Biochimica et Biophysica Acta, 570 (1979) 341—351

BBA 68827

PYRIMIDINE-DEGRADING ENZYMES

PURIFICATION AND PROPERTIES OF β-UREIDOPROPIONASE OF *EUGLENA GRACILIS*

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(Received February 19th, 1979)

Key words: Pyrimidine degradation; β-Ureidopropionase; (Euglena gracilis)

Summary

In photoorganotrophically grown, mid-log phase cells of *Euglena gracilis*, enyzmes of pyrimidine degradation including uracil reductase, dihydrouracil dehydrogenase, dihydropyrimidinase, and β-ureidopropionase, were detected in a crude extract.

β-Ureidopropionase (*N*-carbamoyl-β-alanine amidohydrolase, EC 3.5.1.6) was purified 100-fold by heat treatment, ammonium sulphate fractionation and chromatography using Sepharose 6B and DEAE-Sephadex A-25. The enzyme follows Michaelis-Menten kinetics (K_m of β-ureidopropionase for β-ureidopropionate $3.8 \cdot 10^{-5}$ M, Hill coefficient $n = 1$). Other enzyme properties are: pH optimum 6.25, temperature optimum 60°C, stimulation by Mg^{2+}, inhibition by Cu^{2+}, $M_r \approx 1.5—2 \cdot 10^6$. β-Ureidoisobutyrate, the intermediate of thymine degradation, and β-ureidopropionate are competing substrates of β-ureidopropionase ($K_i = K_m$ of β-ureidopropionase for β-ureidoisobutyrate $1.8 \cdot 10^{-5}$ M).

Structural analogues of β-ureidopropionate, isobutyrate and propionate are competitive inhibitors (K_i of β-ureidopropionase 0.3 and 0.16 mM, respectively).

There were no indications of regulatory function of β-ureidopropionase in pyrimidine degradation.

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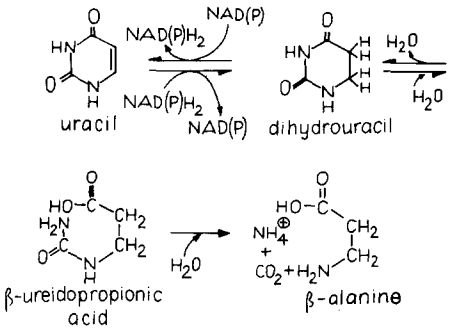


Fig. 1. Reductive degradation of uracil.

Introduction

In most organisms studied, degradation of pyrimidines takes place reductively [1,2]. The three-step sequence starts with reduction in positions 5 and 6 of the pyrimidine ring by NADH or NADPH followed by hydrolytic ring-cleavage between N-3 and C-4 and hydrolytic splitting of the ureido-compounds into CO_2, NH_4^+ and a β-amino acid (Fig. 1). The three reactions are catalysed by NADPH- or NADH-dependent uracil reductase *, dihydropyrimidinase (EC 3.5.2.2), and β-ureidopropionase (*N*-carbamoyl-β-alanine amidohydrolase, EC 3.5.1.6). Detailed enzymatic studies have only been reported for uracil reductase [3,4] as well as for NAD- and NADP-dependent dihydrouracil dehydrogenase (EC 1.3.1.2 and EC 1.3.1.1) of rat liver [3,4] and *Clostridium uracilicum* [5], dihydropyrimidinase from calf and rat liver [6] and β-ureidopropionase of mouse liver [7], rat liver [8], and *Clostridium uracilicum* [9]. But with regard to the 'Molecular Correlation Concept' proposed by Weber [10] or chemical mediation of the excitability of the brain [11] cellular uracil concentration and activities of pyrimidine-degrading enzymes are of importance and show correlation with anabolic reactions of uracil through the salvage pathway. Unfortunately our knowledge of the rate-limiting step in pyrimidine degradation, one prerequisite of Weber's concept, is still poor. According to new results on β-ureidopropionase of mouse liver [7] and rat liver [3] this enzyme might represent the rate-limiting step in the degradation path, while older reports describe the uracil/thymine → dihydrouracil/dihydrothymine reactions as the rate-limiting step (see review [1]). Therefore, β-ureidopropionase was selected for detailed studies during experiments on pyrimidine degradation in the unicellular flagellate *Euglena gracilis* [12—14]. In this plant-like organism pyrimidines are degraded to a different extent by the well-known reductive mechanism [12]. Under in vivo conditions uracil and thymine are degraded in the fixed ratio of 1 : 4 [14], and structural analogues can be used as substrates in pyrmidine degradation [13]. Only 30—40 per cent of the uracil taken up is degraded by photoorganotrophically

