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A novel serine hydroxymethyltransferase from marine bacterium *Alcanivorax* sp. and its application on enzymatic synthesis of L-serine

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ABSTRACT

A novel *glyA* gene from the marine bacterium *Alcanivorax* sp. was cloned and expressed in *Escherichia coli* BL21 (DE3). The recombinant *glyA* encodes a polypeptide of 418 amino acids, which was designated as *Ad*SHMT that shows the highest identity (70%) with a SHMT from *Shewanella algae*. The purified enzyme showed a single band at about 45 kDa by SDS-PAGE analysis. It was found that *Ad*SHMT exhibited the maximal activity at 50 °C and pH 7.0. The K_m, V_{max}, and K_{cat} values of *Ad*SHMT against DL-threo-3-phenylserine were calculated to be 0.097 mol/L, 3.255 μmol/min/mg and 2.451/s, respectively. More importantly, RP-HPLC detection showed that the *Ad*SHMT achieved an 88.37% molecular conversion rate in catalyzing glycine to L-serine, with the final concentration of L-serine being 353.15 mM in the reaction at 35 °C and 22nd hour when the initial concentration of the substrate (glycine) was 0.399 M. The molecular conversion rate of the *Ad*SHMT from the *Alcanivorax* sp. was 1.26-fold that of the *Ec*SHMT from the *E. coli*, which is currently applied in industrial production. Therefore, *Ad*SHMT has the potential for industrial applications due to its high enzymatic conversion rate.

1. Introduction

L-Serine is one of the twenty standard amino acids. Meanwhile, it is required for glycine, L-cysteine, L-tryptophan, phospholipids and hemes synthesis [1]. Additionally, L-serine has an essential role in the generation of activated one-carbon (C_1) units that are required in a variety of metabolic processes including purine and pyrimidine biosynthesis [2]. L-Serine is also required for pharmaceutical purposes [3]. The total annual demand for L-serine is reported to be 300 tons [3], suggesting that L-serine has a great potential for application in our life, and the demand of L-serine will be greater with social and economic development.

L-Serine is usually produced by fermentation [4], chemical synthesis [5] and enzymatic methods [6]. Among these methods, the enzymatic method has the advantages of simple process, short cycle, strong specificity and high yield, and thus has become the most promising method for L-serine manufacturing. In the L-serine production by the enzymatic method, the key reaction within the central metabolism is the reversible interconversion of L-serine and tetrahydrofolate (THF) to glycine and N_5,N_{10}-methylene-THF catalyzed by serine hydroxymethyltrasferase (SHMT; EC 2.1.2.1) [7]. Therefore, a high molecular conversion rate of SHMT for the industrial production of L-serine has always attracted the attention of researchers, and the highest molecular conversion rate (87%) in enzymatic production of L-serine was obtained in 1986 from a SHMT from the recombinant bacterium *Klebisells aerogenes* [6].

SHMT is a pyridoxal 5′-phosphate (PLP)-dependent enzyme [8,9], which is encoded by the *glyA* gene [10] and has been characterized from different organisms, such as bacteria [11], plants [12] and a few mammalian livers [13–15]. For example, SHMT belongs to the abundant proteins in *Bacillus subtilis* [16], *Escherichia coli* [17], *Corynebacterium glutamicum* [18] and *Lactococcus lactis* [19]. SHMT has also been shown to be involved in de novo biosynthesis of thymidylate in the mammals [20]. In plants, the enzyme cooperates with the glycine decarboxylase (GDC) to mediate photorespiratory glycine–serine interconversion [21,22].

In the current study, a *glyA* gene from the marine bacterium *Alcanivorax* sp. was cloned and expressed in *E. coli*, and then the recombinant *Ad*SHMT enzyme was purified and characterized. Additionally, the correlation of the *Ad*SHMT enzyme production and activity with the fermentation time of recombinant *E. coli* was

Abbreviations: SHMT, serine hydroxymethyltransferase; PLP, pyridoxal 5′-phosphate; THF, tetrahydrofolate; GDC, glycine decarboxylase; LB, Luria-Bertani; IPTG, isopropyl-β-D-1-thiogalactopyranoside; GST, glutathione S-transferase; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; CTAB, cetyltrimethyl ammonium bromide; RP-HPLC, reversed phase high performance liquid chromatography; ORF, opening reading frame; OPA, orthophthalaldehyde.

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investigated. The molecular conversion rate of *Ad*SHMT in L-serine enzymatic production was also evaluated.

2. Materials and methods

2.1. Strains, plasmids, mediums and materials

The *E. coli* strains DH5a and BL21 (DE3) were used as hosts to clone and express the target gene, respectively. The vectors, pGEX-6p-1 and pET-15b, were used to express the SHMT gene. The marine bacterium was cultured in marine agar 2216 medium. Luria-Bertani (LB) medium containing ampicillin (100 μg/ml) was used to culture *E. coli* DH5a and *E. coli* BL21.

