Biochimica et Biophysica Acta, 527 (1978) 348—358

BBA 68620

URICASE OF *BACILLUS FASTIDIOSUS*

PROPERTIES AND REGULATION OF SYNTHESIS

G.P.A. BONGAERTS, J. UITZETTER, R. BROUNS and G.D. VOGELS

Department of Microbiology, Faculty of Science, University of Nijmegen, Nijmegen (The Netherlands)

(Received June 27th, 1978)

Summary

Uricase (urate:oxygen oxidoreductase, EC 1.7.3.3) of *Bacillus fastidiosus* was purified to homogeneity in a two-step procedure and was crystallized.

The native molecule had a molecular weight of 145 000—150 000 and was composed of subunits of two kinds (M_r = 36 000 and 39 000) in a 1 : 1 ratio. The quaternary structure of the enzyme was reversibly altered, with concomitant loss of activity, at temperatures between 40 and 60°C. No evidence was found for the involvement of metal ions or coenzymes in the uricase reaction.

The enzyme was inhibited by various metal ions and by cyanide.

The isoelectric point of the enzyme was 4.3, the pH optimum 9.5 and the optimal temperature 30—35°C.

Only uric acid was oxidized by the enzyme and 9-methyluric acid, xanthine, 8-azaxanthine and oxonic acid were competitive inhibitors.

Uricase synthesis was repressed by allantoin and allantoate, even in the presence of uric acid, which induced synthesis of the enzyme.

Molecular oxygen was an important environmental factor in the control of uricase synthesis, probably due to its effect, as cosubstrate in the uricase reaction, in assessing the cytoplasmic concentration of allantoin.

The highest amounts of uricase, up to half of the intracellular soluble protein content, was found in cells growing under limited oxygen supply in media containing uric acid as the main substrate.

Introduction

Bacillus fastidiosus grows well only in media containing uric acid, allantoin or allantoate, as the main or sole source of carbon, nitrogen and energy [1,2]. High levels of uricase (urate:oxygen oxidoreductase, EC 1.7.3.3) were found in crude extracts of cells grown on uric acid [2]. The metallo-enzyme character,

the inhibition by heavy metal ions and a number of other properties of the enzyme appear to vary with the source of the enzyme [3]. We report here the properties of the purified enzyme of *B. fastidiosus*.

In various microorganisms uricase synthesis is regulated by components of the growth medium. Kaltwasser [4,5] observed an increase of uricase activity in *Alcaligenes eutrophus (Hydrogenomonas* H16), *Pseudomonas aeruginosa* and *Micrococcus denitrificans* after transfer into media containing uric acid. Rouf and Lomprey [6] reported that the ability to degrade uric acid and to use it for growth is an inducible property of various bacteria. According to Scazzocchio and Darlington [7] uricase of *Aspergillus nidulans* is induced by uric acid. Uricase formation in a *Streptomyces* species is inhibited in growing cells and the inhibition ceases under conditions of nitrogen or carbon limitation [8]. It was suggested [8] that uricase formation may be controlled by a repression in which a metabolite derived from both the nitrogen and carbon sources may participate.

Uricase of *B. fastidiosus* was reported to be induced by uric acid [1,2]. However, with both uric acid and allantoin present, an increase in uricase activity was followed by a decrease, suggesting the possibility that the presence of allantoin suppressed further uricase formation [1]. We report here that the amount of uricase in cells of *B. fastidiosus* may rise to about 50% of the intracellular soluble protein on oxygen-limited growth of cells in media containing uric acid as the sole organic substrate. Allantoin represses the synthesis of uricase.

Materials and Methods

Strains and culture media. The strains of *B. fastidiosus* used in this study were described previously [2]. If not indicated otherwise *B. fastidiosus* SMG83 was used.

Cells were cultured aerobically in 1-l Erlenmeyer flasks at 37°C with rotatory shaking (200 rev./min) or in a 14 l P.E.C. fermentor (Chemap) heavily aerated (17.5 l air/min) and stirred (1100—1200 rev./min). The components of the growth media were dissolved in trace element phosphate buffer [1] and sterilized at 121°C except for allantoate which was sterilized by Seitz filtration. In flask cultures, the pH increased to 9.0—9.2, whereas in the fermentor cultures the pH was kept constant at pH 8.0 by neutralisation with 1 M HCl solution. Continuous growth was performed in a medium containing per l 1.5 g allantoin and 0.2 M sodium/potassium phosphate buffer (pH 7.4).