* In accordance with Hallock and Yamada [3] the name uracil reductase will be used for the enzyme catalysing the reactions uracil/thymine → dihydrouracil/dihydrothymine and the name dihydrouracil dehydrogenase for the enzyme catalysing the reactions dihydrouracil/dihydrothymine → uracil/thymine.

grown cells [12] whereas 80—90 per cent of thymine is degraded [14]. This remarkable extent of degradation prompted detailed studies on pyrimidine degrading enzymes in order to elucidate their role in pyrimidine metabolism in connection with nucleic acid synthesis during light-induced chloroplast biogenesis of *E. gracilis*.

The present paper describes detection of uracil reductase, dihydrouracil dehydrogenase, and dihyropyrimidinase. It presents for the first time purification procedures and properties of β-ureidopropionase from a plant-like organism.

Materials and Methods

Cell and their culture conditions

The unicellular flagellate *E. gracilis* Klebs var. Z Pringsheim (No. 1224-5/25 from the Algal Collection of the University of Göttingen, G.F.R.) was used in all experiments. Cells were grown photoorganotrophically under permanent illumination at 26° C in 400 or 1400 ml Fernbach flasks containing 100 or 500 ml of culture medium. The culture medium (adjusted to pH 4.0) was that described by Ohmann and Phlak [15]. In this medium glucose, glutamic acid, and ammonium sulphate provided the carbon and nitrogen. Illumination was performed with white light (NARVA, type LS 40 white 20; 3000 lux) at a distance of 40 cm. The cell number was measured with the electronic counter TUR ZG 2 (VEB Röntgenwerk, Dresden, G.D.R.). Growth started without any lag period after inoculation with 2—5 ml mid log phase cells at a concentration of 10^5 cells per ml.

Radiochemicals

[2-^{14}C]Uracil (spec. act. 54.9 Ci/mol) and [2-^{14}C]thymine (spec. act. 53 Ci/mol) were obtained from UVVVR (Prague, CSSR). [2-^{14}C]Dihydrouracil (spec. act. 0.5 Ci/mol) was obtained from ROTOP (Dresden, G.D.R.). [*β-Ureido*-^{14}C]-propionate was prepared from [2-^{14}C]dihydrouracil by the method of Fink et al. [16]. All compounds were tested for radiochemical purity by thin layer chromatography (TLC) and electrophoresis.

Chemicals

Chemicals were obtained as indicated below: NAD, NADP, NADH, and NADPH (VEB Arzneimittelwerk, Dresden, G.D.R.), ATP, AMP, uracil and thymine (Reanal, Budapest, Hungary), UTP, UMP and calibration proteins (combithek®) (Boehringer, Mannheim, G.F.R.), dihydrouracil and dihydrothymine (FERRAK, Berlin-West), various amino acids (Calbiochem, Luzern, Switzerland), Sephadex G-150, Sepharose 6B and DEAE-Sephadex A-25 (Pharmacia, Uppsala, Sweden), β-Ureidoisobutyric acid was prepared from dihydrothymine by the method of Fink et al. [16].

