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Molecular cloning, sequence analysis, and expression in *Escherichia coli* of the cDNA for guanidinoacetate methyltransferase from rat liver

(expression vector)

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ABSTRACT **Five cDNA clones encoding rat liver guanidinoacetate methyltransferase (*S*-adenosyl-L-methionine: guanidinoacetate *N*-methyltransferase, EC 2.1.1.2) were isolated from a λgt11 cDNA library by use of a polyclonal antibody to the purified enzyme. Sequence analysis of the longest cDNA indicated that it consisted of 711 base pairs (bp) of coding region, 51 bp of 5′ noncoding region, and 162 bp of 3′ noncoding region excluding the poly(A) tail. The amino acid sequence deduced from the cDNA contained the sequences of NH_2-terminal and three tryptic peptides. The predicted amino acid composition and molecular weight were in excellent agreement with those obtained with the purified enzyme. Introduction of the cDNA into plasmid pUC118 having the *lac* promoter resulted in a production in *Escherichia coli* of a M_r 26,000 polypeptide in the presence of isopropyl β-D-thiogalactopyranoside. This protein represented as much as 5% of the bacterial soluble protein and showed the guanidinoacetate methyltransferase activity. Sequence analysis and tryptic peptide mapping indicated that the enzyme obtained by the recombinant DNA procedures was structurally identical to the liver enzyme, except for the absence of the NH_2-terminal blocking group. Also, the enzyme showed kinetic properties indistinguishable from those of the liver enzyme.**

Guanidinoacetate methyltransferase (*S*-adenosyl-L-methionine: guanidinoacetate *N*-methyltransferase, EC 2.1.1.2), first found in pig liver by Cantoni and Vignos (1), is the enzyme that catalyzes the last step of creatine biosynthesis. The distribution of guanidinoacetate methyltransferase as well as guanidinoacetate and creatine in the animal kingdom was studied extensively by Van Pilsum *et al.* (2), who showed that methyltransferase occurred in all vertebrates but not in invertebrates. In humans, the biosynthesis of creatine is reported to represent about 75% of the total utilization of methionine through *S*-adenosylmethionine (AdoMet) (3). Guanidinoacetate methyltransferase has been purified to homogeneity from pig and rat liver by Im *et al.* (4) and Ogawa *et al.* (5), respectively, and shown to be a monomeric protein with a relatively small molecular size. From its simple molecular structure, the enzyme is expected to provide a useful system for studying the mechanism of enzymatic methyl group transfer. Guanidinoacetate methyltransferase from rat liver has been shown to possess multiple thiol groups, the integrity of which appears crucial for activity (5).

To clarify the functional role of these cysteines and further define the active site residues that participate in binding and catalysis, knowledge of the primary structure of the enzyme is prerequisite. A relatively low abundance of the enzyme in liver and the difficulty of purifying it in good yield, however, hampered preparation of substantial amounts of the purified protein for sequencing. To avoid this problem we decided to isolate the cDNA clone for the enzyme and deduce the primary structure from its nucleotide sequence. In this communication, we report the isolation and characterization of the cDNA clone for rat liver guanidinoacetate methyltransferase.¶ This paper also describes a method for constructing a recombinant plasmid that produces a protein with guanidinoacetate methyltransferase activity in *E. coli*.

MATERIALS AND METHODS

cDNA Cloning. cDNA clones for guanidinoacetate methyltransferase were screened in a λgt11 library of rat liver cDNAs (6) by using a polyclonal antibody to the enzyme, according to the method of Young and Davis (7) modified by de Wet *et al.* (8) and Ogawa *et al.* (9). The antibody was raised in rabbits and purified by chromatography on a guanidinoacetate methyltransferase-coupled Sepharose 4B column before use. Plaques that produced the chimeric polypeptide were detected by the goat IgG-conjugated peroxidase reaction (Bio-Rad) after the reaction with the monospecific rabbit IgG to the enzyme. Positive clones obtained in the first round of screening were further plaque-purified, and the cDNA inserts were subcloned into the *Eco*RI site of plasmid pBR322. Sequence determination was done by the dideoxy chain-termination method of Sanger *et al.* (10) after subcloning the restriction fragments of the inserts into M13mp18/19 vectors. Restriction enzymes were purchased from Takara Shuzo and Toyobo (Kyoto, Japan) and [α-^{32}P]dCTP (800 Ci/μmol; 1 Ci = 37 GBq) from DuPont/New England Nuclear.

