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Enzymatic Synthesis of *S*-Adenosyl-L-methionine on the Preparative Scale

Jeongho Park,[a] Junzhe Tai,[b] Charles A. Roessner[a] and A. Ian Scott[a,*]

[a]*Center for Biological NMR, Department of Chemistry, Texas A&M University, College Station, Texas 77843-3255, U.S.A.*

[b]*Department of Basic Courses Teaching, Yan Sian Agricultural College, Long Jing 133400, Ji Lin Province, China*

Abstract—The problems inherent in the enzymatic and chemical synthesis of *S*-adenosyl-L-methionine (SAM) led us to develop an efficient, simple method for the synthesis of large amounts of labeled SAM. Previously, we reported that the problem of product inhibition of *E. coli* SAM synthetase encoded by the *metK* gene was successfully overcome in the presence of sodium *p*-toluenesulfonate (*p*TsONa). This research has now been expanded to demonstrate that product inhibition of this enzyme can also be overcome by adding a high concentration of β-mercaptoethanol (βME), acetonitrile, or urea. In addition, a recombinant strain of *E. coli* has been constructed that expresses the yeast SAM synthetase encoded by the *sam2* gene. The yeast enzyme does not have the problem of product inhibition seen with the *E. coli* enzyme. Complete conversion of 10 mM methionine to SAM was achieved in incubations with either the recombinant yeast enzyme and 1 molar potassium ion or the *E. coli* enzyme in the presence of additives such as βME, acetonitrile, urea, or *p*TsONa. The recombinant yeast SAM synthetase was used to generate SAM in situ for use in the multi-enzymatic synthesis of precorrin 2. Copyright © 1996 Elsevier Science Ltd

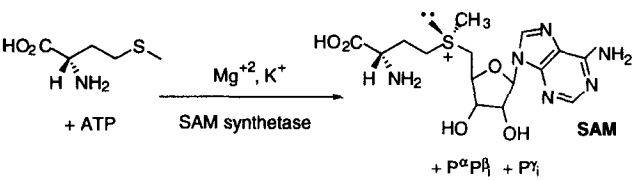
Introduction

S-Adenosyl-L-methionine (SAM) is the major methyl group transfer agent in biological systems. The methyl moiety of SAM is transferred to proteins, lipids, nucleic acids, vitamin B_{12}, etc. by SAM-dependent methyltransferases.[1] In addition to acting as a one carbon donor (the methyl group), SAM also acts as a three carbon donor and a five carbon donor. The three carbon aminopropyl group is incorporated into the polyamines, spermine, and spermidine,[2] and is also a precursor of the plant hormone, ethylene.[3] The five carbon adenosyl moiety is the precursor of the cyclopentenediol of the tRNA wobble base queuosine.[4] SAM also functions as a radical generator in pyruvate formate-lyase,[5] in lysine-2,3-aminomutase,[6] and in ribonucleotide reductase during anaerobic growth.[7] In the clinical field, SAM has potential importance as a therapeutic drug in the treatment of liver disease, as a potential cancer chemopreventive agent,[8] and as an anti-depressant.[9]

The requirement of substantial quantities of isotopically labeled SAM to investigate SAM-dependent enzyme reactions led us to survey existing methods for the preparation of SAM. In fermentation of *Saccharomyces cerevisiae*, SAM accumulates to about 50 μmol per gram of solid yeast,[10] which can be improved by the use of an ethionine-resistance gene[11] or by using other species, (e.g. *S. sake*[12]). However, the yields are still only 15–30%, based on methionine, which represents a major drawback since the labeled isotopomers of methionine are expensive. Chemical coupling reactions between adenosyl homocysteine and methyl group donating agents have also produced biologically active SAM but with poor diastereomeric excess.[13]

SAM has also been synthesized enzymatically[14] using SAM synthetase (EC 2.5.1.6), which acts via the direct attack of the methionine sulfur on the C5′ atom of ATP (Scheme 1). The enzyme also has triphosphatase activity which hydrolyzes the resulting triphosphate to pyrophosphate (PP_i) and phosphate (P_i) before SAM is released.[15]

