BBA 65511

ALLANTOATE AND UREIDOGLYCOLATE DEGRADATION BY *PSEUDOMONAS AERUGINOSA*

F. TRIJBELS AND G. D. VOGELS
Department of Biochemistry, University of Nijmegen, Nijmegen (The Netherlands)

(Received June 13th, 1966)

SUMMARY

1. A single enzyme, allantoicase (allantoate amidinohydrolase, EC 3.5.3.4), catalyzes the conversion of allantoate to (−)-ureidoglycolate. On the other hand, two enzymes were found to be responsible for the degradation of ureidoglycolate by *Pseudomonas aeruginosa* extracts: allantoicase, which degraded (+)-ureidoglycolate 3–4 times faster than (−)-ureidoglycolate, and (−)-ureidoglycolase ((−)-ureidoglycolate amidinohydrolase), which was specific for (−)-ureidoglycolate.

2. Allantoicase and (−)-ureidoglycolase have been purified 15- and 10-fold, respectively.

3. Allantoicase could be protected by Mn^{2+} against heat inactivation at 75°, whereas (−)-ureidoglycolase was completely inactivated under these conditions.

4. The reactions catalyzed by allantoicase and (−)-ureidoglycolase were reversible. The equilibrium constants were 4.8 and 7.4, respectively.

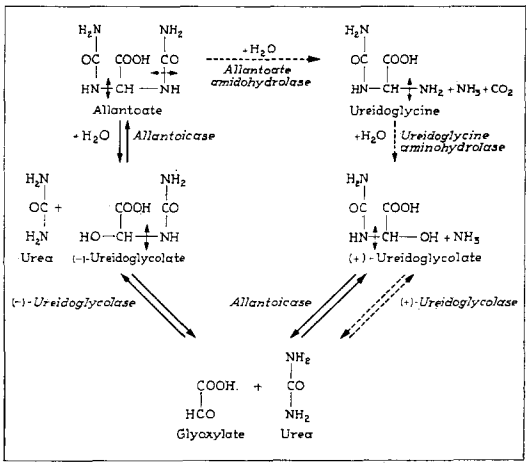
5. The effects of pH and bivalent cations on the enzymic and non-enzymic hydrolysis of ureidoglycolate were investigated.

6. The pathways of allantoate degradation in *P. aeruginosa* and *Pseudomonas acidovorans* were compared.

INTRODUCTION

The conversion of allantoate to glyoxylate is brought about by a two-step mechanism in *P. aeruginosa* and some other microorganisms[1]. Allantoate is degraded by allantoicase (allantoate amidinohydrolase, EC 3.5.3.4) to urea and ureidoglycolate. The latter substance is converted to urea and glyoxylate by ureidoglycolase (ureidoglycolate amidinohydrolase), which is also present in cell-free extracts of *Streptococcus allantoicus*[2,3], *P. acidovorans*[4] and other microorganisms[3]; these organisms, however, contain allantoate amidohydrolase instead of allantoicase. The reactions involved are shown in Scheme 1.

The present communication deals with the purification, stereospecificity and other properties of allantoicase and (−)-ureidoglycolase from *P. aeruginosa*. Allan-



Scheme 1. Degradation of allantoate by *P. aeruginosa* (full lines) and *P. acidovorans* (dotted lines).

toicase degrades allantoate to (−)-ureidoglycolate and urea, but also catalyzes the conversion of (+)-ureidoglycolate to glyoxylate and urea. (−)-Ureidoglycolate formed by the allantoicase reaction is degraded specifically by (−)-ureidoglycolase.

EXPERIMENTAL

Materials and analytical procedures

Cultivation of the microorganisms, preparation of cell-free extracts and synthesis of allantoate and ureidoglycolate were performed as previously described[1].

Protein was determined according to LOWRY *et al.*[5], and allantoate, ureidoglycolate and glyoxylate according to the differential glyoxylate analysis[4]. In the latter procedure ureidoglycolate was transformed to glyoxylate by heating the sample in phosphate buffer (pH 7.0) at 100° for 5 min. Only about 2% of the allantoate was converted to glyoxylate under these conditions.

Optical rotation was determined in a 10-cm cell at 30° in a Perkin–Elmer polarimeter with a sodium-vapor lamp. Polyacrylamide-gel electrophoresis was performed for 3 h at 5 mA, 25–30 V/cm, 4°; cylinder dimensions, 60 mm × 9 mm. The discontinuous buffer system of ORNSTEIN[6] was used.

