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Synthesis of L-*threo*-3,4-Dihydroxyphenylserine (L-*threo*-DOPS) with Thermostabilized Low-Specific L-Threonine Aldolase from *Streptomyces coelicolor* A3(2)

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Abstract Stability-enhanced mutants, H44, 11-94, 5A2-84, and F8, of L-threonine aldolase (L-TA) from *Streptomyces coelicolor* A3(2) (SCO1085) were isolated by an error-prone PCR followed by a high-throughput screening. Each of these mutant, had a single amino acid substitution: H177Y in the H44 mutant, A169T in the 11-94 mutant, D104N in the 5A2-84 mutant and F18I in the F8 mutant. The residual L-TA activity of the wild-type L-TA after a heat treatment for 20 min at 60°C was only 10.6%. However, those in the stability-enhanced mutants were 85.7% for the H44 mutant, 58.6% for the F8 mutant, 62.1% for the 5A2-84 mutant, and 67.6% for the 11-94 mutant. Although the half-life of the wild-type L-TA at 63°C was 1.3 min, those of the mutant L-TAs were longer: 14.6 min for the H44 mutant, 3.7 min for the 11-94 mutant, 5.8 min for the 5A2-84 mutant, and 5.0 min for the F8 mutant. The specific activity did not change in most of the mutants, but it was decreased by 45% in the case of mutant F8. When the aldol condensation of glycine and 3,4-dihydroxybenzaldehyde was studied by using whole cells of *Escherichia coli* containing the wild-type L-TA gene, L-*threo*-3,4-dihydroxyphenylserine (L-*threo*-DOPS) was successfully synthesized with a yield of 2.0 mg/ml after 20 repeated batch reactions for 100 h. However, the L-*threo*-DOPS synthesizing activity of the enzyme decreased with increased cycles of the batch reactions. Compared with the wild-type L-TA, H44 L-TA kept its L-*threo*-DOPS synthesizing activity almost constant during the 20 repeated batch reactions for 100 h, yielding 4.0 mg/ml of L-*threo*-DOPS. This result showed that H44 L-TA is more effective than the wild-type L-TA for the mass production of L-*threo*-DOPS.

Keywords: Thermostability, L-threonine aldolase, *in vitro* mutagenesis, L-*threo*-DOPS

β-Hydroxy amino acids that are found in the skeletons of many natural antibiotics, pharmaceutical drugs, and cosmetics are difficult to synthesize by conventional chemical processes. Enzymatic approaches have thus received great attention as alternatives to the chemical synthesis of β-hydroxy amino acids [3, 13]. However, the enzymatic production of β-hydroxy amino acids on a commercial scale is still limited because of problems associated with this technology such as an incomplete conversion owing to a thermodynamic equilibrium, low diastereoselectivity, and enzyme instability [4, 14, 16].

We have been interested in developing an enzymatic synthesis of L-*threo*-3,4-dihydroxyphenylserine (L-*threo*-DOPS), a powerful drug for the treatment of Parkinson's disease [10], since the commercial production of this drug has so far been accomplished only by a chemical synthesis that consists of very complex multistep reactions including several protection and deprotection reactions [12]. Moreover, chemically synthesized products are a mixture of four different stereo isomers (L-*threo*-DOPS, D-*threo*-DOPS, L-*erythro*-DOPS, and D-*erythro*-DOPS) requiring expensive purification steps to obtain diastereospecifically pure L-*threo*-DOPS. On the other hand, L-threonine aldolase (E.C. 4.1.2.5) is one of the promising candidates for the synthesis of such β-hydroxy amino acids [8, 9]. This enzyme catalyzes the cleavage of L-β-hydroxy amino acids into glycine and corresponding acetaldehydes. Since this aldol reaction is

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reversible, it can also directly synthesize β-hydroxy amino acids by using an equilibrium approach, namely by supplying excess amounts of substrates into the enzyme reaction [17]. One of the main purposes of our work has been to develop thermostable L-TAs suitable for an enzymatic synthesis of L-*threo*-DOPS. So far, one low-specific L-TA isolated from *Escherichia coli* has been shown to exhibit a relatively high thermostability. However, the activity of this enzyme has been modest with a high K_m and a low k_{cat} [7].

