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Bisfunction of propionic acid on purified rat liver β-ureidopropionase

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Propionic acid and isobutyric acid, which are structural analogues of *N*-carbamoyl-β-alanine and *N*-carbamoyl-β-aminoisobutyric acid, respectively, acted as an allosteric activator as well as a competitive inhibitor of purified rat liver β-ureidopropionase. Propionic acid and isobutyric acid had a K_i value of approx. 0.3 mM at pH 7.0. The Hill coefficient for *N*-carbamoyl-β-alanine was 2.0, but the cooperativity decreased to 1.0 in the presence of 1 mM propionic acid. The $K_{1/2}$ value towards *N*-carbamoyl-β-alanine was calculated to be 0.17 mM from Hill plots and the K_m value was determined to be 0.06 mM from replots of the apparent K_m vs propionic acid.

β-Ureidopropionase; Propionic acid; Isobutyric acid; β-Alanine; β-Aminoisobutyric acid; *N*-Carbamoyl-β-alanine

1. INTRODUCTION

β-Ureidopropionase (NCβ-Ala amidohydrolase, EC 3.5.1.6) is the last of the uracil-metabolizing enzymes that catalyze the conversion of uracil to β-alanine [1,2]. The rate-limiting step of uracil catabolism is the conversion of uracil to dihydrouracil by dihydrouracil dehydrogenase [3–5], however β-ureidopropionase also functions in limiting the rate under specific conditions [6,7]. The enzyme was purified from *E. gracilis* cultures [8] and rat liver preparations [9]. The rat liver enzyme has an oligomeric structure [9,10] and exhibits cooperative properties with substrates such as NCβ-Ala and NCβ-AIB [9]. NCβ-Ala produced aggregation of rat liver β-ureidopropionase without significant alteration of enzyme activity and β-alanine caused dissociation concomitant with enzyme inactivation [10]. However, the regulation of β-ureidopropionase activity is nevertheless obscured by physiological conditions.

This paper describes how propionic acid and isobutyric acid, derivatives of the enzyme substrates, act as allosteric activators as well as competitive inhibitors.

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Abbreviations: NCβ-Ala, *N*-carbamoyl-β-alanine; NCβ-AIB, *N*-carbamoyl-β-aminoisobutyric acid

2. MATERIALS AND METHODS

All chemicals used were of analytical grade and purchased from Nakarai Chemicals, Kyoto, unless stated otherwise. NCβ-Ala was a product of Sigma and NCβ-AIB was synthesized according to Fink et al. [11]. Glutamate dehydrogenase in 50% glycerol was from Boehringer Mannheim.

β-Ureidopropionase was purified from rat liver by heat treatment, ammonium sulfate fractionation and preparative chromatography on DEAE-Sepharose CL-6B, hydroxyapatite and Sephacryl S-300 [9]. The enzyme was shown to be homogeneous by polyacrylamide gel electrophoresis in both the presence and absence of SDS, having a specific activity of 1.6 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ under the standard assay conditions. It was stored in 50% glycerol at about 4°C. The specific activity did not vary for at least 2 weeks.

β-Ureidopropionase activity was measured with respect to the rate of formation of ammonia [9]. The standard reaction mixture contained 0.1 M sodium phosphate, pH 7.0, including bovine serum albumin (0.1%), 10 mM $MgCl_2$, 1 mM EDTA, 5 mM 2-mercaptoethanol, and 2 mM NCβ-Ala. Incubation was carried out in a shaking water bath for 30 min at 37°C.

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3. RESULTS

The enzyme preparations were kinetically tested, and the velocity profiles of liver β-ureidopropionase as a function of NCβ-Ala concentration are shown in fig.1a. Enzyme activity with the substrate showed sigmoid kinetics. The cooperativity of the enzyme was calculated, the value of the Hill coefficient being found to be 2.0 (fig.1b). Addition of 1 mM propionic acid to the assay medium changed the velocity curve of rat liver β-ureidopropionate with respect to NCβ-Ala from sigmoidal to hyperbolic with a concomitant change in the Hill coefficient to 1.0. However, the velocity curve in the presence of 1 mM propionic acid did not exceed that in the absence of the effector at the various concentrations of NCβ-Ala examined (fig.1a). The results suggest that propionic acid also acts as an inhibitor of the enzyme activity. The affinity to NCβ-Ala, as shown by the $K_{1/2}$ value, also changed from 0.17 to 0.35 mM in the presence of 1 mM propionic acid. When the same analysis was repeated in the presence of 0.1 and 0.01 mM propionic acid, the Hill coefficients towards the substrate were 1.7 and 2.0, respectively (not shown).