Construction of a Plasmid Expressing Guanidinoacetate Methyltransferase. A plasmid that expresses guanidinoacetate methyltransferase in *E. coli* was constructed as outlined in Fig. 1. The full-length cDNA for guanidinoacetate methyltransferase was inserted in the *Eco*RI site of plasmid pUC118 (Takara Shuzo) to obtain pUCGAT9. The distance between the Shine–Dalgarno sequence (SD sequence) (11) and the initiation codon of the cDNA was made seven nucleotides long as follows. A 25-mer oligonucleotide complementary to the sequence 5′ AGGAAACAGCTATG′AGTTCTTCTGC 3′ was synthesized chemically. The sequence left of the prime corresponds to the sequence including the

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Abbreviations: AdoMet, *S*-adenosyl-L-methionine; IPTG, isopropyl β-D-thiogalactopyranoside.

¶This sequence is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03588).

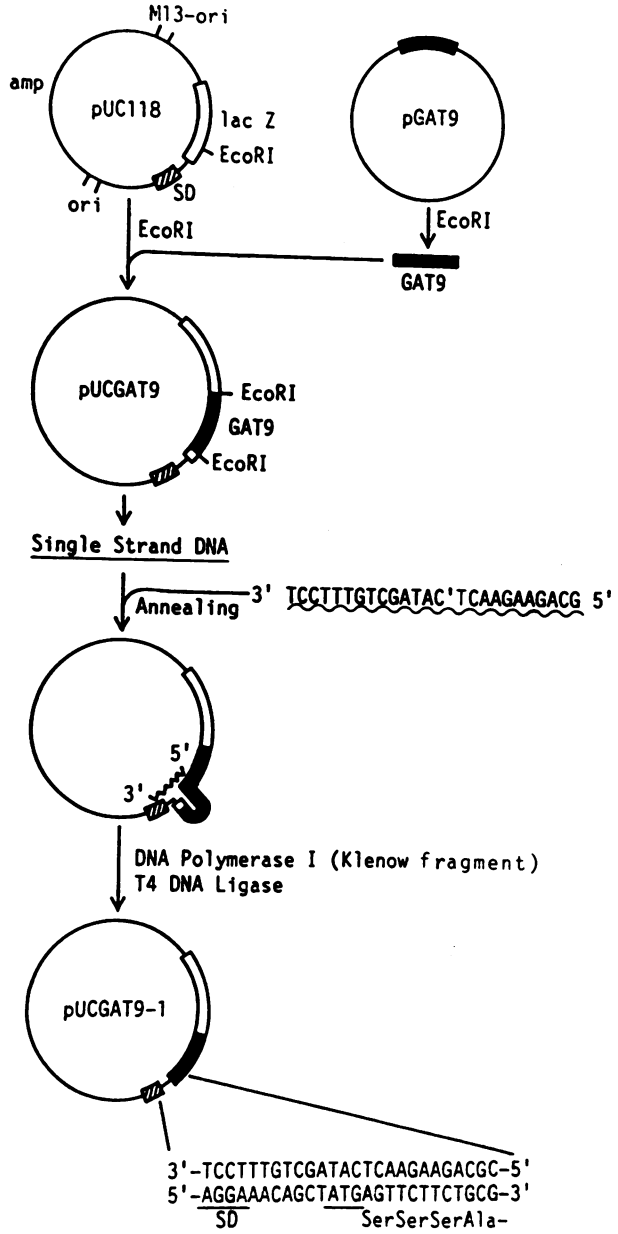

FIG. 1. Schematic representation of the construction of a guanidinoacetate methyltransferase-expressing vector. SD, Shine–Dalgarno.

