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Characterization of D-threonine dehydrogenase homologues of *Escherichia coli*, YbbQ and YhaE

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Abstract

Two hypothetical proteins of *Escherichia coli*, YbbQ and YhaE, show high sequence similarity to D-threonine dehydrogenase. We cloned the genes encoding YbbQ and YhaE into *E. coli* JM109, and purified the expressed proteins to homogeneity from the *E. coli* clones. YbbQ consisted of two identical subunits with a molecular mass of 31 kDa, whereas YhaE was a homotetramer (native molecular mass, 124 kDa). Both enzymes required NAD^+ as a coenzyme, and used serine as a substrate. D-Serine was better substrate than L-serine. YbbQ showed maximum activity at pH 11.0 for the oxidation of D-serine, whereas the optimum pH of YhaE was 10.5. These enzymes also catalyzed the oxidation of glycerate and 3-hydroxyisobutyrate. The V_{max}/K_m values of YbbQ for D-serine, L-serine, D-glycerate, L-glycerate, D-3-hydroxyisobutyrate, and L-3-hydroxyisobutyrate were 1.22, 0.0054, 128, 4.97, 0.0295, and 0.718 $\mu mol\ min^{-1}\ mg^{-1}\ mM^{-1}$, and those of YhaE were 0.690, 0.057, 17.5, 0.650, 0.163, and 0.263 $\mu mol\ min^{-1}\ mg^{-1}\ mM^{-1}$, respectively. Thus, YbbQ and YhaE are NAD^+-dependent dehydrogenases acting on 3-hydroxy acids with 3-carbon chains, and D-glycerate is the best substrate for both enzymes.

Keywords: YbbQ; YhaE; *Escherichia coli*; 3-Hydroxy acid dehydrogenase superfamily; Characterization

1. Introduction

Although a variety of L-amino acid dehydrogenases have been extensively studied [1,2], little attention has been paid to the pyridine nucleotide-dependent D-amino acid dehydrogenases. We have found a novel $NADP^+$-dependent D-threonine dehydrogenase [EC 1.1.1.-], which catalyzes dehydrogenation of the 3-hydroxyl group of D-*threo*-3-hydroxyamino acids such as D-threonine and D-*threo*-3-phenylserine, in cell extracts of *Pseudomonas cruciviae* IFO 12047 and various other Gram-negative bacteria [3]. The enzyme was purified to homogeneity from *P. cruciviae* IFO 12047 and characterized [4]. The primary structure of the enzyme was deduced from the nucleotide sequence of its gene [5]. The primary structure of D-threonine dehydrogenase is homologous with two hypothetical proteins of *Escherichia coli*, YbbQ and YhaE (identities, 40.7 and 34.2%, respectively, similarities, 79.6 and 71.2%, respectively). Neither YbbQ nor YhaE, however, showed D-threonine dehydrogenase activity. To understand their enzymological

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functions, we investigated the activity of YbbQ and YhaE towards each enantiomer of various amino acids. Both enzymes showed significant activities for D-serine in the presence of NAD^+. We purified both enzymes to homogeneity and characterized them.

This paper presents evidence that both YbbQ and YhaE are NAD^+-dependent D-glycerate dehydrogenase, which also acts on 3-hydroxy acids with 3-carbon chains.

2. Experimental

2.1. Materials

NAD^+, $NADP^+$, and NADH were obtained from Kohjin Biochemicals (Tokyo, Japan), L-serine, D-serine, L-glycerate, and D-glycerate were purchased from Sigma Chemical Corp. (St. Louis, Mo., U.S.A), a TSK gel G3000SW column (0.75 cm × 60 cm) was purchased from Tosoh (Tokyo, Japan), and marker proteins for molecular mass measurement was obtained from Oriental Yeast (Osaka, Japan). Both enantiomers of 3-hydroxyisobutyrate were prepared from their methyl esters supplied by Tokyo Kasei Kogyo (Tokyo, Japan).