Preparation of cell-free extracts. Cells were harvested by centrifugation, washed twice with 0.1 M Tris-HCl buffer (pH 8.0) and disrupted by sonication at 0°C for a total of 4.5 min in a MSE 100-W ultrasonic disintegrator with intermittent cooling. The sonicated suspension was centrifuged at 100 000 × *g* for 20 min at 4°C. The supernatant fraction (crude extract) was stored at −20°C.

Biochemical methods. Uricase activity was measured essentially as described by Mahler [9]. The reaction mixture (2.5 ml) was incubated in 50-ml Erlenmeyer flasks placed in a shaking (100 rev./min) water bath at 30°C. The specific activity is defined as μmol uric acid converted per min per mg protein. Ini-

tial conversion rates were determined. Protein was determined by the method of Lowry et al. [10], unless indicated otherwise. The specificity of uricase was determined with solutions of the analogue at the same concentration (10^{-3} M) as used for uric acid. Samples of the reaction mixture were diluted in 0.15 M HCl solution and the extinction was measured at the characteristic maximal absorption wavelength of the analogue. Determinations of dissolved molecular oxygen were performed in a Gilson oxygraph model K-IC. Clear 0.3% uric acid solutions prepared essentially as described previously [2], but buffered with 0.2 M borate buffer (pH 9.0), were used as substrate solution in these measurements.

Purification procedure. Crude extract was applied to a Sephadex G-200 column (75 X 2.5 cm). The fractions containing the highest specific activity of uricase (Fig. 1) were collected and transferred to a DEAE-Sephadex A-50 column. The column was washed with 0.2 M NaCl in 0.1 M Tris-HCl (pH 8.0) and eluted with the same buffer in which the concentration of NaCl increased linearly from 0.2 to 0.4 M. The enzyme left the column in the fractions containing 0.25—0.30 M NaCl. To concentrate uricase the active fractions were diluted to a final concentration of 0.2 M NaCl, transferred to a small DEAE-Sephadex A-50 column (bed volume 1 ml), eluted with 0.4 M NaCl in 0.1 M Tris-HCl (pH 8.0) and dialyzed against 0.1 M Tris-HCl (pH 8.0).

Electrophoresis and isoelectric focusing. Gel electrophoresis was performed according to the method of Davis [12] and protein was stained with Coomassie Blue [13]. Samples containing 10—20 μg protein (for purity check 80 μg) were added to the gels. Isoelectric focusing was performed in polyacrylamide gels or in a sucrose gradient (5—50% sucrose) formed in a LKB-8100 Ampholine electrofocusing column. Ampholine (LKB 1809-101) solutions were used for the pH range from 3.5 to 10.0.

Molecular weight determination. The molecular weight of the native molecule was determined by Sephadex G-200 gel filtration according to Whitaker [14]. Markers were dextran blue ($M_r \geqslant 300\,000$), yeast alcohol dehydrogenase (Boehringer) (M_r = 150 000—152 000) and bovine serum albumin (Merck) (M_r = 68 000). The molecular weight of the subunits was determined by means of 0.1% sodium dodecyl sulphate gel electrophoresis. Markers were bovine liver catalase (Boehringer) (M_r subunits = 60 000), bovine pancreas chymotrypsinogen A (Sigma) (M_r subunits = 24 400), and jack bean urease (Sigma) (M_r subunits = 60 000 and 30 000).

Physicochemical procedures. Analytical studies were carried out with a Spinco model E ultracentrifuge equipped with an electronic recording system for absorption optics. Absorption of the protein solution at 280 nm was 1.1.

EPR measurements were carried out at room temperature with 15 mg powdered enzyme material dialyzed first against a 10^{-3} M EDTA solution and lyophilized before use. The activity before and after these treatments were identical. A Varian EPR spectrometer model E 12 operating at X-band was used. The lyophilized enzyme used in the EPR measurements was redissolved and used for atomic absorption measurements to establish the copper or iron content. A Pye Unicam SP 90 A absorption spectrophotometer was used.

Results

Location of uricase

The results obtained by Mahler [9] indicate that uricase of *B. fastidiosus* is an extracellular soluble protein. The soluble character could be confirmed by Kaltwasser [1] and by us, but in our hands the uricases of all *B. fastidiosus* strains studied, including strain NCIB 10372 of Mahler [9], appeared to be intracellular and were only released into the medium on lysis of the cells.

