The Dual Biosynthetic Capability of *N*-Acetylornithine Aminotransferase in Arginine and Lysine Biosynthesis†

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ABSTRACT: The genes encoding the seven enzymes needed to synthesize L-lysine from aspartate semialdehyde and pyruvate have been identified in a number of bacterial genera, with the single exception of the *dapC* gene encoding the PLP-dependent *N*-succinyl-L,L-diaminopimelate:α-ketoglutarate aminotransferase (DapATase). Purification of *E. coli* DapATase allowed the determination of both the amino-terminal 26 amino acids and a tryptic peptide fragment. Sequence analysis identified both of these sequences as being identical to corresponding sequences from the PLP-dependent *E. coli argD*-encoded *N*-acetylornithine aminotransferase (NAcOATase). This enzyme performs a similar reaction to that of DapATase, catalyzing the *N*-acetylornithine-dependent transamination of α-ketoglutarate. PCR cloning of the *argD* gene from genomic *E. coli* DNA, expression, and purification yielded homogeneous *E. coli* NAcOATase. This enzyme exhibits both NAcOATase and DapATase activity, with similar specificity constants for *N*-acetylornithine and *N*-succinyl-L,L-DAP, suggesting that it can function in both lysine and arginine biosynthesis. This finding may explain why numerous investigations have failed to identify genetically the bacterial *dapC* locus, and suggests that this enzyme may be an attractive target for antibacterial inhibitor design due to the essential roles of these two pathways in bacteria.

Drug resistance in pathogenic bacteria has increased significantly in the past decade, in part due to the widespread use, and misuse, of antibiotics (*1*). The need for new classes of antibacterial compounds, targeting critical biosynthetic pathways unique to bacteria, is apparent. The *meso*-diaminopimelate (Dap)/lysine[1] biosynthetic pathway provides a number of potential targets for inhibition (*2*−*9*), some of which have been shown to be essential for mycobacterial growth (*10*). The products of this pathway, *meso*-Dap and lysine, function as the cross-linking moiety in the peptidoglycan component of Gram-positive, Gram-negative, and mycobacterial cell walls. In addition, lysine is an essential amino acid in mammals, suggesting that inhibition of this pathway would be bactericidal but without effect on lysine metabolism in mammals.

The bacterial DAP/lysine biosynthetic pathway was elucidated by a series of biochemical studies in the 1960s and 1970s (Figure 1) (*11*). More recently, all but one of the genes encoding the seven enzymes which generate L-lysine from L-aspartate semialdehyde have been cloned and sequenced in *E. coli* (*11*), and homologues of these genes have been identified in a large number of Gram-negative, Gram-positive, and mycobacteria. A puzzling exception is the *dapC*-encoded *N*-succinyldiaminopimelate aminotransferase (DapATase, EC 2.6.1.17), a PLP-dependent enzyme that catalyzes the glutamate-dependent transamination of *N*-succinyl-L-2-amino-6-oxopimelate to generate *N*-succinyl-L,L-diaminopimelate (Figure 2). The enzyme was initially purified 110-fold from *E. coli* (*12*), and more recently has been purified from this source 1500-fold to homogeneity (*7*). Early reports of the identification of a *dapC* genetic locus in *E. coli* (*13*) were subsequently shown to encode the *dapD* gene encoding the L-2-amino-6-oxopimelate succinyltransferase (*14*), the enzyme immediately preceding the aminotransferase in the biosynthetic pathway. In all bacteria for which the genome has been sequenced, there are no obvious candidate open reading frames bearing homology to PLP-dependent aminotransferases for which function has not been ascribed.

In this paper, we describe the purification of *E. coli* DapATase. Amino acid sequencing of the amino terminus and an eight residue tryptic fragment, followed by database searching, revealed that these amino acid sequences were identical to appropriate regions of the *E. coli argD* gene. The *argD* gene encodes the PLP-dependent *N*-acetylornithine aminotransferase (NAcOATase), an enzyme involved in arginine biosynthesis (Figure 1) (*15*). The *E. coli argD* gene was PCR-amplified and ligated into an expression vector, and the recombinant, overexpressed enzyme was purified to homogeneity. Our in vitro data demonstrate that this enzyme exhibits both NAcOATase and DapATase activities, with very similar catalytic efficiencies and identical kinetic mechanisms, suggesting that this enzyme can play a role in both lysine and arginine biosynthesis in vivo.

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[1] Abbreviations: DapATase, *N*-succinyl-L,L-diaminopimelate aminotransferase; NAcOATase, *N*-acetylornithine aminotransferase; GDH, glutamate dehydrogenase; Dap, diaminopimelate; NADPH, β-nicotinamide adenine dinucleotide phosphate, reduced form; $NADP^+$, β-nicotinamide adenine dinucleotide phosphate; TAPS, *N*-[[tris(hydroxymethyl)-methyl]-3-amino]propanesulfonic or acid [[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino]-1-propanesulfonic acid; IPTG, isopropylthio-β-D-galactoside; ESI-MS, electrospray ionization mass spectrometry.

