S-Adenosylmethionine Synthetase from Escherichia coli*

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Adenosylmethionine (AdoMet) synthetase has been purified to homogeneity from Escherichia coli. For this purification, a strain of E. coli which was derepressed for AdoMet synthetase and which harbors a plasmid containing the structural gene for AdoMet synthetase was constructed. This strain produces 80-fold more AdoMet synthetase than a wild type E. coli. AdoMet synthetase has a molecular weight of 180,000 and is composed of four identical subunits. In addition to the synthetase reaction, the purified enzyme catalyzes a tripodophosphatase reaction that is stimulated by AdoMet. Both enzymatic activities require a divalent metal ion and are markedly stimulated by certain monovalent cations.

AdoMet synthesis also takes place if adenylyl-5'-yl imidodiphosphate (AMP-PNP) is substituted for ATP. The imidotriphosphate (PPNP) formed is not hydrolyzed, permitting dissociation of AdoMet formation from triphosphate (ATP) formed. Adenosylmethionine synthetase was 180,000 (determined by molecular weight). This paper reports a method for preparation of substantial quantities of homogeneous adenosylmethionine synthetase from E. coli. The amount of AdoMet synthetase in the bacteria was increased by construction of a strain of E. coli, which was derepressed for AdoMet synthetase and which harbored a plasmid containing the structural gene for this enzyme. The large amounts of homogeneous enzyme permitted detailed examination of the physical and kinetic properties of the E. coli adenosylmethionine synthetase. Particular emphasis has been placed on the role of the monovalent cation activator and the use of analogs of ATP for studies of the mechanism of the reaction.

RESULTS

Enzyme Preparation—A strain of E. coli which produces 80-fold more AdoMet synthetase was prepared by incorporating a plasmid containing the metK gene1 into a metJ host. Forty-three milligrams of homogeneous AdoMet synthetase with a specific activity of 2.2 pmol/min/mg were obtained from 50 g, wet weight, of cells (see Table I of the miniprint supplement).

Molecular Weight—The molecular weight of the native AdoMet synthetase was 180,000 (determined by gel filtration). Sodium dodecyl sulfate electrophoresis showed a single band of 43,000 molecular weight, indicating that the native enzyme is a tetramer of identical subunits. The amino acid composition of the enzyme is given in Table II in the miniprint.

Stoichiometry of the Reaction—ATP and methionine react to produce AdoMet and orthophosphate in equimolar amounts (see miniprint). In the presence of added pyrophosphatase, the amount of orthophosphate formed was 3 times the amount of AdoMet formed, indicating pyrophosphate as a third product. Studies with [α-32P]ATP demonstrated that the α-phosphoryl group of ATP is specifically incorporated into AdoMet.

This paper reports a method for preparation of substantial quantities of homogeneous adenosylmethionine synthetase from E. coli. The amount of AdoMet synthetase in the bacteria was increased by construction of a strain of E. coli, which was derepressed for AdoMet synthetase and which harbored a plasmid containing the structural gene for this enzyme. The large amounts of homogeneous enzyme permitted detailed examination of the physical and kinetic properties of the E. coli adenosylmethionine synthetase. Particular emphasis has been placed on the role of the monovalent cation activator and the use of analogs of ATP for studies of the mechanism of the reaction.

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The abbreviations used are: AdoMet, S-adenosyl-L-methionine; A(S)P4P, 5'-mercapto-5'-deoxy-ATP; A(S)P5P, 5'-mercapto-5'-deoxy-ADP; AMP-PNP, adenylyl-5'-yl imidophosphate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; ATPaS, adenosine 5'-O-(1-thiotriphosphate); ATPβS, adenosine 5'-O-(2-thiotriphosphate); ATPγS, adenosine 5'-O-(3-thiotriphosphate); 4',5'-dehydroadenosine, 9-(5-deoxy-β-D-rythropropent-4-enofuranosyl)-adenine; PPNP, imidotriphosphate (O,P-PO2-N-PO3).

Portions of this paper (including all of "Materials and Methods," part of "Results," Figs. 1 to 8, and Tables 1 to IX) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from The Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Maryland 20014. Request Document No. 86M-885, cite authors, and include a check or money order for $5.70 per set of photocopies. Full sized copies are included in the microfilm edition of the Journal that is available from Waverly Press.

1 metK is the structural gene for AdoMet synthetase and is located at 63 min (8-10) on the E. coli chromosomal map (11). A plasmid containing the metK gene was obtained from the Clarke-Carbon Collection (No. 27-37) (12) and was transferred to a metJ host. metJ is a regulatory gene located at 87 min on the E. coli chromosomal map. metJ mutants overproduce the enzymes involved in the biosynthesis of methionine and of adenosylmethionine (13).
into the pyrophosphate produced in the reaction (see miniprint). These results are consistent with the formulation presented for yeast AdoMet synthetase (14):

\[
\text{Ado} - P^+P^+ + L\text{-methionine} + \text{H}_2\text{O} \rightarrow \text{AdoMet} + PP_{\text{H}}^+ + P^+.
\]

**Metal Ion Requirements of Adenosylmethionine Synthetase**—Adenosylmethionine synthetase from yeast requires a divalent cation for activity and is greatly stimulated by certain monovalent cations (5). Fig. 1A in the miniprint supplement shows the effect of divalent metal ion concentration on the activity of the homogenous E. coli enzyme. The divalent cation requirement was satisfied by Mg\(^{2+}\) (relative maximal rate = 1.0), Mn\(^{2+}\) (0.9), Co\(^{2+}\) (1.1), Zn\(^{2+}\) (0.35), Cd\(^{2+}\) (0.09), Ni\(^{2+}\) (0.08), or Ca\(^{2+}\) (0.04) but not by Cu\(^{2+}\) (<0.0001). In the absence of added divalent metal ion, no AdoMet formation was detected (<0.01% of the rate with Mg\(^{2+}\)). With Mg\(^{2+}\), maximal activity was not obtained until the metal ion concentration was at least equal to the ATP concentration (measured at 3 mM, 10 mM, and 20 mM ATP). Since the \(K_m\) for ATP is 0.1 mM in the presence of excess Mg\(^{2+}\) (Table III, miniprint), the Mg\(^{2+}\) activation curves indicate either that free ATP is a potent inhibitor of the reaction or that free divalent cations (present in excess over those bound as MgATP) are required for full activity.