All the Taq DNA polymerases, T4 DNA ligases, restriction enzymes, and DNA markers were purchased from TaKaRa Company (Dalian, China). The primers and fragments were synthesized and sequenced, respectively, by GenScript Co. (Nanjing, China). The protein molecular weight marker was purchased from TIANGEN Co. (Germany) and THFA was synthesized in our laboratory as described by Scrimeour and Vitols [23]. The Kits including DNA purification, Protein Quantification and the GST Bind Purification were purchased from Axygen Co. (USA), Pharmacia Co. (Sweden) and Sangon Biotech Co. (China), respectively. Unless otherwise stated, all the other chemical materials used in this study were purchased from Sigma Company (USA).

2.2. Cloning of the glyA gene encoding the SHMT

The *glyA* gene encoding *Ad*SHMT was amplified by PCR from genomic DNA of the *Alcanivorax* sp. The two pairs of primers used were designed by the sequence of the *glyA* gene from the *Alcanivorax* sp. genomic information in the NCBI (National center for biotechnology information) database (AC no. cp 003466) (http://www.ncbi.nlm.nih.gov/) as follows: (F1-*glyA-Bam*HI: 5′-CGGGATCCATGTTCCCCAAATCCATGTCCAT-3′; R1-*glyA-Xho*I: 5′-CCGCTCGAGTTAGCGCTCATAGACCGGCAACC-3′ and F2-*glyA-Nde*I: GGAATTCCATATGATGTTCCCCAAATCCATGTCCA; R2-*glyA-Bam*H1: CGGGATCCTTAGCGCTCATAGACCGGCAACC). After PCR, the amplified products were cloned into pGEX-6p-1 and pET-15b vector, respectively. Similarly, the *glyA* gene (Gene ID: 947022) from *E. coli* was cloned into vector pET-15b. Finally, the clones were transformed into *E. coli* strain DH5a and confirmed by sequencing. The three recombinant plasmids were designated as pGEX-6p-*AdglyA*, pET-15b-*AdglyA*, and pET-15b-*EcglyA*, respectively.

2.3. Expression and purification of the recombinant enzyme

The *E. coli* strain BL21 (DE3) was transformed with the recombinant plasmid pGEX-6p-*AdglyA* for *Ad*SHMT expression. Initially, a single colony was inoculated into the LB liquid medium containing ampicillin (100 μg/ml) and grown 14–16 h at 37 °C. Subsequently, the culture was transferred into the fresh LB liquid medium (1:100 dilution) with ampicillin (100 μg/ml) for 4–5 h. Then at an optical density (OD_{600}) of 0.4–0.6, isopropyl-β-D-1-thiogalactopyranoside (IPTG) was added into the medium at a final concentration of 0.1 mM, followed by an overnight incubation of the mixture at 18 °C. Finally, the cells were collected by centrifugation and disrupted with High Pressure Homogenizer (NS100IL 2K, Niro Soavi, Germany). The recombinant *Ad*SHMT enzyme was purified by the glutathione S-transferase (GST) fusion protein purification system (GE Healthcare, Sweden). The concentration of the protein was determined by the method of Bradford using the Bradford reagent, and the molecular mass of the purified protein was assessed by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

2.4. Enzyme assay and biochemical characterization

The purified enzyme was used for general characterization of the *Ad*SHMT properties. The SHMT activity was measured as described by Lee and Hsiao [24], with modifications. The 0.3 ml reaction mixture (pH 7.0) consisted of 10 μl of the purified enzyme and 290 μl of Na_2HPO_4 (0.2 M)-citric acid (0.1 M) buffer containing 50 mM DL-threo-3-phenylserine and 50 μM PLP. After 1 h reaction at 50 °C, the benzaldehyde absorption was measured at 279 nm [25]. One unit (U) enzyme activity was defined as the amount of enzyme which catalyzed the release of 1 μmol benzaldehyde per minute. All enzyme activity assays were performed in triplicate and the specific enzyme activity was expressed as the number of units per mg protein.

The optimum temperature of the enzyme activity was determined in the temperature range of 30–70 °C at a 5 °C interval in a Na_2HPO_4-citric acid buffer (pH 7.0). The optimum pH for the purified enzyme activity was determined at various pH values by using 0.2 M Na_2HPO_4/0.1 M citric acid buffer (pH 4.0–8.0) and 0.05 M glycine–NaOH buffers (pH 8.0–10.0). The thermostability of the enzyme was determined at the optimal pH by incubating the enzyme at a temperature range of 35–55 °C (at a 5 °C interval) for 10, 20 and 30 min, and then the residual activity was measured using a standard assay. The effect of pH on the enzyme stability was determined under the standard assay conditions by incubating the enzyme in a range of buffers (pH 6.0–8.0) in the absence of the substrate at 4 °C for 24 h, and then the residual activity was measured. The effects of different metal ions (3 mM) and other chemical reagents (1%) on the recombinant enzyme activity were evaluated at 50 °C for 45 min with a substrate buffer solution (pH 7.0), and the remaining activity was measured under standard conditions. The activity assayed in the absence of metal ions or chemical reagents was defined as 100%.