In this study, we cloned and expressed a putative L-TA from *Streptomyces coelicolor* A3(2). The obtained enzyme was purified to homogeneity and characterized in detail. In addition, we mutagenized the L-TA gene by an error-prone PCR, and obtained several mutant genes that encode L-TAs with an enhanced thermostability. One of the thermostabilized enzymes was used to synthesize L-*threo*-DOPS.

Materials and Methods

Strains and Media

S. coelicolor A3(2) was grown in a Maltose-Bennett's medium [1 g yeast extract, 1 g beef extract, 2 g NZ Amine (type A, Wako Pure Chemical), 10 g maltose per liter] at pH 7.3. *E. coli* JM109 (Takara) was used as the host strain for cloning and selection of thermostable L-TA mutants. Recombinant *E. coli* strains were cultured in a Luria-Bertani (LB) medium (10 g tryptone, 5 g yeast extract, 5 g NaCl per liter), supplemented with ampicillin (100 µg/ml).

Cloning and Expression of L-TA in *E. coli*

A putative L-TA gene on the genome of *S. coelicolor* A3 (SCO1085) was amplified by PCR by using oligonucleotide primers, A (5'-ATATACCATG**GAATTC**AACCCTCCTA-AGACCGACG-3') and B (5'-ATCTAGA**GGATCC**TCA-GCGCGCCATCTCTTCCTTG-3'). These primers add EcoRI and BamHI sites (boldfaced) at the 5'- and 3'-ends, respectively, of the SCO1085 gene. PCR was carried out in 50 µl of a high GC buffer (Takara) containing 0.4 µg of genomic DNA, 2.5 U of LA-Taq polymerase, 2.5 mM each of dNTP, and 25 pmol each of the oligonucleotide for 30 cycles of 94°C for 40 sec, 60°C for 30 sec, and 72°C for 2 min. The PCR reaction amplified a 1,097-bp fragment, which was double digested by EcoRI and BamHI (New England Biolabs). The restricted DNA fragment was recovered from a 1% (w/v) agarose gel with a gel purification kit (QIAGEN), and cloned into the pTrc99A vector by using T4 DNA ligase (Ligation high, TOYOBO). The cloned L-TA gene was used to transform *E. coli* JM109 by a heat-shock procedure, and plasmids from several transformants were sequenced to confirm an intact gene sequence. One of the plasmids thus obtained was named p*lth*-SCA3(2). *E. coli* JM109 containing p*lth*-SCA3(2) was grown in a LB medium containing 100 µg/ml ampicillin. The cloned gene was induced by adding 2 mM IPTG when the absorbance of the culture at 612 nm reached 0.5. The cultivation was further continued for 24 h at 37°C with reciprocal shaking. Subsequently, cells in the culture were harvested by a centrifugation (10,000 ×*g*, 10 min, 4°C), washed twice with 0.85% (w/v) NaCl, and suspended in a Tris-HCl buffer (10 mM, pH 7.0).

In Vitro Mutagenesis of L-TA

Error-prone PCR was performed by a Gene Morph Random Mutagenesis kit (Stratagene) according to the manufacturer's instructions. The Mutazyme DNA polymerase included in the kit is highly effective for introducing a random mutation, and moreover, the mutation rate can be controlled easily by adjusting the template DNA concentrations. To obtain the desired mutation frequencies, mutagenic PCR was done with different amounts of template DNA (50 ng, 250 ng, and 500 ng) in 50 µl of 1× Mutazyme reaction buffer, 200 µM each of dNTP, and 2.5 U of Mutazyme DNA polymerase. The PCR reactions were carried out under the following conditions: 29 cycles of 96°C for 30 sec, 60°C for 30 sec, and 72°C for 75 sec after a denaturing at 96°C for 30 min. Products of the error-prone PCR were cleaned by a QIAquick PCR purification kit (QIAGEN) and double digested by EcoRI and BamHI at 37°C overnight. After separation by a 1% (w/v) agarose gel electrophoresis, the amplified DNA fragments were recovered by a QIAquick gel extraction kit (QIAGEN). The purified DNA was inserted downstream of the *trc* promoter in p*Trc*99A to generate mutant libraries.

