Expression of Secreted His-Tagged S-adenosylmethionine Synthetase in the Methylotrophic Yeast *Pichia pastoris* and Its Characterization, One-Step Purification, and Immobilization

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*S-Adenosylmethionine synthetase* (SAM synthetase) catalyzes the synthesis of *S*-adenosylmethionine (SAM), which plays an important role in cellular functions such as methylation, sulfuration, and polyamine synthesis. To develop a simple and effective way to enzymatically synthesize and produce SAM, a soluble form of SAM synthetase encoded by SAM2 from *Saccharomyces cerevisiae* was successfully produced at high level (~200 mg/L) by the recombinant methylotrophic yeast *Pichia pastoris*. The secreted His-tagged SAM synthetase was purified in a single chromatography step with a yield of approximately 82% for the total activity. The specific activity of the purified synthetase was 23.84 U/mg. The recombinant SAM synthetase could be a kind of allosteric enzyme with negative regulation. The enzyme functioned optimally at a temperature of 35 °C and pH 8.5. The stability of the recombinant synthetase and the effectiveness of different factors in preventing the enzyme from inactivation were also studied. Additional experiments were performed in which the recombinant SAM synthetase was purified and immobilized in one step using immobilized metal-chelate affinity chromatography. The immobilized synthetase was found to be 40.4% of the free enzyme activity in catalyzing the synthesis of SAM from DL-Met and ATP.

### Introduction

*S-Adenosylmethionine synthetase* (ATP: L-methionine S-adenosyltransferase, EC 2.5.1.6, SAM synthetase) is the only enzyme known to catalyze the biosynthesis of *S*-adenosylmethionine (SAM) from L-methionine (Met) and ATP (1). SAM is an important metabolic intermediate in all living cells as a donor of methyl groups in transmethyla-

### Purification

Expression of the *SAM2* gene is induced by the presence of excess methionine in the growth medium, while expression of the *SAM1* gene from *S. cerevisiae* is repressed (16), and there

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is no problem of product inhibition observed in use of the *Escherichia coli* SAM synthetase (17). The synthetase encoded by SAM2 was intracellularly expressed in *E. coli* (18). However, both the levels of expression and the enzyme activity of SAM synthetase in the recombinant strain are lower. Therefore, it is difficult to obtain the recombinant SAM synthetase and the synthetase has not been extensively characterized, which limited the application of SAM synthetase.

Here, a simple and effective way to enzymatically synthesize and produce SAM was developed. We constructed a new recombinant strain of *P. pastoris* that secreted large amounts of SAM synthetase, which was encoded by SAM2 from *S. cerevisiae* and coupled to a hexahistidine tag. The recombinant synthetase was purified using IMAC and enzymically characterized. In the additional experiment, the purification and immobilization of the SAM synthetase were carried out in one step of IMAC. Furthermore, we have successfully used the immobilized SAM synthetase to catalyze the synthesis of SAM from DL-Met instead of the pure L-Met, further reducing the mobilization of the SAM synthetase were carried out in one step of IMAC. All other reagents were of analytical grade and were obtained from commercial sources.

**Construction of Expression Plasmids.** Plasmid pUC18-SAM2 containing the SAM2 gene (GenBank accession number M23368) from *S. cerevisiae* was previously constructed by our laboratory (12). The SAM2 coding region was amplified by PCR using a pair of specific primers, 5′-sense primer, 5′-AATGAATTCAGACAGACCCAGAAGA-3′, and 3′ antisense primer, 5′-TAAGGCGGCCGACGCGATC-3′-AATGAATTCAGACAGACCCAGAAGA-3′-designated according to the nucleotide sequence of SAM2 obtained from GenBank. In the sense primer, an Eco RI restriction site (underlined) was created at the 5′-end, and a His10 tag sequence (bold) was introduced downstream of the initiation ATG codon. In the antisense primer, a Not I restriction site (underlined) was added at the 5′-end, upstream of the termination TAG codon. The 50-μL PCR mixture contained 1 unit of *Pfu* DNA polymerase, 1 × PCR buffer, 200 μM of dNTPs mixture (dATP, dTTP, dCTP, dGTP), 30 μM of each primer, and 2 ng of pUC18-SAM2 plasmid DNA. The thermal cycling program used for amplification consisted of one cycle at 94 °C for 5 min, followed by 30 cycles at 94 °C for 30 s, 59 °C for 45 s, and 72 °C for 90 s, and a final extension step at 72 °C for 10 min. For cloning, the PCR products were gel-purified, digested with Eco RI/Not I, and ligated into the Eco RI/Not I cut in the pPIC9K vector, in-frame to the α-factor secretion signal and downstream of the alcohol oxidase 1 (AOX1) promoter. The expression plasmid pPIC9K-SAM2 was transformed into *E. coli* DH12S and then amplified, purified, and sequenced using an automatic DNA sequencer (ABI-3730, PE Applied Biosystems).

