accounting for the distances being larger than 28 Å or a conformational change and/or intervening group transfer occurs in the succinylation (or acetylation) of the lipoic acid. In particular, the transition dipole of the PBMP probably is significantly displaced (8–10 Å) from the reactive site of the thiamin diphosphate.

The catalytic mechanisms for the α-ketoglutarate and pyruvate dehydrogenase complexes, although similar in many respects, appear to have important differences. Both E_1 and E_2 are different in the two complexes, while the E_3 portion of both complexes is identical (Reed, 1974; Guest & Creaghan, 1973). Future studies will be directed toward a more comprehensive understanding of the structural and mechanistic differences between the two complexes.

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Essential Arginyl Residues in Thymidylate Synthetase from Amethopterin-Resistant *Lactobacillus casei*†

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ABSTRACT: Thymidylate synthetase from amethopterin-resistant *Lactobacillus casei* is rapidly and completely inactivated by phenylglyoxal, a reagent that is highly selective for the modification of arginyl residues. Both dUMP and dTMP afford significant protection, while 5,10-methylenetetrahydrofolate provides little protection against phenylglyoxal-dependent inactivation. Extrapolation to complete inactivation suggests that inactivation by phenylglyoxal correlates with the modification of 1.8 arginyl residues per enzyme subunit, as determined by the incorporation of [7-^{14}C]phenylglyoxal. The presence of either dUMP or dTMP protects approximately 1.0 and 0.7 arginyl residues per enzyme subunit, respectively, against incorporation of [7-^{14}C]phenylglyoxal. In a preliminary study [Cipollo, K. L., & Dunlap, R. B. (1978) *Biochem. Biophys. Res. Commun. 81*, 1139–1144], it was reported that the enzyme is completely inactivated by 2,3-butanedione in borate buffer. Results of amino acid analysis suggest that the complete loss of activity by 2,3-butanedione correlates with the modification of 2.3 arginyl residues per subunit and that dUMP and FdUMP protect 0.7 and 1.1 arginyl residues per subunit, respectively. Similarly, in the ternary complex of enzyme, 5-fluoro-2′-deoxyuridylate, and 5,10-methylenetetrahydrofolate, 1.1 arginines were protected per subunit from modification by 2,3-butanedione. Unlike native enzyme, phenylglyoxal- and butanedione-modified enzyme samples are incapable of forming ternary complex. The results suggest that one arginyl residue per subunit participates in the functional binding of dUMP, presumably through electrostatic interaction with the 5′-phosphate moiety of the nucleotide.

Thymidylate synthetase (EC 2.1.1.45) catalyzes the reductive methylation of dUMP by (+)-5,10-methylene-5,6,7,8-tetrahydrofolate[1] (5,10-CH_2H_4folate) to form dTMP and H_2-folate. This enzyme is of particular interest because it is the target enzyme of the chemotherapeutic agent 5-fluorouracil (Reyes & Heidelberger, 1965). Chemical modification by several sulfhydryl reagents (Plese & Dunlap, 1977; Dunlap et al., 1971; Galivan et al., 1977) has demonstrated a catalytic

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[1] Abbreviations used: 5,10-CH_2H_4folate, 5,10-methylene-5,6,7,8-tetrahydrofolate; H_4folate, 5,6,7,8-tetrahydrofolate; H_2folate, 7,8-dihydrofolate; MMTS, methyl methanethiosulfonate; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); dU, deoxyuridine; FdUMP, 5-fluoro-2′-deoxyuridylate; EDTA, ethylenediaminetetraacetic acid; Mops, 4-morpholinepropanesulfonic acid; CD, circular dichroism.

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role for a cysteinyl residue in thymidylate synthetase from amethopterin-resistant *Lactobacillus casei*. Through the isolation of a peptide fragment containing FdUMP (Bellisario et al., 1976; Pogolotti et al., 1976), the active-site sulfhydryl (or its thiolate anion) has been shown to participate in the formation of a stable covalent ternary complex of enzyme, (+)-5,10-CH_2H_4folate, and FdUMP. Recent ^{19}F NMR investigations have delineated the relative stereochemistry of the ternary complex in the active site of the enzyme (Byrd et al., 1977, 1978).