High-Throughput Screening

Transformants harboring the L-TA gene were picked by using an automatic colony picker (QARRAY lite, X2601, Genetix), transferred into 2 ml of an ampicillin-containing LB medium dispensed in 96-deep-square-well plates, and the plates were incubated overnight at 37°C on a plate orbital shaker (Titramax 1000, HEIDOLPH). Cells in the cultures were harvested by a centrifugation (2,500 rpm, 10 min), and frozen at −80°C for 60 min. For the L-TA assay, cells were suspended in 250 µl of a 10 mM Tris-HCl buffer (pH 7.0) containing 0.5 mg of L-threonine, and incubated at 50°C for 30 min. Then, the same volume of Nash's reagent [15% (w/v) ammonium acetate, 0.5% (v/v) acetic acid, and 20% (v/v) acetyl acetone] was added and the cultures were incubated at 50°C for 30 min. The increase in absorbance at 388 nm (yellow color) was recorded by spectrophotometry (SpectraMax Plus, Molecular Devices) to determine the amount of acetaldehyde produced by the L-TA activity.

Clones showing a L-threonine degrading activity were selected and they were used to inoculate 2 ml of an

ampicillin-containing LB medium dispensed in 96-deep, square-well plates. The plates were incubated, and cells grown in the plates were collected and suspended in 10 mM Tris-HCl buffer (pH 7.0) as described above. Cell-free extracts were then prepared in the 96-deep-square-well plates by using a bead-beater (Biomedical Science, Japan) with 0.1 mm glass beads (Biospec Products, U.S.A.). After a centrifugation, 50-μl portions of the cell-free extracts were transferred to 96-well assay plates followed by a heat treatment at 65°C for 20 min. Subsequently, 50 μl of a 10 mM Tris-HCl buffer (pH 7.0) containing 0.5 mg of L-threonine was added and the plates were incubated at 50°C for 30 min. Then, the same volume of Nash's reagent was added into the heat-treated cell-free extract to screen the residual enzyme activity of L-TA.

Purification

L-TA genes were overexpressed in *E. coli* JM109 by adding 2 mM IPTG as described above. Cells were harvested from two-liter cultures, washed twice with 0.85% (w/v) NaCl, and suspended in a 10 mM Tris-HCl buffer (pH 7.0). Subsequently, a cell-free extract was prepared by a sonification, which was centrifuged at 15,000 rpm for 30 min at 4°C. Nucleotides in the supernatant were removed by a treatment with 1% (w/v) protamine sulfate followed by a centrifugation (15,000 ×*g*, 1 h). The obtained supernatant was loaded onto a Hiprep 16/10 DEAE FF anion-exchange chromatography column (Amersham Pharmacia), which had been equilibrated with a 10 mM Tris-HCl buffer (pH 7.0). Subsequent column chromatography steps were carried out by using FPLC ($AKTA_{FPLC}$, Amersham Pharmacia). Proteins bound to the column were eluted by a linear gradient of NaCl from 0 to 1 M. The L-TA activity was eluted at a 0.37 M NaCl concentration. The active fractions were pooled, precipitated by ammonium sulfate at a 50% saturation, and then loaded onto a HiLoad 16/10 Phenyl Sepharose HP column that had been equilibrated with a 10 mM Tris-HCl buffer containing 7.2 M ammonium sulfate (pH 7.2). The column was eluted by a descending gradient of ammonium sulfate from 7.2 to 0 M. The active fractions eluted from the column were pooled, desalted, and concentrated by Millipore centrifuge concentrators (3,500 ×*g*). The concentrated fraction was further purified by a gel filtration on a Super sepharose column in 10 mM Tris-HCl. The purified enzyme was separated by a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in accordance with the procedure of Nielsen and Reynolds [11]. The native molecular mass of the enzyme was estimated by a gel filtration by using a Superdex 200 HR column.