Regulation of synthesis

The uricase content of cells depends strongly on the growth conditions applied (Table I). A very high specific activity (36.4 μmol/min per mg protein) was found in cells grown at the expense of uric acid under oxygen-limited conditions; then, about half of the soluble protein is uricase, since the specific activity of pure uricase is only twice as high (see below). The high uricase content is also apparent in the elution pattern obtained on Sephadex G-200 gel filtration of crude extracts of cells grown at the expense of either uric acid or alantoin, the product formed from uric acid (Fig. 1). Gel electrophoresis of the active fractions revealed only one prominent protein band, which showed uricase activity and was located at the place where pure uricase was found (Fig. 2). The band was absent in cells grown as batch cultures on allantoin. The data of Table I demonstrate that uricase is induced by the presence of uric acid. No detectable amount of uricase was found in cells grown in the presence of allantoin, unless its concentration is kept very low as was done in the continuous culture. This points to a repressive effect of allantoin. The intracellular ratio of uric acid vs. allantoin and allantoate will be strongly influenced by the presence of molecular oxygen, the cosubstrate of the uricase reaction, when cells grow on uric acid. Therefore, the oxygen supply may strongly affect the specific activity of uricase in the cells (Table I).

The repressive effect of allantoin, and allantoate, on uricase synthesis was

TABLE I

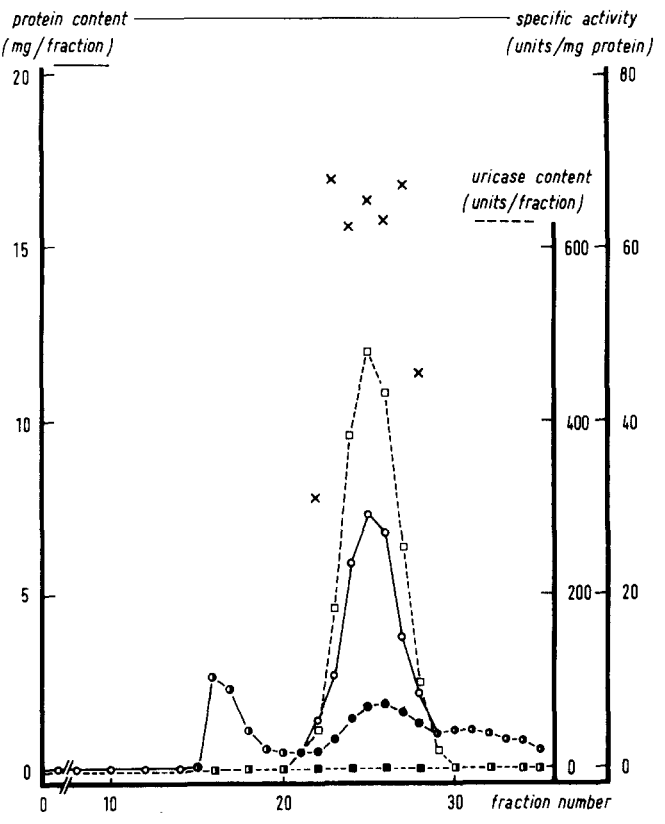
URICASE ACTIVITY IN CELLS OF *B. FASTIDIOSUS* SMG 83 GROWN UNDER VARIOUS CONDITIONS

Growth medium	Conditions *	Specific activity (μmol/min per mg protein)
Uric acid (0.8%) + BHI (0.8%) **	E	36.4
Uric acid (0.8%) + BHI (0.8%)	F	1.5—3.5
Uric acid (S_i = 0.15%; S_e = 0.00%) ***	C (D = 0.11)	3.0—4.5
Allantoin (0.6%) + BHI (0.4%)	E	0.00
Allantoin (S_i = 0.15%; S_e = 0.00%) ***	C (D = 0.14—0.38)	0.10—0.40
BHI (3%)	E	0.20

* E refers to 500-ml cultures in 1-l Erlenmeyer flasks placed in an environmental incubator with rotatory shaking (200 rev./min). The dissolved molecular oxygen became a limiting factor in cultures growing at the expense of uric acid under these conditions; F refers to heavily aerated cultures (10 l) in a 14 l Fermentor; C refers to well-aerated (60 l air/h) continuous cultures (V = 850 ml) with a dilution rate (D; h^{-1}) as indicated.

