Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 4937–4941, July 1986
Microbiology

N^2-Succinylated intermediates in an arginine catabolic pathway of *Pseudomonas aeruginosa*

(*aru* mutants/N^2-succinylarginine/N^2-succinylornithine/metabolic versatility)

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Communicated by Werner Arber, March 5, 1986

ABSTRACT **Arginine-nonutilizing (*aru*) mutants of *Pseudomonas aeruginosa* strain PAO converted L-arginine to N^2-succinylarginine or *N*-succinylglutamate, which were identified by high-voltage electrophoresis and HPLC. Addition of aminooxyacetate, an inhibitor of pyridoxal phosphate-dependent enzymes, to resting cells of the wild-type PAO1 in arginine medium led to the accumulation of N^2-succinylornithine. Enzyme assays with crude *P. aeruginosa* extracts established the following pathway: L-arginine + succinyl-CoA → N^2-succinylarginine → N^2-succinylornithine → *N*-succinylglutamate 5-semialdehyde → *N*-succinylglutamate → succinate + glutamate. Succinyl-CoA may be regenerated from glutamate via 2-ketoglutarate. L-Arginine induced the enzymes of the pathway, and succinate caused catabolite repression. Purified N^2-acetylornithine 5-aminotransferase (N^2-acetyl-L-ornithine: 2-oxoglutarate aminotransferase, EC 2.6.1.11), an arginine biosynthetic enzyme, efficiently transaminated N^2-succinylornithine; this explains the enzyme's dual role in arginine biosynthesis and catabolism. The succinylarginine pathway enables *P. aeruginosa* to utilize arginine efficiently as a carbon source under aerobic conditions, whereas the other three arginine catabolic pathways previously established in *P. aeruginosa* fulfill different functions.**

The pseudomonads are among the most versatile bacteria. They degrade a large number of organic compounds and possess a wide range of biochemical pathways (1, 2). In a single *Pseudomonas* species, several pathways may contribute to the catabolism of one substrate. This situation is illustrated by L-arginine catabolism in the genetically characterized strain PAO of *Pseudomonas aeruginosa*. Oxygen limitation induces the arginine deiminase pathway (3), leading to ornithine and regenerating ATP from ADP (Fig. 1). *P. aeruginosa arc* mutants blocked in this pathway are unable to grow anaerobically with arginine as the only energy source, but they utilize arginine normally as a carbon and nitrogen source under aerobic conditions (4). The arginine decarboxylase pathway produces agmatine and then putrescine (Fig. 1). Arginine induces arginine decarboxylase, whereas agmatine induces agmatine deiminase and *N*-carbamoylputrescine hydrolase (5); *agu* mutants lacking one of the latter enzymes cannot grow on agmatine but are unaffected in aerobic arginine breakdown (6).

2-Ketoarginine and 4-guanidinobutyrate are intermediates in the arginine oxidase pathway (Fig. 1) of *Pseudomonas putida* (7) and good growth substrates for *P. aeruginosa* (8). This pathway might also operate in *P. aeruginosa*; however, an arginine oxidase has not yet been demonstrated. PAO mutants have been isolated that cannot grow on 2-ketoarginine because of defects in 4-guanidinobutyraldehyde dehydrogenase (*kauB*) or guanidinobutyrase (*gbu*). We have constructed *arc agu kau* and *arc agu gbu* triple mutants; these retain the ability to grow on arginine as the only carbon and nitrogen source (unpublished results). This indicates the existence of a further arginine catabolic pathway.

Arginine-nonutilizing (*aru*) mutants can be derived from the wild-type PAO1 in one step (9, 10). As we show here, they are blocked in an arginine catabolic pathway involving *N*-succinyl intermediates. The same pathway has recently been revealed in *Pseudomonas cepacia*, where it accounts for the utilization of arginine as a growth substrate (11).

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions. The mutants PAO957 (*aru-321*) and PAO982 (*aru-247*) were derived from the wild-type PAO1 (12), and the mutants PAO954 (*met-9011 amiE200 aru-292*) (9) and PAO6150 (*met-9011 amiE200 aru-77*) were derived from PAO505 (9), by the methods described (9, 13). Growth was achieved with good aeration at 37°C in the media previously used (3, 6, 13).

