The Fourth Arginine Catabolic Pathway of *Pseudomonas aeruginosa*

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D-Arginine dehydrogenase activity was discovered in *Pseudomonas aeruginosa*. This enzyme was inducible by its substrate, D-arginine, as well as by its product, 2-ketoarginine, but not by L-arginine. The enzyme activity was measured *in vitro*, in the presence of artificial electron acceptors (phenazine methosulphate and iodonitrotetrazolium chloride). 2-Ketoarginine was catabolized further to 4-guanidinobutyraldehyde, 4-guanidinobutyrate and 4-aminobutyrate. Two enzymes involved, 4-guanidinobutyraldehyde dehydrogenase and guanidinobutyrase, were inducible by 2-ketoarginine; the latter enzyme was also strongly induced by 4-guanidinobutyrate. An arginine racemase activity was detected by an *in vivo* test. D-Arginine had the potential to be catabolized via the D-arginine dehydrogenase pathway and, after racemization, via the three L-arginine catabolic pathways previously demonstrated in *P. aeruginosa*. In mutants blocked in the L-arginine succinyltransferase pathway, but not in the wild-type, L-arginine was channelled partially into the D-arginine dehydrogenase pathway. Mutations in the *kauB* locus abolished growth of *P. aeruginosa* on 2-ketoarginine, agmatine and putrescine, and led to loss of 4-guanidinobutyraldehyde dehydrogenase and 4-aminobutyraldehyde dehydrogenase activities. Thus, these two activities appear to be due to one enzyme in *P. aeruginosa*. The *kauB* locus was mapped on the chromosome between *lysA* and *argB* and was not linked to known genes involved in the three L-arginine catabolic pathways. The existence of four arginine catabolic pathways illustrates the metabolic versatility of *P. aeruginosa*.

INTRODUCTION

Three arginine catabolic pathways are known in *Pseudomonas aeruginosa* PAO (Fig. 1). (I) The arginine deiminase pathway converts L-arginine to L-ornithine, with the formation of ATP. The enzymes of this pathway are inducible by energy depletion and by the presence of L-arginine. Mutants which are defective in one of the three enzymes involved (*arcA*, *B*, *C* mutants) cannot grow anaerobically with L-arginine as the energy source (Mercenier *et al.*, 1980*b*, 1982; Vander Wauven *et al.*, 1984). (II) The arginine succinyltransferase pathway is used for aerobic L-arginine degradation. The products are L-glutamate and succinate, and L-arginine induces all enzymes of this route. Mutants blocked in the arginine succinyltransferase pathway (*aru* mutants) grow very poorly if at all on L-arginine as the only carbon and nitrogen source (Jann *et al.*, 1986). (III) The arginine decarboxylase pathway produces putrescine, which can be converted to spermidine, and permits good growth on agmatine and putrescine. The first enzyme, L-arginine decarboxylase (enzyme 12 in Fig. 1), is induced by L-arginine. The synthesis of agmatine deiminase and *N*-carbamoylputrescine hydrolase (enzymes 13 and 14 in Fig. 1) is switched on by agmatine. Putrescine is further catabolized to succinate and ammonia (Jakoby & Fredericks, 1959; Voellmy & Leisinger, 1976; Mercenier *et al.*, 1980*a*). Mutants blocked in the

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Abbreviations: INT, iodonitrotetrazolium chloride; PMS, phenazine methosulphate.

Fig. 1. Known arginine catabolic pathways in *P. aeruginosa*. I, Arginine deiminase pathway; II, arginine succinyltransferase pathway; III, arginine decarboxylase pathway. Numbers refer to enzymes, genotype symbols to *P. aeruginosa* mutants obtained previously (Stalon & Mercenier, 1984; Vander Wauven *et al.*, 1984; Haas *et al.*, 1984; Jann *et al.*, 1986; this study).

conversion of agmatine to putrescine (*aguA*, *B* mutants) cannot grow on agmatine but grow well on L-arginine (Haas *et al.*, 1984).

