Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Molecular Catalysis B: Enzymatic 23 (2003) 265–271
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Purification and substrate characterization of α-ketobutyrate decarboxylase from *Pseudomonas putida*

Hiroyuki Inoue*, Akira Nishito, Shin-ich Eriguchi, Takashi Tamura, Kenji Inagaki, Hidehiko Tanaka

Department of Bioresources Chemistry, Faculty of Agriculture, Okayama University, 1-1-1 Tsushima-naka, Okayama 700-8530, Japan

Received 7 February 2003; received in revised form 18 April 2003; accepted 21 April 2003

Dedicated to Professor Dr. Kenji Soda in honor of his 70th birthday

Abstract

α-Ketobutyrate decarboxylase encoded in the L-methionine catabolism operon of *Pseudomonas putida* is homologous with the E1 component of pyruvate dehydrogenase complex from gram-negative bacteria. The enzyme was purified to homogeneity from the cell extract of an *Escherichia coli* transformant. The purified enzyme was homodimeric with a subunit of M_r 93,000 on SDS-PAGE. The enzyme activity was activated by the addition of both thiamine pyrophosphate (TPP) and a divalent cation, such as Mg^{2+}, Mn^{2+}, and Co^{2+}. The enzyme showed high activity for α-ketobutyrate and α-keto-*n*-valerate rather than pyruvate, but the α-keto acids with increasing length of the side chain as well as branching, such as α-keto-*n*-caproate and α-keto-3-methylvalerate, were not used by the enzyme. The K_m values for α-ketobutyrate and pyruvate were 0.016 and 0.147 mM, respectively, and the k_{cat}/K_m value (10.69 s^{-1} mM^{-1}) for α-ketobutyrate was 29-fold greater than that for pyruvate. Thus, α-ketobutyrate decarboxylase is distinguished from the pyruvate dehydrogenase E1 component with respect to the substrate specificity, although their structural and enzymological properties were similar. These results suggest that the unique substrate specificity of α-ketobutyrate decarboxylase is due to a slight difference in the highly conserved active sites of both enzymes.

Keywords: α-Ketobutyrate decarboxylase; Pyruvate dehydrogenase E1 component; α-Ketobutyrate; Propionyl coenzyme A

1. Introduction

Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), a copolymer of monomers of 3-hydroxybutyrate and 3-hydroxyvalerate, is a biodegradable thermoplastic with proven commercial applications [1,2]. The physical and mechanical properties of PHBV depend on the 3-hydroxyvalerate fraction derived from the condensation of acetyl coenzyme A (acetyl-CoA) and propionyl-CoA, because homopolymeric poly(3-hydroxybutyrate) is a brittle material [3]. Efficient production of PHBV has been achieved by supplying exogenous propionate as a propionyl-CoA precursor to a fed-batch fermentation of *Ralstonia eutropha*, although propionate is expensive [4,5]. The construction of a pathway from succinyl-CoA to propionyl-CoA, which requires the addition of cyanocobalamin to the medium, has also resulted in the PHBV production with significant 3-hydroxyvalerate incorporation in

* Corresponding author. Present address: Institute for Marine Resources and Environment, National Institute of Advanced Industrial Science and Technology, 2-2-2 Hiro-suehiro, Kure, Hiroshima 737-0197, Japan. Tel.: +81-823-72-1935; fax: +81-823-73-3284.
E-mail address: inoue-h@aist.go.jp (H. Inoue).

1381-1177/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S1381-1177(03)00089-4

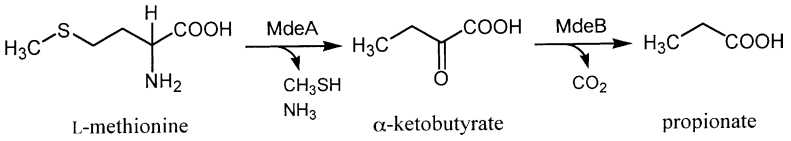

Fig. 1. L-Methionine catabolic pathway in *P. putida*: MdeA, L-methionine γ-lyase; MdeB, α-ketobutyrate decarboxylase.

recombinant *Salmonella enterica*, with a mutation in the ability to metabolize propionyl-CoA [6]. Therefore, propionyl-CoA synthesis from an unrelated carbon source is an important factor in more economical production of PHBV.