2.2. Construction of E. coli overproducers of YbbQ and YhaE

Chromosomal DNA of *E. coli* JM109 was prepared by the method of Saito and Miura [6]. The *ybbQ* gene was amplified by polymerase chain reaction (PCR) with sense (PYBBQ-N) and antisense (PYBBQ-C) primers. The primer sequences were 5′-GTCTGAATTCAGGAAAAGCGAAATT-TAAA-A-3′ (primer PYBBQ-N) and 5′-CGCTGATCAGGC-CAGTTTATGGTTAGCCATT-3′ (primer PYBBQ-C). PCR was performed with AmpliTaq Gold DNA polymerase (Perkin Elmer, USA). The reaction mixture for PCR (100 μl) consisted of 8 μmol of Tris-HCl buffer (pH 8.3), 2 μmol of $(NH_4)_2SO_4$, 0.3 μmol of $MgCl_2$, 20 nmol of each dNTP, 2.5 units of the DNA polymerase, 0.5 μg of the chromosomal DNA (as a template), and each 100 pmol of PYBBQ-N and PYBBQ-C. The reaction mixture was heated at 94°C for 1 min (for denaturation), cooled at 58°C for 1 min (for annealing), and then incubated at 72°C for 4 min (for extension). This programmed temperature shift was repeated 30 times. The amplified DNA fragment (about 0.9 kb) was digested simultaneously with *Eco*RI and *Fba*I, and then ligated into the *Eco*RI–*Bam*HI site of pTrc99A (Pharmacia Biotech, Uppsala, Sweden). We named the constructed plasmid pYBBQ1. The nucleotide sequence of the gene cloned in pYBBQ1 was verified using an Applied Biosystems 373A DNA sequencer with a PRISM-FS kit (Perkin Elmer). The *yhaE* gene was amplified by PCR with sense (PYHAE-N) and antisense (PYHAE-C) primers. Sequences were 5′-GAGCCATGGGGAAAGAGAGATGATTGATAT-GACTATG-3′ (primer PYHAE-N) and 5′-GAGTGAT-CATTAACGAGTAACTTCGACTTTCG-3′ (primer PYHAE-C). Amplification of the *yhaE* gene was performed under the same conditions as described above. The amplified DNA fragment (about 0.9 kb) was digested simultaneously with *Nco*I and *Fba*I, and then ligated into the *Nco*I–*Bam*HI site of pTrc99A. We named the constructed plasmid pYHAE1. The nucleotide sequence of the cloned gene was verified under the above conditions. The constructed plasmid was designated as pYHAE1. Plasmid pYBBQ1 or pYHAE1 was introduced into *E. coli* JM109 competent cells (TaKaRa Shuzo, Kyoto, Japan). The gene products, YbbQ and YhaE, were induced with isopropyl-β-D-thiogalactopyranoside (IPTG) in cells of the *E. coli* clones, *E.coli* JM109/pYBBQ1 and JM109/pYHAE1, respectively.

2.3. Enzyme and protein assays

The standard reaction mixture contained 100 μmol of D-serine, 1 μmol of NAD^+, 200 μmol of glycine-KCl-KOH buffer (pH 11.0), and enzyme in a final volume of 1.0 ml. The substrate was replaced with water in a blank. Incubation was carried out at 30°C in a cuvette with a 1 cm light path. The reaction was started by addition of NAD^+ and monitored by measuring the initial change in A_{340} with a Shimadzu UV-140-02 spectrophotometer. One unit of enzyme was defined as the amount that catalyzed the formation of 1 μmol of NADH per min in the reaction.

Protein was measured by the method of Lowry et al. [7], with crystalline bovine serum albumin as the standard.

2.4. Purification of YbbQ and YhaE

All purification procedures were performed at 0–5°C, and potassium phosphate buffer (pH 7.4) containing 0.02% 2-mercaptoethanol and 10% glycerol was used in the procedures unless otherwise stated.