Enzyme assays

(1) *Uracil reductase*: Standard incubation mixture for the determination in crude extract contained 0.5 mM [2-^{14}C]uracil (0.2 μCi), 10 mM NADPH, 10 mM $MgCl_2$, 10 mM mercaptoethanol, 50 mM Tris/HCl (pH 7.35), 1 mg protein

in a total volume of 150 µl. For the determination of the reverse reaction catalysed by dihydrouracil dehydrogenase test tubes contained 0.5 mM [2-^{14}C]-dihydrouracil (=0.2 µCi) and 10 mM NADP instead of [2-^{14}C]uracil and NADPH. Standard incubation mixture for the purified uracil reductase contained 0.5 mM [2-^{14}C]uracil (=0.1 µCi), 10 mM NADPH, 10 mM $MgCl_2$, 5 mM mercaptoethanol, 20 mM Tris/HCl (pH 7.35), 1 mg protein in a total volume of 150 µl. Incubations were carried out at 30° C in conical centrifugation tubes for 30 min and stopped by protein precipitation in a boiling water bath for 2 min.

(2) *Dihydropyrimidinase*: Standard incubation mixture contained 0.02 mM [2-^{14}C]dihydrouracil (=0.2 µCi), 10 mM $MgCl_2$, 10 mM mercaptoethanol, 50 mM Tris/HCl (pH 7.35), 1 mM protein in a total volume of 150 µl. Incubations were performed at 30° C in conical centrifugation tubes for 30 min and stopped by protein precipitation in a boiling water bath for 2 min. [2-^{14}C]dihydrouracil and [*β-ureido*-^{14}C]propionic acid were separated by TLC using 20—30 µl of the supernatant of the centrifuged incubation mixture and determined radiometrically (see below).

(3) *β-Ureidopropionase*: Standard incubation mixture for the determination in the crude extract contained 0.5 mM [*β-ureido*-^{14}C]propionate (=0.1 µCi), 10 mM $MgCl_2$, 10 mM mercaptoethanol, 50 mM Tris/HCl (pH 7.35), 1 mg protein in a total volume of 1 ml. For the purified enzyme, the standard incubation mixture contained [*β-ureido*-^{14}C]propionate (0.02 µCi, 0.5 mM), 10 mM $MgCl_2$, 5 mM mercaptoethanol 1/15 M Sörensen-buffer (pH 6.25) and $8 \cdot 10^{-5}$ I.U. * of the enzyme in a volume of 1 ml. Incubations were carried out with shaking at 30° C for 30 min in Warburg-vessels containing 1 ml of incubation mixture, 200 µl 20% KOH in the central well and 500 µl 2 N $HClO_4$ in the side arm and were stopped by addition of the $HClO_4$. $^{14}CO_2$ produced was fixed in the KOH, and two aliquotes of 100 µl each were counted by liquid scintillation counting (see below). In the chromatographic analysis of the incubation mixture labelled dihydrouracil could not be detected suggesting no reverse reaction of dihydrouracil cleavage under our conditions. Protein was measured according to Lowry et all [17] using bovine serum albumin as standard.

Radioactivity

Radioactivity was measured in 100-µl portions in vials containing 10 ml toluene/PPO/POPOP scintillation coctail mediated by 5 ml methanol by means of a Nuclear Chicago scintillation Counter. In the case of thin-layer sheets mediation was performed with methanol, too.

Thin-layer chromatography

[2-^{14}C]Dihydrouracil and [*β-ureido*-^{14}C]propionate of the incubation mixture of dihydropyrimidinase were separated by TLC on cellulose sheets Polygram Cell 300 UV_{254} (Machery-Nagel and Co, Düren, G.F.R.) using water-saturated collidine as solvent. Substrate and products of the uracil reductase reaction were separated by continuous TLC on Kieselgel D plates (VEB Chemiewerk, Greiz, Dölau, G.D.R.) (prepared as 0.5 mm thick layers and

* I.U. determined wity 0.1 mM β-ureidopropionate at pH 7.35 and 30° C.

activated at 120°C for 10 min) using chloroform/methanol/acetic acid (100 : 5 : 1; v/v/v) as solvent under sandwich conditions at 4°C for 12 h [18]. Substrate and product of the dihydrouracil dehydrogenase reaction were separated by TLC on Kieselgel G plates prepared as mentioned above using water-saturated collidine as solvent. After localization using thin layer scanner II (Fa. Berthold, Wildbad, G.F.R.) labelled positions were cut out and measured by scintillation counting. In the case of Kieselgel D plates Neatan® (Merck, Darmstadt, G.F.R.) was used to stabilize the thin layer powder after TLC.