Preparation of Cell Extracts and Enzyme Assays. Cells were broken by sonication (9, 14). The following enzyme activities were assayed in crude extracts: arginine N^2-succinyltransferase (EC 2.3.1.-), N^2-succinylarginine dihydrolase (EC 3.5.3.-), N^2-succinylornithine 5-aminotransferase (identical with N^2-acetylornithine 5-aminotransferase, EC 2.6.1.11), and *N*-succinylglutamate desuccinylase (EC 3.5.1.-), all according to ref. 11, except that 0.1 M Tris·HCl (pH 8.2) was used in the succinyltransferase assay. N^2-Acetylornithine 5-aminotransferase (AcOAT; N^2-acetyl-L-ornithine:2-oxoglutarate aminotransferase, EC 2.6.1.11) was measured and purified by the methods of Voellmy and Leisinger (14). *N*-Succinylglutamate 5-semialdehyde dehydrogenase (EC 1.2.1.-) was determined by a coupled assay in a mixture (1.0 ml) containing 5 mM N^2-succinyl-L-ornithine, 5 mM 2-ketoglutarate, 75 μM pyridoxalphosphate, 30 units of purified AcOAT, 1 mM NAD, 50 mM Tris·HCl (pH 8.0), and extract. Formation of NADH was followed spectrophotometrically at 340 nm. Blanks contained no N^2-succinylornithine. One unit is defined as the amount of enzyme that catalyzes the formation of 1 μmol of product per hr. Protein was determined by the Lowry method.

Analysis of Metabolites by High-Voltage Electrophoresis (HVE) and HPLC. Bacteria were grown overnight in minimal medium E and transferred to minimal medium P with 20 mM L-glutamate and 20 mM L-arginine (6); the initial cell density was 2×10^8 cells per ml. After growth at 37°C with shaking for ≈2 generations, $\approx 4 \times 10^8$ cells were washed with 0.9%

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Abbreviations: AcOAT, N^2-acetylornithine 5-aminotransferase (identical with N^2-succinylornithine 5-aminotransferase); HVE, high-voltage electrophoresis.

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NaCl and resuspended in 200 μl of 50 mM K phosphate buffer (pH 7.0) containing 2 mM L-arginine (uniformly, *carboxyl-*, or *guanidino*-^{14}C-labeled; specific activity, 10 μCi/μmol; 1 Ci = 37 GBq; from Amersham). Incubation was at 37°C without aeration. Samples (50 μl) were withdrawn at intervals and treated with 20 μl of 2 M $HClO_4$ at 4°C. Cell debris was sedimented by centrifugation. The supernatant was neutralized with 20 μl of 2 M KOH. After centrifugation at 4°C to remove $KClO_4$, an aliquot of the supernatant was applied to Whatman 3MM paper and electrophoresed in pyridine/acetic acid/H_2O (100:10:890, vol/vol; pH 6.4) at 2500 V for 15–25 min. Metabolites were localized by autoradiography. They could be eluted from paper with H_2O (2 × 1.0 ml) and analyzed by HPLC on a Nucleosil-C_{18} column (Macherey & Nagel, Düren, F.R.G.) in 0.1 M K phosphate (pH 2.0) (details to be reported elsewhere). Excretion products were analyzed as described (11).

Chemicals. N^2-Succinyl-L-arginine was prepared from L-arginine and succinic anhydride (11) and crystallized as the barium salt hydrochloride ($C_{10}H_{16}N_4O_5Ba$·HCl) at pH 4. The ^{1}H NMR spectrum was in agreement with that for the free acid (15). N^2-Succinyl-L-ornithine was synthesized as described (11); it was not crystallized. ^{1}H NMR in ^2H_2O (300 MHz) gave signals at 1.7–2.1 ppm (4H, *m*), 2.69 ppm (2H, *d*), 2.73 ppm (2H, *d*), 3.10 ppm (2H, *t*), 4.49 ppm (1H, *t*). Hydrolysis (6 M HCl, 120°C, 3 hr) produced equimolar amounts of ornithine and succinate. Our *N*-succinyl-L-glutamate (16) preparation contained some succinate (11). Succinyl-CoA, acetyl-CoA, and aminooxyacetic acid were from Sigma; other reagents were from sources indicated in refs. 6, 11, and 14.