The inhibitory effect of organic acids on the enzyme activities was investigated at pH 7.0 (table 1). Propionic, isobutyric and acetic acids were found to be effective inhibitors; in addition, malonic, formic and *n*-butyric acids also inhibited the enzyme activity. The enzyme did not affect other normal 3-carbon compounds such as pyruvic acid, L-lactic acid, β-hydroxypropionic acid, propylamine and propanol. β-Alanine and β-aminoisobutyric acid, which are the metabolic products of NCβ-Ala and NCβ-AIB, respectively, did not inhibit enzymatic activity, at least up to 1 mM under the given conditions.

Table 1

Inhibition of rat liver β-ureidopropionase by organic acids and propionic acid analogues

Inhibitors	Activity (%)
None	100
Formic acid	93
Acetic acid	67
Propionic acid	51
n-Butyric acid	94
n-Valeric acid	100
Isobutyric acid	53
Isovaleric acid	99
Malonic acid	81
Pyruvic acid	100
L-Lactic acid	100
β-Hydroxypropionic acid	98
n-Propylamine	100
n-Propanol	100

All inhibitors were neutralized with 0.1 N NaOH or 0.1 N HCl and were added to give a final concentration of 0.1 mM. NCβ-Ala concentration was 0.2 mM. Other conditions were the same as for the standard assay

The dependence of liver β-ureidopropionase ac-

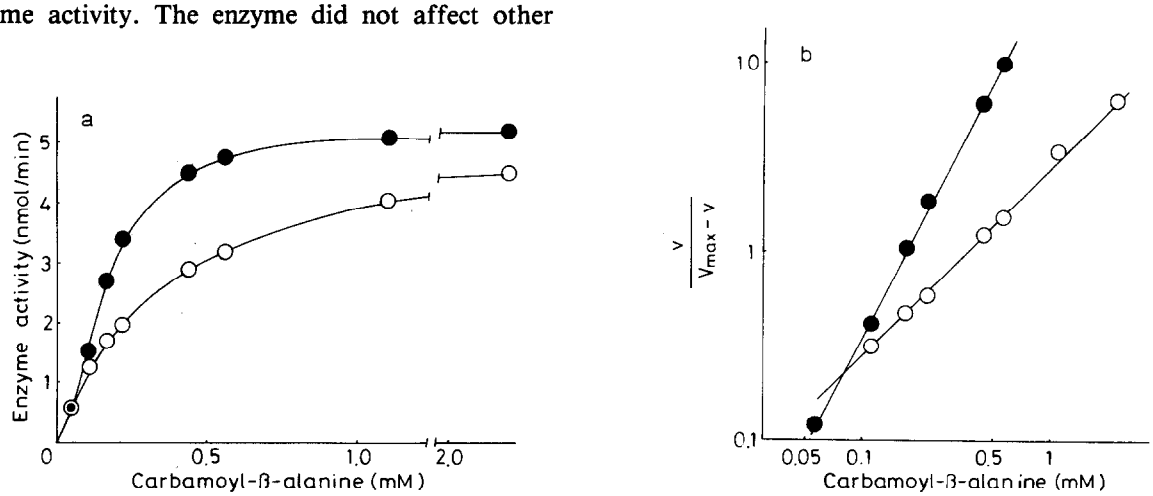

Fig.1. Effect of propionic acid on substrate concentration of β-ureidopropionase activity. Enzyme kinetics are shown as substrate saturation plot (a) and Hill plot (b). (●) Control, (○) 1 mM propionic acid. Other conditions were the same as for the standard assay.

