{N-acetylglutamic } \gamma\text{-semialdehyde} + \text{ADP} + \text{TPN}^{+} + \text{P}_i \quad (3)$$

The enzymatic formation of this semialdehyde thus appears to parallel that of aspartic β-semialdehyde (Black and Wright, 1955).

Enzyme extracts were prepared, at 3°, from E. coli, strain Wc2 (Gorini and Gundersen, 1961), in which several enzymes of arginine synthesis are not repressible by arginine. Cells of this strain (18 g wet weight), grown in glucose-salts medium, were suspended in 5.0 mM Tris, pH 7.5 (36 ml), and disrupted sonically. To the clarified supernatant liquid (42 ml), 20% aqueous dihydrostreptomycin sulfate (13 ml) was added, and the resulting precipitate was removed by centrifugation. The supernatant liquid (50 ml) was treated with ammonium sulfate (8.8 g), and the precipitate was discarded. The material precipitating on the further addition of ammonium sulfate (9.9 g) was collected, dissolved in 5.0 mM Tris buffer, pH 7.4, containing 0.6 mM 2-mercaptoethanol, and dialyzed against the same buffer. The protein solution obtained (10 ml) was chromatographed on a column of DEAE-cellulose (17 x 50 mm, 0.92 milliequivalents/g, California Corp. for Biochemical Research); the eluent was Tris, pH 7.4, at concentrations increasing from 5.0 mM, and containing 2-mercaptoethanol at a constant concentration of

0.6 m<u>M</u>. The kinase and dehydrogenase were eluted at approximately 0.2 and 0.3 <u>M</u> Tris, respectively, and were separately rechromatographed in the same manner. For storage, the eluted enzyme preparations were saturated with ammonium sulfate, and kept as slurries at 3°. For use, the preparations were made up in and dialyzed against 2-mercaptoethanol-containing Tris, pH 7.4.

The reaction mixtures and assay conditions for the kinase and the dehydrogenase are shown in Tables 1 and 2, respectively. With the assay methods given, the kinase preparation proved to be free of detectable dehydrogenase, and vice versa. Table 3 illustrates the conversion of N-acetylglutamate to the corresponding γ-semialdehyde, catalyzed by a mixture of the two enzymes.

Table 1

Essential Components of Kinase Reaction Mixture

The complete system contained: Tris (pH 7.4), 45 μmoles; N-acetyl-<u>L</u>-glutamic acid (adjusted to pH 7.4 with NaOH), 30 μmoles; $MgCl_2$, 15 μmoles; hydroxylamine HCl (adjusted to pH 7.4 with NaOH), 2.1 millimoles; kinase preparation, 78 μg protein; water to 1.5 ml. After incubation at 37° for 60 minutes, the reaction was stopped with 3 <u>N</u> HCl (1.0 ml), and hydroxamate was determined spectrophotometrically according to Lipmann and Tuttle (1945). When hydroxylamine was omitted from the complete reaction mixture, some N-acetyl-γ-glutamyl phosphate was produced, as shown by subsequent hydroxamate formation.

Component omitted	Rate of hydroxamate formation (mμmoles/minute)
None	6.7
N-Acetylglutamate	0.0
ATP	0.0
$MgCl_2$	0.0
Kinase preparation	0.0

Table 2

Essential Components of Dehydrogenase Reaction Mixture

The complete system contained: Tris (pH 8.6), 100 μmoles; N-acetyl-L-glutamic γ-semialdehyde (Vogel, 1953), 1.0 μmole; TPN, 0.25 μmole; K_2HPO_4, 40 μmoles; dehydrogenase preparation, 13 μg protein; water to 1.0 ml. Absorbancy was measured at 340 mμ in a Beckman DU Spectrophotometer (light path, 1 cm; 25°).

Component omitted	Initial rate of TPNH formation (mμmoles/minute)
None	7.1
N-acetylglutamic γ-semialdehyde	0.0
TPN	0.0
K_2HPO_4	0.9
Dehydrogenase preparation	0.0

Equations 1, 2, and 3 are based on the data in Tables 1, 2, and 3, taken together with the following findings: in the kinase reaction, approximately one mole of P_i (determined by the method of Fiske and Subbarow, 1925) is liberated per mole of hydroxamate produced, and ADP (shown by paper electrophoresis) is a reaction product; in the dehydrogenase reaction, approximately one mole of TPNH is formed per mole of N-acetylglutamic γ-semialdehyde (assayed with o-aminobenzaldehyde after acid hydrolysis, A. M. Albrecht and H. J. Vogel, unpublished) consumed, and arsenate may be substituted for P_i; and in the conversion of N-acetylglutamate to N-acetylglutamic γ-semialdehyde, catalyzed by mixtures of the kinase and the dehydrogenase, approximately one mole of TPNH is oxidized per mole of the γ-semialdehyde produced.

Table 3

Conversion of N-Acetylglutamate to N-Acetylglutamic γ-Semialdehyde

The components, added in the order given, were: Tris (pH 8.6), 200 μmoles; N-acetyl-L-glutamic acid (adjusted to pH 7.4 with NaOH), 2.5 μmoles; ATP (disodium salt), 4.0 μmoles; $MgCl_2$, 10 μmoles; TPNH, 0.1 μmole; kinase preparation, 78 μg protein; dehydrogenase preparation, 6.5 μg protein; water to 1.0 ml. Absorbancy was measured at 340 mμ in a Beckman DU Spectrophotometer (light path, 1 cm; 25°).

Component omitted	Initial rate of TPNH utilization (mμmoles/minute)
None	3.9
N-Acetylglutamate	0.0
ATP	0.0
$MgCl_2$	0.1
TPNH	0.0
Kinase preparation	0.0
Dehydrogenase preparation	0.0

Preparations of N-acetyl-γ-glutamokinase and N-acetylglutamic γ-semialdehyde dehydrogenase could also be obtained from E. coli, strain W (from which the above-mentioned Wc2 was derived), or from strain 39A-23R3, which is a W mutant blocked in the synthesis of N-acetylglutamate. When 39A-23R3 was grown in glucose-salts medium supplemented with either N-acetyl-L-glutamate or N^{α}-acetyl-L-ornithine (50 μg/ml; cf. Vogel, 1961), levels of the kinase and of the dehydrogenase were observed comparable to those found for Wc2; when 39A-23R3 or W was grown in the presence of exogenous arginine, repressed levels of the enzymes resulted.

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