Journal of Cellular Biochemistry 29:337–349 (1985)
Biochemical and Molecular Epidemiology of Cancer 9–21

Purification of Human DNA (Cytosine-5-)-Methyltransferase

Keith E. Zucker, Arthur D. Riggs, and Steven S. Smith

Department of Molecular Biology, Beckman Research Institute of the City of Hope, Duarte, California 91010

We have developed a facile procedure for the purification of DNA methyltransferase activity from human placenta. The procedure avoids the isolation of nuclei and the dialysis and chromatography of large volumes. A purification of 38,000-fold from the whole cell extract has been achieved. The procedure employs ion exchange, affinity, and hydrophobic interaction chromatography coupled with preparative glycerol gradient centrifugation. A protein of 126,000 daltons was found to copurify with the activity and was the major band seen in the most highly purified material after SDS gel electrophoresis. This observation, coupled with an observed sedimentation coefficient of 6.3S, suggests that the enzyme is composed of a single polypeptide chain of this molecular weight. Hemimethylated DNA was found to be the preferred substrate for the enzyme at each stage in the purification. The ratio of the activity of the purified product on hemimethylated to that on unmethylated M13 duplex DNA was about 12 to 1. Thus, the purified activity has the properties postulated for a maintenance methyltransferase. The availability of highly purified human DNA methyltransferase should facilitate many studies on the structure, function, and expression of these activities in both normal and transformed cells.

Key words: DNA methyltransferase, 5-methylcytosine, hydrophobic interaction chromatography, maintenance methylase

DNA (cytosine-5-)-methyltransferase (E.C.2.1.1.37) has been partially purified from several mammalian sources [1–6]; however, in no case has the enzyme been purified to homogeneity. The low yields of even the partially purified enzyme limit most *in vitro* studies. As a result, very few of the properties of this important mammalian enzyme have been unambiguously determined. In most studies, the rate of transfer of methyl groups from S-adenosyl-L-methionine (SAM) to hemimethylated native DNA was found to be considerably higher than that to unmethylated native DNA [3,7–10]. Thus, the enzymes possess the properties of the "maintenance methy-

Abbreviations used: Tris, tris(hydroxymethyl) aminomethane; EDTA, ethylenediamine tetraacetic acid; TCA, trichloroacetic acid; PEI, poly(ethylene-imine); SDS, sodium dodecyl sulfate.

Received April 17, 1985; revised and accepted August 2, 1985.

lases" whose existence was originally suggested by Holliday and Pugh [11] and by Riggs [12] as part of a proposal for the somatic inheritance of patterns of DNA methylation at d[CG] doublets. According to these hypotheses fully methylated d[CG] doublets in duplex DNA would give rise to two hemimethylated daughter strands in nascent DNA and the maintenance methylase(s) would rapidly replace the missing methyl groups, thereby regenerating the parental pattern in both daughter cells. Since the time of these proposals it has been convincingly demonstrated that hemimethylated DNA, constructed *in vitro*, is converted to fully methylated DNA *in vivo* following transfection into mammalian cells [13]. Moreover, methylation patterns applied *in vitro* are somatically inherited following transfection [14,15].

Available evidence indicates that methylation patterns can set limits on the transcriptional potential of both normal and transformed cells. A strong negative correlation exists between DNA methylation at critical sites within or near a gene and the potential for the expression of that gene. This has lead to the suggestion that loss of DNA methylation is a necessary but not a sufficient condition for gene expression [16,17]. For recent reviews on these topics see Doerfler [18], Riggs and Jones [19], Nyce et al [20], and Cooper [21].

Abnormal methylation patterns associated with transformed cells have been reported [22–26]. In general, DNA from these cells has been found to be hypomethylated compared to normal tissue, suggesting that at least a transient interruption in normal maintenance methylation often occurs during the generation of transformed cells. Clearly, a key control point in processes associated with oncogenic transformation and normal development might be the DNA methyltransferase(s) that appear(s) to be responsible for somatic inheritance of methylation patterns in DNA. Other cell lineage-specific alterations in methylation pattern [27,28] appear to involve *de novo* methylation and thus might originate with modulation of the activity or specificity of the DNA methyltransferases. To begin to understand the processes that control and modulate DNA methylation patterns, detailed studies of the biochemistry and molecular biology of the enzymes must be performed. However, none of the currently available isolation schemes provide a convenient method for the preparation of amounts of highly purified material. In this report we describe a procedure for the large-scale purification of the enzyme from human placenta.