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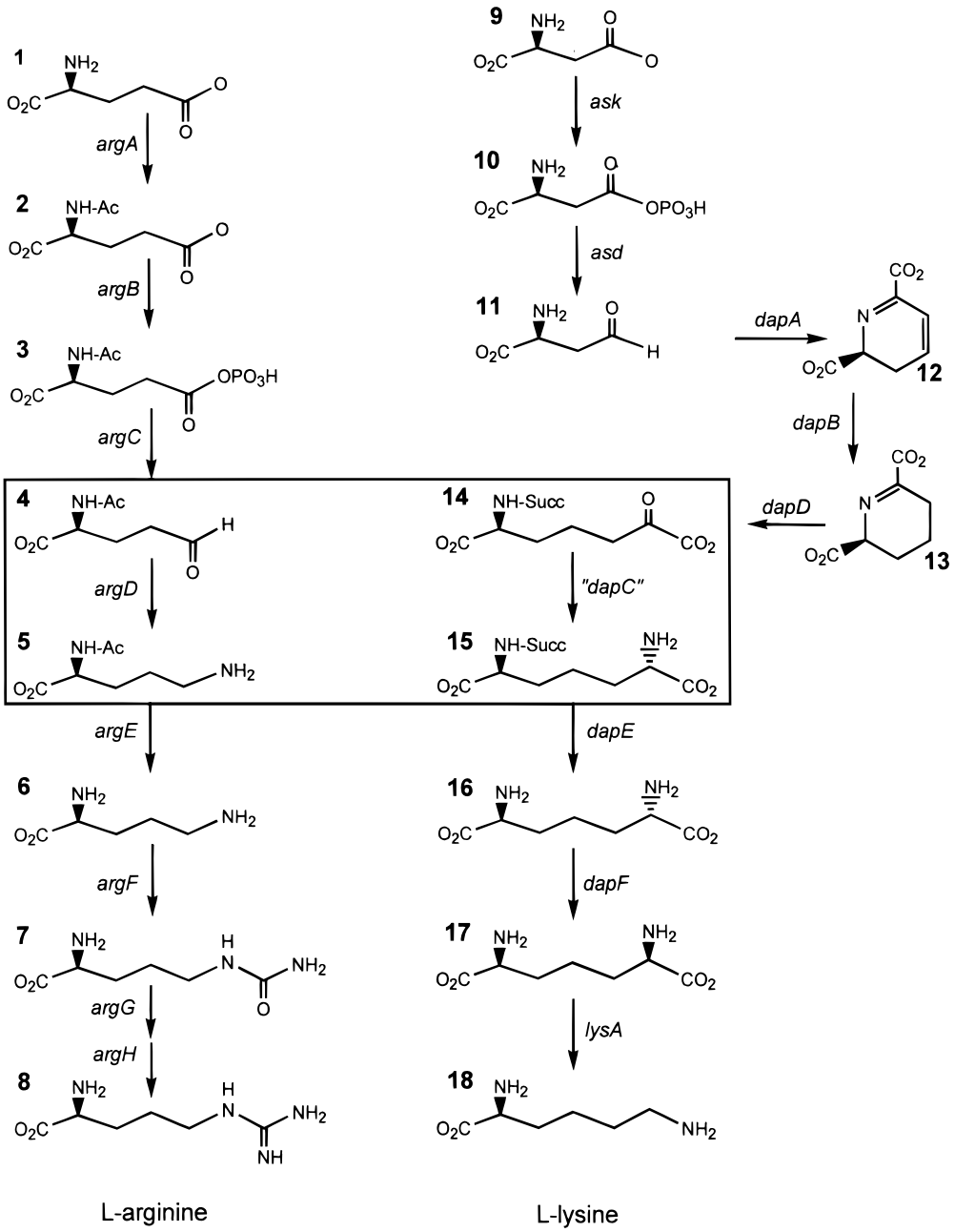

FIGURE 1: Biosynthetic reactions leading from glutamate to arginine and aspartate to lysine. L-glutamate (**1**); *N*-acetyl-L-glutamate (**2**); *N*-acetyl-L-glutamyl-γ-phosphate (**3**); *N*-acetyl-L-glutamate semialdehyde (**4**); *N*-acetyl-L-ornithine (**5**); L-ornithine (**6**); L-citrulline (**7**); L-arginine (**8**); L-aspartate (**9**); L-aspartyl-γ-phosphate (**10**); L-aspartate semialdehyde (**11**); L-dihydrodipicolinate (**12**); L-tetrahydrodipicolinate (**13**); *N*-succinyl-L-2-amino-6-oxopimelate (**14**); *N*-succinyl-L,L-diaminopimelate (**15**); L,L-diaminopimelate (**16**); D,L-diaminopimelate (**17**); L-lysine (**18**).