Standard assay conditions

Standard incubation mixtures contained, per ml, 87 μmoles triethanolamine–HCl buffer (pH 7.5), 0.87 μmole $MnSO_4$, 100 μmoles sodium allantoate or 25 μmoles ureidoglycolate and an appropriate amount of enzymic material. The mixtures were incubated at 30°. Non-enzymic hydrolysis of the substrates was determined in the same incubation mixtures without added enzyme.

One unit of enzyme activity was defined as the amount which will catalyze the transformation of 1 μmole substrate per min. Specific activity is expressed in units per mg of protein.

Purification of allantoicase

Cell-free extract of *P. aeruginosa* was applied to a cooled DEAE-cellulose column (45 cm × 3.3 cm) equilibrated with 0.05 M Tris–HCl buffer (pH 7.5), containing 0.17 μmole EDTA per ml. The column was washed with the same buffer and thereafter with 0.1 M NaCl dissolved in this buffer. During the latter elution 83% of the original allantoicase activity emerged from the column. This fraction was dialyzed against the above-mentioned buffer for 12 h at 4° and applied to a second cooled DEAE-cellulose column (43 cm × 2.1 cm) equilibrated with the buffer. Allantoicase was eluted with the same buffer in which the NaCl concentration was continuously increased to 0.2 M. The active fractions were collected, dialyzed against distilled water for 12 h at 4°, lyophilized and dissolved in 3 ml 0.05 M Tris–HCl buffer (pH 7.5). This solution was applied to a column (74 cm × 2.1 cm) packed with Sephadex G-200 in 0.05 M Tris–HCl buffer (pH 7.5). The gel filtration was performed at room temperature by washing the column with the same buffer. The elution pattern is shown in Fig. 1.

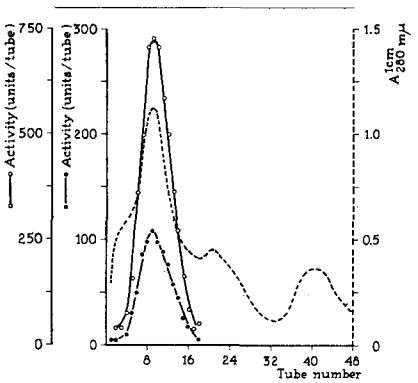


Fig. 1. Gel filtration of allantoicase on Sephadex G-200. The enzymic activity was determined with sodium allantoate (○—○) or ureidoglycolate (●—●) as substrate under standard conditions. Each tube contained 3.2 ml.

Purification of (—)-ureidoglycolase

This enzyme was purified by stepwise DEAE-cellulose chromatography and gel filtration, omitting the gradient elution chromatography. The same procedure was followed for the stepwise DEAE-cellulose chromatography as given for allantoicase, except that the washing with 0.1 M NaCl in buffer was replaced by washing with 0.05 M and 0.15 M NaCl in buffer, successively. The fraction eluted with the buffered 0.15 M NaCl was dialyzed against distilled water for 7 h at 4°, lyophilized and dissolved in 0.05 M Tris–HCl buffer (pH 7.5). Gel filtration of this solution was performed as for allantoicase. The elution pattern is shown in Fig. 2.

RESULTS

Purification of allantoicase and (—)-ureidoglycolase; degradation of ureidoglycolate by allantoicase

Allantoicase and (—)-ureidoglycolase were purified by DEAE-cellulose chroma-

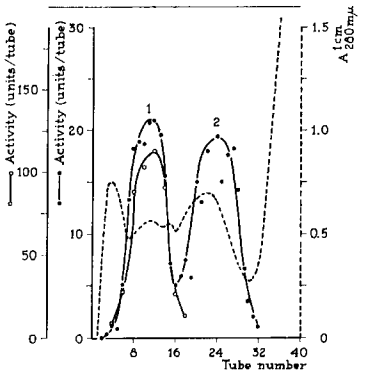


Fig. 2. Gel filtration of allantoicase (1) and (−)-ureidoglycolase (2) on Sephadex G-200. The enzymic activity was determined as described in Fig. 1. Each tube contained 3.2 ml.

tography and gel filtration on Sephadex G-200 (Tables I and II). The specific activities were enhanced 15 and 10 times, respectively. The last step in the purification of (−)-ureidoglycolase was a gel filtration, which yielded two peaks with enzymic activity against (+−)-ureidoglycolate (Fig. 2). The first peak also contained allantoicase activity. The enzymic activities against allantoate and ureidoglycolate in the fractions forming the first peak were distributed in a constant 5:1 ratio; maximal activity for both substrates was obtained at the same elution volume. Subsequently the one peak obtained in the allantoicase preparation (Fig. 1) was found to exhibit activity towards (+−)-ureidoglycolate.