Preparation of the crude enzyme extract

$2 \cdot 10^9$ cells were resuspended in 50 mM Tris/HCl (pH 7.35) and disrupted by ultrasonification with the Branson Sonifier B-12 (Branson Sonic Power Company, Danbury, U.S.A.). Sonification periods of 4 times 1 min each were interrupt by breaks of 1 min. All further procedures were performed at 4°C. After centrifugation at 20 000 × *g* the supernatant was used for enzyme determination.

Purification procedures

After centrifugation the 20 000 × *g* supernatant was exposed to a temperature of 50°C for 1 min and centrifuged. After that the 20 000 × *g* supernatant was fractionated with neutral saturated $(NH_4)_2SO_4$. The fractions between 30 and 40 per cent saturation were subjected to futher purification by column chromatography in the following order:

(1) Sephadex G-150 (column 30 × 340 mm, equilibrated with buffer run at a hydrostatic pressure of 36 cm)

(2) Sepharose 6B (column 30 × 570 mm, using a peristaltic pump at a rate of 30 ml per hour)

(3) ion exchange chromatography on DEAE-Sephadex A 25 (column 9 × 150 mm, after 60 ml of buffer a linear gradient of 0—0.2 M NaCl in buffer was applied at a rate of 30 ml per hour.

The buffer used in all cases was 50 mM Tris/HCl pH 7.35 containing 5 mM mercaptoethanol and 10 mM $MgCl_2$. Absorption of proteins were measured at 280 nm using an Uvicord II (LKB, Bromma, Sweden), collected with an Uvicord fraction collector and concentrated with an Column Eluate Concentrator Model CEC 1® (Amicon, The Netherlands). The prepared β-ureidopropionase was stored at −30°C in 50 mM Tris/HCl pH 7.35 containing 5 mM mercaptoethanol and 10 mM $MgCl_2$ for no more than three days. Under these conditions activity decreases by 10 per cent only. Buffer exchange was carried out according to Determann [19] without detectable loss of activity in a basket centrifuge in presence of 10 mg per ml bovine serum albumin.

Results

Pyrimidine degrading enzymes in the crude extract

The remarkable $^{14}CO_2$ release from [2-^{14}C]uracil (30—40%), thymine (80%) and dihydrouracil (90%), each figure given in per cent of the portion taken up, suggests the presence of an intact reductive pathway in *E. gracilis* [12,14] and

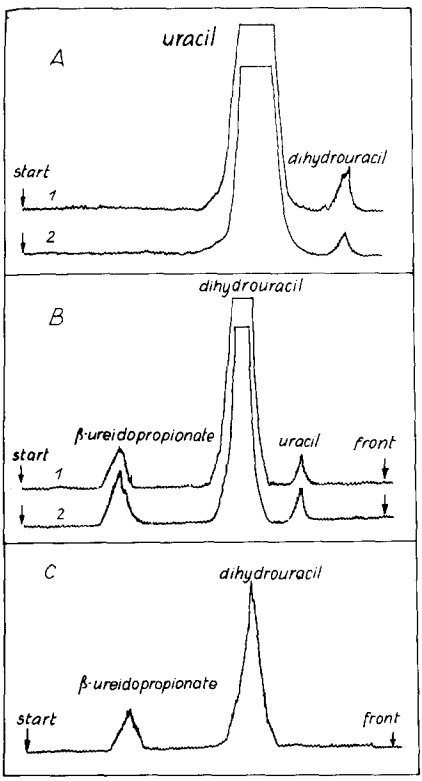


Fig. 2. Detection of uracil reductase (A), dihydrouracil dehydrogenase (B) and dihydropyrimidinase (C) in *E. gracilis*. Crude enzyme extracts of photoorganotrophically grown, mid-log phase cells, were prepared and incubated in the standard assay of each reactions as described in Methods. Labelled substrates and products were localized after TLC as their scanner profile as described in Methods. In the uracil reductase and dihydrouracil dehydrogenase reactions NADPH(H) (1) or NAD(H) (2) were used.