Shine–Dalgarno sequence and the ATG codon of the vector, and the sequence right of the prime represents nucleotides 55–65 of the cDNA (see Fig. 3). The single-stranded pUCGAT9 DNA and the 5′ phosphorylated oligonucleotide were mixed in an equimolar ratio and heated at 100°C for 1 min in a buffer containing 30 mM Tris·HCl, pH 7.9/120 mM NaCl/6 mM $MgCl_2$/10 mM 2-mercaptoethanol. The mixture was then cooled gradually from 60°C to 28°C over a period of 3 hr. The double-stranded DNA was prepared with DNA polymerase I (Klenow fragment) and T4 DNA ligase and was used to transform *E. coli* MV1304. The recombinant *E. coli* clone was selected by colony hybridization (12). For this the filters were incubated in a solution of 0.6 M sodium chloride/60 mM sodium citrate/30% formamide and the ^{32}P-labeled 25-mer probe at 37°C for 12 hr, washed extensively in a solution of 75 mM sodium chloride and 7.5 mM sodium citrate at 45°C, and subjected to autoradiography. The recombinant plasmid was designated pUCGAT9-1.

Purification of the Bacterial Guanidinoacetate Methyltransferase. *E. coli* carrying the vector pUCGAT9-1 was grown in 500 ml of a medium containing 8 g of trypton, 5 g of yeast extract, 2.5 g of sodium chloride, and 17.5 mg of ampicillin. When the cell turbidity measured at 600 nm reached an absorbance of 0.2, isopropyl β-D-thiogalactopyranoside (IPTG) was added to a concentration of 1 mM, and culture was continued for an additional 7 hr. The cells harvested by centrifugation were suspended in 25 ml of 50 mM Tris·HCl, pH 7.5, containing 2 mM EDTA and disrupted by treatment with egg white lysozyme (1 mg/ml) for 30 min in ice. Following freezing-and-thawing and brief sonication, the cell debris was removed by centrifugation. The supernatant was then treated with ammonium sulfate, and the precipitate obtained between 40% and 55% saturation was dissolved in 1 ml of 10 mM Tris·HCl, pH 7.5/1 mM EDTA/1 mM dithiothreitol. The solution was directly loaded on a column of Sephadex G-100 (32 × 980 mm), equilibrated, and eluted with the same buffer. The active fractions were combined and applied to a column of DEAE-cellulose (DE-52, 10 × 50 mm) that had been equilibrated with 10 mM Tris·HCl, pH 7.5/1 mM EDTA. The enzyme was eluted by a linear gradient between 60 ml each of 10 mM Tris·HCl, pH 7.5/1 mM EDTA/1 mM dithiothreitol, and 70 mM potassium phosphate, pH 7.2/1 mM EDTA/1 mM dithiothreitol. The enzyme was concentrated by ultrafiltration and stored at −20°C.

Protein Chemistry. The rat liver guanidinoacetate methyltransferase was purified by the method of Ogawa *et al.* (5), except that 1 mM dithiothreitol was included in all buffers and the isoelectric focusing step was omitted. Hydroxylapatite chromatography in 5 mM potassium phosphate at pH 6.8, rather than at pH 7.2, was effective in separating the enzyme from contaminating proteins, and this obliterated the use of the isoelectric focusing step. Carboxymethylation and proteolytic cleavage of the liver and recombinant guanidinoacetate methyltransferases were done according to standard methods (13, 14). Tryptic peptides were separated by HPLC on a Toyo Soda CCP 8000 liquid chromatograph with a TSK ODS 120T column (4.6 × 250 mm) (Toyo Soda) using a linear gradient from 0.05% trifluoroacetic acid to 0.05% trifluoroacetic acid containing 80% acetonitrile in 60 min at a flow rate of 0.8 ml/min. Purification of peptides was done by rechromatography under the same conditions. Amino acid analysis was done by the precolumn derivatization method using phenylisothiocyanate (15, 16). Amino acid sequence was determined by automated Edman degradation on a 470A gas-phase sequencer (Applied Biosystems, Foster City, CA).