**Transformation and Screening of Multiple Inserts.** Both pPIC9K-SAM2 and pPIC9K (used as a control plasmid) were purified from *E. coli* cells and linearized with the restriction enzyme Sac I. The digested DNAs were transformed into *P. pastoris* GS115 and KM71 by electroperoration according to the instructions supplied with the Multi-Copy Pichia expression kit (Invitrogen). The His+ transformants were then selected on RDB (1 M sorbitol, 2% dextrose, 1.34% YNB, 4 × 10⁻⁵% biotin, 0.005% amino acids without histidine, 2% agar) plates. The recombinant strains GS115/pPIC9K-SAM2 and GS115/pPIC9K, KM71/pPIC9K-SAM2 and KM71/pPIC9K were identified by PCR using genomic DNA as a template and 5′AOX1 and 3′AOX1 as primers. The cells of GS115/pPIC9K-SAM2 and KM71/pPIC9K-SAM2 were plated on YPD-Geneticin (1% yeast extract, 2% peptone, 2% dextrose, and 2% agar) plates containing Geneticin at a final concentration of 0, 0.25, 0.5, 0.75, 1.0, 1.5 mg/mL for screening of multiple inserts.

**Analysis of an Expression Level and Selection of a High Producing Strain.** Colonies of GS115/pPIC9K-SAM2 and KM71/pPIC9K-SAM2 cultured on YPD-Geneticin plates were inoculated into 20 and 100 mL of BMGY medium (1% yeast extract, 2% peptone, 1.34% YNB, 4 × 10⁻⁵% biotin, 100 mM potassium phosphate, pH 6.0, and 1% glycerol) and grown at 30 °C in a shaker incubator for approximately 18 h. When OD₆₀₀ reached 2–6, the cells were harvested by centrifuging the medium for 5 min at 3000 g. The cell pellets were resuspended in 100 and 20 mL of BMMY medium (BMGY medium with 0.5% methanol instead of 1% glycerol) and cultured for 7–8 days at 30 °C. In order to maintain induction, methanol was added every 24 h to keep the final methanol concentration of 0.5%, and 1 mL of culture was withdrawn from each flask. These samples were used to analyze expression levels to determine the optimal harvest time and select the recombinant *P. pastoris* strain with the highest expression level. At the end of incubation, the cultures were centrifuged at 12,000g for 15 min, and the supernatant was stored at 4 °C.

**Purification of the His₆-Tagged SAM Synthetase.** The Ni²⁺-IDA agarose (His-Bind Resin, Ni-charged) column (5 mL of bed volume) was equilibrated with 4 column volumes of buffer A (20 mM Tris-HCl, pH 7.9, 500 mM NaCl). Then 60 mL of the culture supernatant was taken, adjusted to pH 7.9 with 1 M Tris, and applied to the column at a flow rate of 2.0 mL/min. Next, the column was washed with 6 column volumes of buffer A followed by 4 column volumes of buffer B (20 mM Tris-HCl, pH 7.9, 500 mM NaCl, 20 mM imidazole, 0.1% Triton X-100). The N-terminus His-tagged proteins were eluted with 6 column volumes of buffer C (20 mM Tris-HCl, pH 7.9, 500 mM NaCl, 200 mM imidazole, 0.1% Triton X-100, and 0.5 mM PMSF) at a flow rate of 1.0 mL/min. For further assays, the elution fractions were concentrated by ultrafiltration at 4000 g using concentrators with a 10 kDa cutoff limit (Millipore), and the buffer was changed to buffer D (50 mM Tris-HCl, pH 7.0, 1 mM DTT, and 0.5 mM PMSF).

**SDS−PAGE and Determination of Protein Concentration.** Samples were analyzed by SDS−PAGE in a 15% polyacrylamide gel under reducing conditions according to the method of Laemmli (19). The bands were visualized using silver staining. The gels were calibrated using molecular weight markers. The protein concentration in the samples was determined using the Bradford method (20).