2.5. Enzyme kinetic analysis

The K_m, V_{max}, and K_{cat} values of the purified *Ad*SHMT enzyme were determined by measuring the enzymatic activity under the optimum temperature and pH conditions at a substrate concentration range of 20–140 mM. The data were plotted according to the Lineweaver–Burk method.

2.6. Fermentation time and enzyme activity

The recombinant plasmid, pET-15b-*AdglyA*, was transformed into *E. coli* BL21 (DE3). A single colony was picked out from LB solid medium plate and the transformant was grown overnight at 37 °C in the LB liquid medium supplemented with 100 mg/ml ampicillin. The culture was then inoculated into fresh LB medium (1:100 dilution) containing 100 mg/ml ampicillin and grown at 37 °C for 4 h. When the OD_{600} rose to around 0.6, IPTG was added to a final concentration of 0.1 mM, and the culture was incubated at 18 °C for 36 h. Subsequently, an equal volume of culture fluid was collected at a 4 h interval and stored at −4 °C until the end of fermentation. Finally, the optical densities of cultures were measured at OD_{600} under the same conditions. The enzyme activities of the cultures were determined in the same way as described above, except that 0.03% (w/v) cetyltrimethyl ammonium bromide (CTAB) was added when the culture cells were used in the reaction system directly. The expression levels of the recombinant protein were analyzed by 12% SDS-PAGE.

2.7. Enzymatic production of L-serine

The recombinant expression plasmids pET-15b-*AdglyA* and pET-15b-*EcglyA* were introduced separately into *E. coli* BL21 (DE3) for

L-serine production. The two engineering bacteria were inoculated, induced and incubated with the same method and under the same conditions in L-serine production. At the end of fermentation, the bacterial cells were collected by centrifugation at 6000 rpm for 5 min, and then washed twice with phosphate buffer (0.2 M, pH 8.0). After that, the wet cells were divided into equal parts (450 mg for each) and frozen overnight at −80 °C. Subsequently, the *Ad*SHMT (expressed by pET-15b-*AdglyA*) were thawed at 37 °C and re-suspended with phosphate buffer (0.2 M, pH 8.0) in a 15 ml reaction system (I, II, III and IV) containing phosphate buffer (0.2 M, pH 8.0), formaldehyde (23.8 mM), THFA (4 mM), PLP (0.3 mM) and β-mercaptoethanol (0.4 M). The concentrations of the glycine in the four reaction systems (I, II, III and IV) were 0.266, 0.399, 0.533 and 0.666 M, respectively.

The enzymatic reactions were performed in a rotary shaker at 35 °C and 160 rpm for 24 h. 100 μl was sampled at a 2 h interval for 24 h and stored at −80 °C, and the formaldehyde was added to these reaction systems at a 1 h interval at a final concentration of 23.8 mM. The optimum conversion concentration of glycine was determined in reaction system II. Meanwhile, *Ec*SHMT (expressed by pET-15b-*EcglyA*) was used for L-serine production through the enzymatic reactions in reaction system II under the same conditions. Finally, the L-serine and glycine concentration of all the samples were determined by RP-HPLC with pre-column derivatization.

2.8. Sample preparation and RP-HPLC detection of L-serine and glycine concentrations

All the samples were prepared as follows: the standard amino acid and the sample of enzymatic reaction (40 μl) were diluted separately with ultrapure water within a concentration of 50–300 pmol/μl. Subsequently, 100 L of as-prepared enzymatic reaction and standard amino acid solutions were added separately into a 1.5 ml centrifuge tube, with the centrifuge tube containing 600 μl of borate buffer (0.4 M, pH 10.4) and 300 μl of OPA (4 mg/ml) as derivatization reagent. After that, the mixture was filtered through an organic membrane (0.22 μm) and then was thoroughly mixed. Finally, the sample was analyzed with RP-HPLC.

The L-serine and glycine from the enzymatic reaction samples were analyzed on a column of Agilent Eclipse XDB-C18 (250 mm × 4.6 mm, 5 μm) by RP-HPLC (1260 infinity quaternary LC system, Agilent Technologies) with the detector set at 340 nm excitation and 450 nm emission. The detection system was run as follows: the sample (5 μl) was injected into the chromatograph at a flow rate of 1 ml/min at 32 °C (column temperature), and the column was eluted with moving phases A and B by decreasing solvent A from 90% to 72% and increasing solvent B from 10% to 28% at a constant rate during the first 12 min, followed by an increase of solvent B from 28% to 30% at a constant rate in the next 8 min (12–20 min). Finally, the equilibration time was set as 5 min for the next run. Solvent A (pH 5.8) was composed of 25 mM sodium acetate buffer supplemented with tetrahydrofuran (95/5, v/v), and the composition of Solvent B was similar, except that tetrahydrofuran was replaced by the methanol [26].