α-Ketobutyrate, which is the main product in threonine catabolism and a well-known precursor in isoleucine biosynthesis, is one of the attractive precursors for propionyl-CoA production, because amino acids are present in relatively high concentration in the cell without supplement [7]. In this case, a simple, two-step pathway is required to produce propionyl-CoA from threonine: the first step involves a deamination of threonine by threonine dehydratase to produce α-ketobutyrate; and the resulting sufficient amount of α-ketobutyrate is converted to propionyl-CoA by a pyruvate dehydrogenase (PDH) complex [7,8]. Unfortunately, the PDH complex has a low catalytic efficiency for α-ketobutyrate. In a recombinant *Escherichia coli* expressing PHBV biosynthetic genes and the threonine dehydratase gene, when threonine was supplied to the culture medium, 3-hydroxyvalerate levels increased to more than 30 mol% of the polymer [9]. However, the 3-hydroxyvalerate content of the polymer was only 8 mol%, when the endogenous threonine was utilized by this strain, because the production of propionyl-CoA from α-ketobutyrate could compete with the accumulation of other metabolites, such as isoleucine [9] and 2-aminobutyrate [10].

The PDH complex catalyzes the oxidative decarboxylation of pyruvate and consists of multiple copies of three components: pyruvate decarboxylase (E1) utilizing thiamine pyrophosphate (TPP) as a cofactor; dihydrolipoamide acetyltransferase (E2) which contains covalently-bound lipoyl groups; and dihydrolipoamide dehydrogenase (E3) containing tightly bound FAD [11,12]. The substrate-specific E1 component catalyzes the decarboxylation of pyruvate and subsequently the reductive acylation of the lipoamide groups in E2. It is still unknown whether an α-ketobutyrate-specific dehydrogenase complex exists. Thus, a distinct (or an improved) complex including E1 component that possesses increased activity for α-ketobutyrate will be required to efficiently produce the propionyl-CoA from endogenous amino acid.

Pseudomonas putida has an *mde*AB operon involved in L-methionine catabolism [13,14]. This operon encodes the L-methionine γ-lyase (MdeA) that catalyzes the direct conversion of L-methionine into α-ketobutyrate and a novel α-ketobutyrate decarboxylase (MdeB) that catalyzes decarboxylation of α-ketobutyrate into propionate (Fig. 1). The MdeB is homologous with the homodimeric PDH E1 component from gram-negative bacteria, and the MdeB overproducing the *E. coli* transformant has also shown decarboxylase activity with high specificity for α-ketobutyrate rather than pyruvate [14]. For these reasons, the enzyme is considered to be a structural model to design α-ketobutyrate preferring PDH E1. In this paper, we describe the purification and properties of the α-ketobutyrate decarboxylase of *P. putida* expressed in *E. coli*.

2. Experimental

2.1. Bacterial growth conditions

E. coli MV1184 harboring pYH1010, a pUC118-based plasmid carrying *mdeB* [14], was used for overproduction of the α-ketobutyrate decarboxylase. The strain was grown in Luria broth supplemented with 50 mg l^{-1} ampicillin at 24 °C for 35 h under aeration, because only the inactive insoluble enzyme was produced in the transformant grown at 37 °C.

2.2. Purification of α-ketobutyrate decarboxylase

All procedures for enzyme purification were performed at 4 °C. The cell extract of the *E. coli* transformant (32 g) was prepared by sonication in buffer

A (10 mM potassium phosphate buffer (pH 7.2) containing 0.1 mM TPP and 3 mM $MgCl_2$), followed by dialysis against 1000 volumes of buffer A. The precipitate formed during dialysis was removed by centrifugation, and the supernatant solution was applied to a DEAE-Toyopearl 650M (Tosoh) column (4.8 cm × 10 cm) equilibrated with buffer A containing 0.1 M KCl. After the column was washed thoroughly with the same buffer, the enzyme was eluted with the buffer containing 0.3 M KCl. The active fraction was pooled and brought to 2.0 M KCl saturation by the addition of an equal volume of buffer A containing 4.0 M KCl. This enzyme solution was applied to a Butyl-Toyopearl 650M (Tosoh) column (4.8 cm × 14 cm) equilibrated with buffer A containing 2.0 M KCl. The column was washed with the same buffer, and elution of the enzyme was then carried out by a 1.2 l linear gradient of 2.0–0.5 M KCl. Fractions with high specific activities were pooled, and the obtained solution was dialyzed against buffer A containing 0.2 M KCl. Half of the enzyme solution (223 mg) was concentrated and then loaded onto a Sephacryl S-200 (Amersham Biosciences) column (1.5 cm × 90 cm) with buffer A containing 0.2 M KCl at a flow rate of 40 ml h^{-1} equilibrated with the same buffer. The active fractions pooled were applied to a Q-Sepharose FF (Amersham Biosciences) column (3.7 cm × 4 cm) equilibrated with buffer A containing 0.25 M KCl. The column was washed with the same buffer, and the enzyme was eluted with buffer A containing 0.35 M KCl. The active fractions were concentrated, dialyzed against buffer A, and used as the purified enzyme. The enzyme was also stored at −20 °C in 10 mM potassium phosphate buffer (pH 6.7) containing 0.1 mM TPP, 3 mM $MgCl_2$ and 20% (v/v) glycerol until it was needed.