MATERIALS AND METHODS

Materials. All reagents and enzymes were purchased from Sigma (St. Louis, MO) or Aldrich (Milwaukee, WI). Restriction enzymes were purchased from New England BioLabs (Beverly, MA). BL21(DE3) cells and pET overexpression vectors were purchased from Novagen (Madison, WI). Chromatographic supports were purchased from Pharmacia (Piscataway, NJ). *E. coli* K12 cells were obtained from the University of Alabama Fermentation Facility. *N*-Succinyl-2-amino-6-oxopimelate was synthesized as previously described (*7*). *N*-Succinyl-L,L-diaminopimelate was synthesized as previously described (*16*).

Enzyme Assays. All assays were conducted at 25 °C using a UVIKON spectrophotometer equipped with thermospacers and connected to a constant-temperature circulating water bath.

Kinetic parameters for the reaction of L-glutamate and *N*-succinyl-2-amino-6-oxopimelate (the DapATase reaction) were obtained by determining initial rates of NADPH oxidation at 340 nm in a coupled, spectrophotometric assay using glutamate dehydrogenase to convert α-ketoglutarate, formed in the reaction, to L-glutamate in the presence of NADPH and NH_4. Assay conditions were 50 mM TAPS, pH 8.0, 0.3 mM NADPH, 0.1 M NH_4Cl, 20 units/mL GDH, 0.2−5.0 mM glutamate, and 0.2−5.0 mM *N*-succinyl-2-amino-6-oxopimelate.

Kinetic parameters for the reactions of α-ketoglutarate and *N*-acetylornithine (the NAcOATase reaction) were obtained

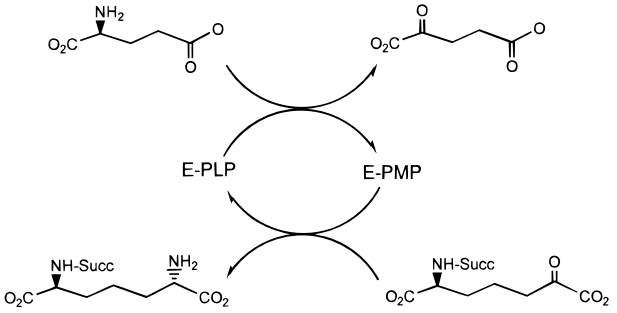

FIGURE 2: Reactions catalyzed by *N*-succinyldiaminopimelate aminotransferase (DapATase). DapATase is a PLP-dependent enzyme that catalyzes the glutamate-dependent transamination of *N*-succinyl-L-2-amino-6-oxopimelate to generate *N*-succinyl-L,L-diaminopimelate.

by measuring the initial rates of NADPH formation at 340 nm in a coupled, spectrophotometric assay using glutamate dehydrogenase to convert L-glutamate, formed in the reaction, to α-ketoglutarate and ammonia in the presence of $NADP^+$. Assay conditions were 50 mM TAPS, pH 8.9, 10 mM $NADP^+$, 55 units/ml GDH, 0.5−2.0 mM, α-ketoglutarate, and 0.2−5.0 mM *N*-acetylornithine. Kinetic parameters were obtained for alternate substrates using the same coupled spectrophotometric assay except that alternate keto acids replaced *N*-succinyl-2-amino-6-oxopimelate, and alternate amino acids and primary amines replaced *N*-acetylornithine.

Initial velocity data were fit to eq 1 which describes hyperbolic, saturation kinetics, while initial velocity data obtained by varying both substrates were fit to eq 2 which describes a ping-pong initial velocity pattern. The Fortran computer programs of Cleland were used (*17*).