** BHI, Brain heart infusion (Oxoid).

*** S_i and S_e indicate the concentrations of the substrate in the influent and effluent, respectively.



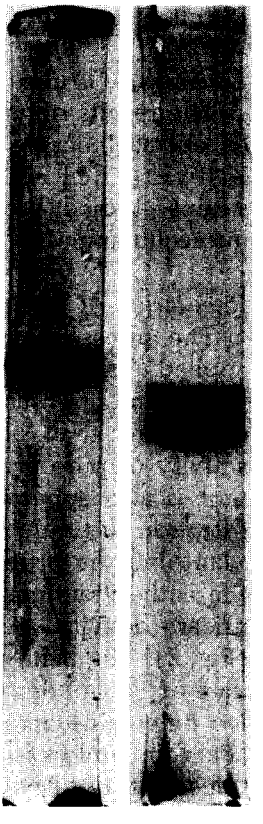


Fig. 1. Sephadex G-200 gel filtration of a crude extract of *B. fastidiosus* SMG 83 cells grown on uric acid (open symbols) or allantoin (closed symbols) in 1-l Erlenmeyer flasks under oxygen-limited conditions at 37°C. The protein content of the fractions was determined according to Warburg and Christian [11]. Symbols used: ○,●, protein content; □,■, uricase content; X, specific activity.

Fig. 2. Gel electrophoresis of purified uricase. Left: 5% polyacrylamide gel after electrophoresis in 0.1 M asparagine adjusted to pH 8.0 with Tris. Right: two bands of the subunits in a 8% gel in the presence of 0.1% SDS.

also apparent in studies on the growth and concomitant degradation of uric acid by cells precultured on allantoin (Fig. 3). A diauxie effect was observed, because allantoin was consumed before the degradation of uric acid started.

Purification and properties

Crude extracts of *B. fastidiosus* cells grown in oxygen-limited flask cultures were purified by means of Sephadex G-200 gel filtration (Fig. 1) and DEAE-Sephadex A-50 chromatography (Table II). Although the enzyme was purified only by a factor 2, the obtained material appeared to consist of highly purified uricase. The activity of uricase was uniformly distributed over one protein peak (Fig. 1). The purified uricase exhibited only one protein band on gel electrophoresis (Fig. 2) performed at pH values from 7.2 to 9, and gel and column isoelectric focusing (data not shown). The isoelectric point was found to be 4.3 at 4°C. The homogeneity of uricase was also evident from ultracentrifugation