3. Results

3.1. Gene cloning and sequence analysis

The DNA fragment, obtained as described in Section 2, was found to code for an opening reading frame (ORF) (1257 bp) starting with an ATG codon and terminating with a TAA codon after sequencing analysis. The G+C content of the ORF was 63.4%, and the sequence encoded a polypeptide of 418 amino acids with a predicted molecular mass of 45.2 kDa. When the amino acid sequence of the putative protein was compared with the sequences of the other *glyA* genes coding in the NCBI database by a protein BLAST search, the amino acid sequence of *Ad*SHMT protein showed the greatest sequence identity with that of the known SHMT proteins from (70%) *Shewanella algae* (GenBank: KC784941) and (69%) *E. coli.* (GenBank: NP_417046).

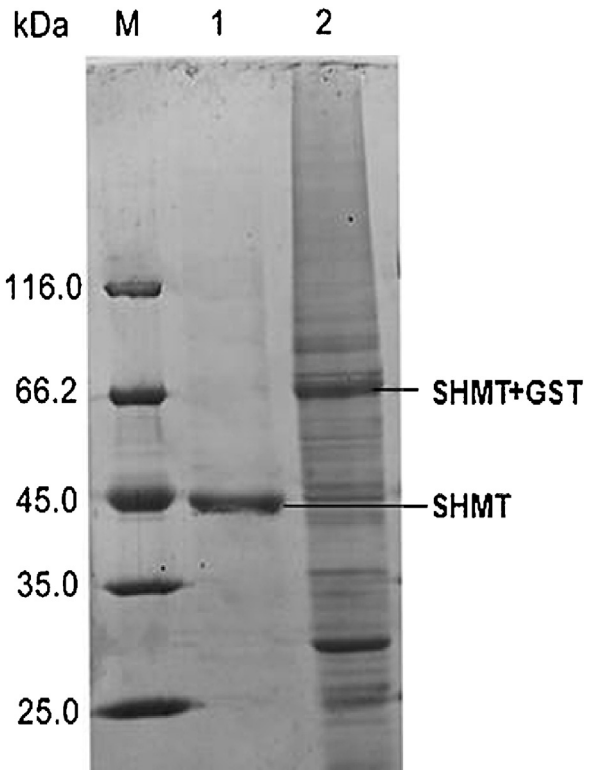

Fig. 1. 12% SDS-PAGE analysis of the purified *Ad*SHMT. Lane M: protein marker. The protein molecular weight ladder is Unstained Protein Molecular Weight Marker (Fermentas, Canada). Lane 1: purified *Ad*SHMT without GST. Lane 2: total protein in recombinant bacterium (harboring pGEX-6p-*AdglyA*) induced by 0.1 mM IPTG.

3.2. Expression and purification of AdSHMT

After the recombinant plasmid pGEX-6p-*AdglyA* was expressed in *E. coli* strain BL21 (DE3) and purified by affinity chromatography, the recombinant *Ad*SHMT was harvested and resolved to a single band of about 45 kDa by the SDS-PAGE analysis (Fig. 1). The concentration of the purified enzyme was determined to be 0.4 mg/ml by the Bradford method.

3.3. Enzyme characterization

*Ad*SHMT exhibited the optimal activity at around 50 °C (Fig. 2a), and retained over 80% activity between 40 and 55 °C. The enzyme could also maintain more than 65% of the maximal activity after 30 min incubation at 35 and 40 °C, but at a temperature above 45 °C, the enzyme activity declined rapidly (Fig. 2b). The optimal pH value for the *Ad*SHMT activity was found to be 7.0 (Fig. 2c), and over 60% of the maximal activity was retained after treatment at pH 6.0–7.5 and 4 °C for 24 h. However, the enzyme displayed less than 20% of its maximal activity at pH 8.0 (Fig. 2d).