$$v = VA/(K + A) \quad (1)$$

$$v = VAB/(K_aB + AK_b + AB) \quad (2)$$

DapATase Purification. Two hundred grams of *E. coli* K12 cells was suspended in 300 mL of 50 mM TEA, pH 7.8, containing 200 mM sucrose and protease inhibitor tablets (1 tablet/50 mL, Boehringer Mannheim). Cells were treated with 0.2 mg/mL lysozyme for 30 min at 4 °C, and osmotically ruptured by the slow addition of an equal volume of ice-cold distilled H_2O. Cell debris was removed by centrifugation for 40 min at 12 000 rpm. Streptomycin sulfate was added (1% w/v, final concentration) to the supernatant to precipitate nucleic acids. Centrifugation at 12 000 rpm for 45 min removed the nucleic acid precipitate, and the resulting supernatant was dialyzed against 14 L of 25 mM TEA, pH 7.8, for 3 h followed by centrifugation at 12 000 rpm for 45 min. The resulting supernatant was applied to a Fast-Flow Q Sepharose anion exchange column equilibrated in 25 mM TEA, pH 7.8. Proteins were eluted with a linear 2 L 0−1 M NaCl gradient. Fractions containing DapATase activity were pooled and made 1 M in ammonium sulfate by the slow addition of solid $(NH_4)_2SO_4$ to the solution. The suspension was centrifuged to remove precipitated proteins, and the clear supernatant was applied to a Phenyl Sepharose column that was equilibrated in 20 mM TEA, pH 7.8, containing 1 M $(NH_4)_2SO_4$. Proteins were eluted with a 1 L linear 1−0 M $(NH_4)_2SO_4$ gradient in 20 mM TEA, pH 7.8. Active fractions were pooled, concentrated, dialyzed against 25 mM TEA, pH 7.8, and applied to a high-performance, anion exchange MonoQ column. Proteins were eluted with a 500 mL linear 0−0.7 M NaCl gradient. Active fractions were pooled, concentrated, and loaded onto a Superdex 200 gel filtration column equilibrated in 25 mM TEA, pH 7.8, containing 0.25 M NaCl. Active fractions eluting from the column were analyzed by SDS−PAGE, revealing a major band at ∼40 kDa, representing ∼75% of the total protein, that coeluted with DapATase activity in these fractions.

Amino Acid Sequencing. The purified protein was electrophoresed on a 12% polyacrylamide slab gel containing 0.1% SDS, and transferred to a Problot membrane. Proteins were stained with Ponceau Red, and the major 40 kDa band was excised. The N-terminal sequence of the 40 kDa band was directly determined using a Procise microsequencer, using standard Edman chemistry.

To obtain the internal amino acid sequence, the protein was electrophoresed as outlined above and stained with Coomassie Blue. The stained 40 kDa band was cut into 1 mm squares and incubated with 200 μL of 30% NH_4OH for 4 h at room temperature. The NH_4OH was decanted, and the gel pieces were vortexed in H_2O for 30 min. The H_2O was decanted, and 2 μg of trypsin in 100 μL of 0.2 M NH_4-CO_3, containing 0.01% Tween 20, was added and incubated with the gel squares overnight at 37 °C. The supernatant was decanted and saved, and 200 μL of 60% aqueous acetonitrile, containing 0.1% TFA, was added to the gel pieces for 30 min at room temperature. This supernatant was decanted and combined with the aqueous supernatant. After lyophilization, the solid was dissolved in H_2O, and the tryptic peptides were separated by reverse phase HPLC on a C_{18} column using a linear 0−60% acetonitrile gradient, containing 0.1% TFA.

Database Searching. A database search was performed with the obtained 26 amino acid amino-terminal sequence of DapATase against protein sequences in the GENEMBL databank using the Wisconsin Genetics Computer Group sequence analysis package (*18*). This search identified the *E. coli argD*-encoded *N*-acetylornithine aminotransferase as exactly matching the determined DapATase amino-terminal sequence. In addition, the sequence of a tryptic fragment of DapATase matched exactly the sequence of the *E. coli* NAcOAT sequence between positions 69 and 77 (position 68 is a lysine residue).

PCR Amplification and Expression of E. coli argD-Encoded Transaminase. The *argD* gene of *E. coli* was amplified using PFU polymerase and PCR, using oligonucleotide primers that contained *Bam*HI and *Nde*I restriction sites at the 5′ and 3′ ends, respectively. The expected 1220 bp PCR product was purified, treated with *Bam*HI and *Nde*I, and ligated into a pET11a plasmid previously treated with *Bam*HI and *Nde*I, to generate a pET11a:*argD* construct containing the *E. coli argD* sequence inserted in-frame with the bacteriophage T7 promotor. The pET11a:*argD* plasmid was used to transform *E. coli* BL21(DE3) cells, and grown on Luria broth (LB) plates containing 50 μg/mL carbencillin. *E. coli* BL21(DE3) cells containing the pET11a:*argD* plasmid were cultured at 37 °C in 6 L of LB containing 50 μg/mL carbencillin until the cultures reached an OD_{600} ∼1. The cultures were then induced with 1 mM (final concentration) IPTG. Cells were collected 3 h later by centrifugation and stored at −20 °C.