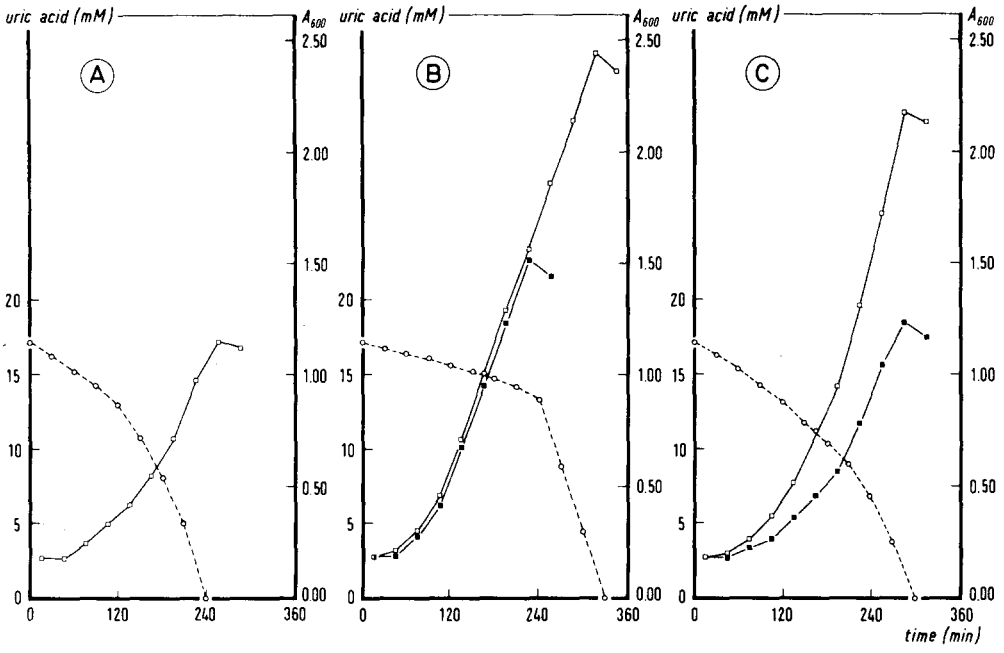


Fig. 3. Consumption of uric acid (dashed lines) and growth (full lines) after inoculation with *B. fastidiosus* SMG 83 cells precultured in an allantoin/brain heart infusion (Oxoid) medium. The cells were inoculated in media containing 17.2 mM uric acid (a), 17.2 mM uric acid and 26.0 mM allantoin (b) 17.2 mM uric acid and 23.0 mM sodium allantoate (c). Growth was followed spectrophotometrically at 600 nm (open squares). Growth of cells was also measured in media containing only allantoin or allantoate (closed squares). Incubation was at 37°C and 200 rev./min. Uric acid was determined at 283 nm after filtration of the samples and subsequent dilution in 0.15 M HCl solution.

studies. The $s_{20,w,c=0}$ value appeared to be 7.1 S, while for pig liver uricase (M_r = 125 000) this value appeared to be 6.8 S [15].

The molecular weight of uricase was determined by means of Sephadex G-200 gel filtration and found to be 145 000—150 000. Gel electrophoresis in the presence of urea (8 M) or sodium dodecyl sulphate (0.1%) showed the presence of two kinds of subunits in the native molecule (Fig. 2). The molecular weights of the subunits were estimated to be 36 000 and 39 000. The native molecule appears to consist of four subunits, probably two of both kinds, because the bands in the gel showed nearly equal density.

TABLE II

PURIFICATION OF URICASE FROM *B. FASTIDIOSUS* SMG 83

Purification step	Activity (units)	Protein (mg)	Specific activity (units/mg protein)	Recovery (%)	Purification
Crude extract	800	22.0	36.4	100	1.00
Sephadex G-200 gel filtration	500	7.7	64.9	62.5	1.78
DEAE-Sephadex A-50 chromatography	370	4.9	75.5	46.3	2.07

The absorption spectrum of the purified uricase exhibited only one peak located at 279—280 nm. No distinct irregularities were observed, which indicates the absence of bound coenzymes like flavins.

Some uricases have been reported [16—18] to be metallo-enzymes containing Cu^{2+} or Fe^{3+}. EPR measurements of 15 mg lyophilized *B. fastidiosus* uricase did not show any detectable amount of paramagnetic copper. The presence of very weak signals in the EPR spectrum indicated that traces of other paramagnetic metal ions were present in the sample. Atomic absorption measurements on a sample of purified uricase showed that the copper : uricase ratio was 1 : 7, and the iron : uricase ratio 1 : 50, which results suggest that these metal ions do not act as functional cofactors.

The amino acid composition of the uricase molecule is given in Table III. No detectable amounts of cysteine could be found by amino acid analysis nor by the 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) method.

The enzyme was crystallized according to the method of Jacoby [21]. At 40% $(NH_4)_2SO_4$ saturation the enzyme crystallized after 3 days as long needles (up to 40 μm). Even after 3 months of storage of the crystals in the $(NH_4)_2SO_4$ solution at 0—4°C nearly all activity (95—100%) was retained.

Enzymatic characterisation of uricase

The enzymatic activity is optimal at pH 9.5 for the uricases of all strains of *B. fastidiosus* tested. Half-maximal activities were observed at pH 8.5 and 10.5. The optimal temperature for the activity of the purified uricase was found to

TABLE III

AMINO ACID COMPOSITION OF PURIFIED URICASE

Amino acids were determined in samples of uricase, hydrolyzed for 24 h in 5.7 M HCl at 105°C, by means of a Jeol 6-AH amino acid auto-analyzer. Tyrosine and tryptophan were determined by the method of Bencze and Schmid [19]. The amount of cysteine in the purified enzyme was determined according to the 5,5′-dithio-bis(2-nitrobenzoic acid) method of Ellman [20].

Amino acid	Weight percentage	Number of amino acid residues per intact molecule
Ala	6.4	130—135
Arg	6.4	59—62
Asx	10.6	133—138
Cys	— *	— *
Glx	14.3	160—166
Gly	4.0	101—105
His	2.8	29—31
Ile	4.6	59—61
Leu	7.0	89—93
Lys	6.1	69—72
Met	1.2	13—14
Phe	7.8	76—80
Pro	2.2	33—34
Ser	5.0	83—86
Thr	6.4	91—95
Trp	1.9	14—16
Tyr	6.7	59—62
Val	6.3	92—96

* Not found.

be 30—35°C (Fig. 4). The decrease of the activity above 35°C was not due to denaturation of the enzyme, since uricase of *B. fastidiosus* is heat-stable up to temperatures of at least 53°C, both in the presence and absence of uric acid (Fig. 4). Probably conformational changes occur in the enzyme at temperatures above 35°C with a concomitant reversible loss of activity.