Enzyme Assay

Under the standard assay conditions, the L-TA activity was determined by measuring the amount of glycine produced from L-*threo*-DOPS in 10 mM Tris-HCl (pH 8.0) at 50°C. The reaction mixture contained 10 μmol L-*threo*-DOPS, 0.5 μmol pyridoxal 5'-phosphate (PLP), and the L-TA enzyme in a total volume of 200 μl. The reaction was done for 10 min and stopped by addition of 100 μl of 30% trichloroacetic acid. One unit of L-TA is defined as the amount of the enzyme producing 1 μmole of glycine per minute under the standard assay conditions. The amount of glycine was quantitatively determined by a HPLC system [HITACHI; column, COSMOSIL 5C18-MS 4.6×150 mm; mobile phase, 0.1% (w/v) 1-heptanesulfonic acid sodium salt in 10% MeOH]. The protein concentration was determined by the Bradford method [1] with bovine serum albumin as a standard.

Stability of L-TAs at Different Temperatures

Each of the purified L-TAs (10 μg/ml) was incubated for 20 min at temperatures ranging from 30 to 70°C. After a cooling on ice, the heat-treated L-TA was serially diluted and the residual L-TA activity was measured at 50°C. The kinetics of the enzyme inactivation was determined by measuring the L-TA activity every 5 min by incubating the purified L-TAs (10 μg/ml) at 63°C.

Synthesis of L-*threo*-DOPS

Cells of *E. coli* harbouring a cloned L-TA gene were grown in 50 ml of LB containing 100 μg/ml of ampicillin, and recombinant L-TA was induced by adding 2 mM IPTG when the absorbance of the culture at 612 nm reached 0.5. The cells were washed with 0.85% (w/v) NaCl, and freezed at −80°C for 1 h. In repeated batch reactions, the cells were mixed with 5 ml of a substrate solution comprising 1 g of glycine, 0.1 g of 3,4-dihydroxybenzaldehyde, 10 μl of 2-mercaptoethanol, 250 μl of a pyridoxal-5-phosphate solution (PLP, 0.6 g/l), 0.0158 g of sodium sulfite, and 0.0375 g of Triton X-100, the pH of the substrate solution being adjusted to 6.5 by 1 N HCl. After mixing by a vigorous vortexing, the synthesis of the L-*threo*-DOPS was carried out at 15°C for 5 h. After the reaction, the *E. coli* cells were recovered by a centrifugation (10000 ×*g*, 10 min) and then mixed with 5 ml of the fresh substrate solution to conduct the second round of reactions. This procedure was repeated 20 times for 100 h.

RESULTS AND DISCUSSION

Cloning and High-Level Expression of the Cloned L-TA

It has been reported that L-TA from *Streptomyces amakusaensis* is stereochemically selective, and more specific to the (2*S*,3*R*) configuration [5]. Thus, we assumed that the L-TA from the *Streptomyces* species might be good sources for L-TAs to be used for the synthesis of L-*threo*-DOPS. Thus, we cloned a putative L-TA gene (SCO1085) from *S.*

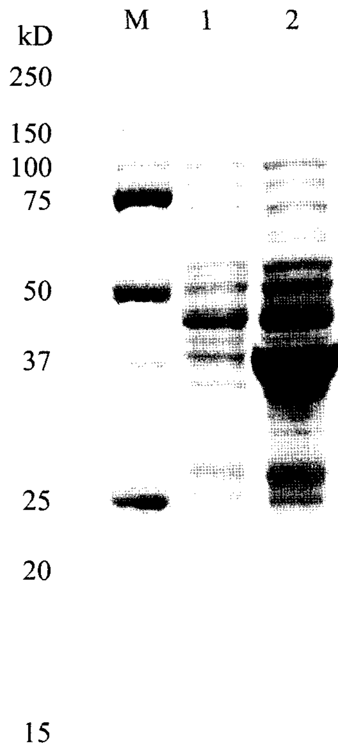

Fig. 1. Expression of L-TA from *S. coelicolor* A3(2) in *E. coli* JM109.
Expressed proteins were analyzed on a 12.5% polyacrylamide gel after staining with Coomassie brilliant blue. Lane M, molecular mass standard; lane 1, cell-free extract of *E. coli*, transformed by the p*lth*-SCA3(1) expression vector, containing the intact L-TA gene; lane 2, cell-free extract of *E. coli*, p*lth*-SCA3(2) expression vector, containing the N-terminal amino acid changed L-TA gene.