In a further attempt to elucidate whether one or two enzymes are involved here, the purified allantoicase preparation was subjected to polyacrylamide-gel electrophoresis. After the electrophoretic run the gel was cut into 26 pieces of about 2 mm each and the pieces were homogenized in 1.5 ml 0.1 M triethanolamine–HCl buffer (pH 7.5) and tested for enzymic activity towards allantoate and (+−)-ureidoglycolate. The two activities were found together in Pieces 21-23, numbered from the origin, in the same 5:1 ratio as obtained in the chromatographic fractionation.

These results strongly suggest that one enzyme, represented by the single peak in Fig. 1 and the first peak in Fig. 2, degraded both allantoate and (+−)-ureidoglycolate. This enzyme will be called allantoicase. In addition there was a second enzyme, represented by the second peak in Fig. 2, which degraded (+−)-ureido-

TABLE I

PURIFICATION OF ALLANTOICASE

Allantoicase activity was tested under standard conditions.

Fraction	*Total protein (mg)*	*Total units*	*Specific activity*	*Recovery units (%)*
Crude extract	850	10 300	12.1	100
DEAE-cellulose (stepwise elution)	154	8 500	55.0	83
DEAE-cellulose (gradient elution)	51	8 500	167	83
Sephadex G-200	31	5 480	177	53

TABLE II

PURIFICATION OF (−)-UREIDOGLYCOLASE

Ureidoglycolate degradation was tested under standard conditions.

Fraction	*Total protein (mg)*	*Total units*	*Specific activity*	*Recovery units (%)*
Crude extract	1130	2230*	1.97*	100
DEAE-cellulose	233	765*	3.28*	34
Sephadex G-200	14.4	300	20.80	13

* (−)-Ureidoglycolase activity was calculated from the difference in enzymic activity before and after heat pretreatment of the material at 70° in the presence of $7 \cdot 10^{-3}$ M $MnSO_4$.

glycolate but was inactive against allantoate. This enzyme will be called ureidoglycolase in this report.

Optical specificity of allantoicase and of ureidoglycolase

With the cell-free extracts of *P. aeruginosa*, previously used[1], (−)-ureidoglycolate was produced from allantoate, and (+−)-ureidoglycolate was degraded completely and without change in optical rotation. When the purified allantoicase preparation was used instead, the degradation of (+−)-ureidoglycolate was accompanied by a change in optical rotation (Fig. 3). From the curves in Fig. 3 it can be calculated that the degradation rate of (+)-ureidoglycolate was about 3–4 times that of the (−)-form. These results indicate that allantoicase produces (−)-ureidoglycolate from allantoate and preferably degrades (+)-ureidoglycolate.

Degradation of (+−)-ureidoglycolate by ureidoglycolase is shown in Fig. 4. This enzyme converted (−)-ureidoglycolate much more rapidly than (+)-ureido-

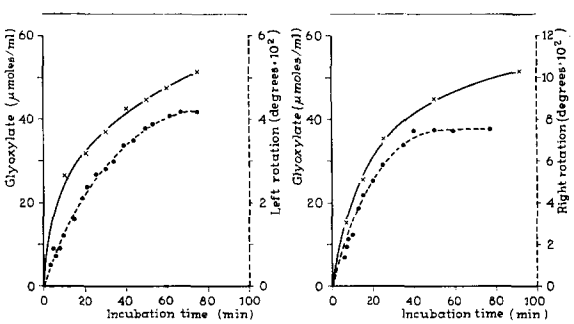


Fig. 3. Degradation of ureidoglycolate by allantoicase. The incubation mixture contained per ml 100 μmoles (+−)-ureidoglycolate, 77 μmoles triethanolamine–HCl buffer (pH 7.5), 0.77 μmole $MnSO_4$ and 91 μg purified allantoicase. The mixture was incubated for 75 min at 30°. The optical rotation was followed with a Perkin–Elmer polarimeter in a 10-cm tube with a sodium-vapor lamp.