The K_m value of the purified enzyme was $1.8 \cdot 10^{-4}$ M uric acid, when tested in atmospheric air. The value is comparable with that of $1 \cdot 10^{-4}$ M, reported for the uricase of *B. fastidiosus* NCIB 10372 [9]. The K_m value for molecular oxygen was found to be $1 \cdot 10^{-3}$ M.

Uricase exhibits a very limited action spectrum and is inhibited by some uric acid analogues [22—26]. The analogues given below were not attacked by the enzyme of *B. fastidiosus* and they inhibited the enzyme in incubation mixtures containing 1.5 mM uric acid and 1.5 mM analogue to an extent as given within parentheses: hypoxanthine (30%), xanthine (83%), 8-azaxanthine (92%), 3-methyluric acid (26%), 7-methyluric acid (40%), 9-methyluric acid (77%), 3,7-dimethylxanthine (theobromine; 8%), 1,3,7-trimethylxanthine (caffeine; 11%) and oxonic acid (2,4-dihydroxy-6-carbohydroxy-1,3,5-triazine; 100%). A strong competitive inhibition was exerted by oxonic acid ($K_i = 3.6 \cdot 10^{-6}$ M), xanthine ($K_i = 2 \cdot 10^{-5}$ M) and 8-azaxanthine ($K_i = 1.85 \cdot 10^{-5}$ M). Müller and Møller [23] reported the K_i value of $3.2 \cdot 10^{-6}$ M oxonic acid for uricase of *Acanthamoeba*. Urea, allantoin, uracil and thymine did not exert any inhibiting effect at the 10^{-3} M level.

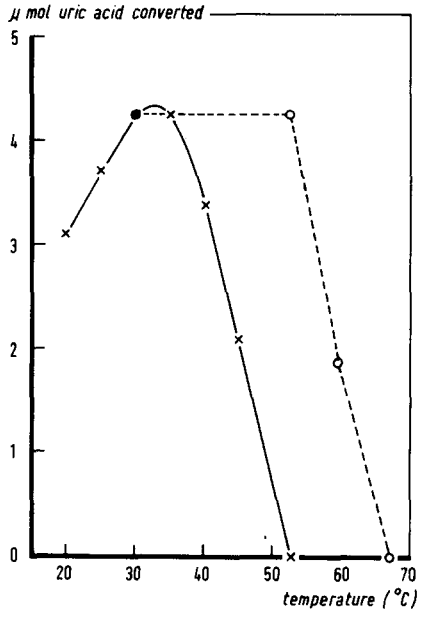


Fig. 4. Effect of the temperature on the activity and stability of uricase. In tests on the activity of uricase (X) a reaction mixture (2.5 ml) containing 4.7 µmol uric acid and 13 µg purified uricase in 0.1 M borate buffer (pH 9.0) was used. Incubation was at the indicated temperature for 10 min. The stability of uricase (○) was tested in solutions (0.7 ml) of purified uricase (26 µg/ml) incubated in preheated tubes at various temperatures for 30 min. Then a sample (0.5 ml) was added to 2 ml uric acid solution and the same reaction mixture was created as used in the activity tests. The activity was measured at 30°C.

The enzyme was partially inhibited in the presence of various cations and the inhibiting effect decreased in the order Zn^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Cd^{2+}, Cr^{3+}, Mn^{2+}, Pb^{2+}, Fe^{2+} (Table IV). No inhibition was exerted by Hg^{2+}, Fe^{3+}, Ca^{2+} and Mg^{2+} tested at the 10^{-3} M level. Similar results were observed in 0.1 M borate buffer (pH 9.0).