YbbQ was purified as follows. Cells of *E. coli* JM109/pYBBQ1 were grown at 30°C in 1.5 l of Luria broth [8] containing ampicillin (50 μg ml^{-1}, final concentration). When the turbidity of the culture at 600 nm had reached 0.6, 1 mM IPTG was added to induce the enzyme production, and cultivation was continued at 30°C for another 12 h. The cells (5.1 g, wet weight) were suspended in 10.2 ml of 0.1 M buffer and disrupted by sonication at 4°C. The supernatant obtained by centrifugation at 12,000 rpm for 1 h was dialyzed against 2 l of the same buffer at 4°C overnight, and the dialyzed solution was used as the cell extract. To the cell extract (total volume, 8.7 ml; total protein, 246.2 mg), solid ammonium sulfate was added to 30% saturation with stirring. After being kept at 4°C for 30 min, the precipitate was removed by centrifugation. The supernatant (13.6 ml) was applied to a Butyl-Toyopearl column (2.3 cm × 10.7 cm) equilibrated with 10 mM buffer (pH 7.4) containing ammonium sulfate (30% saturation). After the column had been washed with the same buffer, the enzyme was eluted with a linear gradient of ammonium sulfate (30–0% saturation). The active fractions were concentrated with an Amicon ultrafiltration unit with a PM-10 membrane filter and dialyzed against 10 mM buffer overnight. The enzyme solution (15.1 ml) was put on a MonoQ HR 10/10 anion-exchange column (1 cm × 10 cm) equilibrated with 10 mM buffer. The column was equipped with a Pharmacia fast protein liquid chromatography system and developed at a flow rate of 4.0 ml min^{-1} with a 70 min linear gradient of NaCl (0–0.35 M). The active fractions collected were concentrated up to 14.4 ml. The enzyme solution was further dialyzed against 10 mM buffer (pH 7.4) containing ammonium sulfate (30% saturation). The solution was put on a Phenyl-Superose HR 5/5 column (0.5 cm × 5 cm), which was equipped with the fast protein liquid chromatography system and equilibrated with the same buffer. The column was developed at flow rate of 0.5 ml min^{-1} with a 60 min linear gradient of ammonium sulfate (30–0% saturation). The active fractions were collected (2.4 ml).

YhaE was purified from 40 ml of a cell extract of *E. coli* JM109/pYHAE1 (containing 1012.8 mg of protein) by a series of ammonium sulfate fractionation, Butyl-Toyopearl hydrophobic column chromatography, and MonoQ HR 5/5 anion-exchange column chromatography steps, under essentially the same conditions above. The enzyme solution was dialyzed against 10 mM buffer and finally concentrated up to 30.7 ml.

2.5. Electrophoresis

SDS-polyacrylamide gel electrophoresis was carried out with 10% polyacrylamide by the method of Laemmli [9].

2.6. Determination of molecular mass

Molecular mass was determined at room temperature by high-pressure liquid chromatography on a TSK gel G3000SW column (0.75 cm × 60 cm) at a flow rate of 0.7 ml min^{-1} with an elution buffer consisting of 0.1 M potassium phosphate buffer (pH 7.0) containing 0.3 M NaCl. A calibration curve was made with the following proteins: yeast glutamate dehydrogenase (290 kDa), pig heart lactate dehydrogenase (142 kDa), yeast enolase (67 kDa), yeast adenylate kinase (32 kDa), and horse cytochrome *c* (12.4 kDa). The molecular mass of the subunit was estimated by SDS-polyacrylamide gel electrophoresis with the following standard proteins: phosphorylase *b* (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20 kDa), and α-lactalbumin (14.4 kDa).

2.7. Analysis of N-terminal amino acid sequence

The *N*-terminal amino acid analysis of YbbQ and YhaE was done by automated Edman degradation with an Applied Biosystems 492 protein sequencer by the method of Matsudaira [10]. The phenylthiohydantoin amino acid derivatives were identified with an Applied Biosystems model 120A phenylthiohydantoin derivative on-line analyzer.

3. Results

3.1. Overproduction and purification of YbbQ and YhaE

To characterize two D-threonine dehydrogenase homologues of *E. coli*, YbbQ and YhaE, we first constructed overproducers of each enzyme, namely *E. coli* JM109/pYBBQ1 and JM109/pYHAE1 (Fig. 1, lanes 1 and 3). We next examined the activity of the enzymes produced toward both enantiomers of hydroxyamino acids naturally occurring in proteins. They showed the highest activity for serine, among the amino acids tested. In particular, the activity for D-serine was significant. Then, both YbbQ and YhaE were purified to homogeneity as monitored by D-serine dehydrogenase activity (Fig. 1, lanes 2 and 4). A summary of the purification of YbbQ and YhaE is shown in Table 1.

