

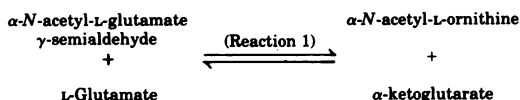
Ornithine δ -Transaminase Activity in *Escherichia coli*: Its Identity With Acetylornithine δ -Transaminase¹

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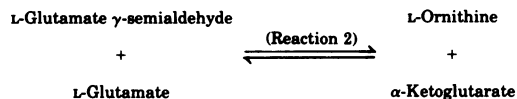
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Procedures that have been developed for the purification of acetylornithine δ -transaminase from *Escherichia coli* W also lead to the simultaneous purification of ornithine δ -transaminase. These two enzymatic activities have the same electrophoretic mobility and are identical immunochemically. Studies of inhibition kinetics demonstrate that the two substrates, acetylornithine and ornithine, compete for the same active site of acetylornithine δ -transaminase; thus, the ornithine δ -transaminase activity in *E. coli* is due to acetylornithine δ -transaminase and not to a separate specific ornithine δ -transaminase.

The biosynthesis of arginine in the procar-
yotic and eucaryotic microorganisms that have
been studied proceeds via a series of *N*-acety-
lated intermediates (5, 7, 10, 12, 15, 16, 20, 21).
The fourth step in this sequence of reactions
is catalyzed by acetylornithine δ -transami-
nase (acetylornithine aminotransferase, EC
2.6.1.11), and this reaction is depicted below:



Many of the microorganisms that have been
studied also possess an ornithine δ -transami-
nase (13) (ornithine-ketoacid aminotrans-
ferase, EC 2.6.1.13). The reaction catalyzed by
this enzyme is shown below:



In organisms that possess an ornithine δ -trans-
aminase, this enzyme does not seem to be in-
volved in arginine biosynthesis, but in the deg-
radation of arginine (22). Eckhardt and Leising-
er have recently demonstrated the presence of
ornithine δ -transaminase activity in *Esche-
richia coli*, strain K-12 (unpublished data).

The synthesis of ornithine δ -transaminase in
the wild-type strain of *E. coli* K-12 has been

shown to be repressible by arginine; by con-
trast, it is inducible by arginine in an *argD*
mutant of *E. coli* K-12 (Eckhardt and Leising-
er, unpublished data). Similarly, in the wild-
type strain of *E. coli* W, the synthesis of acetyl-
ornithine δ -transaminase is subject to repres-
sion by arginine (19), and a mutant of strain W
has been isolated that has an acetylornithine δ -
transaminase that is inducible, rather than re-
pressible by arginine (2). Both the arginine-
repressible and arginine-inducible acetylorni-
thine δ -transaminases from *E. coli* strain W
have been purified to homogeneity (M. Shen,
Ph.D. thesis, North Carolina State Univ., Ra-
leigh, N.C., 1970), and considerable evidence
exists that indicates that these two transami-
nases are different proteins (3, 9).

In view of the similarities in regulation of
synthesis of the ornithine δ -transaminase in
the K-12 strain and the acetylornithine δ -trans-
aminase in the W strain of *E. coli*, the possibil-
ity existed that there were two different trans-
aminase proteins. The purpose of this commu-
nication is to present the evidence that has
accumulated concerning the regulation of these
enzymatic activities, as well as the biochemical
evidence which indicates that in *E. coli* W,
ornithine δ -transaminase activity is due to the
wild-type and the arginine-inducible acetylorni-
thine δ -transaminases and not due to specific
ornithine δ -transaminases.

MATERIALS AND METHODS

Strains and culture conditions. The organisms
used in this study were the wild type *E. coli* strain W
(ATCC 9637) and a mutant of strain W (W2T1M12)
that has an arginine-inducible acetylornithine δ -

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transaminase. The genotype of the wild-type strain is represented by $M^+T^+R^+$. M refers to the location of the mutation that leads to inducibility of the transaminase in the inducible strain, T refers to the wild-type acetylornithine δ -transaminase structural gene, $argD$, and R refers to the arginine regulatory gene, $argR$. The inducible strain was isolated from a transaminase-deficient mutant and its genotype is represented by $M^-T^-R^+$, *met*^{pro} (W2T1M12).

