# ENZYMATIC DEGRADATION OF UREIDOGLYCINE BY PSEUDOMONAS ACIDOVORANS

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ABSTRACT: Ureidoglycine, a postulated intermediate in the metabolism of allantoic acid, has been synthesized and a mutant of Pseudomonas acidovorans, unable to utilize ureidoglycine, has been isolated. The mutant forms ureidoglycine from allantoic acid and is able to use allantoic acid as a source of nitrogen but not as carbon source. The data support the conclusion that a specific enzyme is required for the metabolism of ureidoglycine and that only one of the two pathways which have been proposed for degradation of allantoic acid occurs in P. acidovorans.

Two alternative pathways for the degradation of allantoic acid have been proposed (see Figure 1). Valentine and Wolfe (1) synthesized potassium ureidoglycolate (previously called glyoxylurea) and reported that ureidoglycolic acid was formed from allantoic acid (reaction 1) by Streptococcus allantoicus. Ureidoglycolate synthetase (cf. reaction 4) was purified 135-fold from S. allantoicus and its properties studied (2,3). Vogels (4) proposed that the formation of ureidoglycolic acid involved two reactions catalyzed by the enzymes allantoate amidohydrolase (reaction 2) and ureidoglycine aminohydrolase (reaction 3). Reports of the synthesis of ureidoglycine, the postulated intermediate, or of its identification as a metabolic product have not appeared. Recently, van der Drift, et al. (5) have reported that ureidoglycine can be degraded nonenzymatically by transamination with glyoxylic acid (reaction 5) and that both reactions 2 and 3 are probably catalyzed by allantoate amidohydrolase. They concluded that an enzyme specific for ureidoglycine degradation (ureidoglycine aminohydrolase) need not be postulated.

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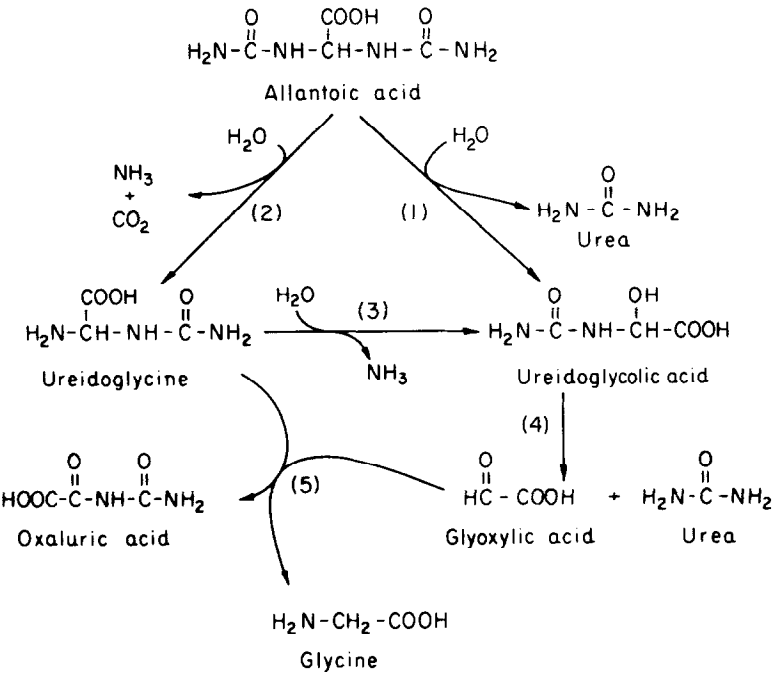


Figure 1 Alternative pathways proposed for the metabolism of allantoic acid by bacteria.

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In this communication, the synthesis of ureidoglycine is reported and evidence is presented to show that reactions 2 and 3 (Figure 1) are separable genetically.