In *P. putida* P2, L-arginine is catabolized to 2-ketoarginine, 4-guanidinobutyraldehyde, 4-guanidinobutyrate and 4-aminobutyrate (Miller & Rodwell, 1971). The first enzyme of this pathway, L-arginine oxidase (Miller & Rodwell, 1971), could not be demonstrated in *P. aeruginosa* (our unpublished results). However, 2-ketoarginine and 4-guanidinobutyrate are good carbon and nitrogen sources and inducers of guanidinobutyrase (= guanidinobutyrate ureohydrolase) in this organism (Stalon & Mercenier, 1984). In *aru* mutants of *P. aeruginosa*, but not in the wild-type, L-arginine induces guanidinobutyrase and gives rise to urea excretion (Vander Wauven, 1985). This suggests that under certain conditions L-arginine can be degraded via 4-guanidinobutyrate in *P. aeruginosa*. Here we demonstrate the existence of this pathway and give evidence for D-arginine dehydrogenase being a key enzyme. We also show that a gene (*kauB*) involved in this pathway is unlinked to the structural genes *arcABC*, *aruABCDE* and *aguAB* of the other three pathways. The *kauB* gene product, an aldehyde dehydrogenase, appears to participate in both the arginine decarboxylase and the D-arginine dehydrogenase pathways.

METHODS

Bacterial strains, plasmids and bacteriophages. *P. aeruginosa* strains are listed in Table 1. Plasmid FP2 (Holloway, 1969) and the generalized transducing phages E79tv-2 (Morgan, 1979) and F116L (Krishnapillai, 1971) were used in genetic manipulations as previously described (Stanisich & Holloway, 1972; Haas *et al.*, 1977).

Chemicals. 2-Ketoarginine (2-oxo-5-guanidinovalerate) was synthesized by oxidative deamination of L-arginine with *Crotalus adamanteus* L-amino acid oxidase (Sigma) in the presence of catalase (Boehringer) as described by Meister (1952, 1954). The product was purified by ultrafiltration through an Amicon PM-10 membrane and chromatography on Dowex 50 x8 (H⁺-form); elution with 0.1 M-HCl gave two peaks which corresponded to 2-ketoarginine and its cyclic form (Cooper & Meister, 1978).

4-Aminobutyraldehyde was prepared by acid hydrolysis of 4-aminobutyraldehyde diethylacetal (Aldrich) as described by Matsuda & Suzuki (1984). The acid solution of the aldehyde, which could be stored at 4 °C for a few days (Jakoby & Fredericks, 1959), was neutralized with NaOH immediately before use.

4-Guanidinobutyraldehyde was synthesized by guanidinylation of 4-aminobutyraldehyde diethylacetal with O-methylisourea (Hasse & Schührer, 1962) followed by acid hydrolysis as above.

D-Arginine was obtained from Fluka, agmatine from Aldrich, aminooxyacetic acid from Sigma and 4-guanidinobutyric acid from Senn Chemicals.

Media and growth conditions. Complete media and minimal medium E have been described before (Stanisich & Holloway, 1972; Haas *et al.*, 1977). In minimal medium P (Leisinger *et al.*, 1972) the carbon and nitrogen sources were: L-glutamate (20 mM), L-arginine (20 mM), D-arginine (20 mM), 2-ketoarginine (5 or 20 mM), 4-guanidinobutyrate (5 or 20 mM), 4-aminobutyrate (20 mM), agmatine (10 mM) or putrescine (20 mM). Minimal medium P was supplemented with 0.1 mM-MnSO₄ when guanidinobutyrase was to be measured (Chou & Rodwell, 1972). Liquid cultures (200 ml) were grown in baffled 1 litre Erlenmeyer flasks with good aeration (Haas *et al.*, 1984).