The *Ad*SHMT activity was affected by most of the metal ions and several chemical reagents tested. Compared with the control, the enzyme activity was strongly inhibited by Cu^{2+} and SDS (1%), but

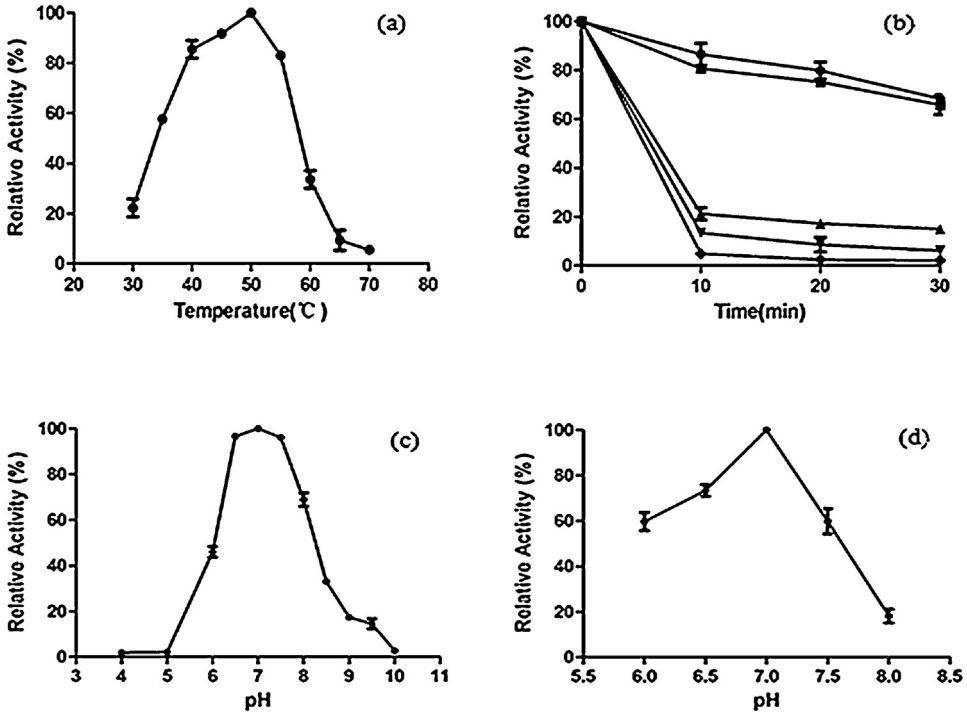

Fig. 2. Effects of temperature and pH on the activity and stability of the purified *Ad*SHMT. (a) The optimum temperature. The enzyme activity was measured at a temperature range of 30–70 °C, and the maximal activity was taken as 100%. (b) Effect of temperature on the enzyme stability. The thermal stability was determined by incubating the enzyme at 35 (●), 40 (■), 45 (▲), 50 (▼) and 55 °C (♦) for 10, 20 and 30 min. The activity of the enzyme without incubation was defined as 100%. (c) The optimum pH. The activity was measured at a pH range of 4.0–10.0, and the maximal activity was taken as 100%. (d) Effect of pH on the enzyme stability. The enzyme stability was determined by incubating the enzyme at a different pH value at 4 °C for 24 h, and the residual activities were measured at pH 7.0. The activity of the enzyme without incubation was defined as 100%. Error bars represent the standard deviation. Results represent the average of three parallel experiments.

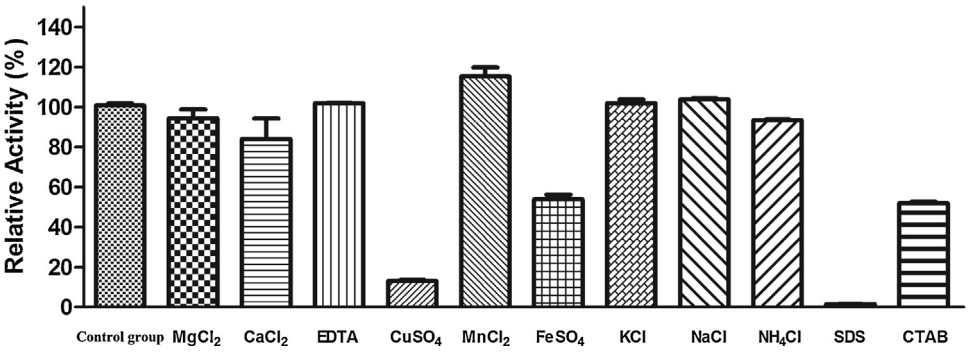

Fig. 3. The effects of different metal ions (3 mM) and other chemical reagents (1%) including SDS and CTAB on the purified *Ad*SHMT activity. Assays were performed under optimum conditions. The activity of the enzyme without metal ions or chemical reagents was defined as 100%. The error bars represent the standard deviation.

increased in the presence of Mn^{2+}. Besides, Mg^{2+}, K^+, Na^+ and EDTA had no significant effect on the enzyme activity (Fig. 3).

3.4. Kinetic analysis

When DL-threo-3-phenylserine was used as the substrate, the values of kinetic parameters in terms of K_m, V_{max} and K_{cat} were calculated to be 0.097 mol/L, 3.255 μmol/min/mg and 2.451/s, respectively, using the Michaelis equation.