The requirement for a monovalent cation for significant activity was satisfied by K\(^+\), NH\(_4^+\), Tris\(^+\), Cs\(^+\), Li\(^+\), and Na\(^+\) but not by Tris\(^-\) or (CH\(_3\))\(_2\)N\(^+\) (Fig. 1B, miniprint). Table III (miniprint) reports the \(K_m\) values for ATP and methionine in the presence of various monovalent cations, the concentrations of monovalent cations required for half-maximal activation, and the relative \(V_{max}\) values with the different monovalent cations. The concentration of Tris\(^+\) required for half-maximal activation is substantially lower than the concentration of K\(^+\) required for half-maximal activation, in common with several other enzymes in which Tris\(^+\) replaces K\(^+\) as activator (15). Fig. 2 (miniprint) shows double reciprocal plots for ATP and methionine at several concentrations of K\(^+\). For both substrates, as the concentration of K\(^+\) increases, the apparent \(V_{max}\) increases, and the \(K_m\) decreases. The data indicate substantial synergism in the binding of ATP and K\(^+\) (approximately 4-fold) and in the binding of methionine and K\(^+\) (approximately 12-fold) (16). In the presence of poor activators, e.g. Li\(^+\) and Na\(^+\), the \(K_m\) values for ATP and methionine (cf. Table III) approach the values expected for zero K\(^+\) concentration (obtained by extrapolation of the data of Fig. 2 (miniprint)), showing that Na\(^+\) and Li\(^+\) do not appreciably synergize substrate binding.

**Tripolyphosphatase Activity of Purified AdoMet Synthetase**—The finding of an AdoMet-stimulated triphosphatase activity in preparations of yeast AdoMet synthetase supported the postulate that triphosphatase might be an intermediate in the AdoMet synthetase reaction (5, 17); consistent with this proposal, a small amount of triphosphatase was isolated from reaction mixtures containing partially purified yeast AdoMet synthetase (17).

Adenosylmethionine synthetase from E. coli co-purifies with an activity which hydrolyzes tripolyphosphate to orthophosphate and pyrophosphate. Evidence that the same enzyme catalyzes both the AdoMet synthetase reaction and the triphosphatase reaction is: (i) the AdoMet synthetase and triphosphatase activities are both present in the electrophoretically homogeneous enzyme; (ii) the ratio of these activities is the same in homogenous AdoMet synthetase purified from E. coli strains which do or do not harbor the plasmid containing the metK gene although the overall purification factor differs by more than 5-fold in the two cases (Table IVC, miniprint) and different purification techniques were used; (iii) identical rates of inactivation of the AdoMet synthetase and triphosphatase activities were obtained by heating and by treatment with various protein modification reagents (Fig. 3, miniprint, and miniprint text); and (iv) the triphosphatase is stimulated by AdoMet and monovalent cations (Table IVA, miniprint). The results with the homogeneous E. coli AdoMet synthetase are consistent with suggestions that a triphosphatase activity is an integral part of the synthetase from various sources (7, 17).

The hydrolytic activity is relatively specific for tripolyphosphate; no activity (<0.1% of the activity with tripolyphosphate) was obtained if pyrophosphate or the cyclic trimetaphosphate was used. ATP (5 mM) is hydrolyzed to ADP at a rate approximately 0.1% of the maximal rate of triphosphatase cleavage.

The triphosphatase activity requires a divalent cation for activity (Table IVB, miniprint) and was stimulated 10- to 20-fold by AdoMet plus monovalent cations (cf. Table IVA, miniprint). The relative ability of monovalent cations to activate the AdoMet-stimulated triphosphatase differs from the pattern observed with the overall AdoMet synthetase reaction, particularly in that Li\(^+\) is the best activator of the triphosphatase, while it is a poor activator of the synthetase reaction. The \(K_m\) for triphosphatase varies substantially with the monovalent cation activator; however, the concentration of adenosylmethionine required for half-maximal activation is 3 μM for all monovalent cations tested and at all concentrations of K\(^+\) tested (Fig. 4, miniprint). Maximal stimulation of the triphosphatase activity requires both AdoMet and a monovalent cation (Table IVA, miniprint), with only a small stimulation by either AdoMet or a monovalent cation alone. With each monovalent cation examined, the rate of triphosphatase hydrolysis in the presence of adenosylmethionine is fast enough for it to be an integral part of the overall AdoMet synthetase reaction (Table V, miniprint).

**Steady State Kinetic Mechanism of the Synthetase Reaction**—Double reciprocal plots for ATP at varying concentrations of methionine and for methionine at varying concentrations of ATP intersect to the left of the 1/\(V\) axis (Fig. 5, A and B, miniprint). Therefore, the reaction proceeds by a sequential mechanism where no products are released until both substrates have been bound (16).

Inhibition experiments (Table VI, miniprint) show that AdoMet, AMP-PNP, and tripolyphosphate compete with ATP and are noncompetitive with methionine. S-Carbamylcysteine, a reported inhibitor of AdoMet synthetase (18), is competitive with respect to methionine and noncompetitive with respect to ATP. Pyrophosphate and orthophosphate are both noncompetitive inhibitors for both ATP and methionine. The inhibition patterns are consistent with random binding of ATP and methionine and ordered product release, pyrophosphate and orthophosphate dissociating before AdoMet. A more detailed discussion of the kinetic mechanism is given in the miniprint section.

**Selenomethionine**—Selenomethionine reacts more rapidly than methionine in the synthetase reaction (Table V, miniprint), as has been reported for AdoMet synthetase from yeast (19). The activity of selenomethionine with various monovalent cations is given in Table VII (miniprint) as well as Table V (miniprint). The \(K_m\) values for selenomethionine in the presence of good activators (K\(^+\) and NH\(_4^+\)) are significantly lower than the \(K_m\) values in the presence of weaker activators (Na\(^+\) and Li\(^+\)), paralleling the results with methionine (Table III, miniprint). Table V (miniprint) presents the effects of different monovalent cations on the synthetase reactions with methionine and selenomethionine and on the AdoMet-stimulated adenosylmethionine synthetase from yeast. The difference of these activities is the same in homogenous AdoMet synthetase purified from E. coli strains which do or do not harbor the plasmid containing the metK gene although the overall purification factor differs by more than 5-fold in the two cases (Table IVC, miniprint) and different purification techniques were used.
lated triphosphatase reaction. In the presence of K⁺, the rate of the AdoMet-stimulated triphosphatase reaction is equal to the rate of the synthetase reaction with selenomethionine, while with other monovalent cations the triphosphatase rate is faster than the synthetase rate.

**Nucleotide Analogs—** Table VIII (miniprint) reports the results obtained with various nucleotides as substrates for AdoMet synthetase. 3'-Deoxy-ATP is a good substrate for the synthetase reaction, showing that the hydroxyl group at the 3' position of the ribose is not intimately involved in the reaction mechanism. The fluorescent ATP analog, formycin triphosphate (20), is also a good substrate for the AdoMet synthetase reaction.