It has been postulated that arginyl residues play a general role as anion recognition sites in enzymes (Riordan et al., 1977; Lange et al., 1977). Since thymidylate synthetase catalyzes a reaction involving both a negatively charged nucleotide and coenzyme, it seemed likely that arginine residues function in substrate recognition. A preliminary report on the modification of thymidylate synthetase by 2,3-butanedione buffer has appeared (Cipollo & Dunlap, 1978). The present study, employing the highly selective arginine modifying reagents 2,3-butanedione in borate buffer (Riordan, 1973) and phenylglyoxal (Takahashi, 1968, 1977), demonstrates that the loss of enzymatic activity correlates with the modification of two arginyl residues per enzyme subunit. Substrate protection studies and the inability of modified enzyme to form ternary complex with FdUMP and (+)-5,10-CH_2H_4folate implicate an arginyl residue at the deoxynucleotide site.

Materials and Methods

Thymidylate synthetase from an amethopterin-resistant strain of *L. casei* was purified to homogeneity in the presence of 10 mM 2-mercaptoethanol following the procedure of Lyon et al. (1975). The enzyme was activated by dialysis against either 50 mM borate, 50 mM Mops, 50 mM phosphate, or 125 mM bicarbonate buffer, pH 7.0, containing 1 mM EDTA and 25 mM 2-mercaptoethanol for 12 h preceding use. The activated enzyme was freed from exogenous thiols by gel filtration on Sephadex G-10 in the appropriate buffer. Enzyme assays were performed spectrophotometrically by following the increase in absorbance at 340 nm using a Beckman Acta CV spectrophotometer. The assay mixture contained 0.1 M potassium phosphate buffer, pH 6.8, dUMP, (±)-5,10-CH_2H_4folate, enzyme (ca. 7×10^{-8} M), and in some cases 25 mM 2-mercaptoethanol (Dunlap et al., 1971). H_4folate was prepared by catalytic hydrogenation of folic acid purchased from Calbiochem (Hatefi et al., 1960). Stock solutions of (±)-5,10-CH_2FH_4 were prepared fresh as previously described (Dunlap et al., 1971). Experiments with phenylglyoxal-modified enzyme required the use of thiol-free cofactor solution which was prepared by omitting 2-mercaptoethanol from the cofactor solution and stored in an argon-filled tube, protected from light in ice.

dUMP, dTMP, dCMP, and 2′-deoxyuridine were obtained from Sigma Chemical Co. Phenylglyoxal monohydrate and 2,3-butanedione were products of Aldrich Chemical Co. and were used without further purification. [7-^{14}C]Phenylglyoxal prepared from [7-^{14}C]acetophenone (ICN) by selenium oxidation (Riley & Gray, 1943) had a specific activity of 0.071 μCi/μmol. [^{14}C]MMTS was prepared by the alkylation of sodium methanethiosulfonate by [^{14}C]methyl bromide (New England Nuclear) following the procedure of Smith et al. (1975) by Dr. Charles A. Lewis and William A. Munroe of our laboratory and had a specific activity of 0.54 mCi/mmol. All other chemicals were reagent grade.

Chemical modification reactions with 2,3-butanedione and phenylglyoxal were carried out under the conditions given in the figure and table legends. All reactions were performed at 25 °C and were initiated by the addition of either freshly prepared 2,3-butanedione or phenylglyoxal to the enzyme solution in the same buffer. When substrates or ligands were used, they were added to the enzyme stock solution. Activity remaining is expressed as a ratio of the activity of the modified enzyme, v, to that of native enzyme (subjected to identical conditions but in the absence of butanedione), v_c, multiplied by 100.