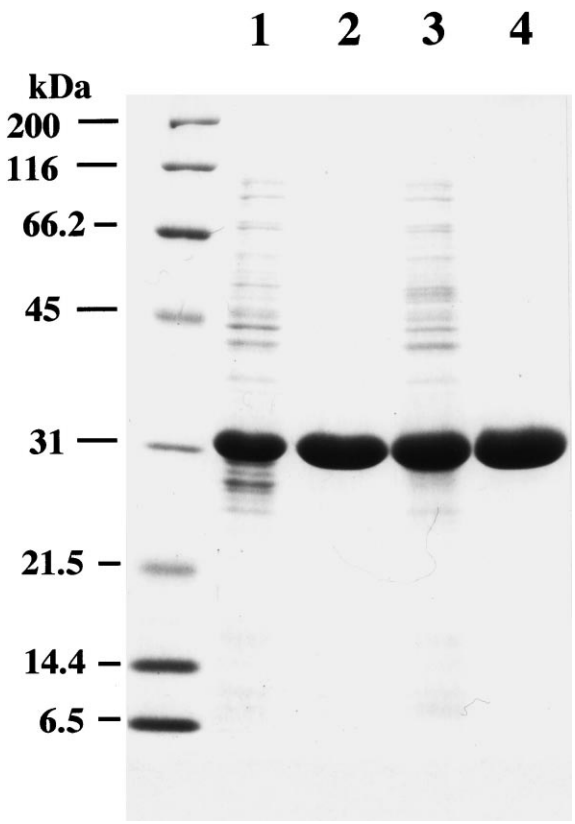

Fig. 1. Overproduction of YbbQ and YhaE. Cell extracts (10 μg of protein) of E. coli clones and the purified YbbQ and YhaE (each 10 μg of protein) were subjected to SDS-polyacrylamide gel electrophoresis (12.5% acrylamide) with the following molecular mass marker proteins: myosin (200 kDa); β-galactosidase (116 kDa); bovine serum albumin (66.2 kDa); ovalbumin (45 kDa); carbonic anhydrase (31 kDa); trypsin inhibitor (21.5 kDa); lysozyme (14.4 kDa) and aprotinin (6.5 kDa). Lane 1, the cell extract of *E. coli* JM109/pPYBBQ1; lane 2, the purified YbbQ; lane 3, the cell extract of *E. coli* JM109/pPYHAE1 and lane 4, the purified YhaE.

3.2. Molecular mass, subunit structure, and N-terminal amino acid sequence

The molecular masses of YbbQ and YhaE were estimated to be 60 and 120 kDa, respectively, by gel filtration on a TSK gel G3000SW. The molecular mass of the YbbQ subunit was about 30 kDa, which is closely similar to that of the subunit of YhaE. Thus, YbbQ is composed of two identical subunits, and YhaE is a homotetramer. The first 15 *N*-terminal amino acids of YbbQ and YhaE were MKLGFIGLGIMGTPM and MIDMTMKVGFIGLGI, respectively. Each sequence is in good agreement with that deduced from the respective nucleotide sequences of the *ybbQ* and *yhaE* genes.

3.3. Absorption spectrum

The absorption spectra of both YbbQ and YhaE in 0.1 M potassium phosphate buffer (pH 7.4) showed an absorption maximum at 275 nm. No absorption peak was detected in the region from 300 to 500 nm.

3.4. Stability

When heated for 10 min in 0.1 M potassium phosphate buffer (pH 7.4) containing 0.02% 2-mercaptoethanol and 10% glycerol, both enzymes were stable at up to 40°C. Upon incubation at 40°C for 10 min in the following buffers (0.1 M) containing 0.02% 2-mercaptoethanol and 10% glycerol: sodium acetate buffer (pH 3.5–5.8), potassium phosphate buffer (pH 6.0–7.0), Tris-HCl buffer (pH 7.0-9.0), and glycine-KCl-KOH buffer (pH 9.0–12.0), YbbQ and YhaE were most stable over a pH range of 6.0–9.5 and 4.5–8.5, respectively.