Due to product inhibition at concentrations >1 mM,[13] the enzymatic synthesis of large amounts of labeled SAM using enzyme from the most readily available source, a recombinant strain of *E. coli*,[16] was not feasible prior to our report that the inhibition was successfully overcome in incubations using 10 mM methionine in the presence of 400 mM *p*TsONa.[17] Further investigations have been carried out to find more efficient additives. In the present study, we describe that the problem of product inhibition in *E. coli* SAM synthetase can also be successfully overcome in cell free incubations containing 10 mM methionine and 8% β-mercaptoethanol, 20% acetonitrile, or 2 M urea. To expand our repertoire of SAM-synthesizing



Scheme 1.

enzymes, we have constructed a recombinant strain of *E. coli* for the expression of yeast SAM synthetase (encoded by the *sam2* gene)[18] and demonstrate that this enzyme does not exhibit substrate inhibition. Since SAM may become the limiting factor in many syntheses requiring SAM as a co-substrate, a SAM generating system would be useful in many enzymatic synthetic schemes. Pursuant to this goal, we report that recombinant yeast SAM synthetase can be used to generate SAM in situ for use in the multi-enzyme synthesis of precorrin 2.

Results and Discussion

The effects of salts, reducing agents, organic solvents, and denaturing agents on *E. coli* SAM synthetase

As previously observed,[17] addition of 400 mM *p*TsONa to a 10 mM scale incubation completely overcame product inhibition of the enzyme (Fig. 1). The effect of *p*TsONa was concentration dependent and total conversion of substrate to SAM was observed within 5 h at concentrations of up to 30 mM methionine in the presence of 1.3 equiv of ATP and 2.6 equiv of $MgCl_2$.[17] The effect of several other inorganic salts was tested (data not shown). The ammonium salt of *p*-toluenesulfonic acid (*p*TsONH$_4$) also overcame product inhibition but a higher concentration was required than for the sodium salt. However, high concentrations of potassium chloride, potassium carbonate, ammonium acetate, or ammonium sulfate resulted in only slight reduction of product inhibition with up to ~40% conversion of 10 mM methionine to SAM in the presence of 200–600 mM KCl or ammonium acetate. None of these salts exhibited the dramatic effect of *p*TsONa in overcoming product inhibition.

Since reduced glutathione (GSH) was previously reported to stimulate the activity of rat liver SAM synthetase,[19] the effect of reducing agents on the activity of *E. coli* SAM synthetase was investigated (Fig. 1). Glutathione (50–500 mM) did not effect the activity, but higher concentrations of both dithiothreitol (DTT) and 2-mercaptoethanol (βME) overcame product inhibition (Fig. 1). Organic solvents have also frequently been used in enzyme catalyzed reactions.[20] Hoffman and coworkers reported the activation of rat liver SAM synthetase (β form) in the presence of 10% DMSO by a factor of 20–30 due to lowering of the $K_{m,Met}$ from 1.3 mM to 33 μM. However, the α form of the enzyme was not affected, the $K_{m,Met}$ (14 mM) value remaining unchanged.[21] The addition of DMSO, acetone, methanol, glycerol, or dimethylformamide to the standard reaction did not effect the enzyme. On the other hand, product inhibition was almost completely overcome with the addition of 20% acetonitrile (3.8 M) (Fig. 2).

Interestingly, addition of 2–4 M urea also completely overcame product inhibition, whereas equivalent concentrations of acetamide did not (Fig. 2). The enzyme has a very rigid conformation as it is only partially denatured in 8 M urea as detected by circular dichroism (Fig. 3), and retains 80% of its activity in 5.45 M urea (Fig. 2). The partial denaturation observed at 2 M urea (Fig. 3) does not effect the active site since the enzyme retains 100% activity.

The conversion (%) of 10 mM methionine to SAM using *E. coli* SAM synthetase in the presence of different additives is summarized in Table 1. Time course experiments in the presence of 3 M urea, 30% acetonitrile, 400 mM *p*TsONa, or 7% βME showed that conversion neared completion after 5 h.