**Enzymatic Activity Determination and Assay of Kinetics of the SAM Synthetase.** SAM synthetase activity was assayed in a total volume of 300 μL containing 20 mM L-Met, 26 mM ATP, 100 mM Tris, 300 mM KCl, 52 mM MgCl₂, and 8 mM reduced glutathione (GSH). The pH of the assay solution was adjusted to 8.5 using 1 M KOH. After 1 h of incubation at 35 °C, the reaction was stopped with 300 μL of 20% HClO₄. The reaction mixture was centrifuged, and the supernatant was analyzed by strong cation exchange HPLC using a mobile phase of 0.5 M of HCOONH₄, pH 4.0, and a flow rate of 2.0 mL/
by comparison with a standard SAM curve. One unit of enzyme activity was defined as the amount of SAM synthetase that catalyzes the formation of 1 μM of SAM in 1 h at 35 °C. For the kinetic analysis the reaction system is the same as for the enzyme activity determination. The concentrations of L-Met or ATP were varied from 0.05 to 1.0 mM.

Optimum Temperature and Optimum pH of the SAM Synthetase. The recombinant SAM synthetase activities were measured at 4, 20, 30, 35, 40, 50, 60, and 70 °C to determine the optimal temperature. The optimum pH of the recombinant synthetase was determined by measuring activity at various pH values between 4.05 and 10.12.

Dependence of the SAM Synthetase Stability on Temperature and pH. For temperature stability measurements, the recombinant SAM synthetase was suspended in 50 mM Tris-HCl buffer, pH 7.0, and incubated at 4, 20, 30, 40, 50, and 60 °C for 2 and 6 h, respectively. After cooling in ice-water for 20 min, the remaining activity was measured. For pH stability studies, the recombinant SAM synthetase was incubated at various pH of 3.5 to 9.5 in universal buffer at 4 °C for 6 h, respectively, and the residual activity of the treated enzyme was assayed. Universal buffer was prepared from a stock solution of 6.001 g citric acid, 3.893 g potassium dihydrogen phosphate, 1.769 g trihexylamine borate, and 5.266 g barbitone in 1 L of water. An aliquot (100 mL) of this solution was adjusted with 0.2 M sodium hydroxide to obtain the desired pH and diluted with water to 1 L.

Effect of Some Ions on Stability of the SAM Synthetase. To test the effect of metals and ions on the stability of the recombinant SAM synthetase, the enzyme was dissolved in 50 mM pH 7.0 Tris-HCl buffer containing a 5 mM concentration of one of several metals and ions (monovalent cations NH₄⁺, Li⁺, Na⁺, K⁺; divalent cations Ca²⁺, Mg²⁺, Ba²⁺, Fe²⁺, Cu²⁺, Zn²⁺, Mn³⁺; monovalent anions Cl⁻, Br⁻, F⁻, I⁻, NO₃⁻, HCO₃⁻, H₂PO₄⁻, CH₃COO⁻), and 0.1 mM Ni²⁺. The samples were incubated at 4 °C for 1 h, and the enzyme activities were determined using the standard assay procedure.

Protection of SAM Synthetase against Thermal Inactivation by Some Materials. To examine the effects of some materials including poly(ethylene glycol) 4000, trehalose, dextran T40, AMP, and various components of the reaction buffer on protection of SAM synthetase against inactivation at room temperature, the recombinant synthetase was dissolved in 50 mM pH 7.0 Tris-HCl buffer containing the materials mentioned above and incubated at 25 °C for 6 h. The residual activities of the enzyme were measured by the standard assay procedure.

Immobilization of the His₆-Tagged SAM Synthetase. A 2.5 mL portion of 50% Ni²⁺-IDA agarose slurry was added to 10 mL of buffer A and mixed gently. Then 50 mL of the culture supernatant containing the recombinant SAM synthetase was taken, adjusted to pH 7.9 using 1 M Tris, and then mixed with the Ni²⁺-IDA agarose slurry by shaking (200 rpm on a rotary shaker) at 4 °C for 1 h. The culture supernatant-Ni²⁺-IDA agarose mixture was centrifuged, and the pellet was washed with buffer B and used as the immobilized enzyme. The eluate was merged with the supernatant for residual activity analysis.