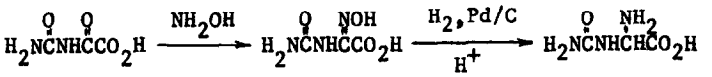
MATERIALS AND METHODS: Organisms: Pseudomonas acidovorans strain 14 den Dooren De Jong (6) was kindly supplied by M. Doudoroff. This organism is capable of growth in minimal medium with potassium allantoate as the sole source of carbon, nitrogen and energy. Strain 14-1, a mutant able to use allantoate as nitrogen source but not as carbon source, was isolated by replica plating after 12 hr exposure to ethyl methanesulfonate.

Cultivation: The minimal salts solution contained (amounts per liter): $Na_2HPO_4 \cdot 7\ H_2O$, 8.2 g; $KH_2PO_4$, 2.7 g; $MgSO_4 \cdot 7\ H_2O$, 0.4 g; $FeCl_3$, 0.5 mg; distilled water to volume. Carbon and nitrogen sources were sterilized separately by filtration and were added aseptically to the minimal salts solution to achieve the desired concentration. Growth was at 30 C with

aeration. The amount of growth was measured as absorbance at 540 mμ in 18 x 150 mm cuvettes, using a Coleman Jr. spectrophotometer.

Gas chromatography: A Hewlett-Packard Model 5750B gas chromatograph equipped with temperature programming, a hydrogen flame detector and F & M Scientific Corp. Model 50 automatic attenuator was used for gas chromatography. The column was 1/8 in x 6 ft and contained 10 per cent silicone gum rubber (methyl vinyl) as the liquid phase. Trimethylsilyl (TMS) derivatives were prepared by treating 10 mg dry material with 1.0 ml anhydrous pyridine, 0.2 ml hexamethyldisilazane and 0.1 ml trimethylchlorosilane at room temperature for 2 hr. Conditions for chromatography were: injector temp, 260 C; detector temp, 290 C; flow rate, helium, 30 ml/min, chart speed, 1"/3 min; oven temperature program: 100 C isothermal 10 min, 6 C/min to 200 C, 10 min hold at 200 C.

EXPERIMENTAL RESULTS: Ureidoglycine was synthesized by the reactions shown below. Details of the synthesis and identification will be reported elsewhere.



Oxaluric acid — Oxaluric acid oxime — Ureidoglycine

Table I shows growth data which identify the site of the enzymatic defect in the mutant, P. acidovorans strain 14-1, as ureidoglycine aminohydrolase. Since one mole of $NH_3$ can be formed from allantoic acid by the action of allantoate amidohydrolase, the mutant is able to utilize allantoate as the source of nitrogen for growth. Growth of the mutant on ureidoglycolate as sole source of carbon and/or nitrogen indicates that inability to grow on allantoate as carbon source is due to lack of ureidoglycine aminohydrolase. Since the mutant is able to use glycine as sole source of nitrogen, it can be concluded that glycine is not formed from ureidoglycine in this organism by nonenzymatic transamination. The wild type parent is able to utilize the synthesized ureidoglycine as the sole source of carbon and/or nitrogen.

If the mutant lacks ureidoglycine aminohydrolase, it should accumulate

TABLE I

UTILIZATION AS CARBON AND/OR NITROGEN SOURCE OF COMPOUNDS RELATED TO ALLANTOIC ACID METABOLISM BY *PSEUDOMONAS ACIDOVORANS*, WILD TYPE AND MUTANT STRAIN 14-1

| Additions to minimal salts, per cent | Absorbance at 540 mμ | |
|---|---|---|
| | Wild type | Mutant |
| Allantoate, 1.0 | 0.30 | 0.03 |
| Succinate, 0.5; allantoate, 0.1 | 0.58 | 0.60 |
| Ureidoglycolate, 0.5 | 0.23 | 0.24 |
| Succinate, 0.5; ureidoglycolate, 0.1 | 0.50 | 0.47 |
| Gluconate, 0.5; urea, 0.1 | 0.67 | 0.65 |
| Succinate, 0.5, $NH_4Cl$, 0.1 | 0.60 | 0.60 |
| Succinate, 0.5; ureidoglycine, 0.1 | 0.46 | 0.02 |
| Ureidoglycine, 0.2 | 0.10 | 0.02 |
| Succinate, 0.5; glycine, 0.1 | 0.70 | 0.63 |