The number of arginines modified by phenylglyoxal was determined by incorporation of [7-^{14}C]phenylglyoxal. Aliquots of thymidylate synthetase reacted with [7-^{14}C]phenylglyoxal in 125 mM bicarbonate buffer were periodically withdrawn and applied to a Sephadex G-10 column with 125 mM bicarbonate buffer, pH 8.0, as the eluent. Immediately following gel filtration, the enzyme was assayed for enzymatic activity and the amount of [7-^{14}C]phenylglyoxal incorporated was determined by using Bray's mixture containing Omnifluor with a Nuclear Chicago Isocap 300 liquid scintillation counter. A stoichiometry of two phenylglyoxals per arginine was used for calculations (Takahashi, 1968).

The number of catalytic sulfhydryl groups after phenylglyoxal modification of thymidylate synthetase was determined by reaction with [^{14}C]MMTS. Thymidylate synthetase, inactivated to 10% of its initial activity by phenylglyoxal in either 50 mM Mops or 125 mM bicarbonate buffer, pH 8.0, was treated with a fourfold molar excess of [^{14}C]MMTS and incubated at room temperature for 20 min. The radiolabeled enzyme was passed down a Sephadex G-10 column to remove excess reagent. The eluted protein was assayed for activity, and the radiolabel was quantitated by liquid scintillation counting in Bray's solution by a Nuclear Chicago Isocap 300.

Polyacrylamide gel electrophoresis of native enzyme, phenylglyoxal-modified enzyme, and the products of the attempted conversion of these enzyme samples to ternary complexes (forms II and III) was performed as previously described (Aull et al., 1974). Following electrophoresis, the 7.5% gels (0.6 × 9.0 cm) were stained with 0.2% Coomassie Blue G.

Fluorescence spectra were measured on a Aminco-Bowman spectrophotofluorometer with excitation at 287 nm. To a 3.0-mL solution of native and butanedione-modified enzyme in 50 mM borate, pH 8.0, a fivefold molar excess of FdUMP and (±)-5,10-CH_2H_4folate were added. A tryptophan solution (3.0 mL) in 50 mM borate, pH 8.0, with an absorbance at 287 nm of 0.2 was used as a reference. The percentage of protein fluorescence quenched by ligands was calculated as previously described (Donato et al., 1976). Circular dichroism spectra were recorded on a Jasco J-40C automatic recording spectropolarimeter. All CD spectra were run with the slit programmed for 2-nm spectral bandwidth.

The amino acid analyses of native and butanedione-modified thymidylate synthetase were performed on a Beckman Model 119 C amino acid analyzer equipped with a Beckman System AA computing integrator. Aliquots (0.6 mL) of the reaction mixture were periodically withdrawn and added to 0.3 mL of 6 N HCl to halt the reaction and to prevent the regeneration of free arginine (Riordan, 1973). Hydrolysis was carried out in 1 mL of constant-boiling 6 N HCl (Pierce) in evacuated, sealed ampules at 110 °C for 24 h.

Results and Discussion

The inactivation of thymidylate synthetase by phenylglyoxal follows pseudo-first-order kinetics and is concentration dependent (Figure 1). Complete inhibition could be achieved with longer incubation times. The rate of inactivation of thymidylate synthetase by 5.9 mM phenylglyoxal at pH 8.0 was affected by the buffer used. Rapid inactivation was ob-

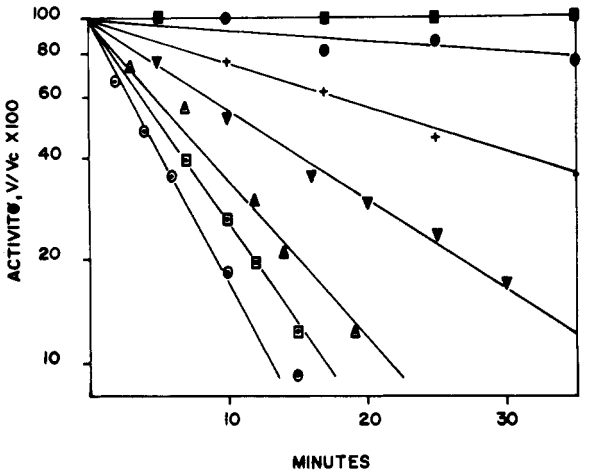

FIGURE 1: Effect of varying concentrations of phenylglyoxal on thymidylate synthetase as a function of time. Enzyme was incubated with 1.1 (●), 2.8 (+), 5.9 (▽), 11 (△), 17 (□), or 22 mM (○) phenylglyoxal in 50 mM Mops (pH 8.0), 25 °C; native enzyme alone in 50 mM Mops buffer (■).