RESULTS

Accumulation of Arginine Derivatives in Arginine-Nonutilizing (*aru*) Mutants. Several PAO mutants unable to grow aerobically on L-arginine as the sole carbon and nitrogen source were obtained after mutagenesis with ethyl methanesulfonate. Their aerobic uptake of L-arginine occurred at the wild-type rate (data not shown). They grew normally in glutamate minimal medium (doubling time, ≈1 hr). Addition of 20 mM arginine to this medium produced a 2- to 10-fold increase in their doubling time but no growth inhibition of the wild-type PAO1. We could not detect any gross enzyme defect related to the three known arginine catabolic pathways (Fig. 1) in the *aru* mutants. We therefore assumed that arginine could be converted to intermediary products via another pathway, and we speculated that the accumulation of intermediates in the mutants might be responsible for the arginine sensitivity.

Resting cells of the arginine-sensitive mutant PAO982 (*aru-247*) accumulated intracellularly an arginine derivative, which was not produced by the wild type. This compound (**1**) was acidic in HVE at neutral pH (Fig. 2*A*) and isotopically labeled with [*carboxy*-^{14}C]-, [*guanidino*-^{14}C]-, or [U-^{14}C]arginine as the precursor, indicating that the carbon skeleton remained intact. Acid hydrolysis of purified compound **1** gave labeled arginine and succinate, according to HVE and HPLC (Fig. 2*B*). Compound **1** was identified as N^2-succinylarginine on the basis of its coelution in HPLC with chemically synthesized compound **1** (Fig. 2*A*). Furthermore, strain PAO982 and the phenotypically similar mutant PAO6150 (*aru-77*) growing in glutamate/arginine medium (100 ml) excreted sufficient quantities of compound **1** to be detected by guanidine-specific reagents after HVE or paper chromatography; as expected, compound **1** was ninhydrin-negative.

Another arginine-sensitive mutant, PAO957 (*aru-321*), accumulated a strongly acidic compound (**2**), which was labeled with [U-^{14}C]- or [*carboxy*-^{14}C]arginine, but not with [*guanidino*-^{14}C]arginine as the precursor (Fig. 2*C*). Glutamate and succinate, both labeled, were found after acid hydrolysis of purified compound **2** (Fig. 2*D*). Synthetic *N*-succinylglutamate had the same retention time in HPLC as compound **2** (Fig. 2*C*). These results are consistent with compound **2** being *N*-succinyl-L-glutamate. Aminooxyacetate, an inhibitor of pyridoxal phosphate-dependent enzymes (17), at 10 mM strongly inhibited growth of strain PAO1. Whole PAO1 cells accumulated several labeled products from [U-^{14}C]arginine in the presence of aminooxyacetate (Fig. 2*E*); one product (**3**), which was not formed with [*guanidino*-^{14}C]arginine, yielded labeled ornithine upon hydrolysis with 6 M HCl (Fig. 2*F*). Synthetic N^2-succinylornithine behaved like compound **3** in HPLC (Fig. 2*E*). Strains PAO982 and PAO6150 excreted compound **3** as a minor product in the stationary phase of growth. In HVE at pH 2 and in paper chromatography, the excreted compound **3** had the same migration as synthetic N^2-succinylornithine and gave a positive ninhydrin reaction.

From these experiments N^2-succinylarginine, N^2-succinylornithine, and *N*-succinylglutamate were inferred to be intermediates in a newly discovered pathway (Fig. 3), which was confirmed by subsequent assays for all enzymes involved.