The mechanism underlying the reversal of product inhibition of *E. coli* SAM synthetase by the above compounds is unknown but most likely is due to the prohibition of SAM binding to the enzyme. Although both SAM and pyrophosphate act as inhibitors of the enzyme, SAM is the most potent inhibitor because it is both a competitive inhibitor against ATP (K_i=0.01 mM) and a noncompetitive inhibitor against methionine (K_i=0.01 mM).[15f] Although pyrophosphate (PP_i),

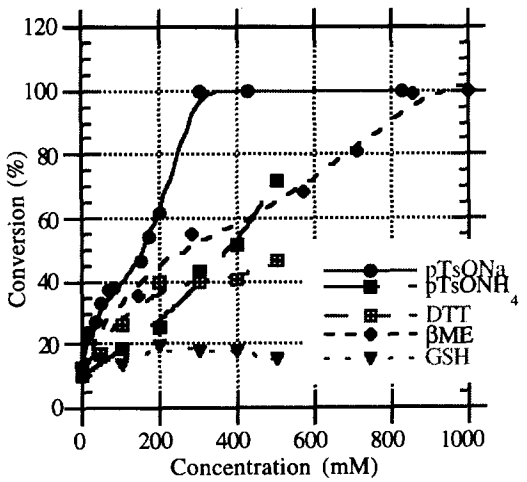


Figure 1. Concentration profiles of *p*TsONa, *p*TsONH$_4$, βME, DTT, and GSH in incubations using *E. coli* SAM synthetase in the presence of 10 mM methionine in buffer B at rt after 16 h.

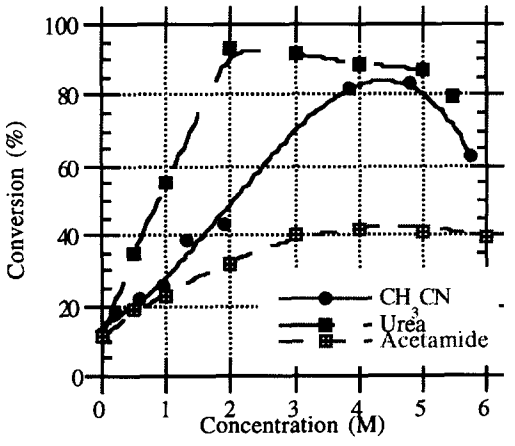


Figure 2. Concentration profiles of acetonitrile, urea, and acetamide in incubations using *E. coli* SAM synthetase in the presence of 10 mM methionine in buffer B (pH 8.0) at rt after 16 h.

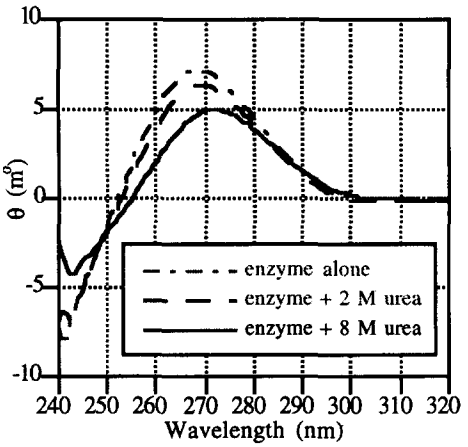


Figure 3. CD spectrum of *E. coli* SAM synthetase alone or in the presence of 2 or 8 M urea.

which is a noncompetitive inhibitor against ATP and methionine ($K_i = 0.4$ mM),[15f] is a weaker inhibitor than SAM, it is still strong enough to show an inhibitory effect in incubations containing >1 mM methionine. In the studies reported here, however, pyrophosphate is hydrolyzed to orthophosphate, which is a weak noncompetitive inhibitor against both ATP ($K_i = 8$ mM) and methionine ($K_i = 12$ mM),[15f] by endogenous pyrophosphatase existing in the cell free lysates.[22] Product inhibition by SAM may result from the formation of inactive SAM-bound-enzyme complexes (E-SAM or E-Met-SAM).[15] The active compounds such as *p*TsONa, βME, acetonitrile, and urea may prevent SAM binding enzyme by ion pair formation with the sulfonium cation, by formation of cation–π complexes,[23] or by forming a solvent cage around the inhibitor. *p*TsONa probably works better than the other tested salts for overcoming product inhibition because the tosyl anion can form not only larger, more stable ion pairs but also cation–π complexes with SAM and thus more efficiently eliminate SAM-enzyme interactions. Alternatively, some of the active compounds may induce a selective partial denaturation of the enzyme which prevents the binding of SAM without effecting catalysis.