3.5. Correlation of fermentation time with AdSHMT activity

The cell concentration and the enzyme activity increased rapidly between 4 and 8 h, and gently subsequently. On the whole, they reached a stable state after 16 h, and still remained in a good state at 36 h (Fig. 4a). Meanwhile, the expression of the *Ad*SHMT enzyme was stable from the 4th to 36th hour by the SDS-PAGE analysis (Fig. 4b).

3.6. Enzymatic reaction of L-serine production

The following values of the enzymatic production of L-serine were acquired by RP-HPLC detection (Fig. 5). The optimum concentration of the substrate (glycine) was 0.399 M based on the L-serine conversion rates of the *Ad*SHMT at 35 °C and the 22nd hour in the four reaction systems (I–IV), which were 77.97, 88.37, 69.87 and 52.45%, respectively (Fig. 6a). Under the same conditions, the maximum molecular conversion rate of the *Ad*SHMT was 1.26-fold that of the *Ec*SHMT (70.25%) at the 22nd hour (Fig. 6b). The L-serine concentrations of two systems were 353.15 mM and 280.74 mM, respectively (Fig. 6c).

4. Discussion

Generally, the methylotrophic microorganisms were isolated from terrestrial environments. In this study, the *glyA* gene from the

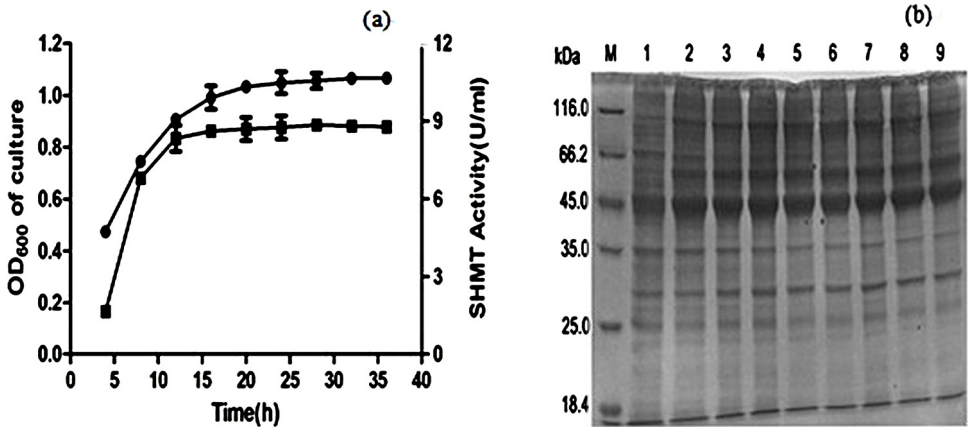

Fig. 4. The correlation of fermentation time with *Ad*SHMT activity, and SDS-PAGE analysis of the *Ad*SHMT expression level. (a) The correlation of fermentation time with *Ad*SHMT activity. The OD_{600} at each time point (●) and the enzyme activity (■) of cultures were measured under the same conditions. (b) The expression analysis of *Ad*SHMT by SDS-PAGE at each time point. Lane M: protein marker. Lanes 1–9: the expression levels of *Ad*SHMT at the 4th–36th hour at a 4 h interval with the band at about 45 kDa. The error bars represent the standard deviation.