Growth was at 30 C with aeration. Absorbance was measured after maximum growth had been attained. Compounds to be tested for use as carbon and nitrogen source were used as single additions to the minimal salts. Compounds tested for use as nitrogen source were used in combination with sodium succinate or potassium gluconate.

ureidoglycine when incubated with allantoate. Strain 14-1 was grown overnight in minimal medium containing 0.5 per cent sodium succinate and 0.1 per cent potassium allantoate. Cells were collected by centrifugation and resuspended in minimal medium containing 0.1 per cent potassium allantoate. The cell suspension, containing $3 \times 10^9$ cells/ml, was incubated at 30 C with shaking. After 6 hr, cells were removed by centrifugation and the supernatant fluid was passed through a membrane filter (Millipore, HA, 0.45 μ pore size). A sample was removed for ultraviolet spectroscopy and the remainder was lyophilized.

The culture supernatant and a 0.1 per cent solution of potassium allantoate were diluted 1:150 with distilled water. Ultraviolet spectra were recorded with a Cary 14 recording spectrophotometer. Figure 2 shows the UV spectra for potassium allantoate, ureidoglycine and the material formed from allantoate by the mutant strain 14-1. The spectrum for the supernatant shows the absence of allantoate and the presence of a compound which is essentially identical to ureidoglycine in UV absorption.

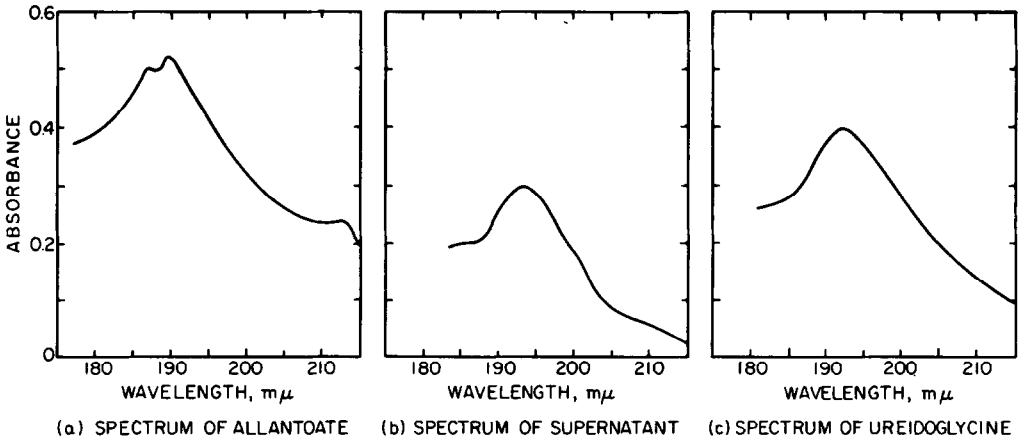



Figure 2 Conversion of allantoate to ureidoglycine by P. acidovorans 14-1.

Cells, 3 x $10^9$/ml, were incubated with 0.1 per cent potassium allantoate for 6 hr and removed by centrifugation and filtration. Spectra for the supernatant fluid, allantoate and ureidoglycine were recorded using a Cary 14 recording spectrophotometer.

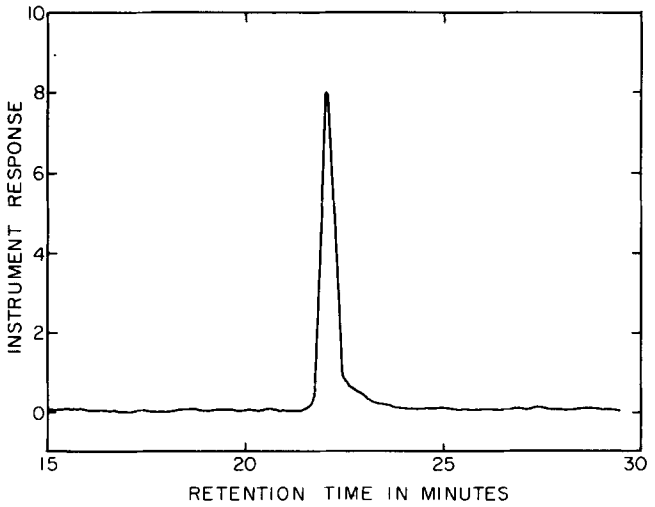



Figure 3 Gas chromatography of the TMS derivative of chemically synthesized ureidoglycine.

