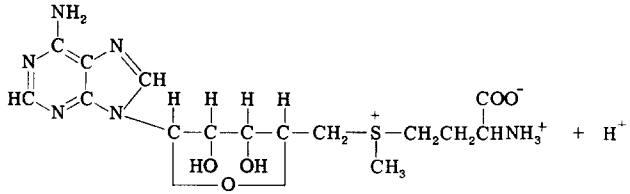
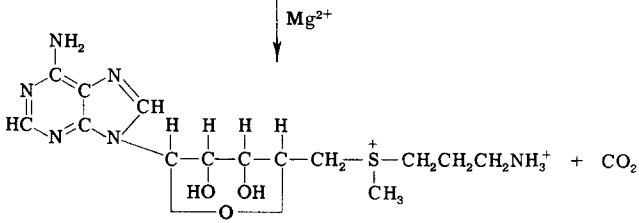
[223] Adenosylmethionine Decarboxylase[1] (*Escherichia coli* W)

By R. B. WICKNER, C. W. TABOR, and H. TABOR



S-Adenosylmethionine



5′-Deoxyadenosyl-(5′), 3-amino-propyl-(1), methylsulfonium salt ("decarboxylated adenosylmethionine")

Assay Method

Principle. The assay depends upon the measurement of $^{14}CO_2$ released from ^{14}COOH-adenosylmethionine.

Reagents

Triethanolamine sulfate, 0.1 *M*, pH 7.4

$MgSO_4$, 1.0 *M*

^{14}COOH-adenosylmethionine (6×10^6 cpm/micromole)[2]

Enzyme: 3×10^{-6} to 10^{-4} unit is used for each assay.

[1]H. Tabor, S. M. Rosenthal, and C. W. Tabor, *J. Am. Chem. Soc.* 79, 2978 (1957); *J. Biol. Chem.* 233, 907 (1958); R. B. Wickner, C. W. Tabor, and H. Tabor, *J. Biol. Chem.* 245, 2132 (1970); see also Vol. V [103a].

[2]Radioactive adenosylmethionine is prepared from ^{14}COOH-DL-methionine using *E. coli* W methionine-activating enzyme (this volume [190]).

Procedure. The reaction is conveniently carried out in the screw-cap vials used for scintillation counting. In each vial are placed 0.8 ml of 0.1 *M* triethanolamine sulfate, pH 7.4; 0.2 ml of 1 *M* $MgSO_4$; an appropriate amount of enzyme; and water to make a total volume of 1.9 ml. When the crude material is assayed, the pH is adjusted to 7.4 with 2 *M* triethanolamine. A 2 cm × 2 cm filter paper square with a drop of Hyamine hydroxide is placed in the cap to trap the $^{14}CO_2$ formed. Five nanomoles (30,000 cpm) of ^{14}COOH-adenosylmethionine in 0.1 ml is added to start the reaction, and the cap is quickly closed. The reaction mixture is incubated at 37° for 5 minutes; 1.0 ml of 1 *M* KH_2PO_4 is then added to stop the reaction and release $^{14}CO_2$. This addition is made rapidly, and the cap is quickly screwed into place. This mixture is shaken for 1 hour at room temperature, and the Hyamine-soaked filter paper is placed in a dioxane-base scintillation fluid[3] and counted.

Definition of Unit and Specific Activity. One unit is defined as that amount of enzyme which will decarboxylate 1.0 micromole of adenosylmethionine in 1 minute under the conditions specified above.

Specific activity is expressed as units per milligram of protein. Protein is measured by a modification of the method of Lowry *et al.*[4]

Purification Procedure

Step 1. Growth and Extraction of Cells. *S*-Adenosylmethionine decarboxylase is prepared from *E. coli* W grown overnight in a 300-liter fermentor, in Vogel-Bonner medium[5] containing 0.2% dextrose. The cells are harvested in the stationary phase with a refrigerated Sharples centrifuge. The yield of cells is approximately 1 kg. The cells are suspended in 4 liters of distilled water at 0° and pressed twice at 8000 psi in a Gaulin laboratory homogenizer. During this procedure, the temperature of the homogenate rises to about 20°, but it is quickly lowered by cooling the homogenate in an ice bath. The crude homogenate is spun in the refrigerated Sharples centrifuge, and the precipitate is discarded. All subsequent procedures are carried out at 0–5° unless otherwise specified.