MATERIALS AND METHODS

DNA Methyltransferase Assay

The standard DNA methyltransferase assay was carried out in polypropylene microcentrifuge tubes in a final volume of 0.10 ml containing 50 mM Tris-acetate, pH 7.8; 10 mM Na_2EDTA; 1.0 mM dithiothreitol; 0.01 mg/ml RNase A; 2.0 μM S-adenosyl-L-[^{3}H]methionine (5–15 Ci/mmol, Amersham Corp. Arlington Heights, IL); 10% (v/v) glycerol; and 0.167 mg/ml heat-denatured *Micrococcus lysodeikticus* DNA (Sigma Chemical Co., St. Louis, MO). After preincubating the mixture for 15 min at 37°C, the reaction was initiated with less than 3 μl of the enzyme preparation. Incubation was continued at 37°C for 1 hr, and the reaction was stopped by placing the tube on ice after an equal volume of 100 mM Tris-acetate, pH 8.0, was added. The resulting mixture was extracted with an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1). One hundred forty microliters of the aqueous phase was removed and immediately precipitated with 5.0 ml of 5% (w/v) TCA containing 5.0

mM sodium pyrophosphate in a 5.0-ml polypropylene tube. Precipitates were collected on GF/C filters (Whatman Inc., Clifton, NJ) and washed three times with 5.0 ml of 2% TCA containing 5.0 mM sodium pyrophosphate. Filters were soaked for 20 min in a tray containing 5% TCA, 5 mM sodium pyrophosphate (about 10 ml per filter) at room temperature, and then rewashed twice as described above. A final wash with 5.0 ml of 100% ethanol dehydrated the filters, which were then dried in an oven at 160°C for 5 min and counted in 10 ml of a tolulene-based scintillation fluid. All sets of assays in which differences in salt concentration were introduced by the enzyme preparation were adjusted with NaCl to the highest salt concentration in the series [29]. After the phosphocellulose-batch step, phenol extration was omitted. A unit of activity is that amount of enzyme that will incorporate-1 pmole of [^{3}H]methyl groups into TCA-insoluable material in 1 hr under the above conditions in a reaction mixture containing 30 mM NaCl. To make direct comparisons of the data with those previously reported by other investigators, we also assayed the enzyme from fractions I through VII under the reaction conditions and with the substrates reported by Pfeifer et al [3] and Wang et al [8].

Enzyme Solubilization

All purification steps were carried out at 4°C unless otherwise indicated. Four full-term placentas were collected shortly after delivery. After removal of the cord and outer membrane, the remaining material was minced with scissors and added to an equal volume of a twofold concentrated solution of buffer A (50 mM Tris-acetate, pH 7.8; 0.1 mM Na_2EDTA; 10 mM 2-mercaptoethanol; 25% glycerol). This crude cell suspension was homogenized in a Waring CB-6 Commercial Blender for 10 min. Cell debris was removed by centrifugation at 8,700g for 10 min. PEI (Polymin P, BASF, Ludwigshafen, Federal Republic of Germany) was added to the supernatant (fraction I) to a final concentration of 0.015% using a 10% solution neutralized with HCl as previously described [29]. After 10 min on ice, a precipitate formed. The supernatant fluid (fraction II) was collected by centrifugation at 18,000g for 30 min.

Phosphocellulose Adsorption and Elution

Phosphocellulose (Whatman, P11) was precycled as described by the manufacturer and equilibrated with 0.10 M NaCl in buffer A. Fraction II was mixed with 0.20 volumes of packed phosphocellulose. The mixture was stirred for 30 min, filtered through a sintered glass funnel under vacuum, and washed with at least five column-volumes of equilibration buffer. The methyltransferase was eluted by adding 100 ml of 0.40 M NaCl in buffer A per placenta and the eluate was collected by filtration. This step was repeated once more and the eluates pooled to give fraction III.