Enzyme Assay. Guanidinoacetate methyltransferase activity was determined spectrophotometrically by a coupled assay with *S*-adenosylhomocysteinase and adenosine deaminase. The enzyme was added to a reaction mixture containing 50 mM potassium phosphate, pH 8.0/20 μM AdoMet [purified by reverse-phase chromatography (17)]/0.5 mM guanidinoacetate and sufficient amounts of *S*-adenosylhomocysteinase and adenosine deaminase, and the resulting decrease in absorbance at 265 nm due to the overall conversion of AdoMet to inosine was followed in a Hitachi 320 spectrophotometer at 30°C. *S*-Adenosylhomocysteinase was purified from rat liver by the method of Fujioka and Takata (18), and adenosine deaminase was obtained from Sigma.

Other Methods. Total RNA was extracted from fresh rat livers by the method of Chirgwin *et al.* (19), and poly(A)-containing RNA was enriched by oligo(dT)-cellulose chromatography (20). Poly(A)-containing RNA was denatured with formaldehyde/formamide (21), run on a 1.5% agarose gel, and blotted on a nylon membrane filter (GeneScreen*Plus*, DuPont/New England Nuclear) according to the method recommended by the manufacturer. The mRNA for guanidinoacetate methyltransferase was detected with the ^{32}P-labeled cDNA probe. $NaDodSO_4$/polyacrylamide gel electrophoresis was done by the method of Laemmli (22). The gel

was stained with Coomassie brilliant blue, and protein bands were quantified by scanning the gel in a Shimadzu CS-910 TLC scanner. Protein content was determined by the method of Lowry *et al.* (23) with the purified guanidinoacetate methyltransferase as the standard.

RESULTS AND DISCUSSION

Cloning and Sequencing of the Guanidinoacetate Methyltransferase cDNA. A λgt11 library containing rat liver cDNAs was screened for plaques that reacted with the purified antibody to guanidinoacetate methyltransferase. Five positive clones were isolated from 10^5 plaques. Because about 800-fold purification is required to obtain pure enzyme from liver (5), and statistically one-sixth of the insert population in the library would have a correct frame and orientation, the frequency of occurrence of positive clones appears reasonable. The insert cDNAs from positive clones were found to be 700–1000 bp in length and had overlapping restriction maps. The sequencing strategy and the complete nucleotide sequence of the largest insert, GAT9, are shown in Figs. 2 and 3, respectively. The insert consisted of an open reading frame of 711 bp spanning the ATG at position 52 and the TGA at 762, a 5′ noncoding sequence of 51 bp, and a 3′ noncoding region with 162 bp excluding the poly(A) tail. The sequence TC-$^{-3}_{A}$CC$^{1}_{A}$TGA is similar to Kozak's consensus CC$^{-3}_{A}$CC$^{1}_{A}$T-GG (24, 25), which is identified as the optimal sequence for initiation by eukaryotic ribosomes. Other ATG codons in this or other frames do not form a part of the consensus. A poly(A) addition signal AATAAA (26) was found at positions 905–910.

Table 1 shows that the overall amino acid composition of the deduced sequence (Fig. 3) is in excellent agreement with that obtained after acid hydrolysis of the purified protein. The calculated M_r of the deduced sequence (excluding the first methionine, see below) is 26,141, which is very close to a value of 26,000 estimated by $NaDodSO_4$/polyacrylamide gel electrophoresis of the protein (5).