Cell extracts. These were prepared at 0–4 °C by sonication (Voellmy & Leisinger, 1975) of exponential-phase cells in 0.1 M potassium phosphate buffer, pH 7.0, containing 2 mM-2-mercaptoethanol and 0.2 mM-MnSO₄. (Mn²⁺ was included to stabilize guanidinobutyrase; Chou & Rodwell, 1972.) Cell debris was removed by centrifugation at 40000 g for 45 min. Extracts were kept at 0 °C and used within 1 d for enzyme assays. Protein concentrations were determined by the Lowry method.

Enzyme assays. All enzymes were assayed at 37 °C. One unit (u) is defined as the amount of enzyme that converts 1 nmol substrate min⁻¹.

D-Arginine dehydrogenase [D-arginine:(acceptor) oxidoreductase (deaminating) EC 1.4.99.-]. The assay mixture contained, in a final volume of 1.0 ml: 0.1 M-Tris/HCl, pH 8.7; 20 mM-D-arginine; 10 mM-KCN; 0.2 mM-FAD (could be omitted without loss of activity); 0.8 mM-iodonitrotetrazolium chloride (INT); 0.8 mM-phenazine methosulphate (PMS); dialysed crude extract (0.02–1.0 mg protein). The reaction was started by the addition of INT + PMS, followed by extract 15 s later. After incubation for 2–4 min, the reaction was stopped by the addition of 0.1 ml 4 M-HCl. After a 1:10 dilution in water, the A₅₀₀ was measured; an absorption coefficient of 11.5 mM⁻¹ cm⁻¹ for reduced INT was used for calculation (Meile & Leisinger, 1982). Blanks did not contain D-arginine. Enzyme activity was a linear function of incubation time up to 4 min and enzyme concentration up to about 120 u ml⁻¹.

Table 1. *P. aeruginosa* strains

Genotype symbols relevant to this work are explained in Figs 1 and 2; other genotype designations are according to Holloway (1986).

Strain	Genotype	Reference or construction
PAO1	Wild-type	Holloway (1969)
PAO303	<i>argB18</i>	Haas <i>et al.</i> (1977)
PAO315	<i>argG9</i>	Haas <i>et al.</i> (1977)
PAO332	<i>argB18 lysA60</i>	Haas <i>et al.</i> (1977)
PAO362	<i>argH32</i>	Haas <i>et al.</i> (1977)
PAO483	<i>pyrE70</i>	Isaac & Holloway (1968)
PAO974	<i>aru-326</i>	Collection of T. Leisinger
PAO983	<i>arcA1 arcB9 aguA9001 gbu-9005 strA nalA</i>	Str ^r Arc ⁻ Agu ⁻ Nal ^r recombinant of PAO987(FP2 ⁺) × PAO4156 (made <i>nalA</i>)*
PAO987	<i>arcA1 arcB9 aguA9001 his16001 strA</i>	Haas <i>et al.</i> (1984)
PAO993	<i>arcA1 arcB9 aguA9001 strA</i>	Haas <i>et al.</i> (1984)
PAO1017	<i>aru-326 kauB6097::Tn5-751</i>	Km ^r Tp ^r Kau ⁻ transductant of E79 <i>tw</i> -2.PAO6097 × PAO974
PAO1021	<i>aru-326 gbu-9005</i>	Pru ⁺ Aru ⁻ recombinant of PAO974 (made <i>thr-6093::Tn5-751</i> ; FP2 ⁺) × PAO4156 (made <i>pru-6087::Tn5-751</i>)†
PAO4155	<i>kauB9001</i> ‡	Kau ⁻ mutant derived from PAO1 by mutagenesis with <i>N</i> -methyl- <i>N'</i> -nitro- <i>N</i> -nitrosoguanidine
PAO4156	<i>gbu-9005</i>	Haas <i>et al.</i> (1984)
PAO6049	<i>met-9011 amiE200 strA</i>	Rella <i>et al.</i> (1985)
PAO6087	<i>met-9011 amiE200 strA pru-6087::Tn5-751</i>	Proline utilization-negative derivative of PAO6049
PAO6093	<i>met-9011 amiE200 strA thr-6093::Tn5-751</i>	Rella <i>et al.</i> (1985)
PAO6097	<i>met-9011 amiE200 strA kauB6097::Tn5-751</i>	2-Ketoarginine utilization-negative Tn5-751 insertion derivatives of PAO6049
PAO6100	<i>met-9011 amiE200 strA kauB6100::Tn5-751</i>	
PAO6206	<i>met-9011 amiE200 strA kauB6206::Tn5-751</i>	
PAO6205	<i>arcA1 arcB9 aguA9001 kauB6097::Tn5-751 strA</i>	
		Km ^r Tp ^r Kau ⁻ transductant of E79 <i>tw</i> -2.PAO6097 × PAO993