Enzymatic Conversion of Arginine to Glutamate and Succinate. Enzyme reactions leading from arginine to N^2-succinylarginine and subsequently to N^2-succinylornithine have been identified in *P. cepacia* (11). The same reactions were found to occur in crude extracts from *P. aeruginosa*. The arginine succinyltransferase activity (reaction A in Fig. 3) could be followed by the disappearance of succinyl-CoA in the presence of L-arginine. Acetyl-CoA was inactive as a substrate. N^2-Succinylarginine was hydrolyzed to N^2-succinylornithine (reaction B in Fig. 3) in extracts from the wild-type or from an *arcA arcB* mutant (6), indicating that the N^2-succinylarginine dihydrolase is different from arginine

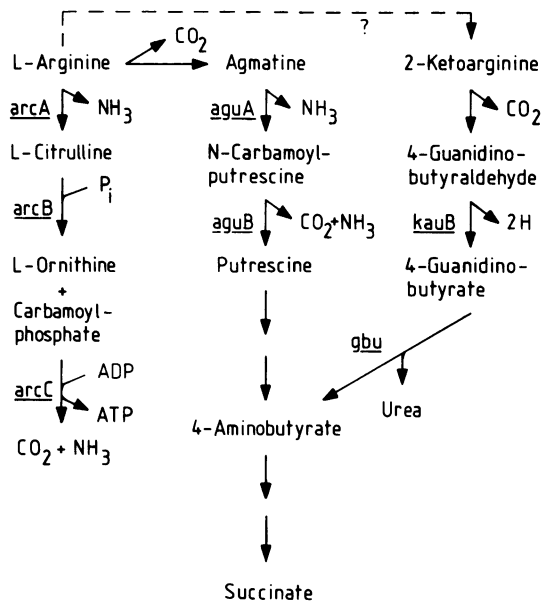

FIG. 1. Arginine catabolic pathways previously established in *P. aeruginosa*. Key enzymes and corresponding genes are as follows: arginine deiminase (EC 3.5.3.6), *arcA*; catabolic ornithine carbamoyltransferase (EC 2.1.3.3), *arcB*; carbamate kinase (EC 2.7.2.2), *arcC*; agmatine deiminase (EC 3.5.3.12), *aguA*; *N*-carbamoylputrescine hydrolase (EC 3.5.3.-), *aguB*; 4-guanidinobutyraldehyde dehydrogenase (EC 1.2.1.-), *kauB*; guanidinobutyrase (EC 3.5.3.7), *gbu*. The arginine decarboxylase (EC 4.1.1.19) reaction leading from arginine to agmatine has been measured but no mutants are available (5). An enzyme converting arginine to 2-ketoarginine has not been measured in *P. aeruginosa* but exists in *P. putida* (7).