Purification of the Recombinant E. coli NAcOATase. E. coli BL21(DE3) cells containing the pET11a:*argD* plasmid (25−30 g wet weight) were suspended in 50 mM TEA, pH 7.8 (1:1 w/v), containing 200 mM sucrose and protease inhibitor tablets (1 tablet/50 mL, Boehringer Mannheim). Cells were treated with 0.2 mg/mL lysozyme for 30 min at 4 °C, and ruptured by the slow addition of an equal volume of ice-cold H_2O. Cell debris was removed by centrifugation for 40 min at 12 000 rpm, and streptomycin sulfate was added (1% w/v, final concentrated) to precipitate nucleic acids. Centrifugation at 12 000 rpm for 45 min removed the nucleic acid precipitate, and the supernatant was dialyzed twice against 14 L of 25 mM TEA, pH 7.8, for 2 h, followed by centrifugation at 12 000 rpm for 45 min. The resulting supernatant was applied to a Fast-Flow Q Sepharose anion exchange column equilibrated in 25 mM TEA, pH 7.8. Proteins were eluted using a linear 2 L 0−1.0 M NaCl gradient. Yellow fractions containing the NAcOATase activity were pooled, concentrated, and dialyzed against 25 mM TEA, pH 7.8, containing 250 mM NaCl, and applied to a Superdex 200 gel filtration column equilibrated in the same buffer. Active fractions were pooled and dialyzed against 25 mM TEA, pH 7.8, and applied to a high-performance anion exchange MonoQ column equilibrated in 25 mM TEA, pH 7.8. The transaminase was eluted with a linear 0−0.8 M NaCl gradient. The recombinant transaminase appeared as a single dense band at ca. 40 kDa on SDS−PAGE gels.

Mass Spectrometry. The molecular mass of the apoenzyme, predicted from the gene sequence, is 43 764.7 daltons. The purified recombinant *E. coli* transaminase exhibited a mass of 43 634 daltons by ESI-MS, corresponding to residues 2−406 of the full-length enzyme, indicating posttranslational amino terminal methionine cleavage.

RESULTS AND DISCUSSION

There are three distinct biosynthetic pathways to generate *meso*-Dap in bacterial lysine biosynthesis, including the succinylase pathway shown in Figure 1. The other two pathways are a mechanistically analogous acetylase pathway, in which *N*-acetylated intermediates are substituted for the succinylated intermediates, and a dehydrogenase pathway, in which the acyclic form of tetrahydrodipicolinate, L-amino-6-oxo-pimelate, is reductively aminated to directly generate *meso*-DAP. The succinylase pathway is the most widely distributed. All of the genes encoding the enzymes of the succinylase pathway have been identified in *E. coli*, except the *dapC* gene, which encodes the *N*-succinyl-L-2-amino-6-oxopimelate:L-glutamate transaminase. PLP-dependent aminotransferases are grouped into four classes according to the mechanistic and structural classification proposed by Mehta et al. (*19*). The reaction catalyzed by DapATase does not easily fit into one of these classes since the α-ketoglutarate-dependent oxidative deamination of amino acids is a common feature of all four classes. Using representative members of each class as a search model, we failed to identify any homologue among the open reading frames in the *E. coli* genome that were functionally unassigned.

Purification of E. coli DapATase, Amino Acid Sequencing, and Database Searching. DapATase activity was purified from *E. coli* using a modification of a previous method (*7*). Four chromatographic steps afforded a significant purification of DapATase, with enzymatic activity and protein profiles

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                                   30
MAIEQTAITRATFDEVILPIYAPAEFIPVK     E. coli argD
 AIEQTAITRATFDEVILPIYAPAEFI        DapATase