Step 2. Streptomycin Step. To the supernatant solution (4380 ml) is added dropwise with constant stirring 500 ml of a freshly prepared 12% solution of streptomycin sulfate. After 4 hours, the solution is centrifuged at 16,000 *g* for 10 minutes or filtered using Whatman No. 802 folded filter paper (50 cm in diameter), and the precipitate is discarded.

[3] J. D. Davidson and P. Feigelson, *Intern. J. Appl. Radiation Isotopes* **2**, 1 (1957).
[4] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951); see also Vol. III [73].
[5] H. J. Vogel and D. M. Bonner, *J. Biol. Chem.* **218**, 97 (1956).

Step 3. Ammonium Sulfate Step. To the supernatant of step 2 is added 2 ml per liter of 0.5 *M* sodium ethylenediaminetetraacetic acid (EDTA), pH 7.4, and 246.5 g per liter (40.5% of saturation) of "Special Enzyme Grade" ammonium sulfate obtained from Mann Research Laboratories. Use of less pure grades of ammonium sulfate results in marked loss of enzymatic activity. The ammonium sulfate is added slowly over 5–10 minutes with stirring, and the suspension is stirred 4–5 hours before centrifugation at 16,000 *g* for 10 minutes or filtration using Whatman No. 12 filter paper. To the supernatant is added slowly with stirring 89.2 g per liter of ammonium sulfate (to a final concentration of 54% of saturation). This is stirred for 4–5 hours and centrifuged or filtered as described above; the precipitate is solubilized in about 500 ml of 0.01 *M* triethanolamine sulfate, pH 7.4.

Step 4. Heat Step. The product of step 3 is heated, 40 ml at a time in 3.5 × 19.5 cm thin-walled glass test tubes. The solution is brought to 66° in less than 1 minute with constant stirring, by immersion of the tubes in a 90–95° water bath. The tubes are placed in a 68° water bath for an additional 4 minutes and then cooled quickly in an ice bath. This material is centrifuged at 16,000 *g* for 10 minutes, and the supernatant is saved. The precipitate is washed with 250 ml of 0.01 *M* triethanolamine sulfate, pH 7.4, and centrifuged; the supernatant is combined with the supernatant saved above. The pooled supernatants are dialyzed against 20 volumes of 0.01 *M* potassium phosphate pH 7.3 containing 10^{-3} *M* EDTA and 10^{-3} *M* 2-mercaptoethanol (buffer A).

Step 5. DEAE-Cellulose Chromatography. Whatman microgranular diethylaminoethylcellulose (DE-52) is washed successively with about 2 liters of 0.5 *N* HCl, 5 liters of water, 2 liters of 0.5 *N* NaOH, and 10 liters of water per 100 g dry weight. A column 3.8 × 40 cm is packed without pressure and equilibrated with buffer A. The product of step 4 is applied to the column. The column is eluted with buffer A containing 0.1 to 0.3 *M* KCl in a 4-liter linear gradient. Fractions with 10-fold or better purification are pooled, ultrafiltered and dialyzed against 10 volumes of buffer A containing 0.2 *M* KCl.

Step 6. DEAE-Sephadex Chromatography. Diethylaminoethyl-Sephadex (Pharmacia A-50) is washed successively with 0.5 *N* HCl, water, 0.5 *N* NaOH, and water as above and equilibrated with buffer A containing 0.2 *M* KCl. A 1.5 cm × 10 cm column is prepared with this material and charged with the product of step 5. The column is eluted with a 500-ml linear gradient of buffer A containing 0.2 *M* KCl to 0.6 *M* KCl. Fractions having specific activities greater than 0.01 U/mg are pooled, concentrated by ultrafiltration to a volume of about 1 ml and dialyzed against 0.05 *M* KCl in buffer A. Glycerol (0.3 ml) is then added.