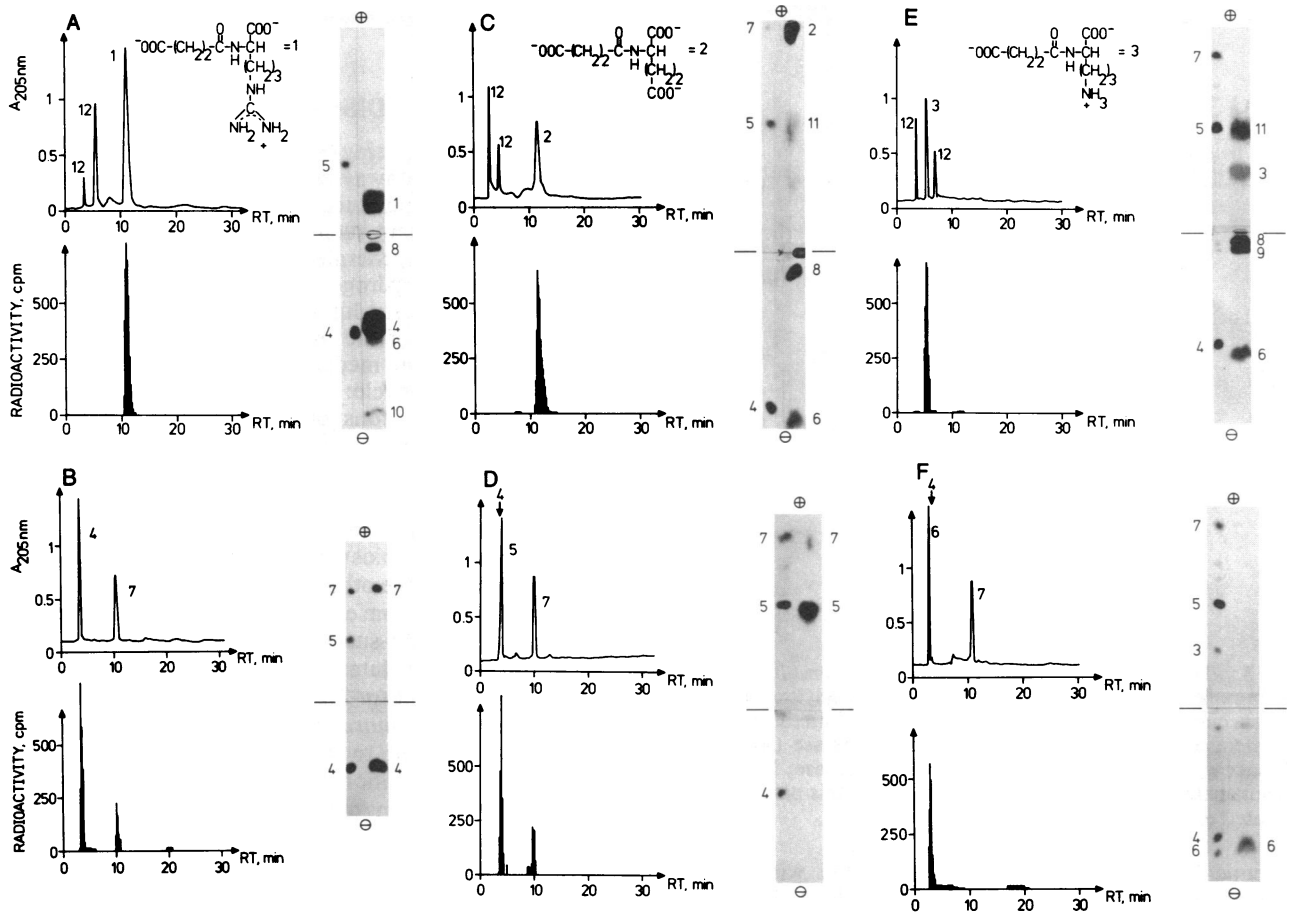

FIG. 2. Detection and identification of succinylated arginine catabolites in *P. aeruginosa*. Resting cells were incubated with [U-^{14}C]arginine for 30–60 min. Products were extracted, separated by HVE at pH 6.4, and revealed by autoradiography. The start (—), the anode (⊕), and the cathode (⊖) are indicated. The left lane contains standards, and the right lane contains the extraction products. The following cells were used: PAO982 (*A*), PAO957 (*C*), and PAO1 treated with 10 mM aminooxyacetate (*E*). The products **1**, **2**, and **3** were eluted from the paper strips shown in *A*, *C*, and *E*, respectively. Product **1** was mixed with synthetic N^2-succinylarginine, **2** was mixed with synthetic *N*-succinylglutamate, and **3** was mixed with N^2-succinylornithine. Then, **1**, **2**, and **3** were subjected to HPLC (details to be described elsewhere) as shown in *A*, *C*, and *E*, respectively. Fractions of 0.2 ml were collected and assayed for ^{14}C radioactivity (by liquid scintillation counting, lower graph) and for absorbance at 205 nm (in arbitrary units, upper graph). RT, retention time. The labeled products **1**, **2**, and **3** were hydrolyzed with 6 M HCl (120°C, 3 hr) and analyzed by HVE and HPLC as shown in *B*, *D*, and *F*, respectively. Products and standards are as follows: **1**, N^2-succinylarginine; **2**, *N*-succinylglutamate; **3**, N^2-succinylornithine; **4**, arginine; **5**, glutamate; **6**, ornithine; **7**, succinate; **8**, citrulline; **9**, 4-aminobutyrate; **10**, agmatine; **11**, unidentified product; **12**, impurities eluted from paper.

deiminase and catabolic ornithine carbamoyltransferase (Fig. 1). N^2-Succinylcitrulline, whose formation from N^2-succinylarginine could be detected by the Archibald color reagent (18), was not a substrate for the dihydrolase but might be an intermediate or byproduct of the reaction.