Table 1. Conversion (%) of 10 mM methionine to SAM using *E. coli* SAM synthetase in the presence of different additives

Additives	Conversion (%)	Additives	Conversion (%)
400 mM *p*TsONa	100	10% DMSO	10
500 mM *p*TsONH$_4$	70	10% Acetone	10
600 mM NH$_4$OAc	40	10% DMF	20
1 M KCl	20	10% MeOH	15
1 M KHCO$_3$	40	10% Glycerol	10
600 mM (NH$_4$)$_2$SO$_4$	30	20% CH$_3$CN	82[a]
8% βME	100[a]	2.0 M Urea	95
500 mM DTT	40	4.0 M Acetamide	38
500 mM GSH	15		

[a]Isolated yield.

Expression and characterization of yeast SAM synthetase

Yeast SAM synthetase encoded by the *sam2*[18] gene was selected for expression since it was previously suggested that yeast SAM synthetase could be used to bypass the problem of product inhibition exhibited by the *E. coli* enzyme.[13,18] In contrast to the enzyme encoded by *sam1*, the specific activity of the enzyme encoded by *sam2* is increased in the presence of methionine.[18] A recombinant strain of *E. coli* [TB1(pUC18:*sam2*)] was constructed for the expression of the *sam2* gene. A new protein band (M_r 43000) corresponding to the reported subunit size of the gene product of *sam2* was demonstrated by SDS–PAGE analysis (Fig. 4).

Lysates of TB1(pUC18:*sam2*) efficiently converted 1.0 mM methionine and 1.3 mM ATP to SAM at pH 7.1 whereas control lysates of TB1(pUC18) had no detectable background SAM synthetase activity. The recombinant yeast SAM synthetase did not exhibit the same level of product inhibition as *E. coli* SAM synthetase at concentrations of above 1.0 mM. It was able to convert 10 mM methionine to SAM with ~40% efficiency under standard conditions (buffer A; 10 mM Met, 13 mM ATP, and 100 mM KCl in 100 mM Tris–HCl at pH 7.1) and ~80% efficiency in the presence of 25 mM ATP. It has a broad pH range (pH 6.5–7.5) with optimum activity at pH 7.0 in incubations using 10 mM methionine. However, the recombinant yeast SAM synthetase incubated with 30 mM ATP in buffer A required up to 20 h to achieve 80% conversion of 10 mM methionine to SAM compared to 5 h when using the *E. coli* enzyme in the presence of *p*TsONa.

This lower level of activity of the lysates containing yeast SAM synthetase may be due to several factors

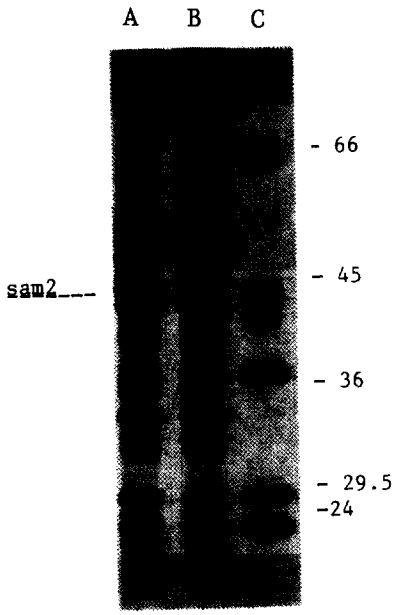


Figure 4. SDS–PAGE analysis of TB1 (pUC18:*sam2*) lysate. A: TB1 (pUC18:*sam2*); B: control (TB1 (pUC18)); C: molecular marker.

including a lower level of expression of the yeast enzyme and the larger K_m reported for the yeast enzyme (0.8 mM compared 0.08 mM for the *E. coli* enzyme).[18a] The requirement for more ATP under standard conditions may result from the slow decomposition of ATP during the long incubation.