3.5. Effect of pH on enzyme activity

YbbQ showed maximum activity at pH 11.0 for the oxidation of D-serine, whereas the optimum pH of YhaE was 10.5 (Fig. 2).

Table 1
Purification of YbbQ and YhaE

Steps	Total protein (mg)	Total units	Sp act (U mg^{-1} of protein)[a]	Yield (%)	Fold
YbbQ					
Cell extract	246.2	216.7	0.88	100	1
$(NH_4)_2SO_4$ fractionation	224.7	337.1	1.50	156	1.70
Butyl-Toyopearl	47.1	143.0	3.04	66.0	3.45
MonoQ HR10/10	6.96	132.5	19.0	61.1	21.6
Phenyl-Superose HR5/5	3.46	95.2	27.5	43.9	31.3
YhaE					
Cell extract	1013	3718	3.67	100	1
Butyl-Toyopearl	702	3187	4.54	85.8	1.24
MonoQ HR10/10	271	2316	8.55	62.3	2.33

[a] The enzyme activity was measured by the method as described in Section 2.

3.6. Effects of coenzymes and metal ions

YbbQ and YhaE required NAD^+ as a coenzyme. $NADP^+$ showed a lower activity than that of NAD^+: YbbQ and YhaE showed 6.79 and 21.8% of the activity obtained with NAD^+, respectively.

Neither enzyme was affected by EDTA and α,α′-dipyridyl (10 mM each). Mg^{2+}, Li^{2+}, and Ca^{2+} (1 mM each) were also without affect. YbbQ lost 40, 68, 23,and 23% of its activity by adding Co^{2+}, Ni^{2+} (1 mM each), Mn^{2+}, and Fe^{2+} (0.1 mM each), respectively. However, YhaE was not affected by these metal ions. YbbQ and YhaE were inhibited 96 and 35%, respectively, by Cu^{2+}.

3.7. Substrate specificity

We examined activity of YbbQ and YhaE toward some organic acids and serine analogues to clarify their function. In addition to D- and L-serine, both enantiomers of glycerate and 3-hydroxyisobutyrate, and 3-hydroxypropionate were oxidized by both enzymes (Table 2). DL-3-hydroxybutyrate, DL-2-hydroxybutyrate, DL-2-hydroxyisocaproate, DL-lactate,

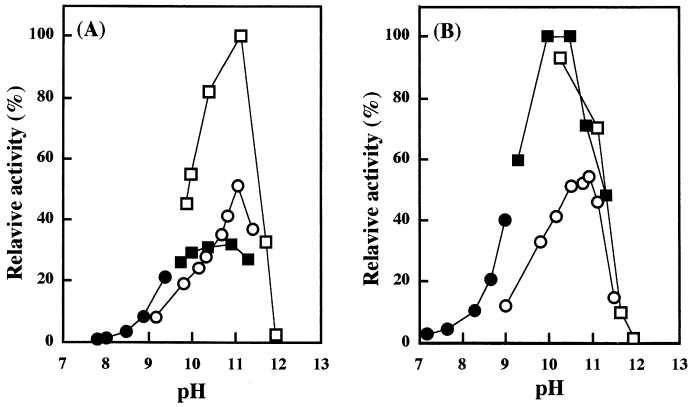

Fig. 2. Effect of pH on activities of YbbQ (A) and YhaE (B). Both enzymes were assayed in the following buffers (0.5 M each): Tris-HCl buffer (●); glycine-KCl-KOH buffer (○); *N*-cyclohexyl-3-aminopropanesulfonic acid buffer (■) and 0.1 M Na_2HPO_4-NaOH buffer (□), by the method described in Section 2. The activities at different pHs are shown in terms of the values relative to the corresponding maximum value taken as 100%.

Table 2
Substrate specificity of YbbQ and YhaE

Substrates[a]	Relative activity (%)[b]	
	YbbQ	YhaE
D-Serine	100	100
L-Serine	7.7	11
D-Glycerate	100	110
L-Glycerate	58	48
D-Hydroxyisobutyrate	24	69
L-Hydroxyisobutyrate	42	88
2-Methyl-DL-serine	52	81
3-Hydroxypropionate	7.4	26

[a] Concentration, 100 mM.
[b] The enzyme activity was measured using these compounds instead of D-serine by the method as described in Section 2. The activities are shown in terms of the values relative to the activity for D-serine taken as 100%.