* Selection for *strA* allowed the introduction of *arcAB* and *aguA*, which are linked to *strA* in FP2 conjugation (Haas *et al.*, 1984).

† Selection for *pru*⁺ permitted the introduction of *aru-326*, which is located in the *oruI* cluster and linked to *pru* in conjugation (Soldati *et al.*, 1982).

‡ The *kauB9001* mutation was formerly designated *ptu-9001* (Holloway, 1986).

4-Aminobutyraldehyde/4-guanidinobutyraldehyde dehydrogenase (EC 1.2.1.19/EC 1.2.1.-). The reaction mixture contained, in 1.0 ml: 0.1 M-potassium phosphate buffer, pH 7.0, 2.5 mM-4-aminobutyraldehyde and 2.5 mM-NAD⁺; or 0.1 M-glycine/NaOH buffer, pH 9.0, 2.5 mM-4-guanidinobutyraldehyde and 2.5 mM-NAD⁺. Similar conditions were used by Jakoby & Fredericks (1959) and Vanderbilt *et al.* (1975). The reaction was initiated by the addition of 10–50 µg protein of crude extracts and the *A*₃₄₀ was measured.

Guanidinobutyrase (EC 3.5.3.7) was assayed under the conditions given by Chou & Rodwell (1972). The urea formed was determined by a modification of the method of Archibald (1945): to a 1.0 ml sample were added 0.5 ml of acid reagent (conc. H₂SO₄/conc. H₃PO₄/H₂O, 1:3:1, by vol.) and 40 µl 4% (w/v) α-isonitrosopropiophenone (in ethanol). After vigorous mixing and boiling for 1 h in the dark, the *A*₅₄₅ was measured.

4-Aminobutyrate aminotransferase = putrescine aminotransferase (EC 2.6.1.19) was assayed according to Voellmy & Leisinger (1976).

RESULTS

Assay of D-arginine dehydrogenase

P. aeruginosa PAO grew on D-arginine as the only carbon and nitrogen source, albeit more slowly (doubling time about 2 h) than on L-arginine (doubling time 1.3 h; Haas *et al.*, 1984). Cells grown on D-arginine contained D-arginine dehydrogenase activity (Table 2). The artificial

Table 2. *Sequential induction of the D-arginine dehydrogenase pathway enzymes*

P. aeruginosa PAO1 was grown aerobically in minimal medium P with the growth substrates listed as sole carbon and nitrogen sources. Enzyme activities were determined in crude extracts.

Growth substrate	Specific enzyme activity [u (mg protein) ⁻¹]			
	D-Arginine dehydrogenase*	4-Guanidino-butyraldehyde dehydrogenase†	4-Amino-butyraldehyde dehydrogenase†	Guanidino-butyrase†
L-Arginine	10	106	29	38
D-Arginine	520	127	38	495
D-Arginine + L-glutamate	1650	NT	NT	486
2-Ketoarginine	387	524	86	3310
4-Guanidinobutyrate	≤ 5	192	45	4720
L-Glutamate	≤ 5	49	15	6
Putrescine	NT	592	92	12
Agmatine	NT	537	122	≤ 5

NT, Not tested.

* Values from a single experiment.