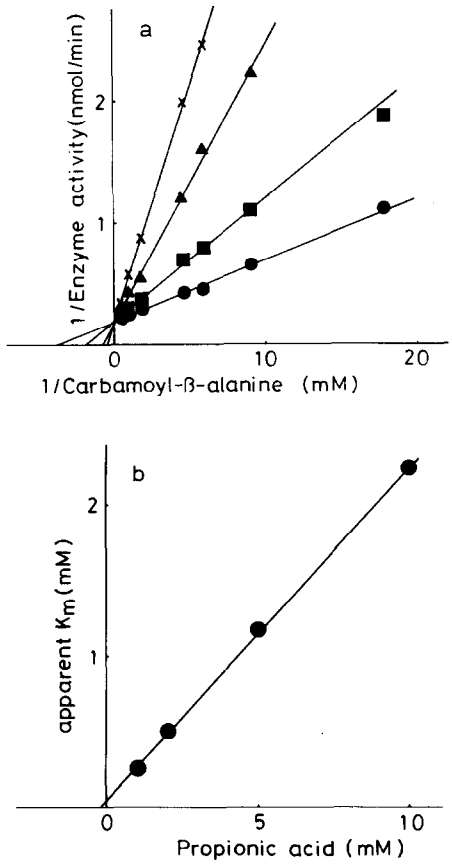

Fig.2. Inhibition of rat liver β-ureidopropionase by propionic acid. (a) Lineweaver-Burk plot. Concentration of propionic acid: (●) 1 mM, (■) 2 mM, (▲) 5 mM, (×) 10 mM. (b) Apparent K_m values for NCβ-Ala as a function of propionic acid concentration.

tivity in the presence of propionic acid on the NCβ-Ala concentration showed hyperbolic kinetics. Lineweaver-Burk plots were linear (fig.2a). However, below 1 mM propionic acid, the Lineweaver-Burk plots were nonlinear (not shown). A replot of the apparent K_m values of each reciprocal plot vs propionic acid concentration gave a straight line (fig.2b). The K_i value was calculated to be 0.3 mM from the intercept on the abscissa and the K_m value was 0.06 mM as determined from the intercept on the ordinate.

Isobutyric acid also had a K_i value of 0.3 mM, indicating competitive inhibition of purified liver β-ureidopropionase, this value agreeing with that obtained for *E. gracilis* [8].

4. DISCUSSION

We previously suggested [9] that β-ureidopropionase from rat liver is an oligomeric enzyme with cooperativity for the substrate. As described here, propionic acid functions as an allosteric effector of enzymatic activity. On the other hand, Matthews and Traut [10] reported no cooperativity for substrate in kinetic studies on β-ureidopropionase from rat liver. This discrepancy might be explained by the fact that we used a purified enzyme and therefore were able to observe the allosteric properties of the enzyme, whereas they used a crude extract prepared by ammonium sulfate fractionation for analysis of the enzyme properties.

The K_m value for β-ureidopropionase with NCβ-Ala was estimated to be 60 μM (fig.2b) in the presence of propionic acid. The $K_{1/2}$ value was 170 μM for NCβ-Ala in the absence of the allosteric effector. Previously reported K_m values for NCβ-Ala varied from 17.4 to 500 μM [2,6,9,10]. As β-ureidopropionase from rat liver is an allosteric enzyme, the concentration of substrate and contamination of allosteric effectors must be determining factors in kinetic experiments.

Our kinetic studies on β-ureidopropionase from rat liver demonstrated two propionic acid-binding sites, one being catalytic and the other allosteric. Matthews and Traut [10] proposed that β-ureidopropionase from liver is regulated in an opposing fashion via dissociation and association by the substrate and product. However, the allosteric site detected here may be different from the regulatory site in the polymerization of β-ureidopropionase, since the presence of 1 mM propionic acid had no effect on the molecular mass of the enzyme [9], but did decrease the Hill coefficient (fig.1b). Since the concentration of propionic acid in living cells is 0.2–0.5 mM [12,13], propionic acid could affect β-ureidopropionase activity in vivo. Detailed studies are necessary to clarify the regulatory effect of β-ureidopropionase in pyrimidine metabolism.

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