The enzyme activity was affected only slightly or not at all by 10^{-3} M chelating agents (bathophenantroline, α,α'-dipyridyl, hydroxychinoline, diethyldithiocarbamate and EDTA) or compounds which are known to react with carbonyl (phenylhydrazine and hydroxylamine), sulfhydryl (*p*-chloromercuribenzoic acid and *N*-ethylmaleimide) and disulfide groups (reduced glutathione, dithiothreitol, cysteine and ascorbic acid). Na_2S and $Na_2S_2O_4$, known as reducing agents, seem to inhibit the enzyme, but their effects are considered as artifacts. $Na_2S_2O_4$ caused a momentaneous lowering of the oxygen concentration, which then becomes a limiting factor. Na_2S itself lowered the oxygen concentration only slowly but it may be transformed to dithionite by the H_2O_2 formed by the uricase action. Uricase of *B. fastidiosus* is completely inhibited by 10^{-3} M KCN. The inhibitory effect of cyanide was previously ascribed to complexation of an essential metal ion in the catalytic center of the uricase, where cyanide would interfere with the binding site of molecular oxygen [16, 18]. This view is not tenable for uricase of *B. fastidiosus*, since this enzyme does not contain essential metal ions and is almost insensitive to various chelating agents. A competing effect of cyanide with molecular oxygen for its binding site, should it occur, cannot be the only effect exerted, since we found an uncompetitive character for the inhibiting effect. The K_i value for cyanide was calculated to be $5 \cdot 10^{-6}$ M in these experiments. The uncompetitive effect may be thought to be composed out of a competitive component due to competition with oxygen and/or uric acid, and a non-competitive one, which can be caused by changes in the tertiary or quaternary structure of the enzyme due to interactions with cyanide. Truscoe [27] suggested that a permanent covalent

TABLE IV

ACTIVITY OF URICASE IN THE PRESENCE OF VARIOUS METAL IONS

The experiments were performed in 2.5 ml 0.1 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (Hepes) buffer (pH 7.5) which contained 4.7 μmol uric acid and 13 μg purified uricase and metal salts as indicated. Incubation was at 30°C. Activity is expressed as the residual activity (%) in the presence of the metal ions as compared with the activity in the absence of the metal ions (0.7 μmol converted in 10 min under suboptimal conditions as to the pH applied).

Metal salt	Activity of uricase		
	10^{-5} M cation	10^{-4} M cation	10^{-3} M cation
$CdCl_2$	97	60	19
$CoCl_2$	88	64	28
$CrCl_3$	104	97	67
$CuSO_4$	72	66	48
$FeCl_2$	100	104	82
$MnCl_2$	94	84	88
$NiSO_4$	70	19	5
$Pb(NO_3)_2$	99	95	86
$ZnSO_4$	37	10	7

modification of the tertiary or quaternary structure of the enzyme was induced by the presence of cyanide, but in our hands the enzymatic activity could be fully recovered by dialysis after treatment of uricase with 0.1 M cyanide for 3 h.

Discussion

The properties of various uricases were recently reviewed [3]. The enzymes of *Alcaligenes eutrophus* (*Hydrogenomonas* H16) [4], *Micrococcus denitrificans* [4], *Pseudomonas aeruginosa* [4,28] and *Pseudomonas testosteroni* [28] are firmly bound to structural components of the cell, whereas the enzymes of *Arthobacter pascens* [18,29], *Fusarium oxysporum* [39] and *B. fastidiosus* are soluble.

The specific activity of pure uricase of *B. fastidiosus* was found to be about 75 as based on measurements of the initial rates in solutions saturated with air and containing $1.5 \cdot 10^{-3}$ M uric acid. This value is 4—10-fold higher than those reported for other purified uricases [3]. About 50% of the intracellular soluble protein consists of this one enzyme when cells were grown on uric acid medium under conditions which limit the oxygen supply. *B. fastidiosus* appears well equipped for immediate and fast growth when uric acid is present in its environment. It may profit optimally from local deposits of uric acid and allantoin present in the urine of various animals or formed from purines by various microorganisms.

The molecular weight of the intact uricase of *B. fastidiosus* amounted to 145 000—150 000, which value is rather high as compared with other uricases, whose molecular weights vary from 93 000 to 125 000 [3]. The native molecule consists of four subunits with molecular weights of 36 000 and 39 000 and probably present in a 1 : 1 ratio. Pitts et al. [15] reported also four subunits, all with a molecular weight of 32 000, for the uricase of pig liver. In contrast to the bacterial enzyme, the pig liver uricase is very resistant to dissociation by sodium dodecyl sulfate. The interactions between the subunits of the enzyme from *B. fastidiosus* are probably influenced in a reversible way at temperatures between 40 and 60°C. A temperature treatment with this range results into a reversible inactivating of the enzyme.