                                   60
GQGSRIWDQQGKEYVDFAGGIAVTALGHCH     E. coli argD

                                   90
PALVNALKTQGETLWHISNVFTNEPALRLG     E. coli argD
      TQGETLXXI                    DapATase
```

FIGURE 3: Comparison of purified DapATase amino-terminal and tryptic peptide sequences with the predicted amino acid sequence of the *E. coli argD*-encoded NAcOATase

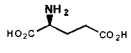
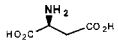
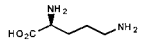
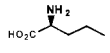
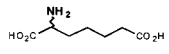
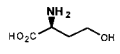
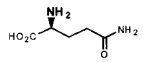
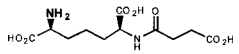
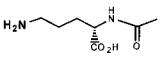
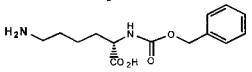
suggesting that the major band observed at ∼40 kDa on SDS−PAGE was DapATase. Amino-terminal protein sequencing and sequencing of a well-resolved tryptic peptide were performed on the purified DapATase.

The 26 residue *N*-terminal sequence aligned exactly with residues 2−27 of the *E. coli argD*-encoded NAcOATase (Figure 3). In addition, the 9 residue internal tryptic peptide sequence aligned exactly with residues 69−77 of the *E. coli argD* sequence (Figure 3). NAcOATase, an enzyme in the arginine biosynthetic pathway, like DapATase, is a PLP-dependent aminotransferase that uses glutamate as an amino donor. There are a number of obvious biochemical parallels between L-lysine and L-arginine biosynthesis, which are highlighted in Figure 1. Early steps in both pathways convert a precursor acidic amino acid (L-aspartate in the case of L-lysine; L-glutamate in the case of L-arginine) to the corresponding aldehyde. This aldehyde is directly transaminated to the corresponding primary amine by NAcOATase in arginine biosynthesis, while three carbon units from pyruvate are first added to aspartate semialdehyde, prior to *N*-succinylation and transamination in lysine biosynthesis. Once the aldehyde or α-ketoacid are transaminated, the *N*-acyl protecting groups, added either in the first step in arginine biosynthesis (*argA*) or after pyruvate addition in lysine biosynthesis (*dapD*), are subsequently removed by the deacylases encoded by either *argE* or *dapE*. Our sequencing results suggested that the purified DapATase was identical to NAcOATase in those amino-terminal regions examined. This surprising result led us to clone, overexpress, and purify the *E. coli argD*-encoded transaminase for biochemical characterization of substrate specificity.

Cloning and Catalytic Properties of the argD-Encoded Aminotransferase. The expressed, homogeneous *E. coli argD*-encoded transaminase catalyzed the *N*-acetylornithine-dependent amination of α-ketoglutarate, exhibiting a $k_{cat} = 37\ min^{-1}$ and a K_m value of 0.15 mM for *N*-acetylornithine (Table 1). The transaminase was also capable of catalyzing the *N*-succinyldiaminopimelate-dependent amination of α-ketoglutarate, exhibiting a $k_{cat} = 18\ min^{-1}$ and a K_m value of 0.075 mM for *N*-succinyldiaminopimelate (Table 1). The k_{cat}/K_m values for the two substrates, a measure of catalytic efficiency, are similar at ∼4000 $M^{-1}\ s^{-1}$ (Table 1). The kinetic constants determined with our overexpressed enzyme differ slightly from those obtained previously (*7*, *12*, *20*) due to differences in assay conditions and the purity of enzyme and substrates.

The kinetic mechanisms of all PLP-dependent transaminases are ping-pong, with the following half-reactions: (a) transfer of ammonia from the amino donor, L-glutamate, to the PLP cofactor to form enzyme-bound PMP; and (b) transfer of ammonia from the PMP form of the enzyme to

Table 1: Kinetic Parameters Determined for Amino Donor Substrates in the DapATase and NAcOATase Reactions[a]

	k_{cat} (sec^{-1})	K_m (mM)	k_{cat}/K_m ($M^{-1}sec^{-1}$)
	0.67 ± 0.07	0.19 ± 0.03	3450 ± 600 (100%)
	0.07 ± 0.01	0.92 ± 0.02	76 ± 8 (2.2%)