In contrast to the *E. coli* enzyme, yeast SAM synthetase activity was not stimulated by the addition of *p*TsONa (Fig. 5). It has been reported that the activity of the enzyme isolated from yeast was increased up to fourfold by addition of 40 mM bicarbonate ion,[18a] so the effect of bicarbonate on the recombinant yeast SAM synthetase was investigated by adding potassium bicarbonate (10 mM–1 M). One molar potassium bicarbonate allowed complete conversion of 10 mM methionine within 10 h in the presence of 1.3 equiv of ATP and 2.6 equiv of $MgCl_2$ (Fig. 5). Potassium chloride showed the same stimulatory effect as potassium bicarbonate. Since equivalent concentrations of sodium chloride or ammonium bicarbonate did not increase the activity (Fig. 5), the observed stimulation apparently results not from ionic strength or an anion effect but from the unique characteristics of potassium ion. High K^+ concentration not only increases the activity (complete conversion is seen in ~10 h in the presence of 1 M potassium bicarbonate) but also eliminates the requirement for higher ATP concentrations, since complete conversion occurs with 13 mM ATP.

Markham has reported that each active subunit of homo-tetrameric *E. coli* SAM synthetase contains one monovalent cation (K^+), two divalent cations (Mg^{2+}), one methionine molecule, and one ATP molecule.[15] He has also shown that monovalent potassium ion not only has synergism for methionine and ATP to bind *E. coli* SAM synthetase but also increases the activity of polyphosphatase, which catalyzes the dissociation of enzyme-bound PPP_i to P_i and PP_i, resulting in faster release of SAM from the active site.[15] Even though the potassium effect on yeast SAM synthetase expressed in *E. coli* might be explained by these results, further investigation is needed to explain why such a high concentration of potassium ion (1 M) is needed to complete the reaction.

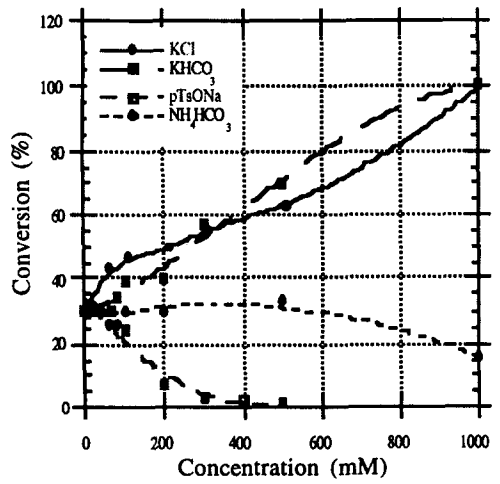


Figure 5. Concentration profiles of $KHCO_3$, KCl, *p*TsONa, and NH_4HCO_3 in incubations using yeast SAM synthetase in the presence of 10 mM methionine in buffer A at rt after 16 h.

Scaled-up production of SAM

The above studies have resulted in several possible methods for overcoming product inhibition of SAM synthetase making larger scale enzymatic synthesis of SAM feasible. Thus a 100 mL reaction containing *E. coli* SAM synthetase under standard conditions (10 mM methionine) in the presence of either 8% βME or 20% acetonitrile afforded 440 and 320 mg of SAM, respectively. Most of the resulting SAM (85–97%) was in the active (−) form and total yields of 80–100% were observed after Dowex 50-W ion exchange chromatography and recrystallization from water:acetone (Fig. 6).

SAM produced in this fashion could be easily isolated in a form that may be used directly in enzymatic reactions by simple recrystallization from acetone. The isolated SAM was successfully used to synthesize precorrin 3 using two methyltransferases in the presence of [4-^{13}C]-δ-aminolevulinic acid (ALA) via a previously described multi-enzyme incubation pathway.[24]

In situ generation of SAM for synthesis of precorrin 2

When yeast SAM synthetase, ATP, and methionine were incubated with the four enzymes required to synthesize precorrin 2 from ALA, approximately 75% conversion of uroporphyrinogen III to precorrin 2 was observed (Fig. 7B). This efficiency (based on the relative peak heights of uroporphyrinogen III and precorrin 2 in the 120 ppm region of the NMR spectrum) was comparable to that obtained when commercially purchased SAM was used in place of the