DL-malate, tartronate, DL-glyceraldehyde, ketomalonate, malonate, glycerol, and glycolate were inert as substrates. Among the serine analogues, 2-methyl-DL-serine functioned as a substrate. *O*-Methyl-DL-serine, *N*-acetyl-DL-serine, DL-homoserine, DL-isoserine, and DL-*threo*-3-phenylserine were not substrates.

3.8. Kinetics

To determine the K_m and V_{max} values of YbbQ and YhaE for D-serine and other substrates, initial velocities were estimated with various concentrations of substrate in the presence of fixed concentrations of NAD^+. Plots of reciprocals of D-serine concentrations gave a family of straight lines. When NAD^+ was used as the variable substrate, similar lines were obtained. These results indicate that the reactions catalyzed by YbbQ and YhaE proceed through a sequential mechanism [11]. The K_m values of YbbQ and YhaE for D-serine and NAD^+ were calculated to be 91 and 0.54 mM and 42 and 0.5 mM, respectively. The kinetic parameters of both enzymes for several substrates are shown in Table 3. Both YbbQ and YhaE showed the highest V_{max} values for D-serine as a substrate, whereas their V_{max}/K_m values for D-glycerate were significantly higher than those for D-serine.

3.9. Reduction of tartronate semialdehyde

D-Glycerate was the best substrate for both enzymes. Thus, the reverse reaction catalyzed by each enzyme was examined at pH 7.0 (50 mM potassium phosphate buffer). Tartronate semialdehyde could not be obtained commercially. It was prepared from hydroxypyruvate using hydroxypyruvate isomerase [12]. When the reaction was started by addition of freshly prepared hydroxypyruvate (50 mM), little oxidation of NADH occurred. When incubated with hydroxypyruvate (50 mM) and hydroxypyruvate isomerase (10 U), however, both YbbQ and YhaE catalyzed the oxidation of NADH. This indicates that both enzymes catalyze reduction of tartronate semialdehyde in the presence of NADH. The rate of reduction of tartronate semialdehyde was more than 10 times that of the oxidation of D-glycerate at pH 7.0, although the exact concentration of tartronate semialdehyde could not be determined due to its instability.

4. Discussion

Two hypothetical proteins of *E. coli*, YbbQ and YhaE, showed NAD^+-dependent D-serine dehydrogenase activity. YbbQ and YhaE are similar in their enzymological properties, such as optimum pH, NAD^+

Table 3
Kinetic parameters of YbbQ (1) and YhaE (2)

Subtsrates	K_m (mM)		V_{max} (U mg^{-1})		V_{max}/K_m (U mg^{-1} mM^{-1})	
	(1)	(2)	(1)	(2)	(1)	(2)
D-Serine	91	42	111	29.0	1.22	0.690
L-Serine	200	67	1.08	3.82	0.0054	0.057
D-Glycerate	0.44	0.6	56.3	10.5	128	17.5
L-Glycerate	7.1	7.4	35.3	4.81	4.97	0.650
D-3-Hydroxyisobutyrate	80	76	2.36	12.4	0.0295	0.163
L-3-Hydroxyisobutyrate	100	60	71.8	15.7	0.718	0.263

requirement, and substrate specificity, but different in subunit structure. The former is a homodimer, whereas the latter is a homotetramer. YbbQ is comparatively more unstable than YhaE. YbbQ (but not YhaE) was inactivated by dialysis against 10 mM potassium phosphate buffer (pH 7.4), though its activity was retained when more than 10% ammonium sulfate was present in the dialysis buffer. Addition of 10 mM malate, malonate or tartronate to the enzyme solution resulted in the stabilization of YbbQ. The stabilization of a dimeric enzyme by dicarboxylates was reported for *Rhodococcus* phenylalanine dehydrogenase [13]. YhaE was not affected by these dicarboxylates. The difference in the stability and subunit structure of the enzymes may be physiologically significant.