Edman degradation of guanidinoacetate methyltransferase failed to release appreciable amounts of phenylthiohydantoin amino acid derivative suggesting that the NH_2 terminus of the enzyme is blocked. To determine the NH_2-terminal sequence the enzyme was digested with trypsin, and the resulting peptides were separated by HPLC and screened for the peptide with a blocked NH_2 terminus (see *Materials and Methods*). The peptide that was eluted at 41 min (data not shown) was found to be resistant to Edman degradation and to have an amino acid composition corresponding to a segment of the translated sequence from serine at position 2 to arginine at position 21. This peptide was further cleaved with chymotrypsin, and an NH_2-terminally blocked peptide with amino acid composition $Ser_4Ala_2ProLeu$ was obtained. Treatment of the octapeptide with acyl amino acid-releasing enzyme (Takara Shuzo) [substrate/enzyme = 100 (molar ratio), 40 hr at 25°C in 0.1 M potassium phosphate, pH 7.2], and subsequent analysis of the resulting peptide on a gas-

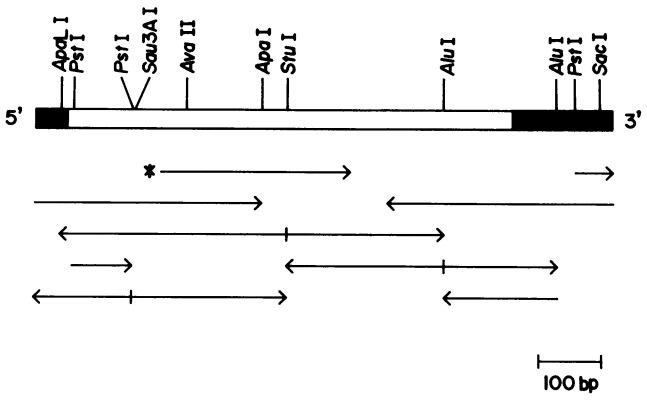

FIG. 2. Partial restriction map and sequencing strategy. The cDNA from plasmid pGAT9 was digested with the restriction endonucleases indicated, and the fragments were subcloned in the multiple cloning sites of M13mp18/19 and sequenced by the method of Sanger *et al.* (10). Arrows indicate direction and extent of the restriction fragments sequenced. Arrow with asterisk, fragment obtained from another clone; open bar, coding region.

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                                                    5'  CCTGGTGGTTCCGCAGCCGTACTCTCCTGGCCTGGTGTGCACAGCCTCACC 51