	0.22 ± 0.01	4.5 ± 0.4	49 ± 5 (1.4%)
	0.49 ± 0.01	30 ± 1.5	16 ± 1 (0.5%)
	0.15 ± 0.01	9.3 ± 0.8	16 ± 2 (0.5%)
	0.70 ± 0.01	73 ± 8	9.6 ± 1 (0.3%)
	0.64 ± 0.01	97 ± 10	6.6 ± 1 (0.2%)
	0.23 ±0.04	36 ± 7	6.4 ± 2 (0.2%)
	0.29 ± 0.02	0.08 ± 0.01	3650 ± 260 (105%)
	0.61 ± 0.01	0.15 ± 0.01	4040 ± 270 (117%)
	0.33 ± 0.04	3.0 ± 0.7	110 ± 25 (3.2%)

[a] α-Ketoglutarate was the cosubstrate in all reactions except when glutamate was the amino donor. In this case, *N*-succinyl-2-amino-6-oxopimelate was the cosubstrate.

the aldehyde or α-ketoacid acceptor. The *E. coli argD*-encoded transaminase yields parallel initial velocity patterns when both the NAcOATase and DapATase reactions are examined (Figure 4).

The ability to use either α-amino acids or primary amines as amino donors is not unprecedented in PLP transamination reactions. All class II PLP-dependent aminotransferases can oxidize either a primary or a secondary amine, or the α-amino group of glutamate. Ornithine aminotransferase, an enzyme involved in proline metabolism, transfers the δ-amino group of ornithine to α-ketoglutarate. Similarly, γ-aminobutyric acid transaminase, an enzyme involved in neurotransmitter regulation, uses either the γ-amino group of γ-aminobutyrate or the α-amino group of glutamate as the amino donor. In the present case, the *dapC*/*argD*-encoded transaminase can use L-glutamate as the amino donor in the physiological, biosynthetic reactions, but catalyzes amino transfer to either the aldehydic carbon of *N*-acetyl-L-glutamate semialdehyde or the keto carbon of *N*-succinyl-L-2-amino-6-oxopimelate.

To probe the substrate specificity of the transaminase, a variety of amino acid and primary amines were tested as alternate amino donors, and a number of keto acids and aldehydes were tested as amino acceptors. These substrate specificity studies extend other studies on the purified *E. coli* transaminase (*7*). In addition to L-glutamate, the L-α-amino acids aspartate, glutamine, ornithine, norvaline, and homoserine are effective amino donors for the transaminase when α-ketoglutarate is the amino acceptor. D-α-Amino acids are not substrates. The specificity for the acyl group at the protected α-amino acid moiety is similarly broad. *N*-Acetyl, *N*-succinyl, and *N*-carbobenzoxy groups at the L-α-amino acid center are accommodated by the enzyme, although the K_m value for L-*N*-Cbz-lysine is ca. 20 times higher than that for the smaller *N*-acylated derivatives of ornithine or DAP (Table 1).

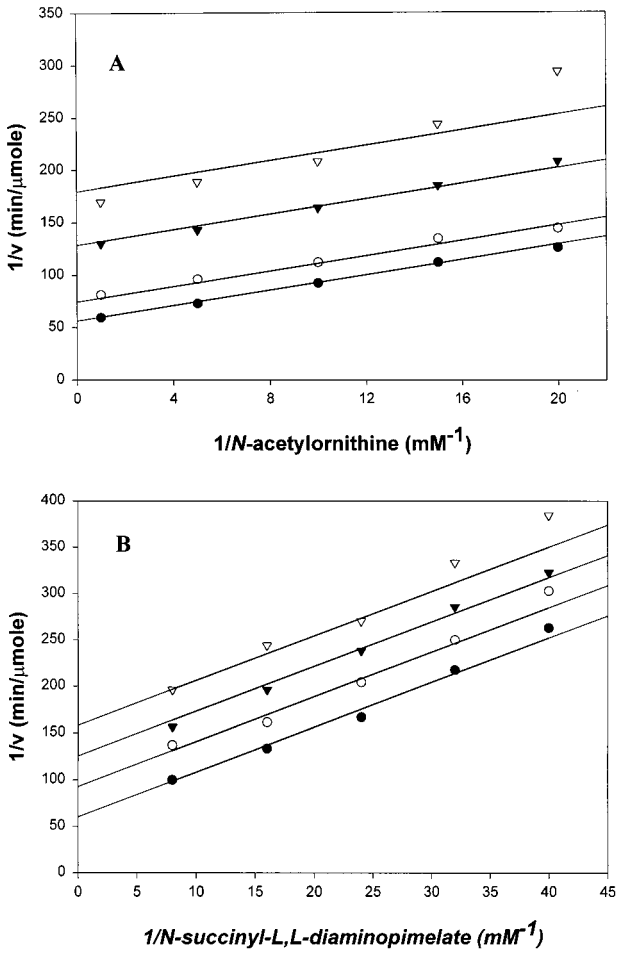
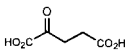
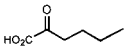
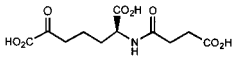


FIGURE 4: Initial velocity patterns of the DapATase (A) and NAcOATase (B) reactions. The data points shown are experimentally determined values, and the straight lines are fits of the data to eq 2. The amino group acceptor in reactions (A) and (B) is α-ketoglutarate.

Unlike the broad specificity for amino donors, the transaminase was more restrictive for compounds that act as amino acceptors. Pyruvate, α-ketocaproate, α-ketoisocaproate, and ketomalonate were poor alternate substrates, exhibiting relative k_{cat}/K_m values that were less than 3% that of α-ketoglutarate (Table 2). The preferred substrates are those specific to the NAcOATase and DapATase reactions (Tables 1 and 2).

Class II PLP-dependent aminotransferases represent the most diverse class of aminotransferases when considering substrate specificity (*19*). The enzymes in the other three classes use only α-amino acids and α-ketoacids as substrates. Perhaps the most unusual feature of the DapATase/NAcOATase is the additional ability to accept a wide variety of α-*N*-acyl substituents. However, primary sequence homology searches of a number of pro- and eukaryotic aminotransferases reveal that the *E. coli argD*-encoded transaminase is most identical in amino acid sequence to the *Pseudomonas aeruginosa aruC*-encoded *N*-succinylornithine aminotransferase involved in arginine degradation (*21*, *22*). The 66% primary sequence identity between these two enzymes is greater than the 60% identity between the *E. coli argD* and