of the enzymes uracil reductase, dihydropyrimidinase and β-ureido-propionase. All three enzymes could be detected radiometrically by TLC of the products (Fig. 2) and by measurement of $^{14}CO_2$ release in the case of β-ureidopropionase using crude extracts of photoorganotrophically grown mid log phase cells of *E. gracilis*. Uracil is degraded through dihydrouracil and β-ureidopropionic acid to CO_2, NH_4^+ and β-alanine while the corresponding compounds in thymine degradation path are dihydrothymine, β-ureidoisobutyric acid and CO_2, NH_4^+ and β-aminoisobutyric acid (Fig. 1). Uracil reductase and dihydrouracil dehydrogenase use NADPH/NADH and NADP/NAD resp. to a comparable extent. The presence of dihydropyrimidinase and β-ureidopropionase in the crude extract results in a further transformation of dihydrouracil after the uracil reductase reaction. Similarly dihydropyrimidinase activity of crude extract results in a $^{14}CO_2$ release. The whole degradation path is detectable in a crude extract as $^{14}CO_2$ release after feeding of [2-^{14}C]uracil (0.8%), [2-^{14}C]-dihydrouracil (2%) and [2-^{14}C]thymine (1.8%) given each in per cent of the portion fed. Therefore the first two enzymes are exactly measurable only after purification steps leading to separation from dihydropyrimidinase and β-ureido-propionase respectively.

The oxidation of dihydrouracil takes place only at high concentrations of NADP or NAD (10 mM). Therefore the influence of these reactions on pyrimidine degradation could be neglected under the conditions used. In the incubation mixture with crude extract containing β-ureidopropionase 3.5% of fed radioactive compound was shown to be converted to $^{14}CO_2$. Because of the absence of labelled dihydrouracil in this incubation mixture the $^{14}CO_2$ release represents the real enzyme activity.

Purification of β-ureidopropionase

For the preparation of purified β-ureidopropionase $4 \cdot 10^9$ photoorganotrophically grown mid-log phase cells were suspended in 20 ml 50 mM Tris/HCl (pH 7.35, 10 mM $MgCl_2$ + 10 mM mercaptoethanol), disrupted by sonification and purified by heat treatment and ammonium sulphate fractionation (see Materials and Methods). After further purification on Sephadex G-150 β-ureidopropionase was eluated with void volume (V_0) and was incompletely separated from dihydropyrimidinase and uracil reductase. The molecular weight of β-ureidopropionase is obviously higher than 200 000 as suggested by elution with V_0. Therefore, further purification was performed on Sepharose 6B (Fig. 3A) resulting in complete separation of β-ureidopropionase from uracil reductase and dihydropyrimidinase. Peak fractions of β-ureidopropionase were purified on DEAE-Sephadex A-25 (Fig. 3B). The whole procedure leads to a 100-fold purification of β-ureidopropionase (Table I). Calculation of molecular weight by molecular sieve chromatography using Sepharose 6B and cytochrome *c*, chymotrypsinogen A, chicken albumin, bovine albumin, aldolase, catalase and ferritin as standard proteins results in a molecular weight of approx. $1.5-2 \cdot 10^6$. Because of this high value further studies on protein properties seems to be necessary.

pH and temperature dependence

In order to analyse the pH dependence of purified β-ureidopropionase, its activity was measured in Tris/HCl and Sörensen buffers in the range of 5.0—