coelicolor A3(2). Because of its high GC content (73.2%), the PCR amplification of the SCO1085 gene was carried out with a high GC buffer included in the LA Taq kit (Takara, Japan). The amplified gene was inserted into pTrc99A and the sequences of the inserted genes were determined for more than 50 transformants. Unexpectedly, nucleotide substitutions were observed at a very high frequency, and only one clone, named p*lth*-SCA3(1), had an intact gene. When the SCO1085 gene was subcloned into the multiple cloning site between NdeI and NotI of the pET21(a) expression vector, the level of the cloned gene was extremely low as shown in Fig. 1 (lane 1) [2]. Next, we inserted the SCO1085 gene into a multiple cloning site of p*Trc*99A between EcoRI and BamHI to construct p*lth*-SCA3(2). In this plasmid, an N-terminal amino acid of the cloned gene changed from Met-Asn (5-GTGAAC-3) to Met-Glu-Phe-Asn (5-ATGGAATTCAAC-3). As shown in Fig. 1, the SCO1085 gene was overexpressed from p*lth*-SCA3(2) but not from p*lth*-SCA3(1). When the cell-free extract obtained from an *E. coli* harboring p*lth*-SCA3(2) was measured for the L-TA activity, glycine was produced successfully from L-threonine and L-*threo*-DOPS. No detectable L-TA was observed from the cell-free extract obtained from an *E. coli* harboring p*lth*-SCA3(1).

Screening of the Thermostable Mutants of L-TA

To obtain thermostabilized L-TA, an error-prone PCR in combination with a high-throughput screening was conducted as described in Materials and Methods. Among the transformants exhibiting the L-TA activity, 20 clones were randomly selected and sequenced. In these clones, 2 to 6 nucleotide substitutions per gene were observed, in which the frequency of the amino acid substitutions was between one and three per gene. Approximately 15,000 clones were screened in total, and 8 mutants named H44, I194, 5A2-84, F8, B1-51, A1-55, H53, and I-58 that displayed higher residual activities than the wild-type L-TA after the heat treatment were selected. The improved thermostability in these mutants was subsequently confirmed by the quantitative L-TA assays described in Materials and Methods. Finally, H44, I1-94, 5A2-84, and F8 were selected for further studies. Sequence analysis showed that each of these mutants had a single amino acid substitution: H177Y for H44, A169T for I1-94, D104N for 5A2-84, and F19I for F8.

Purification and the Properties of the Thermostabilized L-TAs

The wild-type and four thermostabilized L-TAs were purified to homogeneity as described in Materials and Methods. Most of the L-TAs were purified to homogeneity with yields of approximately 5% recovery (data not shown here). The molecular mass of the L-TAs by SDS-PAGE was about 37 kDa, which was almost equal to that expected from the deduced amino acids sequences. Native molecular mass of the enzyme was approximately 150 kDa, according to a gel filtration chromatography, indicating that L-TA is a homotetrameric enzyme. This result is in good agreement with the previous observations that low-specific L-TAs so far characterized are all tetramers [9]. The enzyme activity of the purified enzyme did increase by an addition of pyridoxal-5'-phosphate as shown in Fig. 2D. Optimal enzymatic activity of the recombinant L-TA was observed at pH 7–8 and 50°C as shown in Fig. 2A and 2B.