Fig. 4. Degradation of ureidoglycolate by (−)-ureidoglycolase. The incubation mixture contained per ml 94 μmoles (+−)-ureidoglycolate, 46 μmoles triethanolamine–HCl buffer (pH 7.5), 0.77 μmole $MnSO_4$ and 220 μg purified ureidoglycolase. The mixture was incubated for 90 min at 30°. The optical rotation was followed with a Perkin–Elmer polarimeter in a 10-cm tube with a sodium-vapor lamp.

glycolate and will henceforth be called (—)-ureidoglycolase. Ureidoglycolase from *P. acidovorans* degraded only (+)-ureidoglycolate and will be called (+)-ureidoglycolase[4].

Heat stability of allantoicase and (—)-ureidoglycolase

The influence of heat pretreatment on the enzymic activities was tested in the presence and absence of Mn^{2+}. In its presence allantoicase was stable up to 75°, whereas in its absence the enzyme was inactivated completely within 5 min at 75° (Table III). This protection of enzyme activity by Mn^{2+} against heat denaturation was observed with allantoate as well as with ureidoglycolate as substrate, which further supports the concept that both degradations are brought about by one enzyme.

TABLE III

HEAT STABILITY OF ALLANTOICASE AND (—)-UREIDOGLYCOLASE IN THE PRESENCE AND ABSENCE OF Mn^{2+}

Heat stability of allantoicase was tested under the following conditions. Mixtures containing per ml 50 μmoles Tris–HCl buffer (pH 7.5) and 120 μg purified allantoicase were heated for 5 min at the indicated temperatures in the presence or absence of $8 \cdot 10^{-4}$ M $MnSO_4$. Aliquots of the pretreated enzyme solutions were incubated for 15 min at 30° in mixtures containing per ml 96 μmoles sodium allantoate or 25 μmoles ureidoglycolate, 83 μmoles triethanolamine–HCl buffer (pH 7.5), 0.83 μmole $MnSO_4$ and 10 μg pretreated purified allantoicase. The heat stability of (—)-ureidoglycolase was tested under the following conditions. Mixtures containing per ml 43 μmoles Tris–HCl buffer (pH 7.5) and 185 μg purified (—)-ureidoglycolase were heated for 5 min at the indicated temperatures in the presence or absence of $7.4 \cdot 10^{-4}$ M $MnSO_4$. Aliquots of the pretreated enzyme solutions were incubated for 15 min at 30° in mixtures containing per ml 25 μmoles ureidoglycolate, 83 μmoles triethanolamine–HCl buffer (pH 7.5), 0.83 μmole $MnSO_4$ and 14.2 μg pretreated purified (—)-ureidoglycolase.

Temperature during pretreatment	*Allantoicase*				*(—)-Ureidoglycolase*	
	Allantoate degraded (μmoles/ml)		*Ureidoglycolate degraded (μmoles/ml)*		*Ureidoglycolate degraded (μmoles/ml)*	
	+ Mn^{2+}	*— Mn^{2+}*	*+ Mn^{2+}*	*— Mn^{2+}*	*+ Mn^{2+}*	*— Mn^{2+}*
30°	21.2	22.3	5.05	3.70	3.76	3.20
50°	22.0	18.9	5.18	2.60	3.35	2.95
55°	21.4	21.4	5.28	2.56	3.15	1.90
60°	21.3	20.9	5.83	2.27	2.10	0.90
65°	23.0	10.8	5.21	0.70	0.30	0.20
70°	21.5	1.3	5.11	0.13	0	0
75°	21.6	0	5.16	0	0	0

The effect of heat pretreatment on the enzymic activity of (—)-ureidoglycolase is also presented in Table III. It appears that this enzyme was denaturated more rapidly than allantoicase, even in the presence of $7.4 \cdot 10^{-4}$ M $MnSO_4$. Allantoicase was stable for more than 15 min at 70° in the presence of Mn^{2+}, whereas (—)-ureidoglycolase was completely inactivated under these conditions.