† Mean values from duplicate experiments.

electron acceptors PMS and INT were required for enzyme activity *in vitro* (see Methods). Added FAD, FMN, NAD⁺ or NADP⁺ did not stimulate the reaction. 2-Ketoarginine was tentatively identified by HPLC as a reaction product in crude extracts incubated with D-arginine (Fig. 2). Extracts from D-arginine-grown cells had low dehydrogenase activities towards L-arginine (≤ 1%), L-proline (≤ 2%), D-alanine (≤ 2%) and D-valine (11%) as substrates, 100% being defined as the activity obtained with D-arginine. L-Arginine (Table 2), L-proline, D-alanine and D-valine were not inducers of D-arginine dehydrogenase.

Evidence for an arginine racemase

Arginine auxotrophs of *P. aeruginosa* (e.g. *argB*, *argG* or *argH* mutants; Table 1) were able to utilize D-arginine as a supplement on minimal medium E, and the growth yield was the same as with an L-arginine supplement. This result indicates the presence of an arginine racemase in *P. aeruginosa*. Previously, such an enzyme had been characterized in *P. graveolens* (Yorifuji *et al.*, 1971). Attempts to demonstrate the *P. aeruginosa* arginine racemase *in vitro* were unsuccessful, presumably because both D-arginine and L-arginine were catabolized instantly in extracts.

The arginine auxotrophs could not be complemented by 2-ketoarginine in the presence of L-glutamate, L-aspartate or L-alanine. Thus, there was no evidence for an L-arginine 2-aminotransferase. Furthermore, no such aminotransferase activity could be measured in *P. aeruginosa* extracts under conditions that allow enzymic transamination of L-ornithine, L-lysine, putrescine (Voellmy & Leisinger, 1976) or *N*²-succinylornithine (Jann *et al.*, 1986). Arginine racemase and D-arginine dehydrogenase link L-arginine to the 2-ketoarginine catabolic pathway, which is known to exist in *P. aeruginosa* (Stalon & Mercenier, 1984). The operation of the resulting L-arginine catabolic pathway – pathway IV in *P. aeruginosa* (Fig. 3) – is demonstrated below.

Sequential induction of the D-arginine dehydrogenase pathway

The wild-type strain PAO1 was grown on L-arginine and several intermediates of pathway IV. The specific activities of D-arginine dehydrogenase, 4-guanidinobutyraldehyde dehydrogenase and guanidinobutyrase were determined (Table 2). D-Arginine and 2-ketoarginine were inducers of D-arginine dehydrogenase. 2-Ketoarginine induced 4-guanidinobutyraldehyde dehydrogenase. Guanidinobutyrase was induced to high levels by 4-guanidinobutyrate as previously noted (Stalon & Mercenier, 1984), whereas 2-ketoarginine was weak inducer. This was shown by the low level of guanidinobutyrase [190 u (mg protein)⁻¹] in the 4-guanidinobutyraldehyde-dehydrogenase-negative mutant PAO6097 grown in 2-ketoarginine/

Fig. 2. 2-Ketoarginine as a reaction product of the D-arginine dehydrogenase reaction *in vitro*. D-Arginine was incubated with crude extract in the assay mixture described (Methods) for 15 min. Reaction products were separated by HPLC on a Nucleosil C₁₈ column (4.6 × 40 mm; Macherey-Nagel); elution at 1 ml min⁻¹ was done with 0.1 M-potassium phosphate, pH 2.0. This system is useful for the separation of arginine derivatives (Jann *et al.*, 1986). (a) Complete mixture; (b) mixture without D-arginine. 1, Front including D-arginine; 2, 2-ketoarginine, according to the same retention times of authentic reference products. A_{205} values are given in arbitrary units.

L-glutamate medium; the specific activity for strain PAO1 cultivated in the same medium was 1180 u (mg protein)⁻¹.