Both YbbQ and YhaE showed a stereospecificity for serine and glycerate: D-serine and D-glycerate were far better substrates than the L-enantiomers. We have isolated an $NADP^+$-dependent serine dehydrogenase, which catalyzes the oxidation of 3-hydroxyl group of serine, glycerate, and 3-hydroxyisobutyrate, from *Agrobacterium tumefaciens* [14]. The *A. tumefaciens* enzyme, however, shows comparatively low stereospecificity for these substrates. In this respect, YbbQ and YhaE are obviously different from the *A. tumefaciens* enzyme.

In *E. coli* cells, neither YbbQ nor YhaE is probably involved in D-serine catabolism, because they show low affinity for D-serine and *E. coli* cells defective in D-serine dehydratase gene become highly sensitive to D-serine [15]. The *ybbQ* gene is included in the operon for glyoxylate utilization; in this pathway, one molecule of tartronate semialdehyde is produced from two molecules of glyoxylate. The *yhaE* gene is included in the operon for D-glucarate/galactarate utilization; in this pathway, one molecule each of pyruvate and tartronate semialdehyde are produced from one molecule of 5-keto-4-deoxy-D-glucarate, a catabolic intermediate. It has been believed that tartronate semialdehyde is converted into D-glycerate, followed by the production of 2- or 3-phosphoglycerate from D-glycerate by glycerate kinase, a common member in both catabolic pathways. Both YbbQ and YhaE conserve the structural features of the 3-hydroxy acid dehydrogenase superfamily [16], and showed higher affinity (K_m) and reactivity (V_{max}/K_m) for D-glycerate than for other substrates such as D-serine. Both enzymes catalyzed the reduction of tartronate semialdehyde in the presence of NADH. At physiological pH, the rate of tartronate semialdehyde reduction is much higher than that of D-glycerate oxidation. These results suggest that both YbbQ and YhaE are D-glycerate 3-dehydrogenases (tartronate semialdehyde reductases) and probably function physiologically in conversion of tartronate semialdehyde into D-glycerate.

Since the V_{max} values of YbbQ and YhaE for D-serine are markedly higher than those for D-glycerate, a mutant enzyme showing higher affinity for D-serine could become a potent tool for detection or determination of D-serine. Resolution of the tertiary structure of YbbQ or YhaE and especially, detailed analyses of the region interacting with the functional group on the 2-position of substrate, will provide insight into developing strategies for enzyme engineering and application.

References

[1] T. Ohshima, K. Soda, Adv. Biochem. Eng. Biotechnol. 42 (1990) 187.
[2] W. Hümel, M.-R. Kula, Eur. J. Biochem. 184 (1989) 1.
[3] H. Misono, I. Kato, K. Packdibamrung, S. Nagata, S. Nagasaki, Appl. Environ. Microbiol. 59 (1993) 2963.
[4] H. Misono, Y. Shinagawa, S. Nagata, S. Nagasaki, Agric. Biol. Chem. 51 (1987) 1467.
[5] M. Ashiuchi, K. Packdibamrung, T. Miyaji, S. Nagata, H. Misono, FEMS Microbiol. Lett. 167 (1998) 75.
[6] M. Saito, K. Miura, Biochim. Biophys. Acta 72 (1963) 619.
[7] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, J. Biol. Chem. 193 (1951) 265.
[8] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, 2nd Edititon, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989.
[9] U.K. Laemmli, Nature 227 (1970) 680.
[10] P. Matsudaira, J. Biol. Chem. 262 (1987) 10035.
[11] W.W. Cleland, in: P.D. Boyer (Ed.), The Enzymes, Vol. 2, 3rd Edition, Academic Press, New York, 1970, p. 1.
[12] F.E. de Windt, C. van der Drist, Biochim. Biophys. Acta 613 (1980) 556.
[13] H. Misono, J. Yonezawa, S. Nagata, S. Nagasaki, J. Bacteriol. 177 (1989) 30.
[14] W.K. Maas, R. Maas, E. McFall, J. Bacteriol. 177 (1995) 459.
[15] J.W. Hawes, E.T. Harper, D.W. Crabb, R.A. Harris, FEBS Lett. 389 (1996) 263.
[16] E.K. Chowdhury, K. Higuchi, S. Nagata, H. Misono, Biosci. Biotechnol. Biochem. 61 (1997) 152.