Both allantoicase and (—)-ureidoglycolase were present in crude extract. Allantoate and ureidoglycolate degradation by this extract was measured after heating the extract at 70° in the presence of Mn^{2+} (Fig. 5). The enzymic activity against allantoate was not influenced by this treatment, but 30% of the activity against

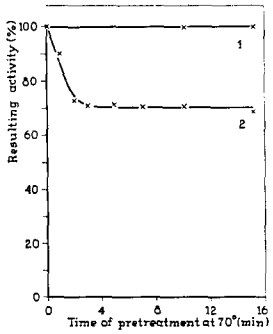


Fig. 5. Influence of heat pretreatment on allantoate (1) and ureidoglycolate (2) degradation by crude cell-free extract of *P. aeruginosa*. A mixture of 7 μmoles $MnSO_4$, crude cell-free extract of *P. aeruginosa* containing 4.86 mg of protein and distilled water in a final volume of 1 ml was incubated for the indicated time intervals at 70°. Aliquots of the pretreated material were incubated at 30° for 30 min in mixtures containing, per ml, 113 μmoles sodium allantoate or 27.3 μmoles (+ −)-ureidoglycolate, 91 μmoles triethanolamine–HCl buffer (pH 7.5), 0.13 μmole $MnSO_4$ and 88 μg of protein. Under the conditions given untreated material degraded 23.4 μmoles allantoate or 7.43 μmoles ureidoglycolate per ml. The activities given in the figure are expressed as percentages of these values.

ureidoglycolate disappeared. From this value the (−)-ureidoglycolase activity was calculated in Table II.

Equilibrium of the allantoicase reaction

Crude cell-free extracts[1] as well as purified allantoicase only partly degraded allantoate (Fig. 6). Previously[1] we suggested this partial degradation to be due to inhibition by one of the products formed from allantoate or to the establishment of an equilibrium. Addition of urease or (−)-ureidoglycolase to the incubation mixture resulted in further degradation of allantoate (Fig. 6). The results obtained with urease were contrary to previous results[1] with crude extracts. This contradiction cannot be explained at present.

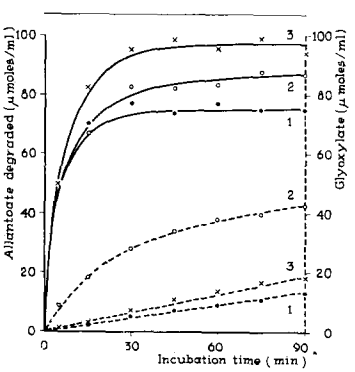


Fig. 6. Effect of urease and (−)-ureidoglycolase on allantoate degradation by *P. aeruginosa* allantoicase. The incubation mixtures contained, per ml, 100 μmoles sodium allantoate, 58 μmoles triethanolamine–HCl buffer (pH 7.5), 0.83 μmole $MnSO_4$ and 200 μg purified allantoicase (1). Moreover, 62 μg purified (−)-ureidoglycolase were present in one experiment (2) and 1.36 mg jack-bean urease in another experiment (3). The mixtures were incubated for 90 min at 30°.

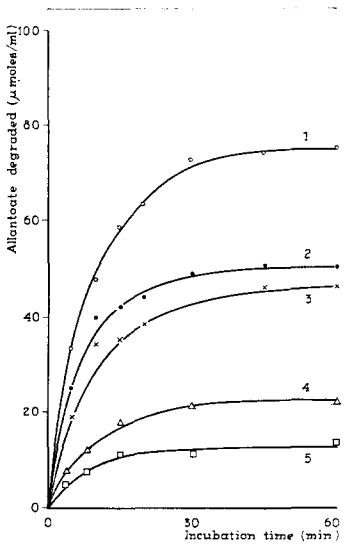


Fig. 7. Influence of urea, methylurea and thiourea on allantoate degradation by allantoicase. The incubation mixtures contained, per ml, 100 μmoles sodium allantoate, 86 μmoles triethanolamine–HCl buffer (pH 7.5) and 86 μg purified allantoicase (1). Besides these additions the mixtures contained, per ml, 950 μmoles methylurea (2), 140 μmoles thiourea (3), 720 μmoles urea (4) or 1440 μmoles urea (5). The mixtures were incubated for 60 min at 30°.