The wild-type cells were grown in minimal salts medium (medium E) of Vogel and Bonner (19) to which glucose, autoclaved separately, was added aseptically to give a final concentration of 0.5%. The growth medium of the mutant was supplemented by the addition of DL-methionine (100 μ g/ml) and DL-proline (25 μ g/ml). Growth was determined turbidimetrically in a Klett-Summerson colorimeter with a no. 66 filter.

In experiments that involved induction and repression of the transaminase activities, samples of overnight cultures that had been grown in medium E supplemented with proline, methionine, and arginine were added to fresh medium. After the cells reached an exponential phase of growth (40 Klett units), they were harvested aseptically by centrifugation at 25°C in a Sorvall RC2-B centrifuge for 15 min at 15,000 rpm.

The packed cells were suspended in 1.5 ml of 0.1 M potassium phosphate buffer, pH 7.0, containing 1.0 mM 2-mercaptoethanol and 0.1 mM ethylenediaminetetraacetic acid. Extracts were prepared from the suspensions by disruption in a Branson model S-75 Sonifier at 0°C. Unbroken cells and cell debris were removed by centrifuging at 15,000 rpm for 15 min, and extracts were assayed immediately for transaminase activity and protein content.

Those experiments in which homogeneous preparations of both the wild-type and the arginine-inducible transaminases were required, 150 ml of a 12-h culture of each of the strains, grown in minimal medium supplemented with the appropriate amino acid mixtures, was used as an inoculum for a 15-liter culture in the same medium. The cells from four 15-liter cultures were harvested with a Sharples continuous-flow centrifuge after 8 to 10 h of incubation without aeration at 37°C. A yield of 1.0 to 1.5 g, wet weight, of cells was usually obtained per liter of medium. The packed cells were suspended in 0.1 M potassium phosphate, pH 7.0 (30 g of packed cells plus 70 ml buffer), containing 1 mM 2-mercaptoethanol and 0.1 mM ethylenediaminetetraacetic acid and disrupted by passage through a French pressure cell. Cell debris and unbroken cells were removed by centrifuging at $78,000 \times g$ for 3 h in a Spinco model L preparative ultracentrifuge. The supernatant fraction was decanted and dialyzed against 30 volumes of 0.1 M potassium phosphate, pH 7.0, and this fraction was then used for further purification of the wild-type transaminase, as well as the inducible acetylornithine δ -transaminase by procedures which have been described previously (9).

Assay of enzymatic activities. The standard assay conditions for acetylornithine δ -transaminase were those described by Albrecht and Vogel (1).

Each reaction mixture contained 50 μ mol of potassium phosphate, pH 8.0; 6.0 μ mol of α -N-acetyl-L-ornithine; 1.7 μ mol of α -ketoglutarate (neutralized with 2.0 M KOH to pH 7.0); 0.05 μ mol of pyridoxal 5-phosphate; and enzyme in a total volume of 0.5 ml. The reaction was initiated by the addition of enzyme and the incubation was carried out for 15 min at 37°C. The reaction was stopped by the addition of 0.3 ml of 6 N HCL. The reaction mixture was then heated at 100°C for 30 min in a boiling water bath and allowed to cool to room temperature. The mixture was neutralized by the addition of 1.0 ml of 3.6 M sodium acetate, and 0.2 ml of aqueous *o*-aminobenzaldehyde (4 mg/ml) was added. The *o*-aminobenzaldehyde reacts with Δ^1 -pyrroline 5-carboxylate, the product of the hydrolytic reaction, which results in the formation of a yellow color. The absorbance (light path, 1 cm) of this yellow solution was determined at 440 nm in a Gilford model 2000 spectrophotometer. Maximum color development occurs within 15 min of incubation at room temperature. In those situations in which turbidity appeared to be present, the solutions were clarified by centrifuging before the determination of the absorbance.

The reaction conditions that were used for the determination of ornithine δ -transaminase activity were exactly the same as those used for acetylornithine δ -transaminase with the exception that ornithine was substituted for acetylornithine at a concentration of 6 μ mol per reaction mixture; and 50 μ mol of sodium-glycinate, pH 9.3, was substituted for potassium phosphate. The final pH of the reaction was 8.8.

Enzyme unit. The unit of Albrecht and Vogel (1), defined as that amount of enzyme that will give an absorbance of 0.1 at 440 nm (light path, 1 cm), was employed.

Protein determination. Protein was determined by the method of Lowry et al. (11).