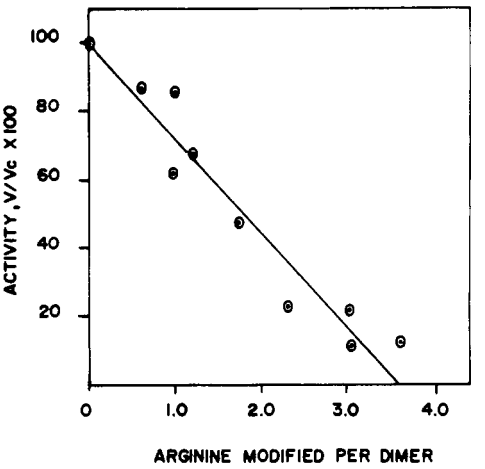

FIGURE 2: Correlation of inactivation of thymidylate synthetase with arginine modification by [7-^{14}C]phenylglyoxal in 125 mM bicarbonate, pH 8.0. Enzyme was treated as described under Materials and Methods, assuming a stoichiometry of two phenylglyoxals per arginine (Takahashi, 1968).

Table I: Effect of Substrates on the Inhibition of Thymidylate Synthetase by Phenylglyoxal in Mops[a]

substrate	concn (M)	act., v/v_c X 100 (%)
none		17
dCMP	8.6×10^{-5}	17
2′-deoxyuridine	8.6×10^{-5}	17
(±)-5,10-CH_2H_4folate	1.7×10^{-4}	25
dTMP	4.3×10^{-5}	71
dTMP	8.6×10^{-5}	79
dUMP	2.2×10^{-5}	64
dUMP	4.3×10^{-5}	74
dUMP	8.6×10^{-5}	87

[a] Modification of enzyme (4.3×10^{-6} M) was performed for 30 min in 5.9 mM phenylglyoxal in 50 mM Mops and 1 mM EDTA, pH 8.0, at 25 °C.

Table II: Substrate Protection against the Incorporation of [7-^{14}C]Phenylglyoxal[a]

ligand	ligand concn (M)	Arg modified[b] per subunit	% act. remaining
none		1.8	12.6
dUMP	5.7×10^{-4}	0.8	87.9
dTMP	7.6×10^{-4}	1.1	76.7

[a] Thymidylate synthetase (1.9×10^{-5} M) was reacted with 10 mM [7-^{14}C]phenylglyoxal in 125 mM bicarbonate buffer and 1 mM EDTA, pH 8.0, for 12.0 min at 25 °C. The phenylglyoxal-modified enzyme was eluted from a Sephadex G-10 column in 125 mM bicarbonate buffer and 1 mM EDTA, pH 8.0, to remove excess reagent. [b] Calculations are based upon the observations of Takahashi (1968) using the stoichiometry of 2 mol of phenylglyoxal per mol of arginine.

served in bicarbonate ($t_{1/2}$ = 6.0 min) and Mops buffers ($t_{1/2}$ = 11.5 min), while much slower rates were found in borate ($t_{1/2}$ = 34.0 min) and phosphate buffers ($t_{1/2}$ = 48.0 min). It is possible that inorganic phosphate interacts with the enzymic dUMP binding site at its 5′-phosphate locus in light of the suggestion by Daron & Aull (1978) that phosphate is a competitive inhibitor of dUMP. Concentrations of inorganic phosphate, however, up to 1.0 mM have little effect on the rate of inactivation either by 2,3-butanedione in borate buffer or by phenylglyoxal in bicarbonate buffer.