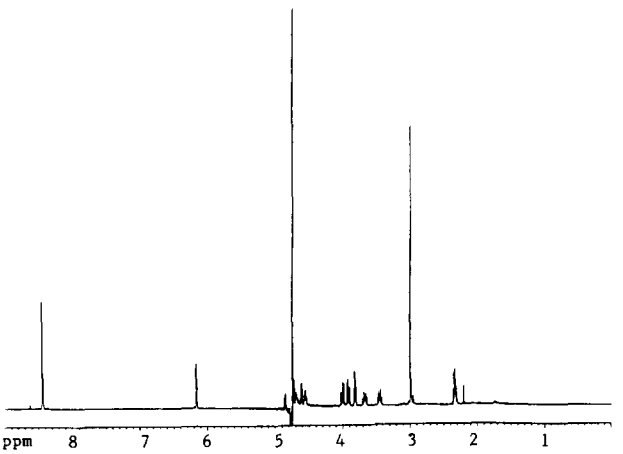


Figure 6. ^{1}H NMR (500 MHz) of SAM isolated from a preparative scale incubation in the presence of 8% βME.

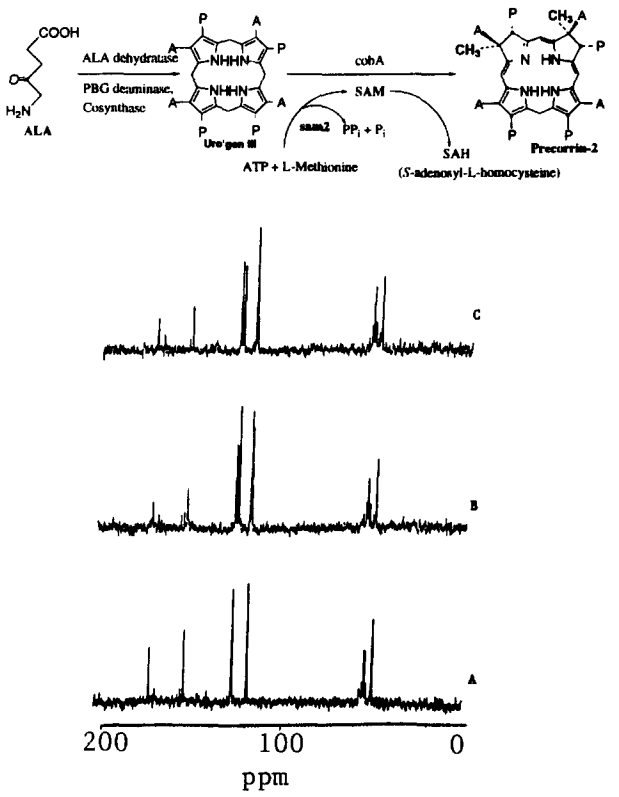


Figure 7. ^{13}C NMR (75 MHz) spectra of the multi-enzymatic synthesis of precorrin 2 from ALA. (A) Authentic spectrum of precorrin 2; (B) spectrum acquired using the in situ SAM generating system; (C) spectrum acquired using commercially purchased SAM.

in situ SAM generating system (Fig. 7C). Yeast SAM synthetase was chosen rather than the *E. coli* enzyme to avoid the problem of product inhibition.

Conclusions

The problem of product inhibition existing in *E. coli* SAM synthetase can be overcome by adding a high concentration of βME, DTT, acetonitrile, urea, or *p*TsONa, and preparative scale incubations in the presence of 8% βME or 20% acetonitrile afford SAM of higher purity and in higher yield than when it is isolated from whole yeast cell incubations. Complete conversion of methionine to SAM can be also achieved in incubations with recombinant yeast SAM synthetase by addition of 1 molar potassium ion. The enzymatic synthesis and purification procedures described above are ideal for the preparation of labeled SAM ($^{13}CH_3$, CD_3, C^3H_3, $CH_3{}^{35}S$, and $^{14}CH_3$). Incubation with crude lysate in the presence of 8% βME may be the most efficient process yet described for the preparative scale synthesis of SAM. The increasing demand for labeled SAM for the study of SAM-dependent enzyme reactions can now be met, for this study provides an efficient method for the synthesis of labeled SAM using *E. coli* SAM synthetase. This is the first report of the use of an in situ generating system to supply the SAM required in a multi-enzyme biosynthetic system.