2.3. Assay for enzyme activity

The α-ketobutyrate decarboxylase activity was measured at 600 nm by an assay involving the reduction of 2,6-dichlorophenolindophenol (DCPIP) as an artificial electron acceptor [15]. In the standard assay, 50 μl of 100 mM α-ketobutyrate was added to 1050 μl of the mixture incubated at 30 °C, containing 0.1 M potassium phosphate (pH 6.7), 0.1 mM DCPIP, 0.2 mM TPP, 0.1 mM $MgCl_2$, and the enzyme. One unit of the enzyme is defined as the amount of the enzyme that reduces 1 μmol of DCPIP per minute. Protein concentration was determined by the method of Lowry et al. with bovine serum albumin as a standard [16].

The influence of varying pH values on the enzyme activity was determined using glycine–HCl, sodium citrate, potassium phosphate, Tris–HCl, and glycine–NaOH buffers (100 mM each) for the pH ranges 2.5–3.5, 3.5–6.3, 6.3–7.5, 7.4–8.5, and 8.5–9.0, respectively. The effect of temperature was examined under the standard assay conditions by varying the temperature (25–80 °C). For the determination of heat stability, the enzyme in buffer A was first incubated for 30 min at different temperatures (25–60 °C). The enzyme solution was subsequently cooled and assayed at 30 °C.

The K_m and V_{max} values were estimated from the intercepts of the Lineweaver-Burk plots. The k_{cat} values were calculated based on M_r 98,082 computed from the amino acid composition of the protein. The concentrations of the substrates used, α-ketobutyrate, pyruvate, and α-keto-*n*-valerate were 0.01–0.5, 0.05–2, and 0.02–2 mM, respectively.

2.4. Molecular mass determination of α-ketobutyrate decarboxylase

The molecular mass of the native α-ketobutyrate decarboxylase was determined by gel filtration through a TSK-G3000sw (Tosoh) column (0.75 cm × 60 cm) equilibrated with 10 mM potassium phosphate buffer (pH 7.2) containing 0.2 M KCl at a flow rate of 0.5 ml min^{-1}. The calibration curve was plotted using the following proteins: thyroglobulin (M_r 670,000), bovine gamma globulin (M_r 158,000), chicken ovalbumin (M_r 44,000) and equine myoglobin (M_r 17,000) (Bio-Rad). The molecular mass of the subunit was determined by SDS-PAGE. An LMW electrophoresis calibration kit (Amersham Biosciences) was used for calibration.

3. Results

3.1. Purification of α-ketobutyrate decarboxylase

A recombinant α-ketobutyrate decarboxylase was extracted as a soluble enzyme from *E. coli* MV1184/

Table 1
Purification of α-ketobutyrate decarboxylase of *P. purida* from *E. coli* MV1184/pYH1010

Step	Total protein (mg)	Total activity[a] (mU)	Specific activity (mU mg^{-1})	Yield (%)
Crude extract	4500	34000	7.6	100
DEAE-Toyopearl 650M	1700	31000	18	90
Butyl-Toyopearl 650M	410	25000	61	74
Sephacryl S-200[b]	190	13000	71	38
Q-Sepharose FF	45	3900	87	11

[a] One unit of the enzyme is defined as the amount of the enzyme that reduces 1 μmol of DCPIP per mm at 30 °C.
[b] Only 223 mg of the Butyl-Toyopearl fraction was used for Sephacryl S-200.

pYH1010 grown at a low temperature (24 °C). Purification of the enzyme resulted in an approximately 11-fold enhancement of the specific activity. Typical results of the purification procedure are summarized in Table 1. Butyl-Toyopearl hydrophobic chromatography was an effective purification procedure due to the high hydrophobicity of the enzyme. Most of the *E. coli* soluble proteins were eluted through the hydrophobic matrix under 2.0 M KCl. In addition, the contaminating high PDH E1 activity from *E. coli* was also removed by this hydrophobic chromatography.