Synthesis of SAM from L-Met. The synthesis reaction was performed in a glass reaction flask (100 mL, φ50 × 60 mm) equipped with a stirrer and an attemperator. The reaction mixture consisted of SAM synthetase immobilized on Ni²⁺-IDA agarose and 50 mL of standard assay buffer containing L-Met instead of L-Met. The stirrer was set to 85 rpm, and the reaction temperature was controlled at 35 °C. Each reaction cycle lasted for 5 h. An aliquot was taken out every hour and immediately diluted with an equal volume of 20% HClO₄ to do analysis. Once a reaction cycle was completed, the immobilized enzyme was immediately separated from the reaction solution by vacuum pumping connected to the filter flask.

Results

Construction and Expression of SAM Synthetase. The DNA sequence encoding SAM synthetase was cloned into the P. pastoris pPIC9K vector in-frame with the yeast α-factor signal sequence for protein secretion. A His₆-tag sequence was added at the N-terminus of the SAM2 gene to facilitate purification and immobilization of expressed protein. The gene cloned into the expression vector was sequenced and confirmed to be correct. In order to screen the strains with high expression of SAM synthetase, both GS115-Mut (Methanol utilization plus) and KM71-Mut (Methanol utilization slow) recombinants were checked. A few Geneticin-resistant transformants that were different in size but similar in colony morphology appeared on the YPD plates containing different Geneticin concentrations. Induction of GS115/pPIC9K-SAM2 and KM71/pPIC9K-SAM2 using methanol for protein expression resulted in the secretion of active SAM synthetase. A 42 kDa protein not present in GS115/pPIC9K or KM71/pPIC9K was clearly visible by SDS-PAGE analysis of culture medium from transformants (Figure 1). In addition, SDS-PAGE analysis indicated that KM71/pPIC9K-SAM2 secreted fewer autologous proteins than GS115/pPIC9K-SAM2 (Figure 1).

Purifications and Yields of SAM Synthetases. His₆-N-tagged SAM synthetases were purified in a single chromatography step using a Ni²⁺-IDA agarose column. The effectiveness of the purification was monitored by SDS-PAGE. The protein yields of GS115/pPIC9K-SAM2 transformant from the 0.75 mg/mL Geneticin screening plate and KM71/pPIC9K-SAM2 transformant from the plate containing 1.0 mg/mL Geneticin were higher than that of the other GS115 and KM71 recombinant strains. The effect of induction time on the yield of SAM synthetase is shown in Figure 2. As shown in Figure 2, the enzyme was accumulated with increase of induction time. The highest yield of SAM synthetase in KM71 recombinant strains was ~200 mg/L, much higher than the highest yield from GS115.
recombinant strains (~49 mg/L). The strain of KM71/pPIC9K-SAM2 obtained from a YPD plate containing 1.0 mg/mL Geneticin was selected as the optimal strain. In the culture supernatant, the specific activity of the recombinant SAM synthetase of this strain was 6.42 U/mg. The purified SAM synthetase appeared as a single band on the silver-stained gels (Figure 3). The activity recovery and the specific activity of the purified SAM synthetase were 82.89% and 23.84 U/mg, respectively (Table 1).

**Optimal Temperature and pH of the SAM Synthetase.** The optimal reaction temperature of the recombinant SAM synthetase was found to be 35 °C. Enzyme activity was significantly reduced at temperatures below 30 °C or above 40 °C (Figure 4A). The highest activities at 35 °C occurred at a pH of 8.5 (Figure 4B). The activity of the enzyme was decreased above pH 9.0, suggesting that highly alkaline condition may have a detrimental effect on enzyme and product stability. The recombinant SAM synthetase was also inactive below pH 7.5, thus, the synthetase is active only in the slightly alkaline condition.

**Kinetics of the SAM Synthetase.** With either L-Met or ATP as the variable substrate, the Hill plotting was employed and Figure 5A and B was obtained. From Figure 5A and B, the apparent $K_m$ values for L-Met and ATP were found to be 0.527 ± 0.003 and 0.631 ± 0.002 mM, respectively, and the Hill coefficients for L-Met and ATP were 0.51 and 0.95, respectively, indicating that the immobilized synthetase as a kind of allosteric enzymes with negative regulation.

**Effect of Temperature and pH on Enzyme Stability.** The activity of the recombinant SAM synthetase was markedly decreased after incubation at temperatures above 4 °C for 2 and 6 h (Figure 6A). The pH stability of the enzyme was assessed by storage at pH 4 °C for 6 h in solutions of universal buffer at pH ranged from 3.5 to 9.5. The results of these experiments indicate that the recombinant synthetase was relatively stable at pH values from 6.5 to 8.5 (Figure 6B).