Compounds and reagents. Pyridoxal 5-phosphate, 2-mercaptoethanol, and ethylenediaminetetraacetic acid were acquired from Calbiochem. α -N-acetyl-L-ornithine was also obtained from Calbiochem and was shown by chromatography and electrophoresis not to contain contaminating impurities of ornithine. *o*-Aminobenzaldehyde was a product from K & K Laboratories. Enzyme-grade ammonium sulfate was purchased from Schwarz/Mann, and Sephadex was purchased from Pharmacia Fine Chemicals; Coomassie brilliant blue was obtained from the Colab Laboratory. All other chemicals were of reagent grade.

Homogeneous preparations of transaminase were used in all experiments reported below except those experiments concerned with induction and repression by arginine. The preparations of enzyme were judged to be homogeneous by analytical disc gel electrophoresis, preparative gel electrophoresis, immunoelectrophoresis, and high-speed sedimentation equilibrium (9).

RESULTS

Identification of glutamic γ -semialdehyde as the product of ornithine transaminase. The

reaction that is catalyzed by the enzyme acetylornithine δ -transaminase (reaction 1) is reversible and will lead to the formation of an *N*-acetylated intermediate acetylglutamic γ -semialdehyde if α -*N*-acetylornithine is the substrate. The presence of the acetyl moiety on the α -nitrogen of α -*N*-acetylglutamic γ -semialdehyde prevents this intermediate from forming a cyclized derivative. Ornithine δ -transaminase (reaction 2), which uses ornithine as the substrate, leads to the formation of glutamic γ -semialdehyde that can readily undergo cyclization to form Δ^1 -pyrroline 5-carboxylic acid. If transamination should occur between ornithine and α -ketoglutarate at the α -amino nitrogen rather than at the δ -amino nitrogen, then α -keto- δ -aminovaleric acid would be formed, which would non-enzymatically cyclize to Δ^1 -pyrroline 2-carboxylic acid. Both Δ^1 -pyrroline 5-carboxylate and Δ^1 -pyrroline 2-carboxylate will react with *o*-aminobenzaldehyde to give colored adducts. To distinguish between these two possibilities in the transamination of ornithine, a proline auxotroph of *E. coli* W (strain 38W-2) was selected. This auxotroph is capable of utilizing Δ^1 -pyrroline 5-carboxylate but not Δ^1 -pyrroline 2-carboxylate as a source of proline for growth. Partially purified preparations of ornithine δ -transaminase were incubated with ornithine and α -ketoglutarate for 4 h and the products of this reaction were separated from the protein by ultrafiltration by using a collodion bag. A product of the reaction that was present in the ultrafiltrate was found to support the growth of the proline auxotroph. The ultrafiltrate that was obtained from a control experiment in which ornithine was omitted from the reaction mixture did not support the growth of this proline-requiring mutant. Neither glutamic acid nor α -ketoglutarate, alone or in combination, was capable of supporting growth of the proline auxotroph; however, extremely slow growth was observed when ornithine was incorporated into the growth medium. The size of the colonies that were observed when ornithine served as the source of proline was extremely small. These results provide evidence that the product of ornithine δ -transaminase is glutamic γ -semialdehyde and not α -keto δ -aminovaleric acid. The extremely slow growth on ornithine demonstrates that some ornithine transaminase activity is apparently present *in vivo*.

Effect of enzyme concentration and time course of aminotransferase activity. The initial reaction velocities of the ornithine and acetylornithine δ -transaminases are linear with

respect to enzyme concentration. The extent of each of these enzymatic reactions was found to be linear with time for at least 2 h. Pyridoxal phosphate is labile at pH 8.8 (the pH of the ornithine transaminase assay) and because of this lability, the concentration of this cofactor must be increased to 0.1 μ M in order to maintain linearity of the reaction over a 2-h period.

Induction and repression of acetylornithine δ -transaminase and ornithine δ -transaminase in *E. coli* W. The data presented in Table 1 show that both the ornithine and acetylornithine δ -transaminases are repressible by arginine in the wild-type strain of *E. coli* W. The concentration of arginine (100 μ g/ml) in the growth medium results in an 80 to 85% repression of both of these transaminase activities. In the inducible strain of *E. coli* W, acetylornithine δ -transaminase is induced approximately fivefold by arginine, whereas ornithine δ -transaminase is only induced approximately twofold by arginine. This difference in the apparent degree of inducibility could be due to instability of the ornithine transaminase at pH 8.8 or the effect of pH on the substrates.