Characterization of *kauB* mutants

Three *P. aeruginosa* mutants (PAO6097, PAO6100 and PAO6206) that were unable to grow on 2-ketoarginine as the carbon and nitrogen source were found among about 5000 Tn5-751 insertion mutants of strain PAO6049 (Rella *et al.*, 1985). These mutants grew on L-arginine, D-arginine, 4-guanidinobutyrate or 4-aminobutyrate, but could not utilize 2-ketoarginine or – unexpectedly – putrescine and agmatine. An additional, chemically induced mutant (PAO4155), which had been isolated as a putrescine-non-utilizer, had the same phenotype. The wild-type strain PAO1 and the four mutants were grown on 4-aminobutyrate and assayed for 4-guanidinobutyraldehyde dehydrogenase activity. Strain PAO1 had 445 u (mg protein)⁻¹, whereas negligible activity [≤ 15 u (mg protein)⁻¹] was detectable in the mutants. This defect appears to cause the inability of the mutants (designated *kauB*) to grow on 2-ketoarginine. It also appears likely that the conversion of 2-ketoarginine to 4-guanidinobutyraldehyde occurs in *P. aeruginosa* in the same way as described for *P. putida*, i.e. by decarboxylation of 2-ketoarginine (Miller & Rodwell, 1971; Vanderbilt *et al.*, 1975), but we did not measure this decarboxylase.

To determine the enzyme deficiency which caused the lack of growth of *kauB* mutants on putrescine or agmatine, we assayed putrescine aminotransferase and 4-aminobutyraldehyde dehydrogenase (enzymes 15 and 16 in Fig. 1). All mutants were impaired in the latter enzyme [≤ 15 u (mg protein)⁻¹]; the specific activity in the wild-type was 82 u (mg protein)⁻¹. Putrescine aminotransferase was present in the one *kauB* mutant tested, PAO6097. Since *kauB* mutations simultaneously affected 4-guanidinobutyraldehyde and 4-aminobutyraldehyde dehydrogenases we conclude that both activities are due to the same enzyme. Further support for such a

Fig. 3. D-Arginine dehydrogenase pathway (pathway IV) in *P. aeruginosa*. The existence of arginine racemase is deduced from the growth of L-arginine auxotrophs on D-arginine. 2-Ketoarginine decarboxylase has been demonstrated in *P. putida* extracts (Vanderbilt *et al.*, 1975) and most probably also occurs in *P. aeruginosa* (see text). All other enzymes were measured in extracts (this work; Voellmy & Leisinger, 1976; Stalon & Mercenier, 1984; Vander Wauven, 1985). The genotype symbols *kauB* and *gbu* refer to available *P. aeruginosa* mutants.

Fig. 4. Transductional mapping of *kauB*. Transducing phage F116L and strains PAO332, PAO4155 and *kauB*::Tn751 derivatives of PAO303 and PAO483 were used. Values indicate percentage cotransduction; 200 transductants were scored. Arrow-heads point to the unselected markers. Three-factor analysis confirmed the marker order (data not shown).

bifunctional dehydrogenase comes from three experiments. (i) The thermal denaturation of both activities was the same when a crude extract was heated to 50 or 60 °C for 5 min (data not shown). (ii) The 2-ketoarginine/putrescine utilization-negative phenotype did not dissociate in transduction of the *kauB*6097::Tn5-751 marker. (iii). Agmatine, putrescine and 2-ketoarginine induced both dehydrogenase activities to a similar extent (Table 2).

The *kauB* mutations in strains PAO6097, PAO6100, PAO6206 and PAO4155 were closely linked in E79*tv*-2-mediated transduction; crosses between *kauB* mutants gave very low frequencies of *Kau*⁺ transductants, i.e. ≤ 3% of the frequencies obtained with a wild-type donor lysate. The *kauB* locus was mapped by transduction between *lysA* and *argB* (Fig. 4) at 10 min on the revised *P. aeruginosa* chromosome map (O'Hoy & Krishnapillai, 1987).