Separation of Adenosylmethionine Formation from Triphosphatase Cleavage with Adenylyl Imidodiphosphate as Substrate—AMP-PNP is a substrate for AdoMet synthetase with a maximal steady-state rate of 0.04% of the maximal rate with ATP. In the reaction with AMP-PNP, AdoMet is formed (Fig. 1), but no orthophosphate or pyrophosphate is detected (<2% of the amount of AdoMet formed), indicating production of imidotriphosphate as the second product.

AMP-PNP + L-methionine → AdoMet + PPNP

The low steady-state rate prompted investigation of the presteady-state kinetics of the reaction (Fig. 1). An initial burst of AdoMet formation occurs immediately after the reaction is initiated, followed by a slower rate of AdoMet production. The same type of curve is obtained whether the reaction is initiated by addition of enzyme or by addition of substrates (Fig. 20). The burst height is proportional to the enzyme concentration but does not change when the concentration of substrates is increased to twice the concentrations used in the reaction for 1.9 nmol of enzyme subunits. Aliquots were removed, at the indicated times mixed with a 50-fold excess of EDTA to stop the production of AdoMet containing the diluted isotope had stopped, while the burst of AdoMet containing label from the undiluted substrate continued (Fig. 7, miniprint). Thus, within 10 s enzyme-bound substrate had completely equilibrated with the isotopically diluted pool of free substrate. Therefore, the reaction was allowed to proceed for 8 h in order to determine whether reversal of the reaction to form dissociable substrates occurred. No change (<2%) in the radioactivity in AdoMet from the diluted isotope occurred even if an 80-fold excess of cold methionine had been added. The rate of the reverse reaction from enzyme-bound AdoMet and imidotriphosphate to a dissociable enzyme-substrate complex was estimated as <2 × 10⁻⁵ of the rate of formation of AdoMet from AMP-PNP and methionine.

Evidence that the slow reaction following the initial burst of AdoMet formation results from slow product release...
from the complex (E-AdoMet-Mg\textsuperscript{2+}-K\textsuperscript{+}-imidotriphosphate) was obtained by gel filtration experiments. The enzyme was incubated with Mg\textsuperscript{2+}, K\textsuperscript{+}, [8-\textsuperscript{3}H]AMP-PNP, and [methyl-\textsuperscript{14}C]methionine (substrate concentrations as given in Fig. 1) and passed through a Sephadex G-25 column which had been equilibrated with 0.1 M Tris-chloride, pH 8.3, 0.1 M KCl, and 5 mM MgCl\textsubscript{2}. Upon elution with the same buffer, both H\textsuperscript{3} and C\textsuperscript{14} co-chromatographed with the enzyme activity, with a constant ratio of H\textsuperscript{3} to C\textsuperscript{14} to enzyme activity across the peak (Fig. 2A). The radioactivity indicated a 1 to 1 correspondence of H\textsuperscript{3} to C\textsuperscript{14}, and greater than 90% of the radioactivity from both isotopes was recovered as AdoMet after a standard assay.

When [\alpha-\textsuperscript{32}P]AMP-PNP and [methyl-\textsuperscript{14}C]methionine were present in the incubation mixture, H\textsuperscript{3} and \textsuperscript{32}P chromatographed in a 1 to 1 ratio with the enzyme peak. To identify the \textsuperscript{32}P-containing compound, protein was precipitated with cold 10% trichloroacetic acid followed by centrifugation. The supernatant was spotted on polyethyleneimine cellulose thin layer plates with internal standards of AMP-PNP, pyrophosphate, orthophosphate, imidodiphosphate, and triphosphate. Plates were developed with 1.5 mM L-Tricthopentane, and the spots cut out and counted. The \textsuperscript{32}P chromatographed with triphosphate, consistent with the presence of [\textsuperscript{32}P]imidotriphosphate. To show the requirements for binding, parallel incubation mixtures were chromatographed in buffer which either lacked KCl or contained 50 mM EDTA in place of MgCl\textsubscript{2}; in neither case did radioactivity co-chromatograph with the enzyme activity. No radioactivity migrated with the enzyme if [8,5'-\textsuperscript{3}H]ATP was substituted for AMP-PNP, if metionine was omitted from the reaction mixture (with either [\alpha-\textsuperscript{32}P]AMP-PNP or [\alpha-\textsuperscript{32}P]PAMP-PNP present), or if after the incubation the enzyme was denatured (e.g. by addition of an equal volume of 10% sodium dodecyl sulfate followed by chromatography in 1% detergent). Thus, the radioactivity co-chromatographing with the enzyme represents AdoMet and imidotriphosphate bound to the enzyme, presumably in conjunction with Mg\textsuperscript{2+} and K\textsuperscript{+}. The complex was not covalently bound to the enzyme since it was dissociated from the enzyme by sodium dodecyl sulfate and no acid-insoluble counts were found when the incubation mixture was treated with cold 10% trichloroacetic acid.

To determine the number of monovalent cations bound per active site, a gel filtration experiment was performed in which \textsuperscript{30}TI\textsuperscript{+} was the activating monovalent cation, and the column was equilibrated with buffer containing 0.4 mM unlabeled TI\textsuperscript{+} instead of K\textsuperscript{+}. Enzyme was incubated with \textsuperscript{30}TI\textsuperscript{+}, [8-\textsuperscript{3}H]AMP-PNP, metionine, and Mg\textsuperscript{2+} and then chromatographed. Upon elution from the column, H\textsuperscript{3} but not \textsuperscript{30}TI\textsuperscript{+} eluted with the enzyme peak. In a second experiment, the enzyme was incubated with \textsuperscript{30}TI\textsuperscript{+}, [8-\textsuperscript{3}H]AMP-PNP, metionine, and Mg\textsuperscript{2+} and then chromatographed on a column containing 0.5 mM \textsuperscript{30}TI\textsuperscript{+} (Fig. 2B). A peak of \textsuperscript{30}TI\textsuperscript{+} chromatographed with the enzyme-bound H\textsuperscript{3} and the ratio of \textsuperscript{30}TI\textsuperscript{+} to H\textsuperscript{3} indicated approximately one TI\textsuperscript{+} bound per bound AdoMet. Equilibrium dialysis gave a similar result with approximately one TI\textsuperscript{+} bound per subunit with a dissociation constant of 0.16 mM (Fig. 8, miniprint). Thus, although a single monovalent cation binds to the complex (E-AdoMet-Mg\textsuperscript{2+}-imidotriphosphate) and is required for binding of AdoMet during gel filtration, the monovalent cation itself exchanges with bulk cations during chromatography.