It has been suggested that the fourth enzyme of arginine biosynthesis, AcOAT, is involved in arginine catabolism because the enzyme is induced by arginine (14) and the AcOAT-negative mutant PAO954 (*aru-292*) grows very poorly on arginine (9). (The *aru-292* marker was formerly designated *argD17* (9) because of an AcOAT involvement in arginine biosynthesis; however, PAO954 is not auxotrophic for arginine.) We now found that the purified AcOAT efficiently utilized N^2-succinyl-L-ornithine as a substrate: the apparent K_m was 1.4 mM (at 15 mM 2-ketoglutarate and pH 9.0), compared to an apparent K_m of 1.1 mM for N^2-acetyl-L-ornithine (14). The specific activities with N^2-succinyl-L-ornithine and N^2-acetyl-L-ornithine were similar and ≈12 times higher than those with L-ornithine as the substrate. These results provide strong support for transamination of N^2-succinylornithine being an intermediate reaction in arginine catabolism (reaction C in Fig. 3). L-Ornithine is unlikely to be a major product in aerobic arginine breakdown (6).

N^2-Succinylglutamate semialdehyde dehydrogenase was measured in a coupled assay with NAD, N^2-succinylornithine, 2-ketoglutarate, purified AcOAT, and crude extract; *N*-succinylglutamate was split enzymatically into glutamate and succinate (reactions D and E in Fig. 3). No evidence was obtained for a succinylarginase or a transsuccinylase (*N*-succinylglutamate + arginine ⇌ N^2-succinylarginine + glutamate; analogous to the arginine biosynthetic acetylornithine transferase reaction).

Induction of the Succinylarginine Pathway and Enzymes Affected in *aru* Mutants. The enzymes of the entire pathway (Fig. 3) were induced by arginine and subject to catabolite repression by succinate (Table 1). The mutants excreting N^2-succinylarginine, PAO982 and PAO6150, had strongly reduced N^2-succinylarginine dihydrolase activities. The strain accumulating *N*-succinylglutamate, PAO957, was blocked in the *N*-succinylglutamate desuccinylase (Table 1). These enzyme defects account for the accumulation products of the mutants. The AcOAT-negative strain PAO954 had very low levels of all enzymes (Table 1), was not sensitive to arginine, and might be a regulatory mutant.

Contribution of Other Arginine Catabolic Pathways to Arginine Utilization in *P. aeruginosa*. The presence of several arginine catabolic pathways in *P. aeruginosa* complicates the

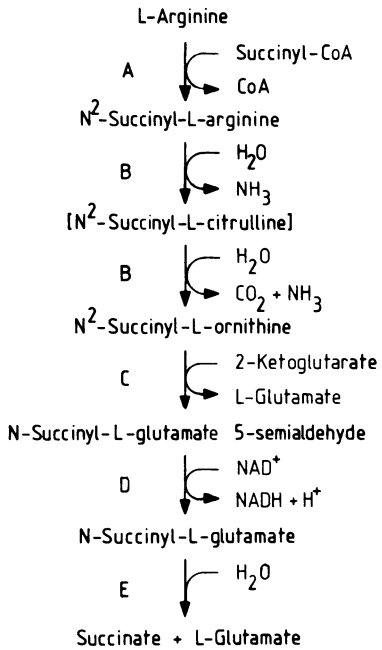

FIG. 3. The succinyltransferase pathway of *P. aeruginosa*. The following enzymes are involved: A, arginine N^2-succinyltransferase; B, N^2-succinylarginine dihydrolase; C, N^2-succinylornithine 5-aminotransferase = N^2-acetylornithine 5-aminotransferase (AcOAT); D, *N*-succinylglutamate 5-semialdehyde dehydrogenase; E, *N*-succinylglutamate desuccinylase. Mutations affecting this pathway are designated *aru* (arginine utilization).