The purified enzyme was shown to be homogeneous by SDS-PAGE, which gave a single band with a molecular weight of approximately 93 kDa. This size is close to the value of 98,082 calculated from the amino acid composition of the protein. N-terminal amino acid sequence analysis of the enzyme exactly matched the *mdeB* DNA sequence together with the lack of an N-terminal methionine residue, indicating that the enzyme is the product of the *mdeB* [14]. The molecular mass of the native enzyme was estimated to be 185 kDa by gel filtration; the enzyme is homodimeric. The purified enzyme was stored at −20 °C for several months without loss of activity, although it was highly sensitive to contaminating proteolytic activity. The enzyme was cleaved into two fragments of about 40 and 53 kDa on the SDS-PAGE when stored on ice for several days in buffer A, but no loss of E1 activity was observed.

3.2. Effect of pH and temperature

The optimum pH of the α-ketobutyrate decarboxylase in the enzyme activity was 6.6. The enzyme was stable when incubated on ice for 30 min at pH 3.5 to 7.2 in various 100 mM buffers containing 0.1 mM TPP and 3 mM $MgCl_2$. When the enzyme was dialyzed overnight against 100 mM citric acid–sodium citrate buffer (pH 4.5), a precipitate was observed. However, the precipitate showed enzyme activity and was solubilized by dialysis against 10 mM potassium phosphate buffer (pH 6.7) without loss of activity. In contrast, the enzyme was unstable under a weak alkaline condition; the remaining enzyme activity was about 15% of the initial activity when incubated at 0 °C for 30 min at pH 8.5. The optimal temperature for the enzyme activity was found at 60 °C. The activity increased linearly as the temperature was raised from 25 to 60 °C and declined over 70 °C. When the enzyme in buffer A (pH 7.2) was heated at various temperatures, it was stable up to 43 °C, but the activity decreased by 50% at 45 °C.

3.3. Component requirements

The enzyme activity decreased significantly (to 9%) on dialysis against buffer A without TPP and $MgCl_2$, whereas their addition to the dialyzed enzyme resulted in partial restoration (58%) of the activity (Table 2). This result indicates that the enzyme is a typical TPP-dependent enzyme. The K_m values for TPP of the enzyme were 17 μM in the presence of a saturating concentration of $MgCl_2$ and α-ketobutyrate. In addition, Mn^{2+} and Co^{2+} were also found to be as good a cofactor as Mg^{2+} (Table 2), suggesting that they were substituted for Mg^{2+}, which interacts with the pyrophosphate part of TPP in the active site of the enzyme.

3.4. Substrate specificity

The substrate specificity of the α-ketobutyrate decarboxylase was examined with various α-keto acids as substrates under the standard condition

Table 2
Effect of metal ions and TPP on the α-ketobutyrate decarboxylase

Metal[a]	Relative activity (%)
Mg^{2+}[b]	100
Mg^{2+}	58
Co^{2+}	68
Mn^{2+}	53
Zn^{2+}	46
Ni^{2+}	45
Ca^{2+}	32
Ba^{2+}	33
Cu^{2+}	14
None (+TPP)	27
None (−TPP)	9

[a] The enzyme reactions were carried out in the standard reaction mixture containing 0.1 mM metal, 0.2 mM TPP, and dialyzed enzyme.
[b] The activity was measured using the enzyme before dialysis.

(Table 3). We found that α-ketobutyrate was the best substrate. α-Keto-*n*-valerate was a better substrate than pyruvate and α-ketoisovalerate. The substrates with a longer side chain, α-keto-*n*-caproate and α-keto-γ-methylthiobutyrate derived from methionine, were not decarboxylated by the enzyme. In addition, no detectable activity was observed in branched-chain α-keto acids, α-ketoisocaproate and α-keto-3-methylvalerate derived from leucine and isoleucine, respectively, as well as in α-ketoglutarate.