The effect of pH on acetylornithine and ornithine δ -transaminase. The data presented in Fig. 1 show that acetylornithine δ -transaminase is active over a wide pH range, from below 7.0 to 9.5, and has maximum enzymatic activity at around pH 8.2. In contrast, ornithine δ -transaminase shows little activity below a pH of 8.0 and displays a pH optimum at approximately 8.7.

Purification of acetylornithine and ornithine δ -transaminases. Crude enzyme extracts

TABLE 1. Induction and repression of acetylornithine δ -transaminase and ornithine δ -transaminase in *E. coli* W^a

Strain	Sp act ^b			
	Minimal medium		Minimal medium + L-arginine	
	Acetylornithine δ -transaminase	Ornithine δ -transaminase	Acetylornithine δ -transaminase	Ornithine δ -transaminase
Wild type	7.0	0.80	0.99	0.11
Inducible	1.4	1.55	7.0	2.94

^a Cells were grown and harvested as described in the experimental procedures and the enzymic activities and protein contents were determined as described in the experimental procedure.

^b The data are expressed as units of transaminase activity per milligram of protein.

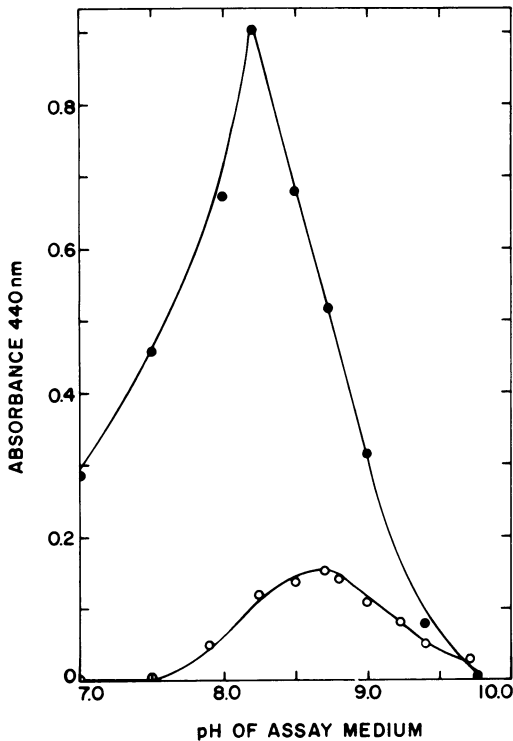


FIG. 1. Effect of pH on acetylornithine and ornithine δ -transaminases in the inducible strain. The reaction medium was the same as that described in the experimental section. Acetylornithine δ -transaminase (26 U) and ornithine δ -transaminase (7 U) were used with 50 μ mol of glycine NaOH (pH 7.0 to 10.0). Symbols: \bullet , Acetylornithine δ -transaminase activity; \circ , ornithine δ -transaminase activity. The pH of each reaction mixture was determined after incubation at 37°C for 15 min.

were obtained from the arginine-inducible strain and were subjected to purification procedures that employed ammonium sulfate fractionation and chromatography using Sephadex G-200, O-(triethylaminoethyl) (TEAE)-cellulose, Sephadex G-100, and diethylaminoethyl (DEAE)-Sephadex (Table 2). The procedures employed for the purification of the ornithine and acetylornithine δ -transaminases show that these two enzymatic activities purified together and that the ratio of the two enzymatic activities remained relatively constant throughout the purification procedures. Those fractions that were obtained from DEAE-Sephadex were subjected to further purification by using preparative disc gel electrophoresis. Analysis of the protein components that were isolated from preparative disc gel electrophoresis demonstrated that both the acetylornithine and ornithine

δ -transaminase activities were present in the same electrophoretic fractions. The fact that these two enzymatic activities from the arginine-inducible strain of *E. coli* purified together suggested that these two enzymatic activities were associated with the same protein.

Because previous evidence had indicated that the arginine-inducible and repressible acetylornithine δ -transaminases were different proteins (3), the possibility existed that the ornithine δ -transaminase and the acetylornithine δ -transaminase would be different proteins in the wild-type strain. The wild-type acetylornithine δ -transaminase was purified according to the same procedures as described for the inducible transaminase in Table 2. Throughout purification it was not possible to detect any fraction that showed ornithine transaminase activity and not acetylornithine δ -transaminase activity. The two transaminase activities purified together and the ratio of activities, acetylornithine/ornithine, remained relatively constant. The specific activity of the purified acetylornithine δ -transaminase was 107 and the specific activity of the ornithine δ -transaminase was 11, giving a ratio of 9.7. The ratio of the two specific activities remained constant throughout purification, which suggests that the two transaminase activities are also associated with the same protein in the wild-type strain. The difference in the acetylornithine/ornithine δ -transaminase specific activity ratios between the inducible strain (6.1) and the wild-type strain (9.7) is most likely due to the fact that the inducible transaminase and the wild-type transaminase are different proteins (3, 9).