Fig. 3A shows the remaining activities of the wild-type and mutant enzymes after treating them at various temperatures ranging from 40°C to 60°C for 20 min. As shown in Fig. 3A, the remaining L-TA activity of the wild-type enzyme after the heat treatment for 20 min at 60°C was only 10.6%. However, the remaining activities in the mutant L-TAs were 85.7% for H44, 58.6% for F8, 62.1% for 5A2-84, and 67.6% for I1-94. Compared with the wild-type L-TA (the half-life was 1.34 min at 63°C), the

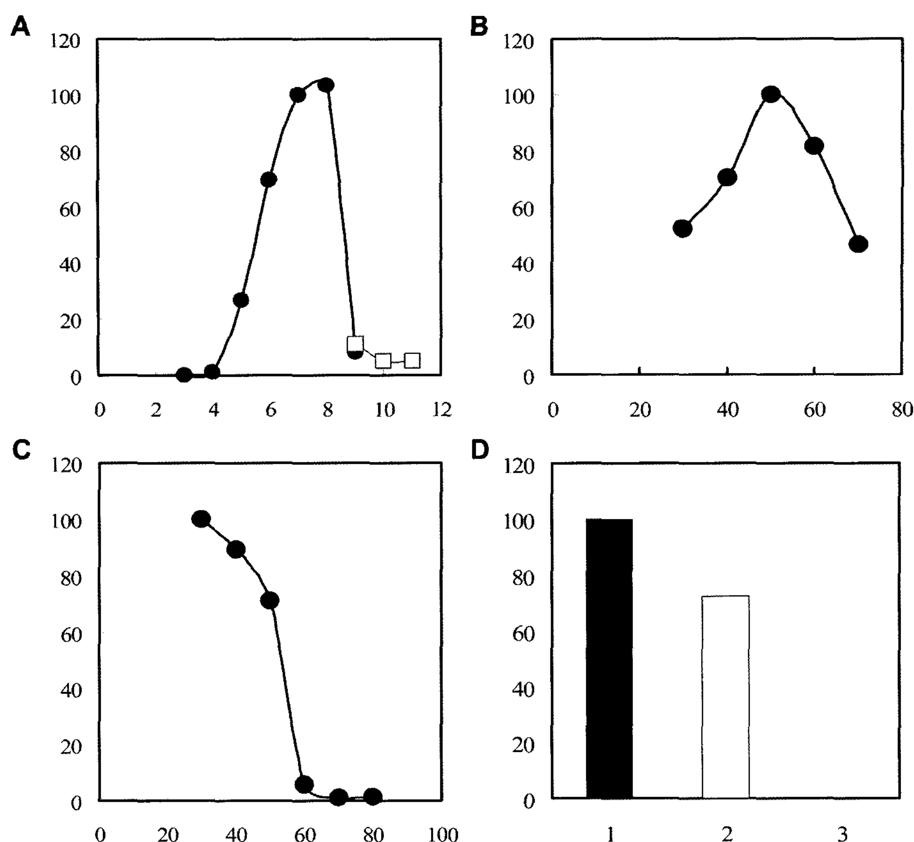

Fig. 2. Characterization of the purified L-TA from *S. coelicolor* A3(2) in *E. coli* JM109.

A. For estimating the optimal pH, the purified enzyme was diluted 10 times (10 μg/ml) in buffer solutions of various pH ranges [pH 3–9, 100 mM Briton-Robinson buffer (filled circles); pH 9–11, 100 mM glycine-NaOH buffer (unfilled squares)], and the L-TA activity was measured at 50°C. **B**. Optimal temperature was determined in a temperature range from 30°C to 70°C. **C**. Thermal stability was determined by measuring the residual L-TA activity after incubating the purified L-TA (10 μg/ml) in a temperature range from 30°C to 80°C. **D**. Effect of PLP addition on the L-TA activity was determined. The purified enzyme (10 μg/ml) was incubated overnight in the presence of PLP (1) or absence of PLP (2) in a buffer solution and the L-TA activity was measured. The intact plasmid pTrc99A was used for negative control (3). All the measurements were conducted in triplicate.