More evidence for the establishment of an equilibrium during the allantoate degradation was obtained from experiments in which urea was added to incubation mixtures (Fig. 7). In the absence of added urea 75% of allantoate was degraded. In the presence of 0.72 M or 1.44 M urea only 22 or 12% of allantoate, respectively, was converted. The equilibrium constant, $K = \frac{[\text{allantoate}]}{[\text{urea}] \cdot [(-)\text{-ureidoglycolate}]}$, calculated from these three experiments gave values of 4.5, 4.9 and 5.1, respectively.

Starting from urea (1 M) and (+−)-ureidoglycolate (0.1 M) allantoate was

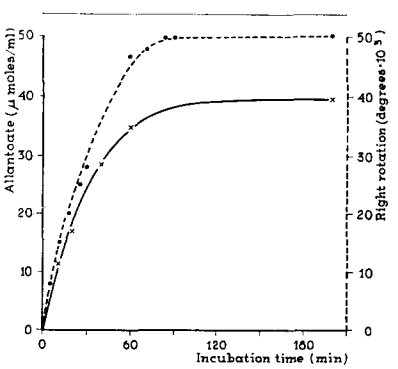


Fig. 8. Production of allantoate from urea and (−)-ureidoglycolate by allantoicase. The incubation mixture contained, per ml, 1 mmole urea, 106 μmoles (+−)-ureidoglycolate, 85 μmoles triethanolamine–HCl buffer and 35 μg purified allantoicase. The pH of the mixture was adjusted to 7.5. A mixture containing no enzyme was prepared as control. Both mixtures were incubated at 30° for 200 min. The change in optical rotation during allantoate formation is given.

formed in the presence of allantoicase (Fig. 8). The change in optical rotation during this conversion indicated that only (—)-ureidoglycolate was transformed into allantoate. This result corroborates the afore-mentioned stereospecificity of the allantoicase reaction. After incubation for 200 min 76% of the (—)-ureidoglycolate was converted to allantoate. The equilibrium constant (3.2) calculated from this experiment was somewhat too low, indicating that equilibrium was not quite reached.

In experiments with crude cell-free extracts[1] the reverse reaction could not be demonstrated because of the presence of (—)-ureidoglycolase. Methylurea, and thiourea, like urea, shifted the position of the equilibrium. In the absence of these substances 75% of allantoate was degraded, but in the presence of 0.95 M methylurea or 0.14 M thiourea only 50 or 46% was degraded, respectively (Fig. 7). Apparently both compounds reacted with (—)-ureidoglycolate, reversing the allantoicase reaction. The enzyme was not specific for allantoate but degraded methyl- and thioallantoate as well. The degradation rate observed on incubation of 5-methylallantoate with the purified allantoicase preparation was, however, 30 times lower than that obtained with allantoate under the same conditions.

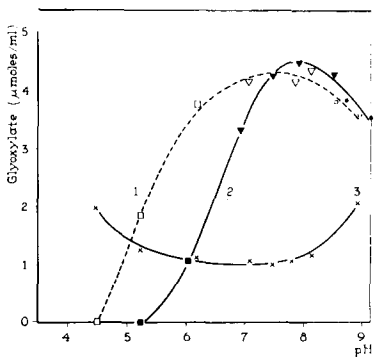


Fig. 9. Effect of pH on enzymic and non-enzymic hydrolysis of ureidoglycolate. The incubation mixtures contained per ml 25 μmoles (+ —)-ureidoglycolate, 0.83 μmole $MnSO_4$, 150 μmoles buffer and 40 μg purified (—)-ureidoglycolase (1) or 19.1 μg purified allantoicase (2). Non-enzymic hydrolysis (3) was measured in a similar mixture from which the enzyme was omitted. The following buffers were used: acetic acid–sodium acetate (pH 4.5–6.2), Tris–HCl (pH 7.0–8.6) and diethanolamine–HCl (pH 8.5–9.2). The mixtures were incubated at 30° for 15 min.

Effect of pH on the enzymic and non-enzymic hydrolysis of ureidoglycolate

The degradation of ureidoglycolate by allantoicase and (—)-ureidoglycolase was measured as a function of the pH of the incubation mixture (Fig. 9). Allantoicase showed optimal activity at pH 8.0; (—)-ureidoglycolase was equally active in the pH range 7.0–8.0. The values for these enzymic hydrolyses had been corrected for non-enzymically formed glyoxylate. This spontaneous cleavage of ureidoglycolate was constant at pH 6.0–8.0 and increased in acidic and alkaline solutions.