Hydroxylapatite Chromatography

Fraction III was mixed with 0.12 volume of packed hydroxylapatite (Bio-gel HTP, Bio Rad Laboratories) that had been equilibrated with 10 mM potassium phosphate in buffer A. The suspension was stirred for 30 min and then loaded into a column. The column was washed with five column-volumes of equilibration buffer and then a six column-volume linear gradient from 10 mM to 500 mM potassium phosphate in buffer A was used to elute the enzyme. Fractions having significant methyltransferase activity were pooled and dialyzed for 12 hr against 25 volumes of

50 mM NaCl in buffer A to give fraction IV. This material could be stored at −20°C for up to 1 week without significant loss of activity. We found it convenient to store material at this stage and have successfully carried out the purification steps described below using the pooled material from two to ten placentas. When the scale of the procedure was changed, column and gradient sizes were proportionally altered.

Cibacron Blue Agarose Chromatography

Fraction IV from four placentas was applied to a 40-ml (2.5 cm × 8 cm) column of Cibacron blue agarose (Bethesda Research Laboratories) equilibrated with 50 mM NaCl in buffer A. After washing with five column-volumes of equilibration buffer, a ten column-volume linear gradient from 50 mM to 2.0 M NaCl in buffer A was applied. Fractions having methyltransferase activity were pooled to give fraction V.

Hydrophobic Interaction Chromatography

Fraction V was adjusted with 5.0 M NaCl to 1.50 M NaCl in buffer A and applied to a 40-ml (1.4 cm × 27 cm) column of dodecyl agarose (Sigma Chemical Co.) equilibrated with the same buffer. The enzyme was eluted with three column-volumes of buffer A containing 1.5 M NaCl. Fractions with significant activity were pooled and immediately applied to a 2.0-ml hydroxylapatite column equilibrated with 10 mM potassium phosphate in buffer B (50 mM Tris-acetate, pH 7.8; 0.10 mM Na_2EDTA; 10 mM 2-mercaptoethanol; 2.0% glycerol). The column was washed with five column-volumes of equilibration buffer and then the methyltransferase was eluted with 0.50 M potassium phosphate in buffer B to yield fraction VI.

Glycerol Gradient Centrifugation

Fraction VI was applied to two linear glycerol gradients (5 to 25% glycerol) in 50 mM Tris-acetate, pH 7.8; 0.10 mM Na_2EDTA; 10 mM 2-mercaptoethanol; and 0.50 M potassium phosphate (12 ml each). Centrifugation was carried out in a Beckman SW 40Ti rotor for 40 hr at 40,000 rpm. Equal-sized fractions (about 0.5 ml each) were collected from the gradients by puncturing the bottom of the tube. Fractions containing significant enzyme activity were pooled from all gradients to give fraction VII.

Analytical Heparin Sephrose Chromatography

Fraction VII was dialyzed for 12 hr against 50 mM NaCl in buffer A and then applied to a 0.50-ml column of heparin sephrose prepared by the method of Iverius [30] and equilibrated with the dialysis buffer. After washing with five column-volumes of the equilibration buffer, the enzyme was eluted with a 5-ml linear gradient from 0.05 M to 2.0 M NaCl in buffer A.

Protein Assays

Protein concentrations were determined by the method of Bradford [31] using bovine serum albumin as standard. PEI does not interfere with this method of protein determination [unpublished observations].

Hemimethylated DNA Synthesis

Hemimethylated DNA was synthesized *in vitro* by the method of Gruenbaum et al [32] using single-stranded M13 phage DNA. Double-stranded unmethylated DNA

was synthesized by the same procedure except that dCTP was used instead of 5-methyl dCTP.

Protein Gel Electrophoresis

The procedures used for sodium dodecyl sulfate slab gel electrophoresis were those of Laemmli [33], with modifications described by Smith and Braun [29]. Dilute protein samples were concentrated by TCA precipitation [29] prior to gel electrophoresis.

RESULTS AND DISCUSSION

Enzyme Solubilization and Poly(ethylene-imine) Treatment

Existing methods for the purification of the methyltransferases from eukaryotic sources have been useful in the characterization of the activity. However, many studies on the structure and function of the enzyme require large amounts of highly purified material. Nuclear isolation and extraction procedures employing high speed centrifugation and dialysis steps prior to column chromatography are part of most published purification procedures [2,3,34]. This sets practical limits on the scale of the purification. The solubilization procedure described here removes these constraints.