**ATP\textsubscript{PS} Diastereoisomers as Substrates of Adenosylmethionine Synthetase**—Derivatives of ATP in which one of the nonbridge oxygens of the \alpha- or \beta-phosphoryl group is replaced by sulfur exist as pairs of diastereoisomers (22). Comparison of these diastereoisomers as substrates for several enzymes has elucidated the absolute stereochemistry of the metal-ATP binding sites (22). With Mg\textsuperscript{2+} (which has a strong preference for coordination to oxygen rather than sulfur ligands) as the activating divalent metal ion, AdoMet synthetase utilized the A isomer of ATP\textsubscript{PS} (as defined by Eckstein (22)) S absolute configuration at the \beta-phosphoryl group (23)) as substrate. The A isomer of ATP\textsubscript{PS} had a K\textsubscript{m} of 0.05 mM and a V\textsubscript{max} equal to 25% of the V\textsubscript{max} of ATP (Table VIII, miniprint). At a concentration of 1 mM, the B diastereoisomer (R absolute configuration at the \beta-phosphoryl group) had no detectable activity (<0.1% the rate of ATP); a K\textsubscript{m} of 0.5 mM was obtained in a Dixon plot.

When Mn\textsuperscript{2+} or Co\textsuperscript{2+}, which coordinate either oxygen or sulfur ligands, replaced Mg\textsuperscript{2+} as the activating divalent cation, AdoMet synthetase utilized both isomers of ATP\textsubscript{PS} as substrates. In the presence of Mn\textsuperscript{2+}, the relative V\textsubscript{max} values for ATP, ATP\textsubscript{PS}(A), and ATP\textsubscript{PS}(B) were 1.0, 0.5, and 0.3, while the respective K\textsubscript{m} values were 0.04 mM, 0.1 mM, and 0.4 mM. With Co\textsuperscript{2+} as the activating metal ion, the relative V\textsubscript{max} values for ATP, ATP\textsubscript{PS}(A), and ATP\textsubscript{PS}(B) were 1.0, 0.14, and 0.04, while the K\textsubscript{m} values were 0.4 mM, 0.3 mM, and 0.2 mM, respectively. The decrease in selectivity toward the diaster-
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eoisomers of ATPβS when metal ions which will coordinate to either oxygen or sulfur ligands replace Mg²⁺ indicates that during the AdoMet synthetase reaction a divalent cation is bound to the β-phosphoryl group of ATPβS and indicates that on the enzyme Mg²⁺ coordinates to the pro-S oxygen atom on the β-phosphoryl group of ATP (23). Diesteroisomers of ATPαS as Substrates of AdoMet Synthetase—The α-sulfur-substituted analogs of ATP were also tested as substrates of AdoMet synthetase. At either 0.5 mM or 2 mM concentration, neither isomer served as substrate (<0.01% of ATP) with Mg²⁺, Mn²⁺, or Co²⁺ as divalent cation. In the presence of Mg²⁺, both isomers gave Kᵢ values of 0.8 mM in a Dixon plot.

In view of recent reports that several enzymes catalyze positional interchange of oxygen atoms of the triplyphosphoryl group of ATP in the absence of an overall reaction (24) and that certain enzymes isomerize thio-phosphoryl derivatives of ATP (25), it was of interest to examine whether ATPαS might be converted to either ATPβS or the C₅S-P, ADPαS; after a phosphatase digestion, diphosphate. The rate of hydrolysis was independent of the presence of methionine but was inhibited by adenosylmethionine. Neither compound showed any activity (<0.01% of ATP) with Mg²⁺, Mn²⁺, or Co²⁺ as divalent cation.

The kinetic experiments presented here suggest that the sequence of events in the AdoMet synthetase reaction involves (i) random addition of methionine and MgATP; (ii) formation of AdoMet and ATPαS which is detectable (<0.01% of ATP) (Table VIII, miniprint). A(S)PPP was, however, rapidly and quantitatively hydrolyzed to the corresponding diphosphate. The rate of hydrolysis was independent of the presence of methionine but was inhibited by adenosylmethionine. The hydrolytic activity was abolished by incubation of the enzyme with 1 mM N-ethylmaleimide which inactivates AdoMet synthetase but was unaffected by incubation with 10 mM iodoacetamide which does not inhibit AdoMet synthetase (cf. miniprint). The ratio of AdoMet synthetase to A(S)PPPase activity was identical in homogenous enzyme prepared from E. coli and yeast enzymes (27).

DISCUSSION

The current work describes the preparation of substantial quantities of homogeneous AdoMet synthetase from E. coli and investigations of the mechanism of the reaction. These studies were facilitated by the use of a metJ E. coli strain containing a metK plasmid, since this strain had an 80-fold greater amount of enzyme than a wild type E. coli.

The only other AdoMet synthetases which have been purified to homogeneity are the yeast isoenzymes (7). AdoMet synthetases from E. coli and yeast catalyze the same overall reaction, both enzymes require divalent cations and monovalent cations for full activity, and both enzymes have a triplyphosphatase activity which is stimulated by adenosylmethionine. However, the AdoMet synthetases from yeast and E. coli differ markedly in molecular weight and subunit composition. The two yeast enzymes have a molecular weight of 115,000, with two nonidentical subunits (55,000 and 60,000 molecular weight) (7). The E. coli enzyme has a molecular weight of 180,000, with four apparently identical subunits. The E. coli and yeast enzymes differ strikingly in their kinetic behavior. No cooperativity is seen in the kinetics of the E. coli enzyme, as contrasted to the negative cooperativity described for the yeast enzymes (27). As opposed to the yeast enzymes, neither a lag phase in the rate of AdoMet formation nor AdoMet stimulation of its own rate of formation has been observed with the E. coli AdoMet synthetase (27). The relatively simple kinetic behavior of the E. coli enzyme has allowed kinetic studies with fewer ambiguities than was possible with the yeast enzyme.

The kinetic experiments presented here suggest that the sequence of events in the AdoMet synthetase reaction involves (i) random addition of methionine and MgATP; (ii) formation of AdoMet and triplyphosphatase; (iii) oriented cleavage of triplyphosphate to yield orthophosphate and pyrophosphate; and finally (iv) product release with orthophosphate and pyrophosphate dissociating before AdoMet. Comparison of the maximal rate of the AdoMet synthetase reaction and the AdoMet-stimulated triplyphosphatase reaction indicates that the predominantly rate-determining step in the overall reaction precedes the triplyphosphatase reaction. The ability of the diasteroisomers of ATPβS to serve as substrates for AdoMet formation depends on which divalent metal ion is present. The results indicate that a divalent cation is bound to the free triplyphosphate chain and that the enzyme Mg²⁺ is bound to the pro-S oxygen on the β-phosphoryl group of ATPβS.