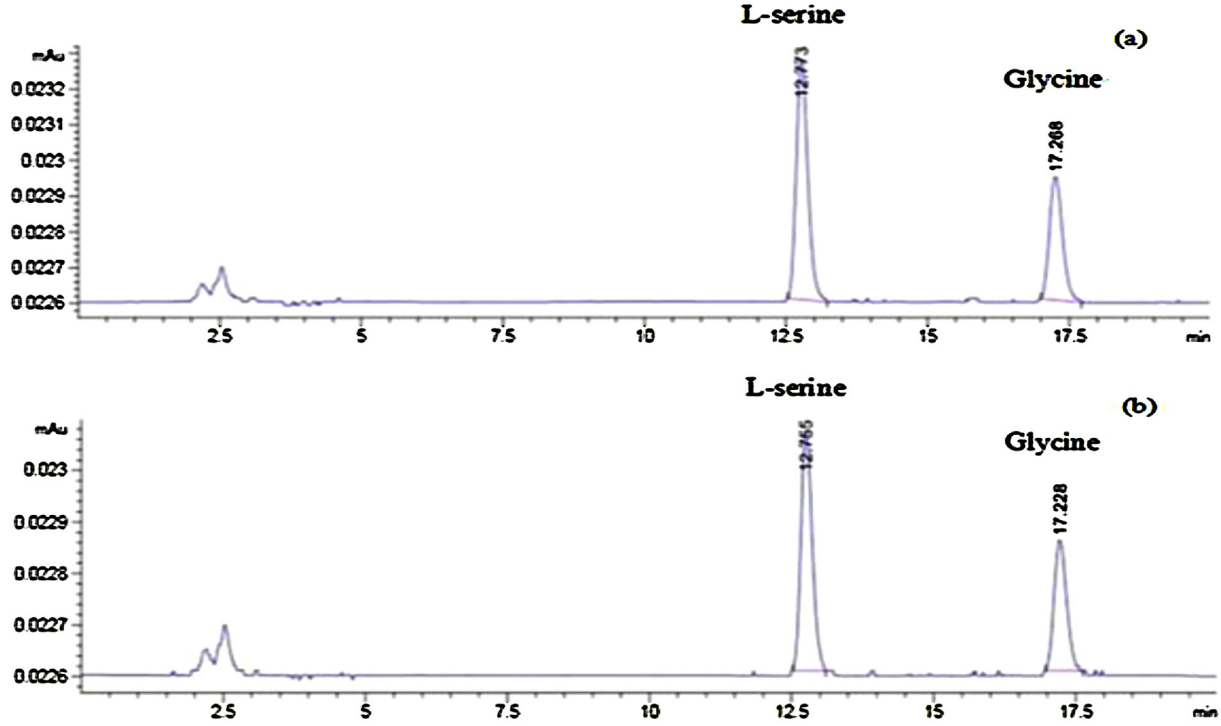

Fig. 5. HPLC analysis of the L-serine concentration. (a) L-serine and glycine standards. (b) Detection of L-serine synthesis by *Ad*SHMT in the enzymatic reaction system II at the 22nd hour.

deep-sea bacterium *Alcanivorax* sp. was cloned and expressed in *E. coli*. To the best of our knowledge, this is the first study of its kind to analyze the protein *Ad*SHMT and the conversion rate of *Ad*SHMT in the enzymatic production of L-serine. The results indicate that *Ad*SHMT has a high potential for industrial applications.

SHMT, which is a ubiquitous enzyme found in Archaea, Eubacteria and Eukarya, belongs to the α-family of PLP-dependent enzymes [27,28]. For the industrial production today, SHMT is useful in the synthesis of the L-serine using glycine and formaldehyde, but the structure of SHMT is rather conserved during evolution [29]. Thus, before finding a better way to improve the production efficiency of L-serine, it is necessary to analyze the characteristics of the enzyme. To this end, we performed the biochemical characterization of the *Ad*SHMT enzyme. The enzyme is resistant to the inactivation induced by several metal ions and chemical reagents. Zhen-yu Zuo *et al.* maintained that the activity of a SHMT enzyme in L-serine production can be enhanced by directed evolution [25,30] to better fit industrial applications.

In the process of L-serine production, gaining large amounts of enzyme with high activity is the key. As shown in Fig. 4a, the biomass increased to a high yield after about 20 h of fermentation. Meanwhile, the enzyme activity also reached a maximum, and showed no significant increase at a later time perhaps due to the protein expression peak at 20th hour. This observation can help reduce the costs greatly and increase productivity in the actual industrial production for the accurate time to obtain a high cell concentration.

The result of the enzymatic production of L-serine shows that different initial concentrations of glycine, whether too high or too low, will affect L-serine production in the same reaction system