Homogenization of placental tissue in buffer A followed by low speed centrifugation to remove cell debris yielded a slightly turbid crude extract. Considerable nucleic acid was solubilized by this procedure. Agarose gel electrophoresis showed that both RNA and DNA were present. Salt extracted from the placental tissue raised the conductivity of fraction I to that of 0.1 M NaCl in buffer A. We compared the enzyme prepared by the method described above with that prepared from nuclei isolated by the method of Wang et al [8]. Detectable activity was considerably higher in these crude extracts than it was in those prepared from isolated nuclei (see Table I). Although the crude extract prepared from isolated nuclei was more highly enriched in methyltransferase than the crude extract prepared by the present procedure, the isolation of nuclei was very time consuming and did not lend itself to larger scale work.

Since it is possible to solubilize substantially more enzyme without the use of nuclear isolation or high salt, the bulk of the enzyme may be loosely associated with the nucleus. This is consistent with the results of Sano et al [4], who reported localizing most of the activity in the cytoplasmic fraction from bovine thymus.

TABLE I. Preparation of Crude Extracts*

Method	Volume (ml)	Activity (U)	Specific activity (units/mg protein)
Low salt whole cell homogenization	621	46,700	1.25
High salt extraction of nuclei	119	29,322	159

*Whole human placentas were dissected and divided into equal portions. One portion was used for the preparation of a crude extract by high salt extraction from isolated nuclei using the method of Wang et al [8]; the other portion was used to prepare a crude extract by the homogenization of whole cells as described in this report. Total enzyme activity and total protein concentration were determined on the crude extracts. The values presented represent the values obtained from a single placenta and are average values obtained for three independent experiments.

PEI treatment efficiently removed nucleic acid from the low salt crude extracts as judged by agarose gel electrophoresis on samples of fraction II (data not shown). However, it also irreversibly inactivated the enzyme at higher concentrations. The optimal amount for removal of nucleic acid was determined by adding increasing amounts of 10% solution of PEI to 1.0-ml aliquots of a representative crude extract [35]. Since the addition of excess PEI resulted in loss of enzyme activity, we found it advisable to determine the optimal PEI concentration required when new lots were prepared. Calibration for each placental preparation has not been necessary.

Phosphocellulose Batch Adsorption and Elution

Because of the large volumes involved at this stage in the purification, batch adsorption and elution is advantageous. Analytical column chromatography of fraction II on phosphocellulose showed that the DNA methyltransferase eluted as a single wide peak at approximately 0.24 M NaCl in buffer A (data not shown). Thus, the enzyme could be adsorbed directly from fraction II to phosphocellulose equilibrated with buffer A containing 0.10 M NaCl. The minimum amount required to adsorb the maximum amount of enzyme was estimated experimentally. Only two thirds of the activity could be removed from the supernatant with excess phosphocellulose. The minimum amount required to adsorb this amount of activity was 0.20 ml packed column material per milliliter of fraction II.

Batch elution with 0.40 M NaCl in buffer A was necessary to recover the bound enzyme. Additional enzyme could not be removed with higher salt washes. This purification step removed more than 99% of the total protein while activating the enzyme to yield over 100% of the total activity found in fraction II, making it a valuable initial step.

Hydroxylapatite Batch Adsorption and Chromatography

Adsorption of fraction III onto hydroxylapatite was also done in batch to facilitate rapid manipulation of large volumes. As much as 1.50 M NaCl in buffer A does not prevent the binding of the DNA methyltransferase to this matrix. Fraction III contained only 0.40 M NaCl and could be bound to hydroxylapatite without dilution or dialysis to lower the salt, making it a convenient purification step. The enzyme from 1.0 ml of fraction III was bound quantitatively by 0.12 ml packed hydroxylapitite.

Analytical column chromatography showed that the DNA methyltransferase could be eluted as a single peak of activity ranging from 0.15 to 0.25 M potassium phosphate in buffer A (data not shown). Extensive washing followed by gradient elution were necessary to obtain maximal purification with hydroxylapatite. The resulting eluate (fraction IV) was purified approximately threefold over that of fraction III, with quantitative recovery of the enzyme activity. The large concentration of enzyme activity that resulted at this step facilitated manipulations at subsequent stages in the purification.