biochemical analysis. Resting cells incubated with [^{14}C]-arginine always produced citrulline and ornithine (Fig. 2 *A*, *C*, and *E*; data for the wild-type PAO1 not shown), which were formed via the arginine deiminase pathway (Fig. 1), and small amounts of agmatine (Fig. 2*A*). In some experiments, traces of putrescine were detected by HVE (not shown). In the accumulated intermediates N^2-succinylarginine (from PAO982) and *N*-succinylglutamate (from PAO957), both the amino acid and succinate were labeled (Fig. 2 *B* and *D*). This means that the ^{14}C label was transferred from arginine to succinyl-CoA, either via the succinyltransferase pathway itself (the *aru* mutations in PAO982 and PAO957 might be leaky), or via one of the other pathways (this seems more likely). N^2-Succinylornithine, which accumulated in the presence of aminooxyacetate, was labeled only in the ornithine but not in the succinate moiety (Fig. 2*F*); the inhibitor also acts on arginine decarboxylase and 4-aminobutyrate aminotransferase (Fig. 1). Since strain PAO954, which is noninducible for the succinyltransferase pathway, grows poorly on arginine as the carbon source, the other catabolic pathways appear to provide only limiting amounts of central metabolites. However, all *aru* mutants could still utilize arginine as a nitrogen source.

DISCUSSION

At first it seems surprising that *P. aeruginosa* should use an arginine catabolic pathway in which the first step requires an energy-rich compound, succinyl-CoA, when other catabolic pathways exist without such a requirement. However, succinyl-CoA can be regenerated from glutamate via the catabolic glutamate dehydrogenase, an arginine-inducible enzyme (19), and 2-ketoglutarate dehydrogenase (20). The arginine sensitivity of the mutants PAO957, PAO982, and PAO6150 in glutamate medium might be due to a disruption of the citric acid cycle: arginine induces arginine succinyltransferase and consumes stoichiometric amounts of succinyl-CoA, which cannot be regenerated because of the *aru* mutations. Thus, growth inhibition by arginine might result from a depletion of the succinyl-CoA pool.

One enzyme of the succinyltransferase route, AcOAT, is shared with arginine biosynthesis and ornithine catabolism (9, 13, 14), whereas the other enzymes appear to be pathway specific and distinct from enzymes previously described. The mutations affecting N^2-succinylarginine dihydrolase (*aru-77*, *aru-247*), *N*-succinylglutamate desuccinylase (*aru-321*), or the entire pathway (*aru-292*) are clustered in the 90-min region of the *P. aeruginosa* chromosome (D.H., unpublished results) and unlinked to other loci involved in arginine catabolism (4, 6). Additional *aru* mutants with similar properties are available in our and Clarke's (10) collection. *N*-Acetylglutamate deacetylase, which enables *P. aeruginosa* to grow on *N*-acetylglutamate, is not induced by arginine (21) and hence is likely to be different from *N*-succinylglutamate desuccinylase.

N^2-Succinylarginine has previously been found in twigs of wintering pear trees where it serves as a storage form of nitrogen (15). *N*-Succinylglutamate accumulates in *Bacillus* during the initial phase of sporulation (16). It is not known how these compounds are synthesized and degraded. The succinyltransferase pathway has been demonstrated so far in *P. aeruginosa*, *P. cepacia* (11), *P. putida*, *P. fluorescens*, *P. mendocina*, *Aeromonas formicans*, and *Klebsiella aerogenes* (22).

In *P. aeruginosa*, each arginine catabolic pathway seems to have a different primary function. The arginine deiminase pathway exploits arginine as an energy source when respiration is restricted (4). The arginine decarboxylase pathway allows agmatine utilization and polyamine synthesis from arginine (5). The arginine oxidase pathway enables the bacterium to grow on 2-ketoarginine and 4-guanidinobutyrate (8). The succinyltransferase pathway uses arginine as an excellent carbon source under aerobic conditions.

Table 1. Regulation of the succinyltransferase pathway enzymes and defects of *aru* mutants

Strain	Relevant mutation	Growth medium	Specific activity, units per mg of protein				
			Enzyme A	Enzyme B	Enzyme C	Enzyme D	Enzyme E
PAO1	—	Succinate/NH_4^+	<1.0	0.2	1.5	ND	0.9
		Arginine	28.4	26.6	41.0	ND	12.4
		Arginine/succinate	6.1	3.1	6.1	ND	0.9
		Arginine/glutamate	14.0	15.5	25.3	4.2	4.3
PAO954	*aru-292*	Arginine/glutamate	<1.0	1.3	0.6	<0.5	3.3
PAO957	*aru-321*	Arginine/glutamate	49.0	8.3	42.7	1.5	<0.2
PAO982	*aru-247*	Arginine/glutamate	13.4	3.7	21.0	4.7	3.8
PAO6150	*aru-77*	Arginine/glutamate	27.8	<0.2	34.3	ND	3.7