Analytical disc gel electrophoresis of ornithine and acetylornithine δ -transaminases.

TABLE 2. Purification of acetylornithine δ -transaminase and ornithine δ -transaminase from the inducible strain of *E. coli* W^a

Fraction	Acetylornithine δ -transaminase	Ornithine δ -transaminase	Ratio of acetylornithine/ornithine activities
	Sp act (U/mg)	Sp act (U/mg)	
Crude	32.9	4.6	7.2
Ammonium sulfate	46.4	8.6	5.4
Sephadex G-200	125.0	18.9	6.6
TEAE-cellulose	366.8	51.2	7.2
Sephadex G-100	405.2	61.1	6.6
DEAE-Sephadex	466.0	76.7	6.1

^a The experimental procedures employed in the purification of the enzymes according to the scheme presented have been described previously (9, 14).

Additional evidence, which demonstrated that the ornithine and acetylornithine δ -transaminase activities are characteristics of the same protein, was obtained by subjecting purified extracts derived from DEAE-Sephadex chromatography to analytical disc gel electrophoresis using 7% polyacrylamide gels (Table 3). After electrophoresis, the acrylamide gels were cut into 1-mm slices, and each of these slices was assayed for both acetylornithine and ornithine

δ -transaminase activities. The data presented in Table 3 show that the ornithine δ -transaminase has the same relative migration as the acetylornithine δ -transaminase from both the wild-type and the inducible strains.

Immunochemical identity of acetylornithine and ornithine δ -transaminases. Purified preparations of acetylornithine δ -transaminases obtained from the arginine-inducible and the wild-type strains were used to prepare antibodies against each of the transaminases. The ornithine δ -transaminases that were purified from the wild-type and inducible strains gave identical cross-reactions with the antibodies that had been prepared against acetylornithine δ -transaminases from the wild-type and inducible strains. The identical cross-reactions between the ornithine δ -transaminases and the antibodies prepared against the acetylornithine δ -transaminases provide additional evidence that the two enzymatic activities are associated with the same protein (Fig. 2).

Evidence that the substrate-binding sites for acetylornithine and ornithine are identical. Because the wild-type and arginine-inducible acetylornithine δ -transaminases carry out a transamination with ornithine as the substrate, the possibility exists that more than one

TABLE 3. *Relative migration of transaminase activities in 7% acrylamide gels subjected to electrophoresis at pH 9.5^a*

Strain	<i>R</i> , relative to bromophenol blue	
	Acetylornithine δ -transaminase	Ornithine δ -transaminase
Wild type	0.38	0.37
Inducible	0.49	0.49

^a Purified extracts of the wild-type and inducible strains of *E. coli* W obtained from DEAE-Sephadex chromatography were subjected to disc gel electrophoresis at pH 9.5 in 7% polyacrylamide gels. The gels were extracted and immediately frozen. The frozen gels were cut into 1-mm slices and the slices were analyzed for acetylornithine and ornithine δ -transaminase activities.