The kinetic parameters for α-ketobutyrate decarboxylase were determined with α-ketobutyrate, pyruvate, and α-keto-*n*-valerate from the Lineweaver-Burk plots (Table 4). The K_m value for α-ketobutyrate (0.016 mM) was 9.2- and 9.3-fold lower compared

Table 3
Substrate specificity of α-ketobutyrate decarboxylase

Substrate[a]	Formula (R–CO–COOH)	Relative activity (%)
Pyruvate	CH_3–	17
α-Keto-*n*-butyrate	CH_3CH_2–	100
α-Keto-*n*-valerate	$CH_3CH_2CH_2$–	49
α-Keto-*n*-caproate	$CH_3CH_2CH_2CH_2$–	<3
α-Keto-γ-methylthiobutyrate	CH_3S–CH_2CH_2–	<3
α-Ketoisovalerate	$CH_3CH(CH_3)$–	16
α-Keto-3-methylvalerate	$CH_3CH_2CH(CH_3)$–	<3
α-Ketoisocaproate	$CH_3CH(CH_3)CH_2$–	0
α-Ketoglutarate	HOOC–CH_2CH_2–	0

[a] α-Keto acids were used at a concentration of 5 mM except for α keto-3-methylvalerate (1.25 mM).

Table 4
Kinetic parameter of straight-chain α-keto acids for α-ketobutyrate decarboxylase

Substrate	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m ($s^{-1}\,mM^{-1}$)
α-Ketobutyrate	0.016	17.1×10^{-2}	10.69
Pyruvate	0.147	5.36×10^{-2}	0.364
α-Keto-*n*-valerate	0.149	12.8×10^{-2}	0.859

with the values for pyruvate and α-keto-*n*-valerate, respectively. Furthermore, the catalytic efficiency (k_{cat}/K_m) for decarboxylation of α-ketobutyrate ($10.69\,s^{-1}\,mM^{-1}$) was 12- and 29-fold greater than those for α-keto-*n*-valerate and pyruvate. These results indicate that α-ketobutyrate is a physiological substrate for the enzyme.

4. Discussion

We have described the physicochemical and catalytic properties of α-ketobutyrate decarboxylase of *P. putida* that was purified to homogeneity from an *E. coli* transformant. Basic enzymological properties of the purified enzyme including molecular mass, subunit structure, pH optimal for the activity, and cofactor requirement, as well as its primary structure, were similar to those of PDH E1s reported for gram-negative bacteria, such as *E. coli* and *Azotobacter vinelandii*, which are homodimers with a subunit mass of approximately 100 kDa [17,18]. It has been reported that a limited proteolysis of *A. vinelandii* PDH E1 with trypsin yielded two main fragments 40 and 54 kDa on the SDS-PAGE [17]. This also appears to correspond to two fragments of about 40 and 53 kDa which resulted from the proteolysis of α-ketobutyrate decarboxylase. These observations suggest that α-ketobutyrate decarboxylase and PDH E1 have similar domain structures; however, it remains unknown whether α-ketobutyrate decarboxylase forms a dehydrogenase complex with the E2 and E3 components in *P. putida*. A slight difference was found in the pH stability of the enzyme. The α-ketobutyrate decarboxylase was unstable under a high pH condition (pH > 8.5), whereas the residual activity of *A. vinelandii* PDH E1 remains high when incubated for 15 min at pH 9.5 [17]. Although a reversible monomerization has also been observed

with *A. vinelandii* and *E. coli* PDH E1s at high pH, an irreversible structural change of subunit, including monomerization, may occur in α-ketobutyrate decarboxylase under the same condition.

The purified α-ketobutyrate decarboxylase was specific for α-ketobutyrate. The enzyme was also able to decarboxylate not only pyruvate but also α-keto-*n*-valerate and α-ketoisovalerate, whereas the latter are known as competitive inhibitors of the *E. coli* PDH complex. The extent of inhibition of the *E. coli* PDH complex decreases with the increasing length of the side chain as well as with branching, and a branched-chain α-keto acid is an even less effective inhibitor [19]. On the other hand, α-ketobutyrate decarboxylase activity also decreased with the increasing length of the side chain. In addition, the enzyme showed higher relative activities for straight-chain α-keto acids than the corresponding branched-chain α-keto acids (Table 3). Therefore, α-ketobutyrate decarboxylase has distinct substrate specificity, but the substrate-binding site (or mechanism) of the enzyme may be correlated with that of PDH E1.