Table 2: Kinetic Parameters Determined for Amino Acceptors in the DapATase and NAcOATase Reactions[a]

	k_{cat} (sec^{-1})	K_m (mM)	k_{cat}/K_m ($M^{-1}sec^{-1}$)
	1.0 ± 0.03	0.12 ± 0.01	8240 ± 60 (100%)
	3.1 ± 0.4	13 ± 3	230 ± 60 (2.8%)
	1.2 ± 0.3	40 ± 11	30 ± 2 (0.4%)
	0.2 ± 0.03	40 ±10	4 ± 1 (.04%)
	1.7 ± 0.04	0.46 ± 0.02	3670 ± 170 (44%)

[a] Glutamate was the cosubstrate in all reactions except when α-ketoglutarate was the amino acceptor. In this case, *N*-acetylornithine was the cosubstrate.

argM-encoded acetylornithine aminotransferases, which function in arginine biosynthesis and degradation, respectively, in this organism. The structural basis for the ability to accommodate these different α-*N*-acyl substituents must await the determination of the three-dimensional structure of this enzyme.

In conclusion, the biochemical data presented here demonstrate that the expressed, homogeneous *E. coli argD*-encoded *N*-acetylornithine aminotransferase is catalytically competent to perform both its namesake reaction as well as the formation of *N*-succinyl-L,L-diaminopimelate from *N*-succinyl-L-2-amino-6-oxopimelate. This suggests that this single enzyme may physiologically function in two critical amino acid biosynthetic pathways, making it a strong candidate for inhibitor design. This conclusion may also explain the inability to identify the *dapC* gene locus, since disruption of the gene would potentially generate auxotrophy to both diaminopimelate and L-arginine. The genetic inactivation of the *E. coli argD* locus and the consequences of this disruption are under investigation.

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REFERENCES

1. Bax, R. P. (1997) *Clin. Infect. Dis. 24*, S151−153.
2. Abbott, S. D., Lane-Bell, P., Sidhu, K. P. S., and Vederas, J. C. (1994) *J. Am. Chem. Soc. 116*, 6513−6520.
3. Song, Y. H., Neiderer, D., Lane-Bell, P., Lam, L. K. P., Crawley, S., Palcic, M. M., Pickard, M. A., Pruess, D. L., and Vederas, J. C. (1994) *J. Org. Chem. 59*, 5784−5793.
4. Williams, R. M., and Yuan, C. (1992) *J. Org. Chem. 57*, 6519−6527.
5. Girodeau, J. M., Agouridas, C., Masson, M., Pineau, R., and LeGoffic, F. (1986) *J. Med. Chem. 29*, 1023−1030.
6. Berges, D. A., DeWolf, W. E., Dunn, G. L., Grappel, S. F., Newman, D. J., Taggart, J. J., and Gilvarg, C. (1986) *J. Med. Chem. 29*, 89−95.
7. Cox, R. J., Sherwin, W. A., Lam, L. K. P., and Vederas, J. C. (1996) *J. Am. Chem. Soc. 118*, 7449−7460.
8. Cox, R. J. (1996) *Nat. Prod. Rep. 13*, 29−43.
9. Cox, R. J., Schouten, J. A., Stentiford, R. A., and Wareing, K. J. (1998) *BioMed. Chem. Lett.*, 945−950.
10. Pavelka, M. S., and Jacobs, W. R. J. (1996) *J. Bacteriol. 178*, 6496−6507.
11. Scapin, G., and Blanchard, J. S. (1998) *Adv. Enzymol. 72*, 279−324.
12. Peterkofsky, B., and Gilvarg, C. (1961) *J. Biol. Chem. 236*, 1432−1438.
13. Bukhari, A. I., and Taylor, A. L. (1971) *J. Bacteriol. 105*, 844−854.
14. Richaud, C., Richaud, F., Martin, C., Haziza, C., and Patte, J.-C. (1984) *J. Biol. Chem. 259*, 14824−14828.
15. Cunin, R., Glansdorff, N., Pierard, A., and Stalon, V. (1986) *Microbiol. Rev. 50*, 314−341.
16. Born, T. L., Zheng, R., and Blancard, J. S. (1998) *Biochemistry 37*, 10478−10487.
17. Cleland, W. W. (1979) *Methods Enzymol. 78*, 103−138.
18. Devereux, J., Herbert, M., and Smithies, O. (1984) *Nucleic Acids Res. 12*, 387−395.
19. Mehta, M. K., Hale, T. I., and Christen, P. (1993) *Eur. J. Biochem. 214*, 549−561.
20. Albrecht, A. M., and Vogel, H. J. (1964) *J. Biol. Chem. 239*, 1872−1876.
21. Itoh, Y. (1997) *J. Bacteriol. 179*, 7280−7290.
22. Jann, A., Stalon, V., Wauven, C. V., Leisinger, T., and Haas, D. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 4937−4941.

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