Table 3. *Properties of aru, kauB and gbu mutants*

Strain	Relevant genotype	Growth on:*				Urea production† (mM)	Specific activity† [u (mg protein) ⁻¹]	
		L-Arginine	D-Arginine	2-Ketoarginine	4-Guanidinobutyrate		4-Guanidino-butyraldehyde dehydrogenase	Guanidino-butyrase
PAO1	(wild-type)	++	+	+	++	≤0.2	72	28
PAO974	<i>aru-326</i>	-	-	+	++	3.0	97	234
PAO1017	<i>aru-326</i>	-	-	-	++	≤0.2	≤15	239
PAO1021	<i>kauB6097::Tn5-751</i>	-	-	-	-	≤0.2	41	≤5
	<i>aru-326</i>	-	-	-	-			
	<i>gbu-9005</i>	++	+	-	-	NT	NT	≤5
PAO4156	<i>gbu-9005</i>	++	+	-	-	NT	≤15	13
PAO6097	<i>kauB6097::Tn5-751</i>	++	+	-	++	NT		

NT, Not tested.

* Minimal medium P with the sole carbon and nitrogen source indicated; ++, growth of single colonies in 1 d; +, growth in 2 d; -, no growth in 2 d. The medium for PAO6097 also contained 1 mM-L-methionine.

† Cells were grown in minimal medium P with 20 mM-L-arginine + 20 mM-L-glutamate. Urea was measured in the culture filtrate after about two generations, with the cell density ranging from 4×10^8 – 2×10^9 cells ml⁻¹; enzyme specific activities were determined in duplicate in crude extracts. Urea was determined as described for the guanidinobutyrase assay.

Channelling of L-arginine into the D-arginine dehydrogenase pathway

The only enzyme that releases urea in arginine catabolism of *P. aeruginosa* is guanidinobutyrase (Figs 1 and 3). Urease is repressed by high NH_4^+ concentration (Janssen *et al.*, 1980) and also by L-glutamate and L-arginine (Früh, 1984) in the growth medium. Therefore, the amount of urea excreted during growth on L-arginine is a rough measure for the operation of the D-arginine dehydrogenase pathway. The wild-type PAO1 did not excrete measurable quantities of urea (Table 3), in agreement with the findings of Vander Wauven (1985). However, when the arginine succinyltransferase pathway was blocked by mutation as in strain PAO974 (Table 3) or in other *aru* mutants (Vander Wauven, 1985), urea was released into the medium after incubation with L-arginine (Table 3) or D-arginine (data not shown). The *aru-326* mutation in strain PAO974 impairs enzymes 5, 7 and 8 (Fig. 1) (V. Stalon, personal communication) and also causes arginine sensitivity of the strain. Two lines of evidence indicate that urea was derived from the guanidino group of L-arginine. (i) Mutations in 4-guanidinobutyraldehyde dehydrogenase (*kauB*) or guanidinobutyrase (*gbu*) abolished urea excretion in derivatives of strain PAO974 (Table 3). (ii) Aminooxyacetate (5 mM), an inhibitor of pyridoxalphosphate-dependent enzymes (Wilson & Holden, 1969), completely inhibited urea production by strain PAO974 in L-arginine/L-glutamate medium. This result is expected if arginine racemase of *P. aeruginosa*, like the enzyme of *P. graveolens* (Yorifuji *et al.*, 1971), requires pyridoxalphosphate for activity.

In *aru-326* strains guanidinobutyrase levels were partially elevated whereas no drastic effect on 4-guanidinobutyraldehyde dehydrogenase could be seen (Table 3). The pattern of enzyme induction in the wild-type (Table 2) and the properties of the *kauB* and *gbu* mutants (Table 3) together indicate that the main function of pathway IV is to allow degradation of 2-ketoarginine, 4-guanidinobutyrate and D-arginine, although the latter compound can also be racemized and catabolized via the three L-arginine catabolic pathways. In *aru* mutants L-arginine catabolism can be channelled partially into pathway IV. When pathways I, III and IV were blocked in strains PAO983 (*arcAB aguA gbu*) or PAO6205 (*arcAB aguA kauB*), good growth on L-arginine was still possible. The doubling time of PAO983 in L-arginine medium was 2 h. This confirms that pathway II is the major aerobic arginine degradation pathway in *P. aeruginosa*.