Adenosylmethionine synthetase requires a monovalent cation, e.g. K⁺, for maximal activity. Ti⁺ is an effective activator for the reaction between the ribose and the triplyphosphate chain enables the hydrolytic activity of AdoMet synthetase to act efficiently on a nucleoside triphosphate. The A(S)PPPase reaction can be monitored continuously at 340 nm by coupling A(S)PP production to NADH oxidation with pyruvate kinase and lactate dehydrogenase (Table IX, miniprint).
of AdoMet synthetase and acts at lower concentrations than the other monovalent cations tested. Through use of $^{32}$Ti, we have established that the enzyme has a single binding site per subunit for a monovalent cation. In the overall reaction, a good activator, such as K', not only increases the maximal velocity of the reaction but substantially increases the affinity of both MgATP and methionine for the enzyme. While weak activators, such as Li" and Na", can produce 50% of the maximal activity obtained with K', the poorer activators do not effectively potentiate substrate binding so that much higher concentrations of substrates are required for maximal activity. The influence of the monovalent cation on the rate of the reaction is shown by comparison of the reactions with methionine and selenomethionine as substrates. In the presence of K', selenomethionine is a 2-fold better substrate than methionine, and the rate of the overall reaction with selenomethionine is equal to the rate of the triphosphophatase. With poorer activators, however, selenomethionine is closer to methionine in activity, and the rate of the overall reaction with both amino acids is less than the rate of the AdoMet-stimulated triphosphophatase.

The involvement of enzyme-bound triphosphophosphate as an intermediate in the AdoMet synthetase reaction, first suggested by Mudd (17), has been of particular interest in studies of the mechanism of the enzyme (7, 17, 19, 27). The hydrolytic activity of the E. coli AdoMet synthetase is relatively specific for triphosphophosphate, with hydrolysis of ATP occurring at only 0.1% the rate of triphosphophosphate cleavage. The hydrolysis of triphosphophosphate is stimulated by AdoMet and monovalent cations. The stimulation is most effective when both types of activators are present together. The ATP analog A(S)PP is hydrolyzed to the corresponding diphosphate (A(S)PP) at a rate which is only attained with triphosphophosphate in the presence of AdoMet.

Use of the ATP analog adenyl imidodiphosphate permits the dissociation of AdoMet formation from the subsequent hydrolytic reaction which occurs with ATP as substrate. When AMP-PNP replaces ATP in the AdoMet synthetase reaction, AdoMet is rapidly formed, but the other product of the reaction, imidotriphosphate, is not hydrolyzed. Product release from the 'E-Mg"-AdoMet-imidotriphosphate-K' complex occurs 2500-fold more slowly than the turnover rate with ATP. The burst of AdoMet formation is accompanied by the concentration of enzyme subunits, indicating that the equilibrium of the reaction on the enzyme strongly favors formation of AdoMet and imidotriphosphate. The stoichiometry of one AdoMet formed per subunit further indicates that the E. coli AdoMet synthetase is composed of functionally identical subunits. The burst rate decreases when K' is replaced by Li"; however, with either of these monovalent cations, the rate of product formation is not significantly altered when selenomethionine replaces methionine. The failure of the rate of product formation to increase when selenomethionine replaces methionine in the reaction with AMP-PNP is in contrast to the reaction with ATP as substrate and suggests that with AMP-PNP as substrate the rate-determining step for the burst reaction may be a step whose rate depends on the monovalent cation present, but which precedes AdoMet formation. The possibility does remain that with AMP-PNP as substrate the reaction is unable to proceed at a faster rate than obtained with methionine, and, thus, the rate of the burst of product formation could reflect the actual AdoMet-forming reaction. Despite tests of many compounds as potential intermediates (28), no evidence for an intermediate between substrates and AdoMet has yet been obtained.

The inability to obtain significant reversal of the AdoMet synthetase reaction has been previously noted, and the rate of ATP formation from free AdoMet and triphosphopshate was estimated as 5 x 10^-7 the rate of the forward reaction of yeast AdoMet synthetase (29). Pulse-chase experiments show that the rate of the reverse reaction from enzyme-bound AdoMet and imidotriphosphate is <2 x 10^-4 the rate of the forward reaction, and, thus, the essentially irreversible step occurs after reversible substrate binding but before product release and may be the formation of AdoMet. In light of the results with AMP-PNP, which yields a nonhydrolyzable analog of triphosphophosphate as a product, the triphosphophatase reaction which normally occurs during the catalytic cycle of AdoMet synthetase may be rationalized as acting to allow the enzyme to turn over efficiently by converting the tightly bound product, triphosphophosphate, to the more weakly bound orthophosphate and pyrophosphate.

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5'-Deoxyadenosylmethionine Synthetase from Escherichia coli

George D. Marsden, Edmund W. Defax, Celia White Tabor, and Herbert Tabor

This section contains Materials and methods, references, figures, and tables.

**Materials and Methods**

ATP, methionine, adenosylmethionine, dithiothreitol, glycine, and sodium tripolyphosphate were from Sigma Chemical Co. (St. Louis, Mo.). Sodium pyrophosphate, adenosyl methionine, and adenosine-5'-triphosphate were from Calbiochem-Behring Corp. (La Jolla, Calif.). Sodium butyrate and sodium orthosilicate were from BDH Chemical Co. (London). 8-Bromo-ATP was a gift from Dr. W. W. Knowles, The Rockefeller University. 3'-Deoxy-ATP was synthesized from 5'-iodo,5'-deoxy-adenosine and was a generous gift of Dr. B. O. M. Alton, University of California, San Diego. Cyclic AMP was a gift from Dr. G. H. Featherstone, University of Connecticut. 33-P orthophosphate was from New England Nuclear Corp. (Boston, Mass.). 32-Porthophosphate was from Schwarz BioResearch Inc. (Orangeburg, N.Y.). All other reagents were of reagent grade and were obtained from commercial sources.

**Supplementary Material**

**Table I**

| Buffer | Components |
|--------|------------|
| Buffer A | NaCl, 1 M; MgCl₂, 5 mM; Tris-chloride, pH 7.5 |
| Buffer B | NaCl, 0.1 M; MgCl₂, 5 mM; Tris-chloride, pH 7.5 |

**Figure 1**

A gel electrophoresis pattern of the dithiothreitol-disulfide exchange reaction of adenosylmethionine synthetase. The reaction mixture contained 0.1 M Tris-chloride, pH 7.5, 5 mM MgCl₂, 10 mM NaCl, 10 mM dithiothreitol, 10 mM ATP, and 30% (v/v) glycerol. The reaction was started by the addition of adenosine-5'-triphosphate, and the reaction was monitored by the decrease in absorbance at 250 nm. The reaction was stopped after 10 minutes by the addition of 25% (v/v) glycerol. The mixture was then centrifuged, and the supernatant was assayed for adenosylmethionine synthetase activity. The reaction was carried out at 0.5 mg of enzyme per ml. The reaction was monitored by the decrease in absorbance at 250 nm. The reaction was stopped after 10 minutes by the addition of 25% (v/v) glycerol. The mixture was then centrifuged, and the supernatant was assayed for adenosylmethionine synthetase activity. The reaction was carried out at 0.5 mg of enzyme per ml.