An earlier study by Bisswanger suggested that the *E. coli* PDH complex possesses a binding site for pyruvate consisting of a groove close to the methyl group rather than a pocket that would completely exclude long and bulky side chains in substrate analogues [19]. Recently, the crystal structure of *E. coli* PDH E1 has been determined at a resolution of 1.85 Å; it was revealed that the active site cavity of the enzyme has dimensions of 18 Å × 8 Å × 21 Å with a wide mouth and very deep cleft between the two subunits [20]. Surprisingly, all 21-key residues involved in cofactor binding and likely to interact with the substrate and/or reaction intermediates are conserved in the primary sequence of α-ketobutyrate decarboxylase, except for Val192 of *E. coli* PDH E1, which corresponds to Gly202 (Fig. 2). This finding suggests that the active site of α-ketobutyrate decarboxylase is very similar to that of PDH E1. The similar K_m values for TPP found in the α-ketobutyrate dehydrogenase (17 μM) and the resolved *E. coli* PDH E1 (34 μM [21]) also are compatible with the involvement in cofactor binding of the conserved active site. Nevertheless, the *E. coli* PDH complex accepts α-ketobutyrate as a substrate with 10-fold less affinity ($K_m = 3$ mM) than pyruvate [19]. Thus, the difference between Val192 of *E. coli* PDH E1 and Gly202 of α-ketobutyrate decarboxylase is notable for α-ketobutyrate acceptance.

Val192 is conserved in the sequences of all known homodimeric PDH E1s (Fig. 2), except for a second PDH E1 (PdhE) of *A. eutrophus* whose physiological role is unclear [22]. In the active site of *E. coli* PDH E1, the main chain oxygen of Val192 forms a conserved hydrogen bond to the 4′-amino group of TPP. Although the side chain of this residue seems close to the phenolic ring of Tyr177 contributing to stabilization of some transition state in the reaction pathway [20,23], no contribution of Val192 to substrate binding has been reported. Interestingly, the other TPP enzymes

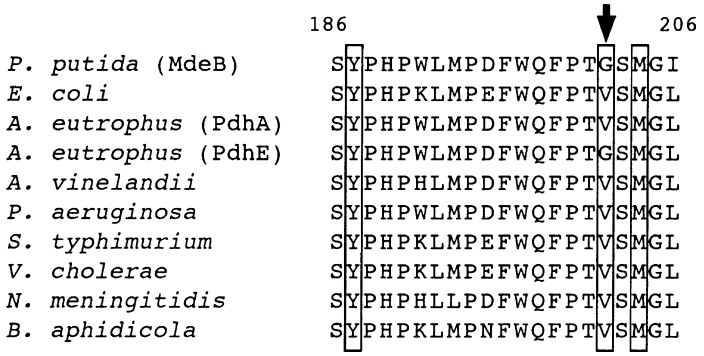

Fig. 2. Sequence alignment of α-ketobutyrate decarboxylase and PDH E1s in the glycine 202 region of α-ketobutyrate decarboxylase. Gly202 is shown with an arrow. Putative key residues involved in the cofactor and substrate binding in PDH E1 are boxed [20]. The GeneBank/EMBL accession numbers are as follows: *P. putida* (MdeB), D89015; *E. coli*, P06958; *A. eutrophus* (PdhA), Q59097; *A. eutrophus* (PdhE), S65474; *A. vinelandii*, CAA75394; *Pseudomonas aeruginosa*, Q59637; *Salmonella typhimurium*, CAB89841; *Vibrio cholerae*, D82079; *Neisseria meningitidis*, E81094; *Buchnera aphidicola*, P57301.

utilizing pyruvate, pyruvate oxidase and acetohydroxy acid synthase possess a conserved valine residue that is very close to the reactive C-2 of the TPP thiazolium ring [24]. It has been suggested that the valine residue of pyruvate oxidase plays a role in interaction with the methyl group of pyruvate [25]. The Val-mutants of *E. coli* pyruvate oxidase replaced by a smaller residue (Gly, Ala and Ser) have shown a markedly decreased activity by 14–288-fold with pyruvate but retained full activity (1.5–2.4-fold decrease) with α-ketobutyrate [25]. In contrast, glyoxylate carboligase, an analogous protein of acetohydroxy acid synthase, allows interaction with glyoxylate, which is a smaller substrate than pyruvate. This enzyme possesses an isoleucine residue in the positions corresponding to the valine residue [24,25]. From these reports, we also predict that the unique substrate specificity of α-ketobutyrate decarboxylase is caused by a slight difference in the active site between α-ketobutyrate decarboxylase and PDH E1: the smaller Gly202 at the position of the conserved valine residue of PDH E1 may be a key residue to form the extended active site with high affinity for α-ketobutyrate. Finally, the α-ketobutyrate decarboxylase will provide a variable model for structural modification of the homodimeric PDH E1 from the PHBV-producing bacteria engineered metabolically, such as *E. coli*, *S. enteria*, and *R. eutropha*. Further studies are necessary to evaluate the altered substrate specificity using a modified PDH complex and the economical production of PHBV via propionyl-CoA from endogenous threonine or methionine.