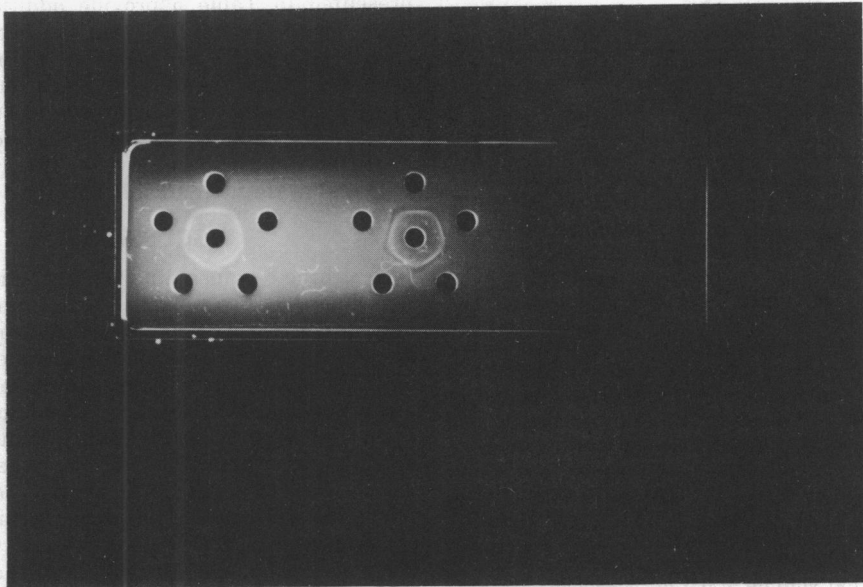


FIG. 2. Ouchterlony immunodiffusion analysis of ornithine transaminase with anti-acetylornithine δ -transaminase antibody. Left: In the center well was placed antibody prepared against the wild-type acetylornithine δ -transaminase. In the wells at 12, 5, and 7 o'clock was placed wild-type acetylornithine δ -transaminase. The wells at 2 and 10 o'clock contained wild-type ornithine transaminase. Right: In the center well was placed antibody prepared against the inducible acetylornithine δ -transaminase. In the wells at 12, 5, and 7 o'clock was placed inducible acetylornithine δ -transaminase. The wells at 2 and 10 o'clock contained inducible ornithine δ -transaminase.

substrate-binding site is present on each enzyme and that these binding sites are separate and specific for acetylornithine and ornithine, respectively.

The results presented in Table 4 provide evidence that suggests that acetylornithine and ornithine bind at the same catalytic center. The absorbance that was obtained in experiment 1 with 5.4 mM ornithine as the δ -amino substrate was 0.056. Boiling of the reaction mixture in the presence of hydrochloric acid did not decrease nor increase the absorbance. The absorbance observed in experiment 2 with 1.4 mM acetylornithine as the δ -amino substrate was 0.247, and this absorbance was observed only after hydrolysis by hydrochloric acid. In experiment 3 (Table 4), it can be seen that acetylornithine interferes with the enzyme's reaction with ornithine. The presence of 1.4 mM acetylornithine caused approximately 48% inhibition

TABLE 4. The inhibition of acetylornithine and ornithine δ -transaminase activities of the arginine-inducible acetylornithine δ -transaminase by ornithine and acetylornithine^a

Expt	Substrate concn (mM)		Transaminase act (absorbance at 440 nm)	
	Ornithine	Acetylornithine	Expected	Observed
1	5.4			0.056
2		1.4		0.247
3	5.4	1.4	0.056	0.029
4	5.4	1.4	0.303	0.172

^a Acetylornithine δ -transaminase from the wild-type and inducible strains obtained from TEAE-cellulose chromatography was used for inhibition studies. The development of the yellow color that results from the reaction between *o*-aminobenzaldehyde and Δ^1 -pyrroline 5-carboxylate cannot occur when acetylornithine is used as a substrate until after acid hydrolysis of the reaction mixture. On the other hand, when ornithine is used as substrate, the product of the transamination will spontaneously cyclize and will react with *o*-aminobenzaldehyde without hydrolysis of the reaction mixture. The activity that was due to acetylornithine δ -transaminase was determined after hydrolysis by boiling in the presence of 6 N HCl and was measured as the increase in absorbance above that which was present before hydrolysis. The reaction medium contained a final volume of 0.5 ml; various concentrations of ornithine and acetylornithine are as described below; glycine NaOH (final pH 8.8), 50 μ mol; α -ketoglutarate, 1.7 μ mol; pyridoxal 5-phosphate, 0.05 μ mol; and enzyme, 19 U. The activity of ornithine δ -transaminase was measured in experiment 3 and the activity of acetylornithine δ -transaminase was measured in experiment 4.

of ornithine transamination. In experiment 4, acetylornithine and ornithine were also present in the reaction mixture, and the mixture was hydrolyzed at the end of the reaction. If there were two independent reaction centers, the sum of the absorbances obtained for experiment 1 and 2 (Table 4) should be observed in experiment 4. The absorbance value of 0.172 after hydrolysis is well below the additive absorbance of 0.303. If the absorbance due to ornithine transamination (0.029) is subtracted from the observed absorbance after hydrolysis (0.172), the absorbance that is actually due to the acetylornithine transamination is 0.143, which represents a 42% inhibition of acetylornithine transamination by ornithine.