DISCUSSION

Several types of microbial enzymes have been described which catalyse the conversion of L-arginine to 2-ketoarginine: L-arginine oxidase in *P. putida* (Miller & Rodwell, 1971), L-arginine:2-oxoglutarate transaminase in *Arthrobacter simplex* (Tachiki *et al.*, 1980), and an extracellular, basic L-amino acid deaminase in *Neurospora crassa* (DeBusk & Ogilvie, 1984). None of these types of enzymes could be demonstrated in *P. aeruginosa*. Instead, a D-arginine-inducible, D-arginine dehydrogenase activity was found. In terms of substrate and inducer specificity, this enzyme appears to be distinct from other amino acid dehydrogenases previously reported in *P. aeruginosa*, namely D-valine dehydrogenase, which has a broad substrate spectrum (Marshall & Sokatch, 1968; Manoharan & Jayaraman, 1978), and three more specific enzymes, D-alanine dehydrogenase (Pioli *et al.*, 1976; Waters & Venables, 1986; Magor & Venables, 1987), allohydroxy-D-proline dehydrogenase (Bater *et al.*, 1977; Manoharan & Jayaraman, 1979) and L-proline dehydrogenase (Meile & Leisinger, 1982).

The physiological electron acceptor and the cofactor of D-arginine dehydrogenase have not been determined yet. If molecular oxygen is the acceptor, as in the case of a number of D-amino acid oxidases (Sallach & Fahien, 1969), FAD or FMN might be used as the cofactor and the enzyme would have to be classified as a D-arginine oxidase.

The racemase (epimerase)/D-amino acid dehydrogenase motif occurs in several amino acid catabolic pathways of *Pseudomonas*, e.g. in the degradation of L-lysine (Chang & Adams, 1974; Payton & Chang, 1982), hydroxy-L-proline (Adams, 1970; Manoharan & Jayaraman, 1979), L-alanine and β -alanine (Waters & Venables, 1986). In arginine catabolism of *P. aeruginosa*, the L-arginine succinyltransferase pathway seems to be favoured over the D-arginine dehydrogenase pathway, as indicated by the properties of the *kauB* and *gbu* mutants and the induction patterns of the pathways (Table 2; Yorifuji *et al.*, 1983; Stalon & Mercenier, 1984; Jann *et al.*, 1986).

When the succinyltransferase route is blocked, L-arginine is directed into the D-arginine dehydrogenase pathway. It may seem surprising that the *aru* mutant PAO974 did not grow on D-arginine (Table 3). This can be explained by the high arginine sensitivity of this and other similar *aru* mutants (Jann *et al.*, 1986). In L-glutamate medium both L- and D-arginine caused growth inhibition of those *aru* mutants which were blocked after N²-succinylarginine, presumably because succinyl-CoA was consumed and not regenerated (Jann *et al.*, 1986).

The *kauB* locus at 10 min was clearly separated from other arginine catabolic genes in *P. aeruginosa*, *arcABC* (8 min; Vander Wauven *et al.*, 1984), *aguAB* (19 min; Haas *et al.*, 1984) and *aruABCDE* (66 min; Soldati *et al.*, 1982; Jann *et al.*, 1986); these map locations are according to the revised chromosome map of O'Hoy & Krishnapillai (1987). The *kauB* product was a bifunctional 4-aminobutyraldehyde/4-guanidinobutyraldehyde dehydrogenase, like similar enzymes in plant leaves (Matsuda & Suzuki, 1984) and in *P. putida* (Yorifuji *et al.*, 1986). An additional, 4-guanidinobutyraldehyde-specific dehydrogenase has been found in *P. putida* (Yorifuji *et al.*, 1986).

Evidence for four arginine catabolic pathways has also been obtained in *P. putida* (Stalon *et al.*, 1987), but no arginine catabolic mutants have been reported in this organism. The network of arginine catabolic pathways in *P. aeruginosa* described here is a further example of the well-known metabolic versatility of *Pseudomonas*.

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