Sample size was 1.0 μl. Conditions are given in the text.

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Trimethylsilyl derivatives were prepared as described using ureidoglycine and the lyophilized supernatant. Figure 3 shows the elution pattern for the

TMS derivative of the chemically synthesized ureidoglycine. The elution pattern for the TMS derivative prepared from lyophilized supernatant was identical to that for the TMS derivative of synthetic ureidoglycine. Figure 4 shows the elution pattern for a mixture containing equal amounts of the TMS derivatives of ureidoglycine and the lyophilized supernatant. Retention time was 22.0 minutes in each case and only a single, well-defined peak was obtained. Allantoate reacted slowly with the silylating reagents used and two peaks were obtained, appearing at 11.0 and 20.4 min under the same conditions.

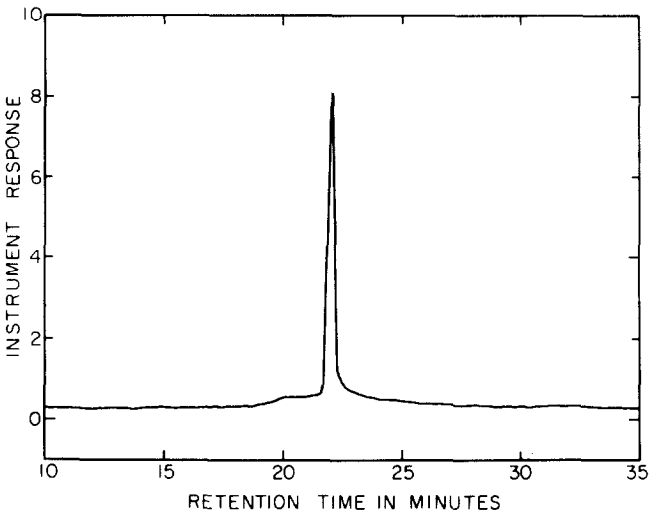


Figure 4 Gas chromatography of a mixture of TMS derivatives of chemically and biologically synthesized ureidoglycine.

The sample contained equal amounts of the TMS derivatives of the synthetic compound and the product formed by cells incubated with potassium allantoate (see text for details). Total sample size was 1.0 µl. Conditions are given in the text.

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DISCUSSION: Gaudy and Bruce (7) reported that the allantoicase pathway, as proposed by Valentine, et al. (8) is the sole pathway for degradation of allantoic acid in *Pseudomonas aeruginosa*. Mutants lacking allantoicase activity were unable to utilize allantoic acid. The properties of strain 14-1 of *P. acidovorans* support the conclusion that the allantoate amidohydrolase pathway is the sole route for metabolism of allantoic acid in this organism, as

proposed by Trijbels and Vogels (9). However, the more recent proposal (5) that only allantoate amidohydrolase is required for metabolism of allantoic acid seems incorrect, since data presented herein show that the ability to utilize ureidoglycine can be lost by a mutation which does not affect ability to utilize allantoate as a nitrogen source.

If a single enzyme, allantoate amidohydrolase, were responsible for both reactions 2 and 3 (Figure 1), it would be necessary to conclude that it is possible to alter the molecule by mutation in such a way that only one of the two reactions catalyzed by the enzyme is eliminated. The properties of the mutant 14-1 are more readily explained if it is assumed that the two activities reside in separate molecules, although the possibility that these are non-identical subunits of an enzyme aggregate cannot be eliminated at present.

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