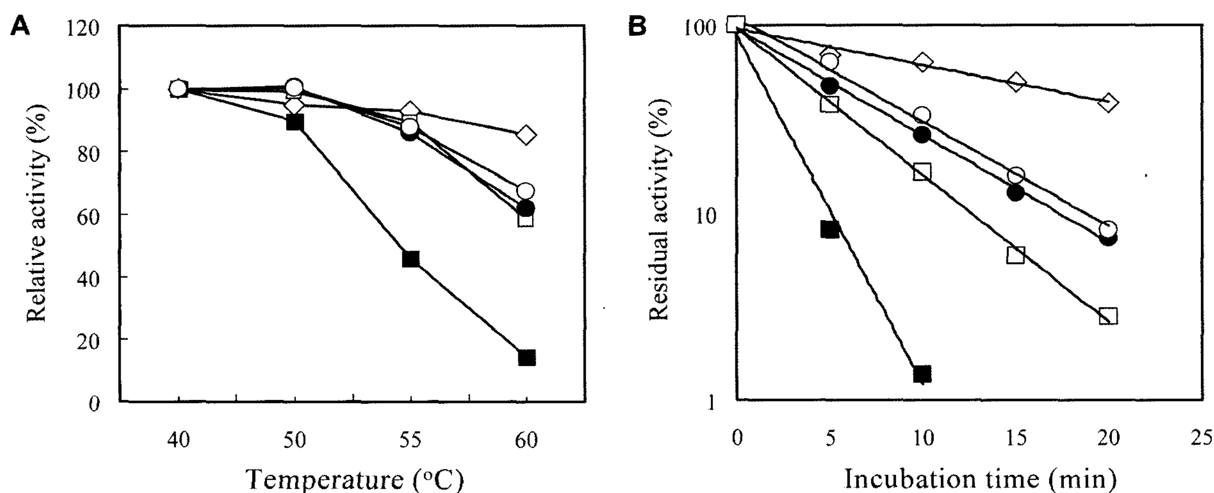

Fig. 3. Thermostability of mutant enzymes with wild-type L-TA.

A. Residual enzyme activities of the L-TAs were measured after heat treating them at each temperature. **B**. Half-lives of each mutant and the wild-type enzymes were calculated after measuring the residual enzyme activities after incubating them until no enzyme activity could be detected at 63°C. Symbols: unfilled diamonds, H44 mutant (H177Y); unfilled circles, 11-94 mutant (A169T); filled circles, 5A2-84 mutant (D104N); unfilled squares, F8 mutant (F181); filled squares, wild-type L-TA.

Table 1. Kinetic constants of the most thermostable L-TAs (H44) and recombinant wild-type L-TA from *S. coelicolor* A3(2).

Enzyme	V_{max} (U/mg)			K_m (mM)			k_{cat} (s^{-1})			k_{cat}/K_m ($mM^{-1}s^{-1}$)		
	L-Threonine	L-Phenylserine	L-*threo*-DOPS	L-Threonine	L-Phenylserine	L-*threo*-DOPS	L-Threonine	L-Phenylserine	L-*threo*-DOPS	L-Threonine	L-Phenylserine	L-*threo*-DOPS
Recombinant	170	210	312	1.8×10^{-4}	2.0×10^{-6}	2.0×10^{-4}	1.95×10^{2}	1.07×10^{4}	2.2×10^{4}	1.08×10^{6}	5.35×10^{9}	1.1×10^{8}
H44	188	233	330	1.6×10^{-4}	2.6×10^{-6}	2.3×10^{-4}	1.78×10^{2}	1.13×10^{4}	2.3×10^{4}	1.32×10^{6}	5.7×10^{9}	1.26×10^{8}

Kinetic constants were determined. Measuring of the enzyme activity was done by an HPLC system equipped with a Shimazu fluorescence detector. L-*threo*-DOPS concentrations for the K_m value were measured with varying concentrations of L-*threo*-DOPS from 0.1 μM to 0.8 μM in a 50 mM Tris-HCl buffer (pH 8.0) including 0.5 mM PLP with 10 U of L-TA.