Phosphate buffers were not used in the tests as the spontaneous hydrolysis of ureidoglycolate was much larger in these buffers[4].

Influence of bivalent cations on the non-enzymic and enzymic hydrolysis of ureidoglycolate and on the enzymic hydrolysis of allantoate

The rate of non-enzymic hydrolysis of ureidoglycolate was strongly enhanced

TABLE IV

INFLUENCE OF BIVALENT CATIONS ON THE ENZYMIC AND NON-ENZYMIC HYDROLYSIS OF UREIDOGLYCOLATE AND ON THE ENZYMIC HYDROLYSIS OF ALLANTOATE

The incubation mixtures contained, per ml, 20 μmoles sodium allantoate or 20 μmoles ureidoglycolate, 67 μmoles triethanolamine–HCl buffer (pH 7.5), cations to a final concentration of $2.5 \cdot 10^{-4}$ M and 220 μg (+)-ureidoglycolase of *P. acidovorans* (2), 8.3 μg (−)-ureidoglycolase of *P. aeruginosa* (3), 4 μg allantoicase of *P. aeruginosa* (4 and 5). The mixtures were incubated for 30 min at 30°. In the experiments without added cations 0.44 (1), 1.98 (2), 3.21 (3), 0.64 (4) or 4.42 (5) μmoles of substrate were degraded per ml. Rates of hydrolysis are expressed as percentages of these values. Corrections had been made for the non-enzymic hydrolysis of ureidoglycolate.

Cations ($2.5 \cdot 10^{-4}$ *M*)	*Rate of hydrolysis (%)*				
	Ureidoglycolate				*Allantoate*
	Non-enzymic (1)	*(+)-Ureidoglycolase (2)*	*(−)-Ureidoglycolase (3)*	*Allantoicase (4)*	*Allantoicase (5)*
—	100	100	100	100	100
Mg^{2+}	105	98	93	86	114
Ca^{2+}	119	96	93	107	99
Cd^{2+}	135	98	65	266	177
Mn^{2+}	176	106	105	315	196
Ni^{2+}	207	85	85	94	94
Co^{2+}	226	91	96	143	130
Pb^{2+}	246	89	45	218	110
Hg^{2+}	336	—	—	—	—
Zn^{2+}	390	70	60	127	129
Cu^{2+}	505	30	60	140	156

in the presence of several bivalent cations (Table IV). All cations were tested at a concentration of $2.5 \cdot 10^{-4}$ M. The following sequence gives the cations in the order of increasing catalytic activity: $Mg^{2+} < Ca^{2+} < Cd^{2+} < Mn^{2+} < Ni^{2+} < Co^{2+} < Pb^{2+} < Hg^{2+} < Zn^{2+} < Cu^{2+}$. This arrangement resembles the "Irving–Williams sequence" ($Ca^{2+} < Mg^{2+} < Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$) which is typical for non-enzymic catalysis of polaric reactions[7].

The non-enzymic hydrolysis of ureidoglycolate was tested at different concentrations ($0–5 \cdot 10^{-3}$ M) of Cd^{2+}, Mn^{2+}, Co^{2+} and Zn^{2+}. The rate of hydrolysis was directly proportional to the concentration of these cations. The same was true for the catalytic influence of 0–0.17 M phosphate (pH 7.5) on ureidoglycolate hydrolysis. In assays of the enzymic hydrolysis of ureidoglycolate, corrections were always made for non-enzymic hydrolysis.

Table IV shows the influence of bivalent cations ($2.5 \cdot 10^{-4}$ M) on the activity of allantoicase tested with allantoate or ureidoglycolate as substrate. None of the cations tested inhibited the reaction; Mg^{2+}, Ca^{2+} and Ni^{2+} had little or no effect. All other cations enhanced both activities of allantoicase, Mn^{2+} and Cd^{2+} being the strongest activators. On the other hand, (+)-ureidoglycolase from *P. acidovorans* and (−)-ureidoglycolase from *P. aeruginosa* were not activated by any of these cations. Several ions did not influence the reaction rate, but Cd^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+} and Cu^{2+} were inhibitory. (−)-Ureidoglycolase seemed to be more sensitive to inhibition by Cd^{2+} and Pb^{2+} ions than (+)-ureidoglycolase.