The effects of substrates on phenylglyoxal inactivation are summarized in Table I. dUMP and dTMP afford substantial protection against inactivation at low concentrations, while (±)-5,10-CH_2H_4folate provides little protection. In contrast, dCMP and 2′-deoxyuridine, which do not function as substrates for the enzyme, do not afford protection. Results of a parallel study showed that both dUMP and dTMP afford significant protection against inactivation by 2,3-butanedione in borate buffer (Cipollo & Dunlap, 1978). The greater protection observed for dUMP over dTMP with both modifying reagents is consistent with the K_m values reported by Daron & Aull (1978). The inability of 5,10-CH_2H_4folate to protect against inactivation might be explained by kinetic data, which are consistent with an ordered sequential mechanism, where dUMP adds to the enzyme before 5,10-CH_2H_4folate and H_2folate is released before dTMP (Daron & Aull, 1978).

The modification of arginyl residues by phenylglyoxal was calculated from the incorporation of [7-^{14}C]phenylglyoxal, assuming a stoichiometry ot two phenylglyoxals per arginine (Takahashi, 1968, 1977). A plot correlating the loss of activity with the incorporation of [7-^{14}C]phenylglyoxal extrapolates to the modification of 1.8 arginines per enzyme subunit (Figure 2). After long incubation periods, additional arginyl residues are modified. In separate experiments, thymidylate synthetase was reacted with 10 mM [7-^{14}C]phenylglyoxal for 12.0 min, in both the absence and presence of ligands, and monitored for incorporation of radiolabel (Table II). The enzyme was inactivated to 13% of its native activity in the absence of ligands with the concomitant modification of 1.8 arginines per subunit. In contrast, dUMP and dTMP afford significant protection against both inactivation and arginine modification with 0.8 and 1.1 arginines modified per subunit, respectively.

The modification of thymidylate synthetase by 2,3-butanedione in borate buffer, followed by amino acid analysis, indicates that arginine is the only amino acid modified. A plot correlating the loss of activity and the loss of arginine extrapolates to the modification of 2.3 of the 12 arginyl residues per enzyme subunit (Figure 3). In separate experiments, thymidylate synthetase was reacted with 10 mM 2,3-butanedione for 90 min in both the presence and absence of ligands. The enzyme was inactivated to 15% of its initial activity in the absence of ligands with the concomitant modification of 2.1 arginines per subunit. When enzyme was treated with 2,3-butanedione in the presence of dUMP or FdUMP, 0.7 and 1.1 arginyl residues per subunit, respectively, were protected from modification (Table III). Furthermore,

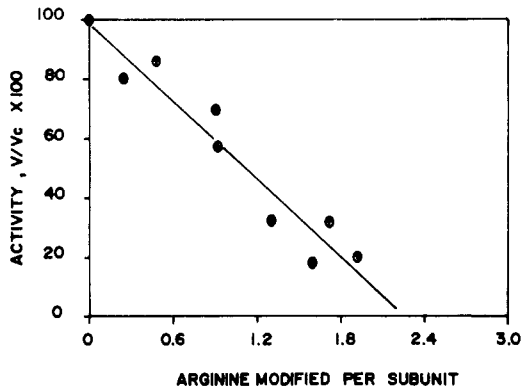

FIGURE 3: Correlation of inactivation of thymidylate synthetase with arginine modification by 2,3-butanedione in 50 mM borate and 1 mM EDTA, pH 8.0. Enzyme was treated as described under Materials and Methods.

Table III: Substrate Protection against 2,3-Butanedione Modification Followed by Amino Acid Analysis[a]

ligand	ligand concn (M)	Δ Arg	% act. remaining
none		2.1	14
dUMP	1.4×10^{-4}	1.4	82
FdUMP	1.0×10^{-3}	1.0	
FdUMP, (±)-5,10-CH_2H_4folate	1.0×10^{-3}, 9.3×10^{-5}	1.1	

[a] Thymidylate synthetase (7.1×10^{-6} M) was reacted with 10 mM 2,3-butanedione in 50 mM borate buffer and 1 mM EDTA, pH 8.0, for 90 min at 25 °C.

thymidylate synthetase in the covalent ternary complex was protected from the modification of 1.1 arginines per subunit. Thus, the results of the amino acid analyses of 2,3-butanedione-modified enzyme and the findings of the [7-^{14}C]-phenylglyoxal incorporation studies strongly suggest that a single arginyl residue is involved in the functional binding of dUMP, presumably through electrostatic interaction with the nucleotide 5′-phosphate moiety.