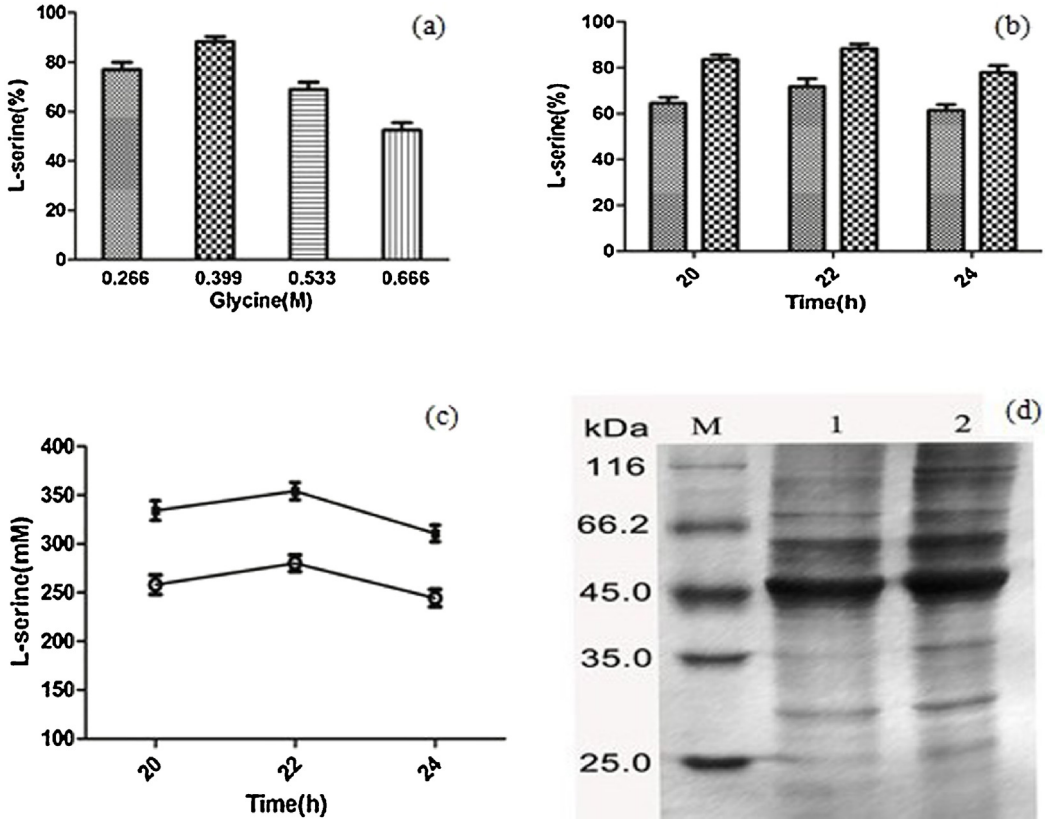

Fig. 6. The L-serine molecular conversion rates and SDS-PAGE analysis of *Ad*SHMT and *Ec*SHMT. (a) The maximum molecular conversion rate of *Ad*SHMT at a different substrate (glycine) concentration. HPLC was used to analyze the L-serine concentration followed by the calculation of the molecular conversion rates in enzymatic reaction systems I (▩), II (▩), III (▬) and IV (▬) at 35 °C and 22nd hour. (b) Comparison of L-serine molecular conversion between *Ad*SHMT and *Ec*SHMT at the optimum substrate (glycine) concentration. HPLC was used to analyze the L-serine molecular conversion rates in the *Ad*SHMT (▩) and *Ec*SHMT (▩) enzymatic reaction systems at a substrate (glycine) concentration of 0.399 M. (c) Comparison of the L-serine concentrations between *Ad*SHMT and *Ec*SHMT reaction systems. HPLC was used to analyze the L-serine concentration in the *Ad*SHMT (■) and *Ec*SHMT (○) enzymatic reaction systems at 35 °C and at the 20th, the 22th and the 24th hour. (d) SDS-PAGE analysis of the induced *Ad*SHMT and *Ec*SHMT. Engineering bacteria pET-15b-*AdglyA* and pET-15b-*EcglyA* were treated under the same conditions. Lane M: protein marker. Lane 1: *Ad*SHMT (about 45 kDa) from the *Alcanivorax* sp. Lane 2: *Ec*SHMT (about 45 kDa) from the *E. coli*. The error bars represent the standard deviation.

(Fig. 6a). This phenomenon may be related with the reaction itself, because in this reaction, the pathway can interchange between serine and glycine in the presence of the SHMT and the other two cofactors of THF and PLP [31]. A too high or too low initial glycine concentration resulted in a low conversion rate of the *Ad*SHMT in the enzymatic L-serine production, indicating that an appropriate concentration of glycine (0.399 M in the current study) can be beneficial to the enzymatic L-serine production.

Researchers usually pay more attention to the conversion rate of the enzyme in the actual industrial production. In previous studies, Hsiao et al. [6] used a SHMT enzyme from a recombinant strain of *Klebsiella aerogenes* as a crude extract to study the production of L-serine, resulting in a serine titer of 160 g/L with a 70% molecular conversion rate of glycine. However, in the current study, the L-serine conversion rate was as high as 88.37% in the enzymatic reaction. While the expression level of *Ad*SHMT was very similar to that of *Ec*SHMT under the same conditions (Fig. 6d), the molecular conversion rate of the *Ad*SHMT was 1.26-fold that of the *Ec*SHMT, which is currently applied in the industrial production.

In addition, we obtained a novel SHMT from the deep-sea bacterium *Alcanivorax* sp., while almost all the microorganisms used in the studies reported were from the terrestrial environment. To date, only a few reports have been published on L-serine production from glycine and methanol by methylotrophic bacteria with the serine pathway [32,33]. Although the *glyA* gene from *Alcanivorax* sp. encoding SHMT was reported [34], this is the first report about the characteristics and the conversion rate of *Ad*SHMT in L-serine production. Additionally, the kinetic analysis indicates that *Ad*SHMT has the potential to be further transformed by directed evolution, based on the reduction of the K_m (97 mM) and the K_m values from 33.4 to 24.5 mM as described by Zuo et al. [25].

5. Conclusion

The results show that the purified *Ad*SHMT has the optimal activity at 50 °C and pH 7.0. Moreover, the enzyme is resistant to the inactivation induced by several metal ions and chemical reagents. The maximum molecular conversion rate of the *Ad*SHMT was 1.26-fold that of the *Ec*SHMT, and the high conversion rate of the *Ad*SHMT in enzymatic L-serine production suggests that this enzyme has the potential for industrial applications.

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