References

[1] A. Steinbüchela, H.E. Valentin, FEMS Microbiol. Lett. 128 (1995) 219.
[2] G. Braunegg, G. Lefebvre, K.F. Genser, J. Biotechnol. 65 (1998) 127.
[3] P.A. Holmes, Phys. Technol. 16 (1985) 32.
[4] D. Byrom, Trends Biotechnol. 5 (1987) 246.
[5] Y. Poirier, C. Nawrath, C. Somerville, BioTechnology 13 (1995) 142.
[6] I.S. Aldor, S.W. Kim, K.L. Prather, J.D. Keasling, Appl. Environ. Microbiol. 68 (2002) 3848.
[7] A.C. Eschenlauer, S.K. Stoup, F. Srienc, D.A. Somers, Int. J. Biol. Macromol. 19 (1996) 121.
[8] T.K. Van Dyk, R.A. LaRossa, Mol. Gen. Genet. 207 (1987) 435.
[9] H.E. Valentin, D.L. Broyles, L.A. Casagrande, S.M. Colburn, W.L. Creely, P.A. DeLaquil, H.M. Felton, K.A. Gonzalez, K.L. Houmiel, K. Lutke, D.A. Mahadeo, T.A. Mitsky, S.R. Padgette, S.E. Reiser, S. Slater, D.M. Stark, R.T. Stock, D.A. Stone, N.B. Taylor, G.M. Thorne, M. Tran, K.J. Gruys, Int. J. Biol. Macromol. 25 (1999) 303.
[10] D.J. Ager, T. Li, D.P. Pantaleone, R.F. Senkpeil, P.P. Taylor, I.G. Fotheringham, J. Mol. Catal. B: Enzym. 11 (2001) 199.
[11] A. de Kok, A.F. Hengeveld, A. Martin, A.H. Westphal, Biochim. Biophys. Acta 1385 (1998) 353.
[12] V. Bunik, J. Mol. Catal. B: Enzym. 8 (2000) 165.
[13] H. Inoue, K. Inagaki, M. Sugimoto, N. Esaki, K. Soda, H. Tanaka, J. Biochem. 117 (1995) 1120.
[14] H. Inoue, K. Inagaki, S. Eriguchi, T. Tamura, N. Esaki, K. Soda, H. Tanaka, J. Bacteriol. 179 (1997) 3956.
[15] L.S. Khailova, R. Bernkhardt, G. Khiubner, Biokhimiya 42 (1977) 113.
[16] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, J. Biol. Chem. 193 (1951) 265.
[17] A.F. Hengeveld, A.H. Westphal, A. de Kok, Eur. J. Biochem. 250 (1997) 260.
[18] H. Saumweber, R. Binder, H. Bisswanger, Eur. J. Biochem. 114 (1981) 407.
[19] H. Bisswanger, J. Biol. Chem. 256 (1981) 815.
[20] A.F. Hengeveld, C.P. van Mierlo, H.W. van den Hooven, A.J. Visser, A. de Kok, Biochemistry 41 (2002) 7490.
[21] J. Yi, N. Nemeria, A. McNally, F. Jordan, R.S. Machado, J.R. Guest, J. Biol. Chem. 271 (1996) 33192.
[22] S. Hein, A. Steinbuchel, Eur. J. Biochem. 237 (1996) 674.
[23] N. Nemeria, Y. Yan, Z. Zhang, A.M. Brown, P. Arjunan, W. Furey, J.R. Guest, F. Jordan, J. Biol. Chem. 276 (2001) 45969.
[24] M. Ibdah, A. Bar-Ilan, O. Livnah, J.V. Schloss, Z. Barak, D.M. Chipman, Biochemistry 35 (1996) 16282.
[25] Y.Y. Chang, J.E. Cronan Jr., Biochem. J. 352 (2000) 717.