The ability of phenylglyoxal-modified enzyme to interact with FdUMP and 5,10-CH_2H_4folate to form a ternary complex was investigated by gel electrophoresis. Native enzyme forms a ternary complex that is stable to gel electrophoresis (Aull et al., 1974), while the phenylglyoxal-modified enzyme has lost the capacity to form ternary complex under identical conditions. Similar results were observed in parallel studies with the butanedione-modified enzyme (Cipollo & Dunlap, 1978).

The stability of butanedione-modified thymidylate synthetase in borate buffer can be exploited to investigate the ability of the enzyme to form a ternary complex as monitored by the relatively mild techniques of fluorescence and CD. The fluorescence intensity of native enzyme is considerably quenched by the formation of the FdUMP–5,10-CH_2H_4-folate–enzyme ternary complex (relative fluorescence intensity of 0.177). Donato et al. (1976) have attributed this quenching to either a direct involvement of the enzymic tryptophan residues in ternary complex formation or a conformational change that alters the environment of tryptophan residues such that emission is less efficient. Significantly, butanedione-modified enzyme, which retained 15% of its initial activity, has a relative fluorescence intensity of 0.876 in the presence of FdUMP and (±)-5,10-CH_2H_4folate.

The CD spectra of native and butanedione-modified thymidylate synthetase were found to be identical from 500 to 250 nm with a single positive ellipticity at 290 nm. Formation of the FdUMP–5,10-CH_2H_4folate–native enzyme ternary complex results in considerable alterations in this region, producing positive ellipticity at 365 nm, negative ellipticity at 327 nm, and a positive shoulder at 305 nm (Donato et al., 1976). Importantly, on addition of excess FdUMP and (±)-5,10-CH_2H_4folate to butanedione-modified enzyme, which was inactivated to 13% of its native activity, the $\Delta\theta_{365}$ was 11% of that of native enzyme ternary complex.

When probing enzymes with phenylglyoxal, it is important to exclude sulfhydryl modification as the basis for inactivation due to the potential formation of thiohemiacetals (Schubert, 1935; Takahashi, 1968). Treatment of phenylglyoxal-modified enzyme, retaining 10% of its native activity, with [^{14}C]MMTS, in either 125 mM bicarbonate or 50 mM Mops buffer, pH 8.0, yielded 1.7 titratable sulfhydryl groups. The modification of 1.7 sulfhydryls is consistent with the previously reported results of Lewis et al. (1978) and Plese & Dunlap (1977), in which the incorporation of radiolabel was correlated with loss of both enzymatic activity and the ability to form a ternary complex. Consequently, the formation of thiohemiacetals as the basis of inactivation by phenylglyoxal can be dismissed.

Although the data in this study support the participation of arginine in nucleotide binding, the possibility of arginyl residues participating in folate recognition through hydrogen bonding with the carboxylate anions of the coenzyme cannot be discounted. Crystallographic studies of model compounds (Adams & Small, 1974) and enzyme systems have demonstrated that the guanidino group of arginine is well suited for interaction with carboxyl groups. In the *Escherichia coli* and *L. casei* dihydrofolate reductases, the α-carboxyl group of the folate analogue, methotrexate, is hydrogen bonded to the side chain of arginine (Matthews et al., 1977, 1978). In the absence of added deoxynucleotides, thymidylate synthetase does not bind 5,10-CH_2H_4folate when examined by either equilibrium dialysis (Galivan et al., 1976a) or circular dichroism (Galivan et al., 1975). In contrast, the enzyme is capable of binding 5,10-methylenetetrahydropteroyltetraglutamate (Kisliuk et al., 1974; Galivan et al., 1976b), which is the predominant folate found in *L. casei* (Baugh et al., 1974). Because the folyl-polyglutamates are believed to be the true substrates of thymidylate synthetase, the possibility exists for arginine to interact with the charged polyglutamyl side chain. The modification of such an arginyl residue would not be obvious in this study since the monoglutamate form of the folate coenzyme was used. We are presently performing experiments to further delineate the role of arginine in thymidylate synthetase.