**Effect of Some Ions on Enzyme Stability.** The effects of some ions on the recombinant synthetase stability were examined. Compared to the activity of the enzyme incubated in Tris-HCl buffer, pH 7.0, the activity of the recombinant synthetase was not significantly affected by NH₄Cl, KNO₃, MgCl₂, KHCO₃, or KH₂PO₄ (relative activity 103.72~97.83%), but its activity was partly inhibited by KBr, CH₃COOK, KF, KI, CaCl₂, KCl, LiCl, NaCl, MnCl₂, and BaCl₂ (relative activity 93.33~70.70%).

| Table 1. Purification of the Recombinant SAM Synthetase from BMMY Culture Supernatant |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|------------------|
| purification step               | total protein (mg) | total activity (U) | specific activity (U/mg) | recovery of total activity (%) | purification factor |
| supernatant                     | 54.54            | 350.21           | 6.42             | 100             | 1                |
| 200 mM imidazole                | 12.18            | 290.29           | 23.83            | 82.89           | 3.71             |

70.70%, and was greatly inhibited by FeCl₂, CuCl₂, and ZnCl₂ (relative activity 59.22~22.17%) (Table 2). To quantify the influence of Ni²⁺ during the protein purification and immobilization process, the effect of adding NiSO₄ was also examined. There was no obvious effect (relative activity 99.13%) on the enzyme activity when the concentration of NiSO₄ was very low (0.1 mM).

**Prevention of Inactivation of SAM Synthetase by Some Chemicals.** As shown in Figure 6A, the recombinant synthetase incubated 25 °C for 6 h markedly inactivated, and only 33.98% of the enzyme activity was retained. Thus, how to stabilize the enzyme is a serious problem. We found that KCl could produce significant protection for the synthetase activity at room temperature (relative activity 99.22%), while poly(ethylene glycol) 4000, trehalose, dextran T40, L-Met, and MgCl₂ had a little effect (relative activity 63.11~40.62%). AMP, ATP, and GSH caused complete inactivation of the enzyme (Figure 7).

**Characterization of Immobilized SAM Synthetase and Its Application in the Synthesis of SAM from DL-Met.** The His₆-tagged SAM synthetase was immobilized on Ni²⁺-IDA agarose at 4 °C. The coupling efficiency and recovered activity of the immobilization reaction were 97.9% and 39.5%, respectively. The activity of the immobilized SAM synthetase was 9.63 U/mg, approximately 40.4% of the free enzyme activity. To reduce cost, racemic DL-Met was employed as the substrate instead of L-Met for the synthesis of SAM by the immobilized SAM synthetase, and the enzymatic activity was decreased to about 78% of that for L-Met as substrate. When DL-Met and ATP were used as the substrates and the Hill plotting was employed, Figure 8A and B was obtained. The apparent $K_m$ values for DL-Met and ATP were 1.303 ± 0.001 and 1.641 ± 0.004 mM, respectively. Hill coefficients for DL-Met and ATP were 0.42 and 0.71, respectively, indicating that the immobilized synthetase was also an allosteric enzyme with negative regulation when
DL-Met was used as substrate instead of L-Met. The immobilized SAM synthetase could be used to continuously synthesize SAM. The yield of SAM from one cycle was less than that from the anterior cycle. Figure 9 depicts the results from the continuous synthesis of SAM (4 cycles) catalyzed by the immobilized SAM synthetase.

Discussion

So far it is not practical to synthesize SAM in larger scale using enzymes because it is difficult to obtain sufficient SAM synthetase. We have achieved the expression, purification, and characterization of a soluble form of recombinant SAM synthetase from P. pastoris. The methylotrophic yeast secreted a high level of SAM synthetase (~200 mg/L). The SAM synthetase was secreted in a soluble form. The recombinant SAM synthetase was secreted in a soluble form, and the activity of the enzyme incubated in Tris-HCl buffer, pH 7.0 was defined as 100%.