Experimental

Chemicals and enzymes

[^{13}C-Me]Methionine was purchased from Cambridge Isotope Laboratories. ATP was purchased from United States Biochemical. *S. cerevisiae* genomic DNA and inorganic pyrophosphatase from *E. coli* were purchased from Sigma. Restriction enzymes and T4 DNA ligase were purchased from Bethesda Research Laboratory, New England Biolabs, or Boehringer–Mannheim Biochemicals and used as directed by the supplier. *Taq* polymerase was purchased from Promega. DNA was purified with a Gene Clean Kit purchased from *Bio101*. DEAE–Sephadex was purchased from Pharmacia. Other chemicals were of the highest purity obtainable from commercial sources. Protein concentrations were determined with a dye binding assay purchased from Bio-Rad.

Bacterial strains and plasmids

E. coli strain TB1 was obtained from Dr Tom Baldwin, Texas A&M University. Plasmid pUC18 (*lac* promoter) was purchased from Bethesda Research Laboratories. *E. coli* strain DM50 (pK8) bearing the *metK* gene in pBR322 was kindly provided by Dr G. Markham, Fox Chase Cancer Institute.

Molecular biology techniques

Molecular biology techniques were performed by using previously described procedures.[25] The polymerase chain reactions contained, in a final volume of 100 μL, 10 μL of 10× reaction buffer, 16 μL of 1.25 mM dNTP solution, 40 pmol of each primer, 2.5 units of *Taq* polymerase, 5 μL of 25 mM magnesium chloride, and 500 ng of *Saccharomyces cerevisiae* genomic DNA. One cycle of the polymerase chain reaction consisted of denaturation (1.5 min at 94 °C), annealing (2.5 min at 55 °C), polymerization (2.5 min at 72 °C), and cooling (0 min at 45 °C), and forty cycles were carried out.

Construction of recombinant strain of *E. coli* for the expression of yeast SAM synthetase

Plasmid pJH108 (pUC18:*sam2*) for the expression of yeast SAM synthetase was constructed using an expression cassette polymerase chain reaction technique to provide cloning sites and optimum translational signals for the expression of foreign proteins in *E. coli*.[26] The 5′ PCR primer contained a 5′ *BamH*I restriction site (GGATCC), a strong ribosome biding site (AGGAGG), an optimum spacer element (AATTTAAA), a translational initiation codon (ATG), and the codons for the first nine amino acids of SAM synthetase encoded by the *sam2* gene. The sequence of the 5′ primer was 5′>GGT ACC CGG GGA TCC AGG AGG AAT TTA AAA TGT CCA AGA GCA AAA CTT TCT TAT TTA CC<3′. The 3′ primer consisted of the codons for the last 10 amino acids of the *sam2* gene, a translational stop signal (TTA), and 3′ *Pst*I (CTGCAG) and *Xho*I (CTCGAG) restriction sites.

The sequence of 3′ primer was 5′>AAG CTT CTC GAG CTG CAG **TTA** AAA TTC CAA TTT CTT TGG TTT TTC CCA TGA<3′. The PCR product (1.18 kb) and plasmid pUC18 were digested with *BamHI* and *Pst*I, purified, ligated, and transformed into TB1.

Expression of *E. coli* and yeast SAM synthetase and preparation of cell lysates

For preparation of SAM synthetase, TB1 (pJH108) or DM50 (pK8) were grown overnight with good aeration at 37 °C in Luria–Bertani (LB) medium containing 50 μg/mL ampicillin or 30 μg/mL tetracycline, respectively. The cells (~30 g from 8 L) were collected by centrifugation and resuspended in 100 mL of 100 mM Tris–HCl buffer (pH 8.0) containing 1 mM EDTA. Lysozyme was added to 50 μg/mL and the suspension was incubated at room temperature for 30 min. Phenylmethylsulfonyl fluoride (PMSF) was added to a final concentration of 0.1 mM, the cells were lysed by sonication at full power (three times) in an ice bath maintaining the temperature below 15 °C, and the lysate was centrifuged for 20 min at 35000 rpm in a Beckman Ti 45 rotor to remove unlysed cells and cell debris. The supernatant was either used immediately or could be stored at −20 °C until needed.