**Figure 2**

A gel electrophoresis pattern of the dithiothreitol-disulfide exchange reaction of adenosylmethionine synthetase. The reaction mixture contained 0.1 M Tris-chloride, pH 7.5, 5 mM MgCl₂, 10 mM NaCl, 10 mM dithiothreitol, 10 mM ATP, and 30% (v/v) glycerol. The reaction was started by the addition of adenosine-5'-triphosphate, and the reaction was monitored by the decrease in absorbance at 250 nm. The reaction was stopped after 10 minutes by the addition of 25% (v/v) glycerol. The mixture was then centrifuged, and the supernatant was assayed for adenosylmethionine synthetase activity. The reaction was carried out at 0.5 mg of enzyme per ml. The reaction was monitored by the decrease in absorbance at 250 nm. The reaction was stopped after 10 minutes by the addition of 25% (v/v) glycerol. The mixture was then centrifuged, and the supernatant was assayed for adenosylmethionine synthetase activity. The reaction was carried out at 0.5 mg of enzyme per ml.

**Figure 3**

A gel electrophoresis pattern of the dithiothreitol-disulfide exchange reaction of adenosylmethionine synthetase. The reaction mixture contained 0.1 M Tris-chloride, pH 7.5, 5 mM MgCl₂, 10 mM NaCl, 10 mM dithiothreitol, 10 mM ATP, and 30% (v/v) glycerol. The reaction was started by the addition of adenosine-5'-triphosphate, and the reaction was monitored by the decrease in absorbance at 250 nm. The reaction was stopped after 10 minutes by the addition of 25% (v/v) glycerol. The mixture was then centrifuged, and the supernatant was assayed for adenosylmethionine synthetase activity. The reaction was carried out at 0.5 mg of enzyme per ml. The reaction was monitored by the decrease in absorbance at 250 nm. The reaction was stopped after 10 minutes by the addition of 25% (v/v) glycerol. The mixture was then centrifuged, and the supernatant was assayed for adenosylmethionine synthetase activity. The reaction was carried out at 0.5 mg of enzyme per ml.

**Figure 4**

A gel electrophoresis pattern of the dithiothreitol-disulfide exchange reaction of adenosylmethionine synthetase. The reaction mixture contained 0.1 M Tris-chloride, pH 7.5, 5 mM MgCl₂, 10 mM NaCl, 10 mM dithiothreitol, 10 mM ATP, and 30% (v/v) glycerol. The reaction was started by the addition of adenosine-5'-triphosphate, and the reaction was monitored by the decrease in absorbance at 250 nm. The reaction was stopped after 10 minutes by the addition of 25% (v/v) glycerol. The mixture was then centrifuged, and the supernatant was assayed for adenosylmethionine synthetase activity. The reaction was carried out at 0.5 mg of enzyme per ml. The reaction was monitored by the decrease in absorbance at 250 nm. The reaction was stopped after 10 minutes by the addition of 25% (v/v) glycerol. The mixture was then centrifuged, and the supernatant was assayed for adenosylmethionine synthetase activity. The reaction was carried out at 0.5 mg of enzyme per ml.

**Figure 5**

A gel electrophoresis pattern of the dithiothreitol-disulfide exchange reaction of adenosylmethionine synthetase. The reaction mixture contained 0.1 M Tris-chloride, pH 7.5, 5 mM MgCl₂, 10 mM NaCl, 10 mM dithiothreitol, 10 mM ATP, and 30% (v/v) glycerol. The reaction was started by the addition of adenosine-5'-triphosphate, and the reaction was monitored by the decrease in absorbance at 250 nm. The reaction was stopped after 10 minutes by the addition of 25% (v/v) glycerol. The mixture was then centrifuged, and the supernatant was assayed for adenosylmethionine synthetase activity. The reaction was carried out at 0.5 mg of enzyme per ml. The reaction was monitored by the decrease in absorbance at 250 nm. The reaction was stopped after 10 minutes by the addition of 25% (v/v) glycerol. The mixture was then centrifuged, and the supernatant was assayed for adenosylmethionine synthetase activity. The reaction was carried out at 0.5 mg of enzyme per ml.

**Figure 6**

A gel electrophoresis pattern of the dithiothreitol-disulfide exchange reaction of adenosylmethionine synthetase. The reaction mixture contained 0.1 M Tris-chloride, pH 7.5, 5 mM MgCl₂, 10 mM NaCl, 10 mM dithiothreitol, 10 mM ATP, and 30% (v/v) glycerol. The reaction was started by the addition of adenosine-5'-triphosphate, and the reaction was monitored by the decrease in absorbance at 250 nm. The reaction was stopped after 10 minutes by the addition of 25% (v/v) glycerol. The mixture was then centrifuged, and the supernatant was assayed for adenosylmethionine synthetase activity. The reaction was carried out at 0.5 mg of enzyme per ml. The reaction was monitored by the decrease in absorbance at 250 nm. The reaction was stopped after 10 minutes by the addition of 25% (v/v) glycerol. The mixture was then centrifuged, and the supernatant was assayed for adenosylmethionine synthetase activity. The reaction was carried out at 0.5 mg of enzyme per ml.