Equilibrium of the ureidoglycolase reaction

Degradation of ureidoglycolate, like that of allantoate, proceeded until equilibrium was reached. The equilibrium constant for this reaction was determined in the presence of Zn^{2+}, because of the catalytic effect of Zn^{2+} on the rate of hydrolysis of ureidoglycolate. The reaction was followed in mixtures containing per ml 20 μmoles ureidoglycolate, 70 μmoles triethanolamine–HCl buffer (pH 7.5), 10 μmoles $ZnSO_4$ and either 100, 67, or 33 μmoles of urea and sodium glyoxylate. After 60 min at 30° equilibrium was achieved: 77, 60 or 41 μmoles glyoxylate were present per ml, respectively. From these results the equilibrium constant, $K = \frac{[\text{ureidoglycolate}]}{[\text{urea}] \cdot [\text{glyoxylate}]} = 7.4$, was calculated. This value is in good agreement with the results of GAUDY AND WOLFE[8] who found a value of 7.6.

DISCUSSION

P. aeruginosa converts allantoate to glyoxylate *via* (−)-ureidoglycolate by a route distinctly different from that in *P. acidovorans*[4]. The two enzymes involved in the allantoate degradation by *P. aeruginosa* have been purified and separated from each other. It appears that allantoicase formed (−)-ureidoglycolate from allantoate, but degraded (+)-ureidoglycolate. This result can be understood by comparing the structural resemblance between the two conversions shown in the reaction scheme given in INTRODUCTION. The two activities could not be separated by DEAE-cellulose chromatography, gel filtration on Sephadex G-200 or polyacrylamide-gel electrophoresis. Testing under standard conditions, we found in the crude extract and after each purification step the same 5:1 ratio between the activities of allantoicase against allantoate and (+)-ureidoglycolate. The same was true after heat pretreatment of the enzyme in the presence of Mn^{2+} up to 75°.

In crude cell-free extracts of *P. aeruginosa* (+−)-ureidoglycolate was degraded completely by the action of (−)-ureidoglycolase and allantoicase. The two activities could be distinguished because at 70° in the presence of Mn^{2+} allantoicase was stable, while (−)-ureidoglycolase activity was completely destroyed after 5 min at 70°.

The effects of bivalent cations on allantoicase, (−)-ureidoglycolase and (+)-ureidoglycolase (*P. acidovorans*) were compared. Allantoicase activity was enhanced by the addition of several bivalent cations. It appeared that the effect of most ions was greater on the ureidoglycolate degradation than on the allantoate degradation by this enzyme. The influence of bivalent cations on (+)- and (−)-ureidoglycolase was similar, but differed from that on allantoicase. Both enzymes were inhibited by several cations and none of the ions tested, except Mn^{2+}, enhanced their activity.

The conversion of allantoate to glyoxylate involves two reversible reactions. This might be important in plants which accumulate large amounts of allantoate or transport nitrogen in this form. At the moment it is not known whether allantoate is formed in these plants *via* purines or directly from glyoxylate and urea.

ACKNOWLEDGEMENTS

We wish to thank Professor Dr. S. L. BONTING for his helpful criticism of the manuscript. The skillful technical assistance of Miss N. TIETHOF is acknowledged.

REFERENCES

1 F. TRIJBELS AND G. D. VOGELS, *Biochim. Biophys. Acta*, 118 (1966) 387.
2 E. GAUDY, R. BOJANOWSKI, R. C. VALENTINE AND R. S. WOLFE, *J. Bacteriol.*, 90 (1965) 1525.
3 G. D. VOGELS, *Thesis*, Institute of Technology, Delft, 1963.
4 F. TRIJBELS AND G. D. VOGELS, *Biochim. Biophys. Acta*, 113 (1966) 292.
5 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
6 L. ORNSTEIN AND B. J. DAVIES, *Disc Electrophoresis*, Part 2, Distillation Products Industries, Eastman Kodak, Rochester, 1962, p. 6.
7 H. BRINTZINGER, *Z. Naturw.-Med. Grundlagenf.*, 2 (1965) 188.
8 E. GAUDY AND R. S. WOLFE, *J. Bacteriol.*, 90 (1965) 1531.