Conclusion

The results of parallel studies on the inactivation of thymidylate synthetase with butanedione and phenylglyoxal are complementary and permit the conclusion that an arginyl residue participates in the deoxynucleotide binding site of this enzyme. In this connection, it is of particular interest that the enzyme employs dUMP, but not 2′-deoxyuridine, as a substrate and that FdUMP is a potent inhibitor, while the corresponding nucleoside is without effect.

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Purification and Characterization of *S*-Adenosyl-L-methionine Decarboxylase from Mouse Mammary Gland and Liver†

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ABSTRACT: *S*-Adenosyl-L-methionine decarboxylase, a key enzyme in polyamine biosynthesis, has been purified to apparent homogeneity from mouse mammary gland and liver by a combination of ammonium sulfate fractionation, DEAE-cellulose and methylglyoxal bis(guanylhydrazone)–Sepharose 4B affinity chromatographies, and gel filtration. Crucial factors for good yield of the pure enzyme include the use of putrescine, pyridoxal phosphate, a detergent (0.01% deoxycholate), and high salts as stabilizing agents at various stages of purification. The purified enzyme from both tissues has essentially identical specific activities and other properties examined and is completely free of propylamine transferase activity (spermidine or spermine synthase). The apparent molecular weight of native enzyme as determined by gel filtration and by sucrose density centrifugation was approximately 68 000 and 74 000, respectively, whereas the subunit molecular weight of 32 000 was obtained with sodium dodecyl sulfate–polyacrylamide gel electrophoresis, suggesting that the enzyme is a dimer. The enzyme has an isoelectric point of 5.9 and a pH optimum of 7.5. The activity of purified enzyme is stimulated by physiological concentrations of putrescine (K_a = 0.5 μM), which decreases the apparent K_m for the substrate from 1.1×10^{-4} to 2×10^{-5} M and also prevents inactivation of the enzyme. Another related polyamine, spermine, but not spermidine, inhibits the enzyme activity by reducing the V_{max} of the enzyme reaction at physiological concentrations (K_i = 0.5 mM). By use of the purified liver *S*-adenosyl-L-methionine decarboxylase, a monospecific mouse antibody to this enzyme has been raised in rabbits. The antibody cross-reacts with the enzyme from mammary gland, inactivates the enzyme, and forms a single precipitation line with *S*-adenosine-L-methionine decarboxylase from both tissues as shown by an Ouchterlony double-diffusion test. Immunoproduct analysis by sodium dodecyl sulfate gel electrophoresis of the antigen synthesized by mouse mammary explants in vitro demonstrated the presence of a sharp band which comigrated with the subunit of authentic (pure) enzyme. The antibody was used in measuring levels of antigen in the mammary gland of mice treated with methylglyoxal bis(guanylhydrazone), and the results indicated that the increased accumulation of the enzyme activity was primarily due to alterations in synthesis and degradation of enzyme molecules.

S-Adenosyl-L-methionine decarboxylase (*S*-Ado-Met decarboxylase)[1] serves an essential role for polyamine biosynthesis by catalyzing the formation of *S*-methyladenosyl-L-homocysteamine (decarboxylated *S*-Ado-Met), the sole donor of the propylamine moiety for the biosynthesis of spermidine

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[1] Abbreviations used: MGBG, methyl glyoxal bis(guanylhydrazone); *S*-Ado-Met, *S*-adenosyl-L-methionine; $NaDodSO_4$, sodium dodecyl sulfate; BSA, bovine serum albumin; ADH, alcohol dehydrogenase; LDH, lactate dehydrogenase.