**Figure 7**

A gel electrophoresis pattern of the dithiothreitol-disulfide exchange reaction of adenosylmethionine synthetase. The reaction mixture contained 0.1 M Tris-chloride, pH 7.5, 5 mM MgCl₂, 10 mM NaCl, 10 mM dithiothreitol, 10 mM ATP, and 30% (v/v) glycerol. The reaction was started by the addition of adenosine-5'-triphosphate, and the reaction was monitored by the decrease in absorbance at 250 nm. The reaction was stopped after 10 minutes by the addition of 25% (v/v) glycerol. The mixture was then centrifuged, and the supernatant was assayed for adenosylmethionine synthetase activity. The reaction was carried out at 0.5 mg of enzyme per ml. The reaction was monitored by the decrease in absorbance at 250 nm. The reaction was stopped after 10 minutes by the addition of 25% (v/v) glycerol. The mixture was then centrifuged, and the supernatant was assayed for adenosylmethionine synthetase activity. The reaction was carried out at 0.5 mg of enzyme per ml.
Adenosylmethionine Synthetase

**FIG. 1.** (A) Divalent metal ion activation of the AdoMet synthetase reaction. Reaction mixtures contained 15 mM ATP, 1.2 mM methionine, 0.05 M HCl, 0.12 M NaCl, 0.18 M KCl, 0.12 M Tris-chloride, pH 8.3. The symbols represent: ○ ○ ○ ○ ○, NiCl2; Δ Δ Δ Δ Δ, MgCl2; △ △ △ △ △, MnCl2; □ □ □ □ □, CaCl2; ○ ○ ○ ○ ○, ZnCl2; △ △ △ △ △, CdCl2; ○ ○ ○ ○ ○, MgCl2; △ △ △ △ △, MnCl2; □ □ □ □ □, CaCl2; ○ ○ ○ ○ ○, ZnCl2. The symbols represent: A-A, no divalent metal ion added; B-B, 0.18 M KCl in the absence of added divalent metal ion or with CdCl2 in this concentration range. (B) Monovalent cation activation of the AdoMet synthetase reaction. Solutions contained 15 mM ATP, 20 mM MgCl2, 1.2 mM methionine, and 0.1 M Tris-chloride, pH 8.3. The symbols represent: ○ ○ ○ ○ ○, K+; ○ ○ ○ ○ ○, Na+. Other conditions were as in (A).

**FIG. 2.** (A) Double reciprocal plot for ATP at various concentrations of KCl. The symbols represent: ○ ○ ○ ○ ○, 0.05 M KCl; ○ ○ ○ ○ ○, 0.18 M KCl; ○ ○ ○ ○ ○, 0.66 M KCl; ○ ○ ○ ○ ○, 2.0 M KCl; ○ ○ ○ ○ ○, 2.4 M KCl. The units of v are moles AdoMet per minute. Symbols A-A and B-B represent: A-A, no added monovalent cation; B-B, 5 mM NaCl. Other conditions were as in (A).

**FIG. 3.** Inactivation of AdoMet synthetase by ATP at various concentrations of KCI. The symbols represent: ○ ○ ○ ○ ○, 0.05 M KCl; ○ ○ ○ ○ ○, 0.18 M KCl; ○ ○ ○ ○ ○, 0.66 M KCl; ○ ○ ○ ○ ○, 2.0 M KCl; ○ ○ ○ ○ ○, 2.4 M KCl. Other conditions were as in (A).

**FIG. 4.** Adenosylmethionine synthetase activity at various concentrations of KCl. The symbols represent: ○ ○ ○ ○ ○, no added monovalent cation; △ △ △ △ △, 1 mM KCl; ○ ○ ○ ○ ○, 0.6 M KCl. Other conditions were as in (A).

**FIG. 5.** (A) Reaction of [8-3H]AMPPNP with L-methionine (10 mM) in the presence of L-cysteine, pH 8.3. The symbols represent: ○ ○ ○ ○ ○, 0.1 mM L-cysteine; ○ ○ ○ ○ ○, 1.0 mM L-cysteine; ○ ○ ○ ○ ○, 2.0 mM L-cysteine; ○ ○ ○ ○ ○, 3.0 mM L-cysteine; ○ ○ ○ ○ ○, 4.0 mM L-cysteine. Other conditions were as in (A).

**FIG. 6.** (A) Reaction of [8-3H]AMPPNP with methionine (10 mM) at various concentrations of MgCl2 in the presence of L-cysteine, pH 8.3. The symbols represent: ○ ○ ○ ○ ○, 0.25 mM MgCl2; ○ ○ ○ ○ ○, 0.59 mM MgCl2; ○ ○ ○ ○ ○, 0.84 mM MgCl2; ○ ○ ○ ○ ○, 1.09 mM MgCl2. Other conditions were as in (A).

**FIG. 7.** Pulse-chase experiments showing reversible formation of an enzyme-substrate complex in the AdoMet synthetase reaction. Solutions contained 0.45 mM AMPPNP, 0.5 mM [1-14C]methionine, 0.1 M KCl, 20 mM MgCl2, in 0.1 M Tris-chloride, pH 8.3. The symbols represent: ○ ○ ○ ○ ○, AdoMet synthetase activity; A-A, tripolyphosphate activity. Other conditions were as in (A).
### Table 1: Purification of Adenosylmethionine Synthetase

| Step | Vol. (mL) | Protein (mg) | Total Activity (nmol/min) | Specific Activity (nmol/min/mg) | Yield (%) |
|------|-----------|--------------|--------------------------|---------------------------------|-----------|
| DEAE-850 Sephadex | 150 | 71,775 | 167 | 0.235 | 73 |
| Sephadryl S-300 | 27 | 133 | 162 | 0.23 | 73 |
| Sepharose 6B-400 | 47 | 41 | 76 | 1.2 | 13 |

Note: Purified enzyme was used as a single band in polyacrylamide gel electrophoresis under the presence and absence of sodium dodecyl sulfate. (1) The purified enzyme was stable indefinitely at -70°C and for at least several months at 4°C.

### Table 2: Table II: Adenosylmethionine Synthetase (AdoMet), ATP, and S-Adenosylhomocysteine (SAH) Requirements

| Residue | Number | Residue | Number | Residue | Number | Residue | Number |
|---------|--------|---------|--------|---------|--------|---------|--------|
| AAA     | 46     | AIA     | 46     | TNE    | 10     |
| GIA     | 10     | GIA     | 12     | GIA    | 10     |
| THI     | 12     | THI     | 12     | THI    | 12     |
| PEN     | 45     | PEN     | 41     | PEN    | 41     |
| SER     | 45     | SER     | 45     | SER    | 45     |

Note: Determined by method of Edelhoch (42).

### Table 3: Table III: Mononuclear cation activation of the AdoMet synthetase reaction

| Mononuclear cation | | Relative max |
|--------------------|----------------|----------------|
| $^{2+}$             | 0.5            | 0.11           |
| $^{2+}$             | 0.6            | 0.11           |
| $^{2+}$             | 1.0            | 0.11           |
| $^{2+}$             | 0.15           | 0.11           |
| $^{2+}$             | 0.2            | 0.11           |
| $^{2+}$             | 0.3            | 0.11           |

Note: Concentration of mononuclear cation required for half maximal activation.