The isoelectric point of the intact molecule is 4.3, which value is lower than those found for uricases from pig liver [31], *Aspergillus flavus* [32] and *Candida utilus* [17], which exhibit values of 6.3, 6.2 and 5.4, respectively.

No detectable amounts of cysteine were found in the enzyme. In accordance with this result, the enzyme is hardly or not al all sensitive to heavy metal ions and sulfhydryl reagents. EPR and atomic absorption studies and experiments with chelating agents revealed no evidence for the presence of Cu^{2+}, Fe^{3+} or other functional metal ions. It remains an interesting question how an enzyme, which appears to act in the absence of cofactors and bound or free coenzymes, can perform such a complex reaction sequence, like uricase does, viz. electron transfer to oxygen and reactions involving the decarboxylation and hydrolysis of uric acid and intermediary products.

Acknowledgements

The authors are indebted to Dr. J. Walters (Department of Biophysical Chemistry) for running the enzyme in the analytical ultracentrifuge, to A. Klaassen (Department of Physical Chemistry) for the performance of the EPR measurements, and to P. Koonen (Department of Anorganic Chemistry) for the performance of the atomic absorption measurements.

References

1 Kaltwasser, H. (1971) J. Bacteriol. 107, 780—786
2 Bongaerts, G.P.A. and Vogels, G.D. (1976) J. Bacteriol. 125, 689—697
3 Vogels, G.D. and van der Drift, C. (1976) Bacteriol. Rev. 40, 403—468
4 Kaltwasser, H. (1968) Arch. Mikrobiol. 60, 160—171
5 Kaltwasser, H. (1969) Arch. Mikrobiol. 65, 288—302
6 Rouf, M.A. and Lomprey, Jr., R.F. (1968) J. Bacteriol. 96, 617—622
7 Scazzocchio, C. and Darlington, A.J. (1968) Biochim. Biophys. Acta 166, 557—568
8 Watanabe, Y., Ohe, T. and Morita, M. (1976) Agric. Biol. Chem. 40, 131—139
9 Mahler, J.L. (1970) Anal. Biochem. 38, 65—84
10 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
11 Warburg, O. and Christian, W. (1941) Biochem. Z. 310, 384—421
12 Davis, B.J. (1964) Ann. N.Y. Acad. Sci. 121, 404—427
13 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406—4412
14 Whitaker, J.R. (1963) Anal. Chem. 35, 1950—1953
15 Pitts, O.M., Priest, D.G. and Fish, W.W. (1974) Biochemistry 13, 888—892
16 Mahler, H.R., Hübscher, G. and Baum, H. (1955) J. Biol. Chem. 216, 625—641
17 Itaya, K., Fukumoto, J. and Yamamoto, F. (1971) Agric. Biol. Chem. 35, 813—821
18 Nose, K. and Arima, K. (1968) Biochim. Biophys. Acta 151, 63—69
19 Bencze, W.L. and Schmid, K. (1957) Anal. Chem. 29, 1193—1196
20 Ellman, G.L. (1959) Arch. Biochem. Biophys. 82, 70—77
21 Jacoby, W.B. (1968) Anal. Biochem. 26, 295—298
22 Fridovich, I. (1965) J. Biol. Chem. 240, 2491—2494
23 Müller, M. and Møller, K.M. (1969) Eur. J. Biochem. 9, 424—430
24 Watanabe, Y., Yano, M. and Fukumoto, J. (1968) J. Agric. Chem. Soc. Jap. 42, 621—628
25 Baum. H., Hübscher, G. and Mahler, H.R. (1956) Biochim. Biophys. Acta 22, 514—527
26 Roush, A.H. and Shieh, T.R. (1963) Fed. Proc. 22, 292
27 Truscoe, R. (1968) Enzymologia 34, 325—336
28 Bongaerts, G.P.A., Sin, I.L., Peters, A.L.J. and Vogels, G.D. (1977) Biochim. Biophys. Acta 499, 111—118
29 Arima, K. and Nose, K. (1968) Biochim. Biophys. Acta 151, 54—62
30 Maruyama, Y. and Alexander, M. (1962) J. Bacteriol. 84, 307—312
31 Mahler, H.R. (1963) in The Enzymes (Boyer, P.D., Lardy, H. and Myrback, K., eds.), 2nd edn., Vol. 8, p. 285—296, Academic Press, New York
32 Laboureur, P. and Langlois, C. (1968) Bull. Soc. Chim. Biol. 50, 811—825