NMR spectroscopy

^{1}H and ^{13}C NMR spectroscopy were performed either at 300 MHz on a Bruker WM-300 spectrometer equipped with an ASPECT 2000 data system or at 500 MHz on a Bruker AM-500 spectrometer equipped with an ASPECT 3000 data system with 45° pulse, 1 sec relaxation delay, and waltz decoupling. Spectra were taken at room temperature unless otherwise stated. Deuterium oxide (10–20%) was added as lock solvent.

Assay of SAM synthetase

SAM synthetase was assayed by monitoring the conversion of [^{13}C-Me]methionine (δ 15.2 ppm) to [^{13}C-Me]SAM (δ 24.6 ppm) by ^{13}C NMR spectroscopy as previously described.[17] Standard reaction conditions for yeast SAM synthetase (assay buffer A) were 4 mM ^{13}C-Met, 6 mM ^{12}C-Met, 100 mM Tris–HCl buffer, pH 7.1, 100 mM KCl, 26 mM $MgCl_2$, 13 mM ATP, 1 mM EDTA, 10% deuterium oxide, and 0.1% 2-mercaptoethanol (βME) in a volume of 2.7 mL. Standard reaction conditions for *E. coli* SAM synthetase (assay buffer B) were 100 mM Tris–HCl buffer, pH 8.0, 50 mM KCl, 26 mM $MgCl_2$, 13 mM ATP, 1 mM EDTA, 10% deuterium oxide, and 0.1% βME in a volume of 2.7 mL. The reactions were initiated by adding 300 μL of lysates containing ~5 mg of total protein.

Scaled-up preparations of SAM

Reactions with *E. coli* SAM synthetase were carried out for 5 h with vigorous stirring at room temperature in 90 mL of buffer B (10 mM Met) containing 8% βME and or 20% acetonitrile and 10 mL (170 mg of total protein) of a lysate derived from DM50 (pK8). The 100 mL reaction mixture was either acidified with 14 mL of perchloric acid or lyophilized and redissolved in 100 mL of 1 N HCl. Precipitated protein was removed by centrifugation and SAM was isolated from the supernatant by chromatography on Dowex-50W with elution with HCl as previously described.[27] Alternatively, the cation exchange column chromatography step could be eliminated if small molecules were removed from the lysate by dialysis against 50 mM Tris–HCl containing 1 mM EDTA, 0.1 mM βME, and 50 mM KCl prior to use in SAM-synthesizing reactions. The reaction mixture was adjusted to 1 M HCl and the precipitated protein removed by centrifugation. The supernatant was lyophilized and the residue dissolved in a minimal amount of water. The crude product was recrystallized by adding acetone and the white solid was washed three times with acetone. Sometimes a gummy product was obtained. In this case the mother liquor was decanted, the product washed with acetone, redissolved in water, and lyophilized. This procedure gives quantitative yields of SAM containing very small amounts of impurities (mainly Tris–HCl) as determined by ^{1}H NMR spectroscopy.

Coupled enzymatic synthesis of precorrin 2 from ALA and L-methionine

^{13}C-Labeled precorrin 2 was synthesized from [4-^{13}C]-δ-aminolevulinic acid (ALA) using a modification of a multi-enzyme synthetic protocol in which the last reaction is a SAM dependent bismethylation. Briefly, 10 mg of ALA was incubated as previously described[24] with ALA dehydratase, porphobilinogen deaminase, uroporphyrinogen III synthase, uroporphyrinogen III methyltransferase, and cofactor SAM in 100 mL of total volume. The SAM was either added directly to the reaction (20 mg) or was generated in situ by the addition of 15 mg of methionine (1 mM), 80 mg of ATP (1.3 mM), 53 μL of 4.9 M $MgCl_2$ (2.6 mM), 375 mg of KCl (50 mM), and 10 mL of a lysate (260 mg of total protein) derived from TB1 (pJH108) (7 g of wet cells harvested from 2 L culture) containing yeast SAM synthetase. The products were isolated and analyzed by NMR as previously described.[24]

Acknowledgments

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