Dye-Ligand Chromatography

The results of chromatography on Cibacron blue agarose have not been previously reported for the human enzyme. Gradient elution resulted in a single symmetrical peak at about 1.10 M NaCl in buffer A. The enzyme was enriched about fivefold by this chromatographic step with a yield of slightly over 100%. Fraction V consists

of at least 100 different polypeptide chains (as shown in Fig. 4), suggesting that considerable further purification is required at this point. When we tested column matrices that had been successfully used to purify the enzyme in other published procedures, no more than a threefold purification beyond fraction V was obtained. Affinity steps like DNA cellulose and S-adenosyl-L-homocysteine agarose also gave little purification at this stage.

Hydrophobic Interaction Chromatography

Chromatography of fraction V on hydrophobic interaction columns of increasing carbon chain lengths revealed that a chain length of 12 carbons was useful for DNA methyltransferase purification. In experiments like that shown in Figure 1, the enzyme was applied to the column at 1.5 M NaCl. Under these conditions, the material that did not bind tightly to the column contained more than 80% of the input activity and was highly purified. Two major peaks of protein are seen in the weakly bound material. One class of protein, including DNA methyltransferase, eluted after about 3.5 column-volumes, whereas a second class of protein eluted after about seven column-volumes. However, more than 90% of the input protein was retained by the

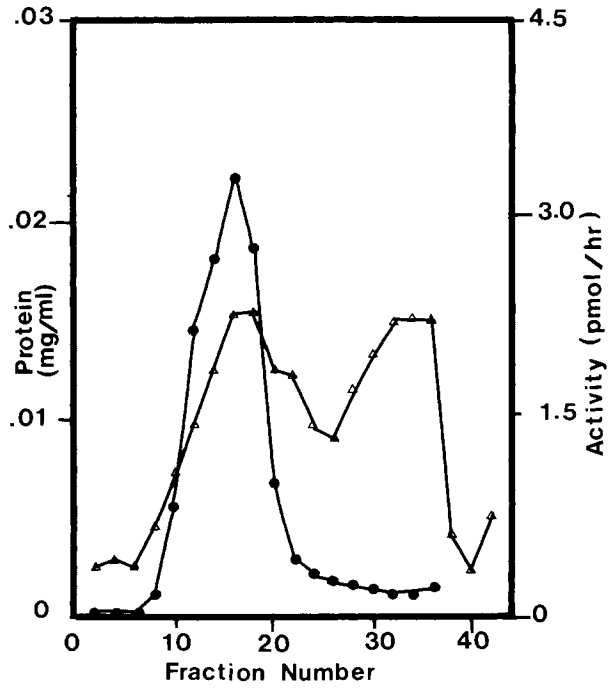

Fig. 1. Dodecyl agarose chromatography. The pooled active fractions from the Cibacron blue agarose column (fraction V) were adjusted to 1.5 M NaCl in buffer A and applied to a 1.4 cm × 27 cm column of dodecyl agarose equilibrated with the same buffer. After the sample was applied, the column was washed with three column-volumes of the same buffer. Fractions (8 ml) were collected at a flow rate of 40 ml/hr. (△), Protein concentration; (●), methyltransferase activity.