In view of the mutual inhibition of transamination of the two δ -amino substrates by both acetylornithine and ornithine, it appears that each of the substrates serves as a competitive inhibitor of the other. However, the possibility still remains that each of these substrates has a different binding site and that each substrate serves as an allosteric inhibitor of the other.

The data presented in Fig. 3 and 4 show that acetylornithine and ornithine are competitive both in the case of the wild-type and in the case of arginine-inducible transaminases. The data presented in Table 5 provide additional evidence for the existence of only one binding site for acetylornithine and ornithine for both wild-type and arginine-inducible transaminases. The K_i values for acetylornithine and ornithine are similar to the K_m values for acetylornithine and ornithine, respectively, for both the wild-type and arginine-inducible enzymes.

DISCUSSION

Ornithine δ -transaminase activity in *E. coli* W is due to acetylornithine δ -transaminase, not to a distinct enzyme. In the wild-type strain, the ratio of activities of acetylornithine δ -transaminase and ornithine δ -transaminase remains essentially constant throughout purification. In the arginine-inducible strain in which the synthesis of acetylornithine δ -transaminase is induced by arginine, the ratio of activities of acetylornithine δ -transaminase and ornithine δ -transaminase also remains constant throughout purification. The Michaelis constants of ornithine and acetylornithine for the transamination reaction are essentially the same as their respective inhibition constants (Table 5), which indicates that both substrates compete for the same active site.

The K_m for ornithine in the ornithine δ -transaminase assay (9.6 mM) is 20 times greater

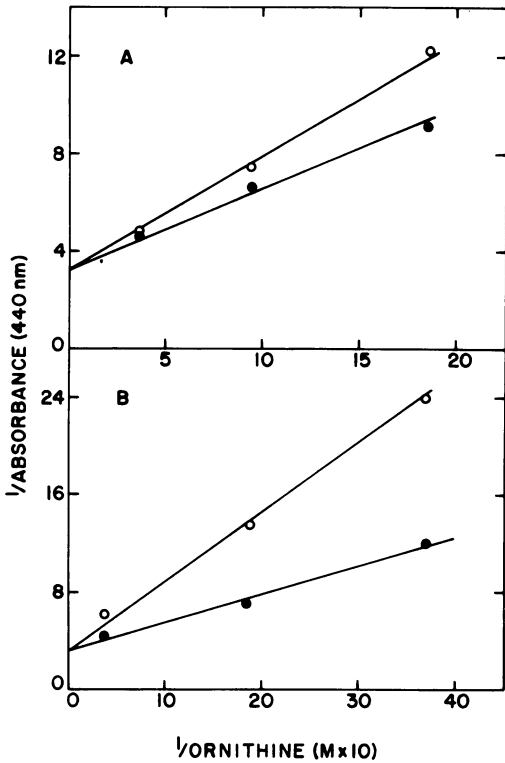


FIG. 3. Competitive inhibition by ornithine of the acetylornithine δ -transaminase isolated from the wild-type and arginine-inducible strains. The reaction conditions were the same as those described in Table 4. (A) represents the wild-type acetylornithine δ -transaminase activity. The acetylornithine concentrations were 0.14, 0.7, and 1.4 mM; \bullet , no ornithine was added; \circ , 27 mM ornithine was added. (B) represents the acetylornithine δ -transaminase activity from the inducible strain. The acetylornithine concentrations were 0.7, 1.4, and 7mM; \bullet , no ornithine was added; \circ , 27 mM ornithine was added.