ATG AGT TCT TCT GCA GCC AGC CCG CTT TTC GCG CCT GGC GAG GAC TGC GGC CCC GCG TGG 111
Met Ser Ser Ser Ala Ala Ser Pro Leu Phe Ala Pro Gly Glu Asp Cys Gly Pro Ala Trp
     1                                  10
CGC GCG GCC CCC GCG GCC TAT GAT ACG TCT GAC ACG CAC CTG CAG ATC CTG GGC AAG CCA 171
Arg Ala Ala Pro Ala Ala Tyr Asp Thr Ser Asp Thr His Leu Gln Ile Leu Gly Lys Pro
20                                 30
GTA ATG GAG CGT TGG GAG ACC CCC TAC ATG CAT TCG CTG GCG GCT GCT GCT GCC TCC AGA 231
Val Met Glu Arg Trp Glu Thr Pro Tyr Met His Ser Leu Ala Ala Ala Ala Ala Ser Arg
40                                 50
GGG GGC CGG GTC CTG GAA GTG GGC TTT GGG ATG GCC ATT GCA GCC TCC AGG GTG CAG CAG 291
Gly Gly Arg Val Leu Glu Val Gly Phe Gly Met Ala Ile Ala Ala Ser Arg Val Gln Gln
60                                 70
GCC CCC ATA AAG GAA CAC TGG ATT ATT GAA TGC AAC GAT GGG GTC TTC CAG CGT CTA CAA 351
Ala Pro Ile Lys Glu His Trp Ile Ile Glu Cys Asn Asp Gly Val Phe Gln Arg Leu Gln
80                                 90
AAC TGG GCC CTG AAG CAG CCA CAT AAG GTT GTT CCC TTG AAA GGC CTG TGG GAG GAG GAG 411
Asn Trp Ala Leu Lys Gln Pro His Lys Val Val Pro Leu Lys Gly Leu Trp Glu Glu Glu
100                                110
GCA CCT ACA CTG CCT GAT GGT CAC TTT GAT GGG ATT CTA TAC GAC ACA TAT CCA CTG TCT 471
Ala Pro Thr Leu Pro Asp Gly His Phe Asp Gly Ile Leu Tyr Asp Thr Tyr Pro Leu Ser
120                                130
GAA GAG ACC TGG CAC ACT CAC CAG TTC AAC TTT ATT AAG ACT CAT GCT TTC CGT TTG CTG 531
Glu Glu Thr Trp His Thr His Gln Phe Asn Phe Ile Lys Thr His Ala Phe Arg Leu Leu
140                                150
AAG CCT GGG GGT ATC CTC ACT TAC TGC AAC CTC ACG TCC TGG GGG GAA CTC ATG AAG TCC 591
Lys Pro Gly Gly Ile Leu Thr Tyr Cys Asn Leu Thr Ser Trp Gly Glu Leu Met Lys Ser
160                                170
AAG TAC ACA GAC ATC ACT GCC ATG TTT GAG GAG ACT CAG GTG CCT GCA CTG CTG GAA GCT 651
Lys Tyr Thr Asp Ile Thr Ala Met Phe Glu Glu Thr Gln Val Pro Ala Leu Leu Glu Ala
180                                190
GGC TTC CAG AGA GAA AAC ATC TGT ACA GAG GTG ATG GCG CTG GTG CCC CCA GCC GAC TGC 711
Gly Phe Gln Arg Glu Asn Ile Cys Thr Glu Val Met Ala Leu Val Pro Pro Ala Asp Cys
200                                210
CGC TAC TAT GCC TTC CCT CAG ATG ATC ACA CCC CTG GTC ACC AAG CAC TGA GCGGCTGGCCC 773
Arg Tyr Tyr Ala Phe Pro Gln Met Ile Thr Pro Leu Val Thr Lys His
220                                230                 235
AGGGCTACAAGGAGAATATGTCCTCCTCAGTGCCTTTGTAGCTGGAGTGTGGCTCCAGCCTCTCCACTATCCCTGCAGT 852

GTGACATCCTAACCTCTGCCTGGTACGGCCATCTCCCCAGAGCTCAGGAGTAAAATAAATGCTACCAAGACTAAAAAAA 931

AAAAAAAAAA 3'                                                                    941
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FIG. 3. Nucleotide sequence of the cDNA for rat guanidinoacetate methyltransferase and deduced amino acid sequence. The nucleotide sequence numbers are shown at right margin, and amino acid sequence numbers are shown below the amino acids. Peptides for which the sequences are determined are underlined. A poly(A) addition signal is boxed.

Table 1. Amino acid composition of guanidinoacetate methyltransferase

Amino acid	Number of residues per enzyme molecule: Derived from cDNA sequence	Amino acid analysis of purified protein*
Aspartic acid	9	13
Asparagine	5	
Threonine	16	15
Serine	11	12
Glutamic acid	17	30
Glutamine	10	
Proline	18	20
Glycine	15	16
Alanine	26	27
Half-cystine	5	3
Valine	11	12
Methionine	7	5
Isoleucine	11	10
Leucine	21	21
Tyrosine	8	6
Phenylalanine	10	9
Histidine	9	8
Lysine	10	10
Arginine	9	9
Tryptophan	7	5

*From ref. 5.

phase sequencer revealed the sequence Ser-Ser-Ala-Ala-Ser-Pro-Leu, corresponding to residues 3–9 of the deduced sequence. Thus, the NH_2-terminal sequence is identical to that deduced from the cDNA except for the absence of methionine. Lack of methionine in the protein suggests that the NH_2-terminal methionine is removed by posttranslational modification and that the new NH_2-terminal serine is then modified. Carboxypeptidase A digestion of the enzyme [substrate/enzyme = 100 (molar ratio), 60 min at 25°C in 0.1 M *N*-ethylmorpholine acetate, pH 8.5], resulted in the release of leucine, valine, threonine, lysine, and histidine in equimolar amounts. These amino acids occur at the COOH-terminal end of the cDNA-derived sequence. Furthermore, three peptides of known sequence are found in the translated sequence (Fig. 3). From the above evidence the cDNA sequence appears to encode the entire amino acid sequence of guanidinoacetate methyltransferase.