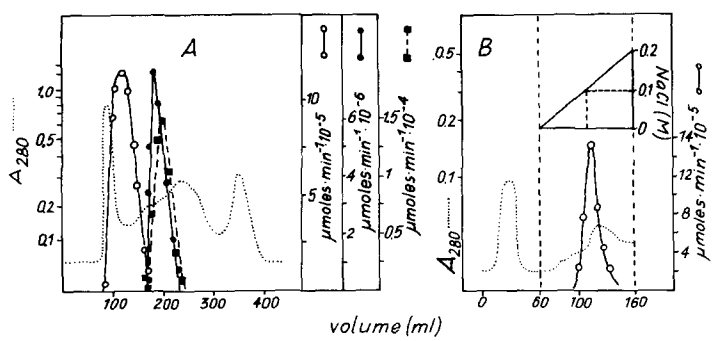


Fig. 3. Purification of β-ureidopropionase by chromatography on Sepharose 6B (A) and DEAE-Sephadex A-25 (B). Separations were performed in 50 mM Tris/HCl buffer pH 7.35 containing 5 mM mercaptoethanol and 10 mM $MgCl_2$ (A) and with 60 ml of this buffer followed by a linear gradient of 0—0.2 M NaCl in this buffer (B) as described in Methods. Protein absorbance was measured at 280 nm (· · · · · ·). Enzyme activity of uracil reductase (■———■), dihydropyrimidinase (●———●), and β-ureidopropionase (○———○) were determined in each fraction using standard assay as described in Methods.

TABLE I

PURIFICATION OF β-UREIDOPROPIONASE OF *E. GRACILIS*

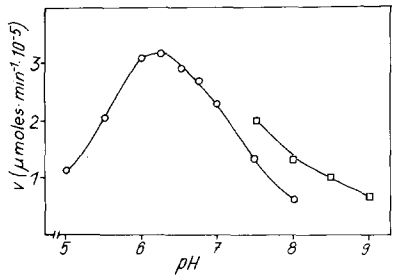
(Procedure described in Methods).

Fraction	Volume (ml)	Protein content (mg · ml^{-1})	Activity (I.U. × 10^{-3})	Spec. act. (μmol · min^{-1} · mg^{-1} × 10^{-3})	Purification factor
Crude extract	23	12.0	37	0.154	1
Heat treatment	23	7.4	42	0.25	1.6
Ammonium sulphate fractionation	5	8.4	33.6	0.8	5.2
Sepharose 6B	28.5	0.115	15.8	4.8	31.1
DEAE-Sephadex A-25	13	0.044	8.4	14.7	95.5

9.0. This way the pH optimum was shown to be 6.25 (Fig. 4). The enzyme exhibits a temperature optimum at 60°C.

v/s characteristics

The substrate conversion by β-ureidopropionase follows Michaelis-Menten kinetics as seen in the *v/s* plot (Fig. 5a). According to the Lineweaver-Burk plot (Fig. 5B) the K_m valuewas calculated to 3.8 · 10^{-5} M. The non-cooperativity of



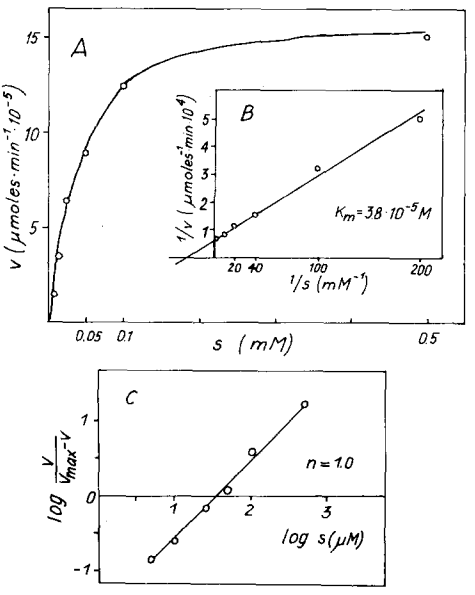


Fig. 4. pH dependence of purified β-ureidopropionase from *E. gracilis*. Photoorganotrophically grown, mid-log phase cells, were used, and 100-fold purified enzyme was prepared by heat treatment, ammonium sulphate fractionation and chromatography on Sepharose 6B and DEAE-Sephadex a-25 as described in Methods. Incubations were performed in 50 mM Tris/HCl (□———□) and 1/15 M Sörensen buffer (○———○) as described in Methods.