Table 2. Effects of Some Ions on the Stability of the Recombinant SAM Synthetase

<table>
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<th>relative activity (%)</th>
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* The activity of the enzyme incubated in Tris-HCl buffer, pH 7.0 was defined as 100%.
E. coli expression systems for the production of heterologous thylotrophic yeast, SAM synthetase from eukaryotic genomes, whereas the other post-translational modifications limits the expression of the inability to correctly fold the foreign protein and perform 18 SAM2 encoded by the liver SAM synthetase was expressed in but it is difficult to isolate the enzymes from these sources. Rat SAM in small scale. For larger-scale synthesis, the enzyme from was also used to express the S. cere 21 E. coli stra...tion conditions. For these reasons, engineered P. pastoris may be further improved by enlarging the fermentation volume and optimizing the fermenta-
dation. In addition, the sequence of SAM2 gene has no N-linked glycosylation site, which can reduce the probability of over-
glycosylation. It is an advantage for secretory expression. P. pastoris expression systems, besides having the capability of producing large quantities of SAM2 SAM synthetase, also permit the recombinant protein to be secreted in soluble form. For P. pastoris expression systems, the levels of protein obtained from shaken flasks are generally 10-fold lower than can be achieved with fermentors as the cell density is lower and the extent of aeration is often more limited (22). Therefore, the yield of the recombinant SAM synthetase may be further improved by enlarging the fermentation volume and optimizing the fermentation conditions. For these reasons, engineered P. pastoris represents a very useful and cost-effective expression system for basic research and industrial production of SAM synthetase.

In S. cerevisiae, the SAM synthetases encoded by SAM1 and SAM2 consist of two and four identical subunits, respectively (23, 24), and this enzyme from other organisms is usually a tetramer or dimer (21, 25, 26). So deviations from Michaelis-Menten kinetics were observed for SAM synthetases from S. cerevisiae, human liver, and E. coli (23, 27, 28). The same phenomenon is also observed for the recombinant SAM synthetase, and the Hill coefficients of the synthetase were less than 1 (Figures 5 and 8). Therefore, the recombinant SAM synthetase should be confirmed to be an allosteric enzyme with negative regulation by its product. The apparent Kₘ values of the recombinant synthetase, both for l-Met and ATP, are higher than those of the endogenous S. cerevisiae SAM synthetase (for l-Met 0.14 ± 0.01 mM, for ATP 0.047 ± 0.003 mM) (23); the difference could presumably be due to the estimation at a different range of substrate concentrations.

Comparison between the endogenous S. cerevisiae SAM synthetase and the recombinant SAM synthetase reveals both similarities and differences. Both synthetases require an alkaline pH condition for optimum activity. The optimal pH value of the endogenous SAM synthetase is near pH 7.6 (29), while the value of the recombinant synthetase is pH 8.5. However, the product SAM is slowly decomposed in neutral or alkaline solution at room temperature (30). It is therefore necessary to acidify the reaction mixture with HClO₄ in order to stop the reaction and stabilize the product. In addition, both proteins are...
markedly sensitive to temperature. For example, when the endogenous SAM synthetase was immersed in a water bath for 13 min at 55 °C, 7% of the initial activity was retained (29), and when the recombinant synthetase was incubated at 50 °C for 2 h, only 3% of the enzyme activity was retained. GSH could prevent the endogenous SAM synthetase from thermal inactivation, whereas KCl could significantly protect the recombinant synthetase.

As the recombinant SAM synthetase is unstable at room temperature, the operation of the enzyme has to be curtailed to maintain full activity. Addition of a His6-tag at the N-terminal of SAM synthetase and adopting the IMAC methodology permitted the purification and immobilization of the enzyme in a single chromatographic step, which effectively shortens operating time. In the meantime, immobilization of SAM synthetase obviously improved the stability of the enzyme. The residual activity of the free enzyme after preincubation at 35 °C for 6 h was about 0% (Figure 6A), whereas the enzymatic activity of the immobilized enzyme was kept constant during the first cycle (Figure 9). It is very important in the industrial application. The immobilized enzyme may be desorbed from the Ni2+-IDA agarose during the cycle and between 2 cycles, further investigation is needed to solve this problem. However, it is easy to replace an old catalyst with a new one.

Although dl-Met could be used as one of substrates instead of L-Met, the apparent Km values for dl-Met was increased and the special activity of the recombinant synthetase was decreased. But, the price of dl-Met is much lower than that of L-Met; therefore, from the viewpoint of industrial production, use of dl-Met to produce SAM instead of L-Met could be a significant improvement in production process.

Acknowledgment

We extend special thanks to Dr. Dongyang Li for his support and valuable discussion.

References and Notes