Enzyme A, arginine N^2-succinyltransferase; enzyme B, N^2-succinylarginine dihydrolase; enzyme C, N^2-succinylornithine 5-aminotransferase; enzyme D, *N*-succinylglutamate semialdehyde dehydrogenase; enzyme E, *N*-succinylglutamate desuccinylase. Enzyme C was measured with N^2-succinylornithine as a substrate. ND, not determined.

We thank Hugo Grossenbacher for help with HPLC, Annick Mercenier for uptake data, and Marta Kley and Patrizia Eppler for technical assistance. This work was supported by the Swiss National Foundation for Scientific Research (Projects 3.050-0.81, 3.041-0.81, and 3.620-0.84) and by the Fonds de la Recherche Fondamentale Collective (Projet 2.4521.83). V.S. is a Research Associate of the Fonds National de la Recherche Scientifique (Belgium).

1. Clarke, P. H. & Ornston, L. N. (1975) in *Genetics and Biochemistry of Pseudomonas*, eds. Clarke, P. H. & Richmond, M. H. (Wiley, London), pp. 191–340.
2. Haas, D. (1983) *Experientia* **39,** 1199–1213.
3. Mercenier, A., Simon, J.-P., Vander Wauven, C., Haas, D. & Stalon, V. (1980) *J. Bacteriol.* **144,** 159–163.
4. Vander Wauven, C., Piérard, A., Kley-Raymann, M. & Haas, D. (1984) *J. Bacteriol.* **160,** 928–934.
5. Mercenier, A., Simon, J.-P., Haas, D. & Stalon, V. (1980) *J. Gen. Microbiol.* **116,** 381–389.
6. Haas, D., Matsumoto, H., Moretti, P., Stalon, V. & Mercenier, A. (1984) *Mol. Gen. Genet.* **193,** 437–444.
7. Vanderbilt, A. S., Gaby, N. S. & Rodwell, V. W. (1975) *J. Biol. Chem.* **250,** 5322–5329.
8. Stalon, V. & Mercenier, A. (1984) *J. Gen. Microbiol.* **130,** 69–76.
9. Voellmy, R. & Leisinger, T. (1976) *J. Bacteriol.* **128,** 722–729.
10. Rahman, M., Laverack, P. D. & Clarke, P. H. (1980) *J. Gen. Microbiol.* **116,** 371–380.
11. Vander Wauven, C. & Stalon, V. (1985) *J. Bacteriol.* **164,** 882–886.
12. Holloway, B. W. (1969) *Bacteriol. Rev.* **33,** 419–443.
13. Früh, R., Haas, D. & Leisinger, T. (1985) *Arch. Microbiol.* **141,** 170–176.
14. Voellmy, R. & Leisinger, T. (1975) *J. Bacteriol.* **122,** 799–809.
15. Kasai, T. & Sakamura, S. (1984) *Phytochemistry* **23,** 19–22.
16. Aubert, J. P., Millet, J., Pineau, E. & Milhaud, G. (1961) *Biochim. Biophys. Acta* **51,** 529–537.
17. Wilson, O. H. & Holden, J. T. (1969) *J. Biol. Chem.* **244,** 2737–2742.
18. Archibald, R. M. (1944) *J. Biol. Chem.* **156,** 121–141.
19. Früh, R. (1984) Dissertation (Eidgenössische Technische Hochschule, Zürich).
20. Mitchell, C. G. & Dawes, E. A. (1982) *J. Gen. Microbiol.* **128,** 49–59.
21. Früh, H. & Leisinger, T. (1981) *J. Gen. Microbiol.* **125,** 1–10.
22. Stalon, V. (1985) in *Evolution of Prokaryotes*, eds. Schleifer, K. H. & Stackebrandt, E. (Academic, London), pp. 277–308.