Step 7. Preparative Disc Gel Electrophoresis. A preparative acrylamide disc gel electrophoresis system, slightly modified from the pH 6.5–7.5 histidine system of Chrambach described by Jakoby and Kohn,[6] is used for the final purification step; the Canalco preparative disc gel electrophoresis apparatus (PD2/320) is used. The lower gel has 0.4% bisacrylamide, 7.5% acrylamide, a pH of 7.5, and a total volume of 6.6 ml. Before enzyme addition, electrophoresis of the cast lower gel is carried out for 4 hours at 10 ma, with 0.15 M histidine HCl, pH 6.9, in the electrode and elution chambers. The stacking (upper) gel (2.5% acrylamide, pH 6.5, total volume 2.0 ml) is then cast, and the enzyme solution from Step 6 is layered over the stacking gel. The electrophoresis is run for 5 hours at 4° with a current of 10 to 15 ma. During the entire electrophoresis, the gel is eluted at about 0.4 ml per minute with Buffer A containing 0.05 M KCl. The first protein detected in the eluate is adenosylmethionine decarboxylase. The purified enzyme, which is unstable if allowed to remain dilute, is concentrated to about one mg/ml by ultrafiltration as soon as it has been eluted from the gel.

A typical purification is summarized in the table.

PURIFICATION OF ADENOSYLMETHIONINE DECARBOXYLASE FROM *Escherichia coli*[a]

Step	Volume (ml)	Total protein (mg)	Total units	Specific activity (U/mg) × 10^5
1. Crude extract	4080	57,900	1.52	2.6
2. Streptomycin supernatant	4040	51,700	1.56	3.0
3. $(NH_4)_2SO_4$ step	890	16,300	1.23	7.6
4. Heat step	1190	4,260	0.59	15
5. DEAE-cellulose chromatography	800	224	0.38	160
6. DEAE-Sephadex	60	9.8	0.13	1320
7. Acrylamide gel electrophoresis	4.7	3.0	0.061	2000

[a]Overall yield, 4%; overall purification, 770-fold.

Properties

Stability. The final purification product slowly loses activity at 4° with a half-life of around 6 months. It is inactivated rapidly by low ionic strength or dialysis in the absence of EDTA.

Purity. The active fractions of the final step show no evidence of contamination as judged by sedimentation equilibrium centrifugation or by

[6]W. B. Jakoby and L. D. Kohn, *A Method of Enzyme Purification*, Academic Press in preparation.

analytical disc gel electrophoresis at pH 6.5, 7.5, or 9.5 in 7.5% or 20% gels.

Molecular Weight. Meniscus depletion sedimentation equilibrium experiments carried out by Dr. W. R. Carroll and Mr. E. R. Mitchell indicated that the molecular weight is about 115,000.

Inhibitors and Activators. The enzyme shows a requirement for Mg^{2+}. The enzyme activity is abolished by carbonyl reagents, such as cyanide, phenylhydrazine, borohydride, semicarbazide, and hydroxylamine. There is no activation of the enzyme by pyridoxal phosphate at any stage in the purification.

Prosthetic Group. Pyridoxal phosphate is absent as shown by the absence of absorption peaks above 280 mμ in the purified enzyme, and, after reaction with phenylhydrazine, the absence of the peak at 410 mμ characteristic of pyridoxal phosphate phenylhydrazone. The enzyme phenylhydrazone spectrum does, however, show a peak at 323 mμ consistent with the presence of enzyme-bound pyruvate phenylhydrazone. This is confirmed by the identification of tritiated lactate in the hydrolyzate of borotritide-reduced purified enzyme.[7]

Kinetics. The enzyme obeys classical Michaelis-Menten kinetics, has a K_m of 0.1 mM and a V_{max} of 40 micromoles per minute per unit. The usual assay is performed at substrate concentrations much below the K_m; thus, the pure material has a relatively low specific activity when assayed under standard conditions.

Other sources. Adenosylmethionine decarboxylase has also been purified from rat prostate.[8]

[7] An acid-stable, nondialyzable contaminant was found in many commercial preparations of sodium borotritide, often containing as much as 0.1% of the radioactivity. This material, when present in sufficiently high concentration, resulted in large amounts of radioactivity in an enzyme-free blank. This problem was avoided either by using low concentrations of sodium borotritide or by diluting the borotritide with sodium borohydride. Occasional preparations of borotritide seemed to be free of this contaminant.

[8] J. Jänne and H. G. Williams-Ashman, *Biochem. Biophys. Res. Commun.* **42**, 222 (1971).