half-lives of the mutant L-TAs were 14.6 min for H44, 3.7 min for 11-94, 5.8 min for 5A2-84 and 5.0 min for F8 at 63°C (Fig. 3B). Since the H44 enzyme exhibited the highest resistance against the heat inactivation, this enzyme was further characterized. The kinetic parameters of the wild-type and H44 enzymes are shown in Table 1. The Michaelis-Menten kinetic parameters of the wild-type and H44 enzymes were almost identical, indicating that the thermostability of the H44 enzyme was improved without affecting its catalytic abilities. Although a crystal structure of L-TA from *Thermotoga maritima* has only been elucidate until now, it only showed a very low (29%) homology to L-TA from *S. coelicolor* A3(2). Thus, it is almost impossible to build a model structure in order to find where the obtained mutation is located. However, the fact that a significantly enhanced thermal stability was obtained without affecting any other properties implies that this mutation is not related with an activity site, but it might be related with a strengthening of the oligomeric structure [15, 18]. The determination of the three-dimensional structure of a low-specific type L-TA will help to elucidate the functions of the obtained mutation.

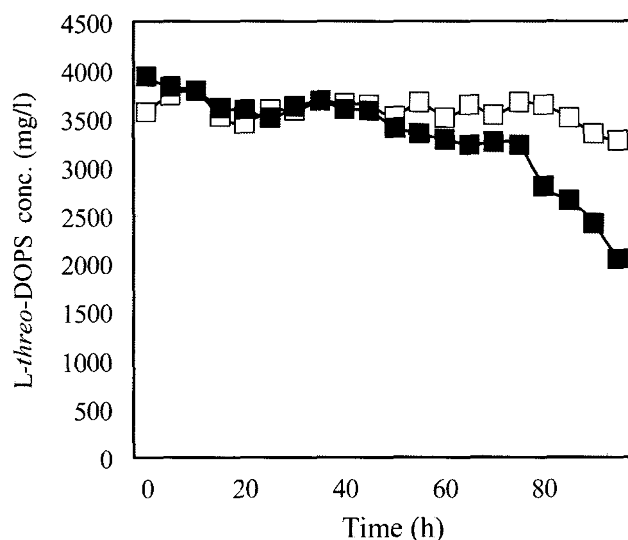

Fig. 4. Synthesis reaction of L-*threo*-DOPS by a whole-cell conversion reaction.

The enzymatic conversions of glycine and 3,4-dihydroxybenzaldehyde to L-*threo*-DOPS by *E. coli* cells harboring the wild-type L-TA (filled squares) or H44 mutant enzyme (unfilled squares) were performed in 50-ml falcon tubes with vigorous shaking at 15°C for 5 h. *E. coli* cells were harvested by a centrifugation (10,000 rpm, 10 min), and resuspended in a substrate solution, followed by continuous shaking at 15°C for the next whole-cell conversion reaction.

Synthesis of L-*threo*-DOPS

Before use in the conversion of glycine and 3,4-dihydroxybenzaldehyde to L-*threo*-DOPS, *E. coli* cells were frozen at −80°C for 1 h with the expectation that the permeability of the *E. coli* cells to the substrates may increase with the freeze-thawing treatment. No decrease in the enzyme activity was observed in the frozen cells without any stabilizer; however, purified L-TA was inactivated by a freezing at −80°C for 1 h without any stabilizer. When cells expressing the wild-type enzyme were used for the aldol condensation, L-*threo*-DOPS was successfully synthesized with a yield of 2.0 mg/ml in 20 repeated batch reactions. However, its L-*threo*-DOPS synthesis activity was dramatically decreased as the condensation reaction was repeated. Compared with wild-type L-TA, the H44 mutant maintained its L-*threo*-DOPS synthesizing activity during the 20 repeated batch reactions for 100 h, yielding 4.0 mg/ml of L-*threo*-DOPS (Fig. 4). This result showed that the H44 mutant is more effective for the construction of a L-*threo*-DOPS mass production process than the wild-type L-TA.

In this study, we have reported that single amino acid substitutions of L-TA from *S. coelicolor* A3(2) improved the thermostability of this enzyme without affecting its catalytic properties. Moreover, a thermostabilized L-TA, H44, showed a higher performance during an enzymatic aldol condensation for the synthesis of L-*threo*-DOPS.

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