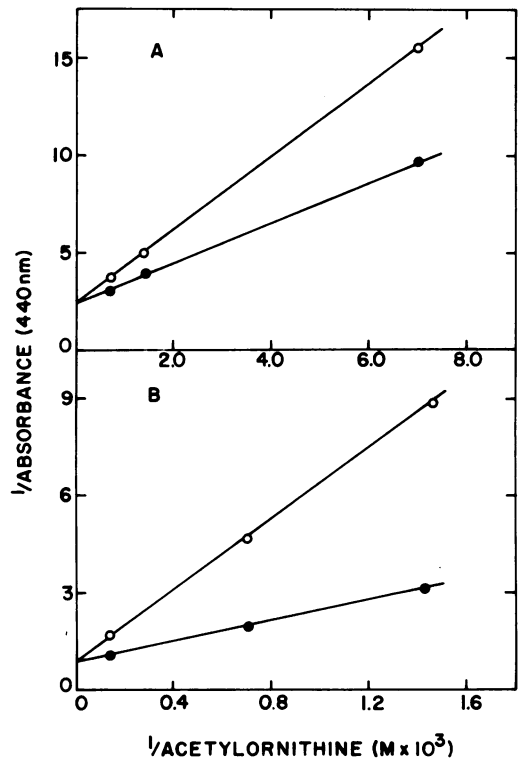


FIG. 4. Competitive inhibition by acetylornithine of ornithine δ -transaminase activity from the wild-type and arginine-inducible strains. The experimental procedure is exactly the same as that given in the legend to Table 4. (A) represents the wild-type ornithine δ -transaminase activity in which the ornithine concentrations were 5.4, 10.8, and 27 mM. Symbols: \bullet , No acetylornithine was added; \circ , 0.14 mM acetylornithine was added. (B) represents the ornithine transaminase activity of the inducible strain in which the ornithine concentrations were 2.7, 5.4, and 27 mM. Symbols: \bullet , No acetylornithine was added; \circ , 1.4 mM acetylornithine was added.

than the K_m for acetylornithine (0.48 mM) in the acetylornithine δ -transaminase assay. The ornithine δ -transaminase assay is carried out at a pH of 8.8, whereas the acetylornithine δ -transaminase assay is assayed at pH 8.0. The increase in pH of the assay medium of the ornithine δ -transaminase is most likely responsible for the difference in K_m for the two substrates since the K_m for acetylornithine in the acetylornithine δ -transaminase assay is about 15-fold higher at pH 8.8 than it is at pH 8.0. The increase in K_m of both of these substrates would not be primarily due to a change in the degree of protonation of the δ -amino group, but is most likely due to a change in the ionic environment in and around the active center of the enzyme.

TABLE 5. Michaelis constants and inhibition constants of acetylornithine and ornithine for the wild-type and arginine-inducible acetylornithine δ -transaminases^a

Strain	Acetylornithine		Ornithine	
	K_m , mM	K_i , mM	K_m , mM	K_i , mM
Wild type	0.48	0.33	9.65	12.37
Inducible	1.54	1.20	7.5	6.9

^a The procedures followed were the same as those described in the legends of Fig. 3 and 4. The data were analyzed using a computer program originally written by W. W. Cleland (4).

The fact that the enzyme recognizes ornithine as a δ -amino substrate in addition to acetylornithine is most likely due to the deprotonation of the α -amino group of ornithine at pH 8.8. At a pH of 8.0, 82% of the α -amino group of ornithine is protonated and at this pH, there is no detectable ornithine δ -transaminase activity. At a pH of 8.8, about 42% of the α -amino group of ornithine is unprotonated and this increase in the unprotonated form could explain the increase in ornithine transamination that takes place. The α -amino group of acetylornithine is acetylated and therefore is not easily protonated.

Accordingly, the ornithine transamination reaction is probably not normally of physiological significance in *E. coli*. The fact that a glutamic acid γ -semialdehyde-responding mutant will grow, at a reduced rate, in the presence of ornithine indicates that ornithine transaminase activity is present and could be useful for the utilization of arginine if the cell is forced to grow on arginine as a sole source of carbon and nitrogen. The finding that the arginine-inducible acetylornithine δ -transaminase of *E. coli* is active with ornithine as the substrate, however, suggests a possible evolutionary relationship of this enzyme to the ornithine δ -transaminase of other microorganisms, especially since ornithine δ -transaminase is inducible by arginine in *Bacillus subtilis* (8), *Neurospora crassa* (6), and *Aspergillus nidulans* (14). Very recently the synthesis of acetylornithine δ -transaminase has been shown to be induced by arginine in *Pseudomonas aeruginosa* and this enzyme also has ornithine δ -transaminase activity (17). It has been proposed that the arginine-inducible enzyme plays a dual role in that it is active in the biosynthesis, as well as in the degradation of arginine. It will be of interest to compare the structure of the inducible acetylornithine δ -transaminase of *E. coli* with the structures of ornithine transaminases from other microorganisms, as well as from mammalian species.

The first indication that ornithine δ -transaminase activity was present in *E. coli* was observed in the K-12 strain. The work presented here does not preclude the possibility that there are two distinct transaminases in the K-12 strain; but, the antibodies prepared against the strain W acetylornithine δ -transaminases do cross-react with partially purified preparations of ornithine and acetylornithine δ -transaminases from the wild-type and arginine-inducible strains of *E. coli* K-12 and there has been no evidence to date of two distinct transaminases in the K-12 strain (unpublished data).

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