### Table 4: Table IV: Mononuclear cation activation of the AdoMet synthetase reaction

| Mononuclear cation | $K_{n}$ (mM) | $K_{n}$ (ATP) | Relative max |
|--------------------|---------------|---------------|---------------|
| $^{2+}$             | 0.5           | 0.11          | 0.1           |
| $^{2+}$             | 0.6           | 0.11          | 1.0           |
| $^{2+}$             | 1.0           | 0.11          | 0.11          |
| $^{2+}$             | 0.15          | 0.11          | 0.2           |
| $^{2+}$             | 0.2           | 0.11          | 0.3           |
| $^{2+}$             | 0.3           | 0.11          | 0.5           |

Note: Concentration of mononuclear cation required for half maximal activation.

### Table 5: Table V: Comparison of the rates of the reactions catalyzed by AdoMet synthetase

| Experiment | Source of enzyme | Synthetase | Triphosphatase |
|-----------|-----------------|------------|---------------|
| A         | E. coli         | 20         | 2.2           |
| B         | E. coli         | 13         | 2.1           |

Note: Reactions were measured in the presence of 10 mM methionine and 3.2 mM ATP.
Adenosylmethionine Synthetase

**TABLE VI**

Nonradioactive amino activation of the adenosylmethionine reaction

| Nontemplate cation | K* (mM) | K** (mM) | Relative * / ** |
|--------------------|---------|---------|-----------------|
| Na+                | 5       | 0.6     | 1.0             |
| K+                 | 1       | 0.6     | 1.1             |
| Ca2+               | 0.1     | 1.4     | 0.8             |
| Mg2+               | 0.1     | 3.0     | 0.6             |
| Li+                | 0.1     | 5.0     | 0.6             |
| Na+                 | 0.1    | 20.0     | 0.6             |

* K* is the concentration of cations required for half-maximal activation, measured with 1.2 mM ATP, 5 mM MgCl2, and 0.2 mM [methyl-14C]methionine. The Km for ATP in the presence of 0.2 mM [methyl-14C]methionine, 5 mM MgCl2, and 0.1 M KC1 was 0.05 mM.

**TABLE VII**

Nucleotide substrates for adenosylmethionine synthetase

| Compound | Vmax (nmol/h/mg) | Km (mM) | K* (mM) | K** (mM) | Relative * / ** |
|----------|-----------------|---------|---------|---------|-----------------|
| ATP      | 100             | 0.12    | 0.13    | 0.1     | 1.1             |
| GTP      | 42              | 0.11    | 0.12    | 0.1     | 1.1             |
| F-AMP     | 50              | 0.11    | 0.12    | 0.1     | 1.1             |
| ADP      | 3               | 0.01    | 0.01    | 0.01    | 1.0             |
| GMP      | 4               | 0.01    | 0.01    | 0.01    | 1.0             |
| AMP      | 1               | 0.01    | 0.01    | 0.01    | 1.0             |

* Determined from a Dixon plot in the presence of 0.1 mM ATP. Other conditions as given above.

**TABLE VIII**

Comparison of ASDH and ADH synthetase activities in AdoMet synthetase purified from wild-type x y and y mutant containing protocatechuate pool strains

| Experiment | Enzyme Source (mol AdoMet/mg) | AdoMet synthetase reaction | Ratio |
|------------|-----------------------------|---------------------------|-------|
| A          | YAPI205/pCC27-63             | 0.18                      | 1.0   |
| B          | SMM1205                      | 0.11                      | 0.9   |

* Determined from a Dixon plot in the presence of 0.1 mM ATP. Other conditions as given above.

**TABLE IX**

Comparison of ASDHase and ADH synthetase activities in AdoMet synthetase purified from wild-type x y and y mutant containing protocatechuate pool strains

| Experiment | Enzyme Source (mol NADH/mg) | AdoMet synthetase reaction | Ratio |
|------------|-----------------------------|---------------------------|-------|
| A          | YAPI205/pCC27-63             | 0.18                      | 1.0   |
| B          | SMM1205                      | 0.11                      | 0.9   |

* Determined from a Dixon plot in the presence of 0.1 mM ATP. Other conditions as given above.

**TABLE X**

Comparison of ASDHase and ADH synthetase activities in AdoMet synthetase purified from wild-type x y and y mutant containing protocatechuate pool strains

| Experiment | Enzyme Source (mol NADH/mg) | AdoMet synthetase reaction | Ratio |
|------------|-----------------------------|---------------------------|-------|
| A          | YAPI205/pCC27-63             | 0.18                      | 1.0   |
| B          | SMM1205                      | 0.11                      | 0.9   |

* Determined from a Dixon plot in the presence of 0.1 mM ATP. Other conditions as given above.

**TABLE XI**

Comparison of ASDHase and ADH synthetase activities in AdoMet synthetase purified from wild-type x y and y mutant containing protocatechuate pool strains

| Experiment | Enzyme Source (mol NADH/mg) | AdoMet synthetase reaction | Ratio |
|------------|-----------------------------|---------------------------|-------|
| A          | YAPI205/pCC27-63             | 0.18                      | 1.0   |
| B          | SMM1205                      | 0.11                      | 0.9   |

* Determined from a Dixon plot in the presence of 0.1 mM ATP. Other conditions as given above.

**TABLE XII**

Comparison of ASDHase and ADH synthetase activities in AdoMet synthetase purified from wild-type x y and y mutant containing protocatechuate pool strains

| Experiment | Enzyme Source (mol NADH/mg) | AdoMet synthetase reaction | Ratio |
|------------|-----------------------------|---------------------------|-------|
| A          | YAPI205/pCC27-63             | 0.18                      | 1.0   |
| B          | SMM1205                      | 0.11                      | 0.9   |

* Determined from a Dixon plot in the presence of 0.1 mM ATP. Other conditions as given above.

**TABLE XIII**

Comparison of ASDHase and ADH synthetase activities in AdoMet synthetase purified from wild-type x y and y mutant containing protocatechuate pool strains

| Experiment | Enzyme Source (mol NADH/mg) | AdoMet synthetase reaction | Ratio |
|------------|-----------------------------|---------------------------|-------|
| A          | YAPI205/pCC27-63             | 0.18                      | 1.0   |
| B          | SMM1205                      | 0.11                      | 0.9   |

* Determined from a Dixon plot in the presence of 0.1 mM ATP. Other conditions as given above.

**TABLE XIV**

Comparison of ASDHase and ADH synthetase activities in AdoMet synthetase purified from wild-type x y and y mutant containing protocatechuate pool strains

| Experiment | Enzyme Source (mol NADH/mg) | AdoMet synthetase reaction | Ratio |
|------------|-----------------------------|---------------------------|-------|
| A          | YAPI205/pCC27-63             | 0.18                      | 1.0   |
| B          | SMM1205                      | 0.11                      | 0.9   |

* Determined from a Dixon plot in the presence of 0.1 mM ATP. Other conditions as given above.

**TABLE XV**

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