Protein sequences similar to guanidinoacetate methyltransferase were not found in a search of the National Biomedical Research Foundation data base (10.0 release), using the program of Lipman and Pearson (27). Primary structures of several eukaryotic AdoMet-dependent methyltransferases, including rat glycine *N*-methyltransferase (28), bovine hydroxyindole *O*-methyltransferase (29), and bovine phenylethanolamine *N*-methyltransferase (30), are now known. No significant sequence similarity is found even among methyltransferases. No particular region common to methyltransferases appears to exist, at least on the level of primary structure.

Size of the Guanidinoacetate Methyltransferase mRNA. Poly(A)-containing RNA from fresh rat liver was denatured, size-fractionated on agarose gel, and blotted on a membrane filter. Hybridization with ^{32}P-labeled cDNA showed that guanidinoacetate methyltransferase mRNA was composed of a single component with ≈1200 nucleotides (Fig. 4).

Expression of the cDNA Sequence in *E. coli*. It is empirically known that the most ideal distance between the Shine–Dalgarno sequence and the initiation codon in mRNAs of *E. coli* is seven nucleotides (11). Because the 5′ noncoding sequence of guanidinoacetate methyltransferase cDNA is obviously too long for efficient expression when introduced to expression vector pUC118, we deleted a part of the sequence to give the desired length of seven nucleotides as described (Fig. 1). *E. coli* transformed with the recombinant plasmid thus prepared (pUCGAT9-1) was grown for 7 hr in the presence of IPTG, and cells were lysed by treatment with lysozyme/EDTA and sonication. The extract was found to catalyze the guanidinoacetate methyltransferase reaction. $NaDodSO_4$/polyacrylamide gel electrophoresis of the extract showed that an M_r 26,000 protein was induced in the presence of IPTG (Fig. 5, lane 3), which amounted to as much as 5% of the bacterial soluble protein.

Purification and Characterization of the Bacterial Enzyme. The protein having guanidinoacetate methyltransferase activity was purified from *E. coli* extract by ammonium sulfate fractionation, Sephadex G-100 gel filtration, and DEAE-cellulose chromatography as described. On $NaDodSO_4$/polyacrylamide gel electrophoresis the enzyme preparation after DEAE-cellulose chromatography showed a single protein band at M_r 26,000, indistinguishable from the purified enzyme from rat liver (Fig. 5, lanes 6 and 7). About 5 mg of the homogeneous enzyme could be obtained from a 1-liter culture. The identical behavior of the bacterial and liver enzymes on a Sephadex G-100 column indicates that the bacterial enzyme is also monomeric.

The bacterial enzyme had apparent K_m values of 2.7 μM and 32.2 μM for AdoMet and guanidinoacetate, respectively, and a V_{max} value of 130 nmol of AdoMet disappeared per min per mg of protein at pH 8.0 and 30°C. The corresponding values for the liver enzyme were 2.6 μM, 31.3 μM, and 122 nmol per min per mg of protein. Thus, kinetic properties are indistinguishable between the two enzymes.

In contrast to the liver enzyme, Edman degradation of the recombinant enzyme sequentially released phenylthiohydantoin-amino acids. The first eight cycles of degradation gave an amino acid sequence identical to the cDNA-derived

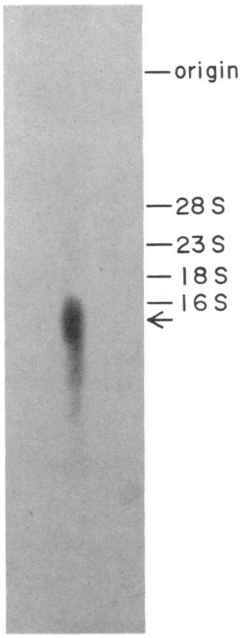

FIG. 4. RNA blot analysis of guanidinoacetate methyltransferase mRNA. Ten micrograms of poly(A)-containing RNA was transferred to a nylon sheet and hybridized with ^{32}P-labeled cDNA as described. The sheet was autoradiographed at −80°C for 16 hr using an intensifying screen. Ribosomal RNAs from rat liver and *E. coli* were used as size markers.