Fig. 5. *v/s* characteristics of β-ureidopropionase from *E. gracilis*. The enzyme was 100-fold purified and assayed as described in Methods using β-ureidopropionate as the substrate. Enzyme kinetics are shown as substrate saturation plot (A), Lineweaver-Burk plot (B) and Hill plot (C).

β-ureidopropionase is suggested by the Hill plot (Fig. 5C) giving a value of $n = 1$.

Influence of cations and selected metabolites

The influence of some cations was tested on purified enzyme with their chlorides in the absence of $MgCl_2$. Except the stimulatory effect of Mg^{2+} only an inhibitory effect of Cu^{2+} is remarkable while K^+, Na^+, Ca^{2+}, Mn^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+} were ineffective in the concentration of 1 mM.

For the evaluation of the regulatory function of β-ureidopropionase the effect of pyrimidine metabolites as well as certain adenylate compounds and coenzymes on the enzyme was of interest. Except for β-ureidoisobutyric acid the following compounds tested were without any effect on the range of 0.3–1 mM: NH_4^+, β-alanine, β-aminoisobutyric acid, ureidosuccinic acid, uracil, thymine, dihydrouracil, dihydrothymine, ATP, ADP, AMP, UTP, and NAD(P)(H). The inhibitory effect of β-ureidoisobutyric acid as an intermediate of thymine degradation raises the question of substrate specificity of β-ureidopropionase.

Substrate specificity of β-ureidopropionase and the inhibitory influence of some structural analogues

The inhibition of β-ureidopropionase by β-ureidoisobutyrate is competitive with respect to β-ureidopropionate (Fig. 6). β-Ureidoisobutyrate is a substrate of β-ureidopropionase as suggested by the following experiment. Peak fractions of uracil reductase and dihydropymidinase, after separation on Sepharose 6B free of β-ureidopropionase, were incubated with [2-^{14}C]thymine as substrate. In this case $^{14}CO_2$ release could not be observed but after TLC of the incubation mixture labelled dihydrothymine and β-ureidoisobutyrate became detectable. After addition of peak fractions of β-ureidopropionase $^{14}CO_2$ release took place suggesting β-ureidoisobutyrate degradation.

Under these conditions the K_i value equals the K_m value. Thus $K_i = K_m$ of

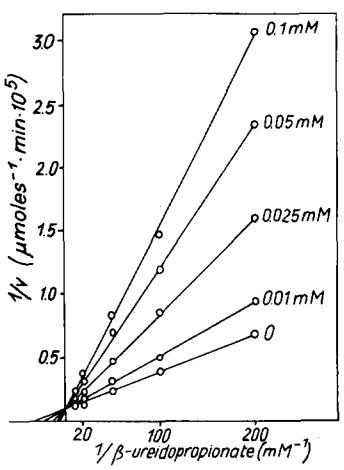


Fig. 6. Inhibitory effect of β-ureidoisobutyrate in the β-ureidopropionase reaction. Using labelled β-ureidopropionase, the activity of 100-fold purified β-ureidopropionase was measured in presence of 0.01, 0.025, 0.05, and 0.1 mM β-ureidoisobutyrate or without any addition as described in Methods.

$1.8 \cdot 10^{-5}$ M for β-ureidoisobutyrate determined by the Lineweaver-Burk plot (Fig. 6) and the Dixon plot was similar K_m of β-ureidopropionase for β-ureidopropionate. According to the Lineweaver-Burk plot linearity of K_m (β-ureidopropionate) vs. β-ureidobutyrate concentration suggests competition between both substrates.

Competitive inhibition was observed with some structural analogues of β-ureidopropionate: isobutyrate (K_i of 0.3 mM), propionate (K_i of 0.16 mM), α-fluoroacetate (K_i of 0.35 mM), and acetate (K_i of 0.7 mM). The following compounds containing the ureido group were ineffective in a concentration of 1 mM: urea, biuret, thiourea, monophenylurea, methylurea, acetylurea, allantoin, allantoic acid, and L-canavanine sulphate Similarly ineffective were the following compounds: β-aminoisobutyric acid, γ-aminobutyric acid, β-DL-amino *n*-butyric acid, α-amino *n*-butyric acid, α-amino isobutyric acid, β-alanine, and α-L-alanine (1 mM each).