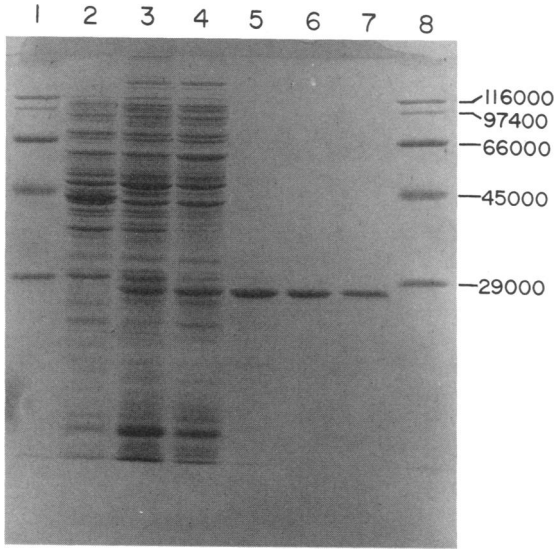

FIG. 5. $NaDodSO_4$/polyacrylamide gel electrophoresis of bacterial guanidinoacetate methyltransferase at each stage of purification. Each sample was denatured by heating at 100°C for 1.5 min in the presence of 1% $NaDodSO_4$ and 2.5% 2-mercaptoethanol, run on a 12.5% $NaDodSO_4$/polyacrylamide gel, and stained with Coomassie brilliant blue. *E. coli* MV1304 cells carrying the plasmid pUCGAT9-1 were grown with or without IPTG, and cell extracts were prepared as described. Lanes: 1 and 8, size markers; 2, extract of *E. coli* grown without IPTG; 3, extract of *E. coli* grown with IPTG; 4, ammonium sulfate fraction; 5, eluate from a Sephadex G-100 column; 6, eluate from a DEAE-cellulose column (1 μg); and 7, purified rat liver enzyme (1 μg).

NH_2-terminal sequence lacking the first methionine. HPLC of the tryptic peptides from the bacterial enzyme showed an elution profile identical to that obtained with the liver enzyme except for one peak (data not shown). The peak at 41 min in the latter, which represents the NH_2-terminal peptide (see above), was replaced by a peak at 39.5 min. The amino acid composition of the 39.5-min peptide, which was susceptible to Edman degradation, agreed with that of the NH_2-terminal peptide from residues 1–20 of Fig. 3. It appears that lack of the NH_2-terminal blocking group makes the peptide more hydrophilic so that it eluted earlier in the chromatography. Thus, we concluded that, in the bacterial environment, the NH_2-terminal methionine is cleaved but modification at the new NH_2 terminus does not occur. The results presented above indicate that the recombinant enzyme is structurally identical to the native rat liver enzyme except for the absence of the NH_2-terminal blocking group.

By use of the recombinant DNA procedures described in this communication it is possible to achieve a high-level expression of rat liver guanidinoacetate methyltransferase in *E. coli*. About 5 mg of the pure protein can easily be obtained from a 1-liter culture by a three-step purification procedure. This contrasts with the yield of the enzyme from the liver; only about 1 mg of the homogeneous enzyme is obtained from 300 g of rat liver by a rather laborious procedure (5). Although the recombinant enzyme has no NH_2-terminal blocking group, it appears to be otherwise identical in structure to the liver enzyme. Catalytic properties are also indistinguishable between the two enzymes. Thus, abundant supply of the material should help elucidate the structure–function relationships of guanidinoacetate methyltransferase through the techniques of x-ray crystallography, chemical modification studies, and site-directed mutagenesis.

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