Discussion

Reductive degradation of pyrimidines in *E. gracilis* suggested from tracer experiments with intact cells [12] is now demonstrated by detection of the respective enzymes. Uracil reductase uses uracil and thymine as substrates. This is in accordance with reports on rat liver enzyme [4] in which thymine and uracil are substrates to a comparable extent after purification by disc-electrophoresis. There were similar reports on the enzyme of *Hydrogenomonas facilis* [20].

Similar to the rat liver enzyme [4] the dihydrouracil dehydrogenase observed in *E. gracilis* uses dihydrouracil as well as dehydrothymine. Without any doubt, under cellular conditions dihydrouracil oxidation has no metabolic function because of the high K_m of dihydrouracil dehydrogenase for dihydrouracil ($1.5 \cdot 10^{-3}$ M in rat liver [4]), the location of dihydropyrimidine degrading enzymes in the cytosol [3,4] and the irreversibility of the β-ureidopropionase reaction. In line with these observations we find no incorporation of [2-^{14}C]dihydrouracil into uracil and acid soluble nucleotides by intact cells of *E. gracilis* and, after feeding of [2-^{14}C]uracil, labelled dihydrouracil is detectable by TLC only after inhibition of the last step of uracil degradation [14]. The previously reported incorporation of [*β-ureido*-^{14}C]propionic acid into acid soluble nucleotides [21] seems to be doubtful.

In view of the proposed role of β-ureidopropionase in the degradation path [1] more data on its kinetics and properties would be useful. β-Ureidopropionase of *E. gracilis* follows Michaelis-Menten kinetics (Fig. 5). Noncooperativity is suggested by the Hill coefficient $n = 1$ (Fig. 5C). The K_m of β-ureidopropionase for β-ureidopropionate ($3.8 \cdot 10^{-5}$ M) is significantly lower than that of rat liver enzyme ($5 \cdot 10^{-4}$ M) [8] or the enzyme of *Clostridium uracilicum* ($6.3 \cdot 10^{-4}$ M) [9]. Degradation of both β-ureidopropionate and β-ureidoisobutyrate was shown for the enzyme of *E. gracilis*. The competition between both substrates in the β-ureidopropionase reaction was demonstrated by the Lineweaver-Burk plot (Fig. 6), the Dixon plot, and linearity of K_m (β-ureidopropionate) vs. β-ureidoisobutyrate. Kinetics of purified enzyme suggests that degradation of both substrates occurs to a similar extent. In intact

cells under comparable conditions the ratio of thymine and uracil degradation is 4 : 1 [14]. The unspecific degradation of uracil and thymine and their intermediates by pyrimidine degrading enzymes seems to be a more general phenomenon [1]. This is emphasized by the recently demonstrated degradation of cyclic imides of different ring size by dihydropyrimidinase [22—24].

Indications as to a function of β-ureidopropionase as rate-limiting enzyme in pyrimidine degradation are not supported by the clear Michaelis-Menten kinetics of the *E. gracilis* enzyme and its insensitivity to selected metabolites. Because of the location of uracil and its degradation products in different pools [Waternack, unpublished) compartmentation of pyrimidine degradation seems to play a predominant role in this eucaryotic cell.

Structural analogues in pyrimidine degradation are of special interest to enhance the inhibitory effect of some halouracils in chemotherapy. Most of analogues studied in this respect are inhibitors of the transformation of uracil → dihydrouracil [13,25,26]. Similar inhibitory effects of selected structural analogues of β-ureidopropionate on the β-ureidopropionase reaction in vivo are not useful because of their low affinity for the enzyme.

Acknowledgement

We would like to express our thanks to Dr. K.-P. Rindt for greatly improving the text.

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