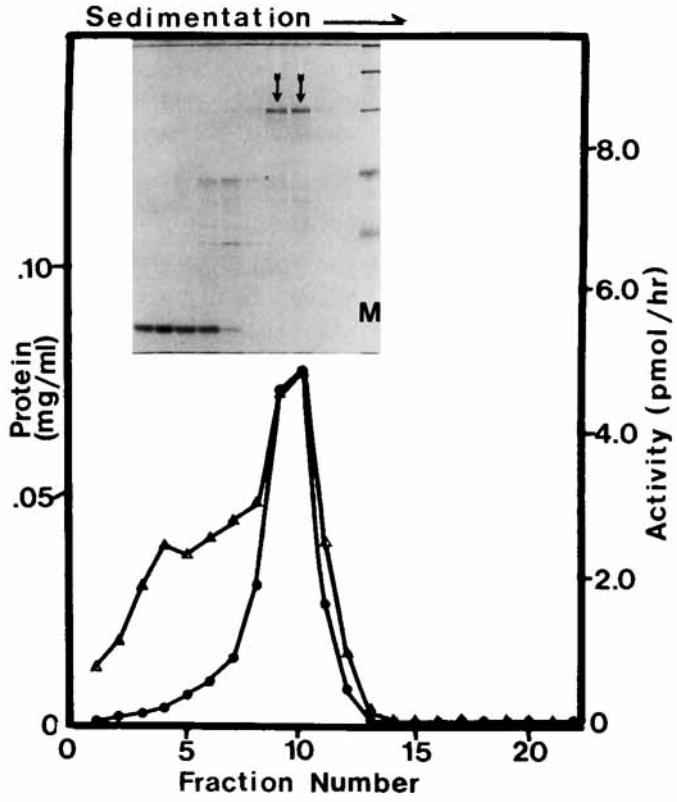

Fig. 2. Preparative glycerol gradient sedimentation. DNA methyltransferase (0.9 ml of fraction VI) was layered onto a 5 to 25% glycerol gradient (12 ml) in 50 mM Tris-acetate, pH 7.8; 0.10 mM Na_2EDTA; 10 mM 2-mercaptoethanol; and 0.5 M potassium phosphate and sedimented as described in Materials and Methods. The gradients were fractionated into about 23 equal 0.5-ml fractions. Total activity and protein were determined for each of the fractions in the gradient. (△), Protein concentration; (●), methyltransferase activity. Inset: SDS gel electrophoretic profile for fractions 3 through 12 from the gradient. The last lane on the right of the photograph (M) contained molecular weight markers as follows: myosin, 205,000; β-galactosidase, 116,000; bovine serum albumin, 66,000; and ovalbumin, 45,000. The two peak fractions are marked with vertical arrows.

column. These columns were not reused. Alternatively, the enzyme could be bound at salt concentrations just above 1.5 M and eluted with a gradient to lower salt.

When the chromatography is carried out at high salt, as shown in Figure 1, dialysis of fraction V can be avoided. Further, because the enzyme does not bind to the column, it is possible to perform this step very rapidly. Since more than 90% of the protein is removed and the volume increases slightly, the methyltransferase emerges from the column at very low protein concentration. It does not store well at this stage and should be concentrated immediately. Hydroxylapatite provides a rapid concentration method. The specific activity of the enzyme actually decreases about 40% after this concentration step. The overall purification obtained over the Cibacron blue pool is still tenfold. It is possible to elute the enzyme from hydroxylapatite in a buffer containing only 2% glycerol, permitting direct loading of fraction VI onto the glycerol gradients.

TABLE II. Large-Scale Purification of DNA Methyltransferase From Human Placenta*

Fraction	Volume (ml)	Total units (U)	Total protein (mg)	Specific activity (U/mg)	Yield purification %	Yield purification Fold	Activity ratio
Fraction I, crude extract	2,640	206,400	137,000	1.50	100	1.0	—
Fraction II, PEI	2,452	141,200	122,400	1.20	68	0.77	—
Fraction III, phosphocellulose	1,372	168,000	932	180	81	120	10.4
Fraction IV, hydroxylapatite	170	168,400	343	490	82	328	8.9
Fraction V, Cibacron blue	87	173,600	68	2,600	84	1,690	5.2
Fraction VI, dodecyl-HAP[a]	2.0	34,360	1.5	24,000	17	15,200	10.0
Fraction VII, glycerol gradient	2.9	15,680	0.27	58,000	7.6	38,300	12.3

*All data are averages for three independent preparations. The activity ratio is defined as the enzyme activity on hemimethylated duplex M13 RF DNA divided by the enzyme activity on unmethylated duplex M13 RF DNA under the conditions described in Materials and Methods.

[a]Dodecyl-HAP, pooled material after dodecyl agarose chromatography and hydroxylapatite concentration.

Size Fractionation Using Glycerol Gradient Centrifugation

The last step in the purification procedure involved size fractionation of fraction VI. A typical glycerol gradient profile is shown in Figure 2. The DNA methyltransferase activity sedimented at a rate faster than any other protein in the mixture. Fraction VII showed a purification of about 2.5-fold with slightly less than 50% of the total activity retained, on average. Analytical glycerol gradients using internal marker enzymes confirmed the results of Pfeifer et al [3] by showing that the DNA methyltransferase had a sedimentation coefficient of approximately 6.3S (data not shown). The specific activity of the peak fractions in glycerol gradients from independent preparations was constant. Moreover, this peak was resolved from other protein in the profile and appears to be composed largely of active enzyme.

Evidence for Enzyme Purity

The specific activity of the most highly purified enzyme was 58,000 units mg of protein under the standard assay conditions. The specific activity of fraction VII was also determined using the reaction conditions described by Wang et al [8] or by Pfeifer et al [3] in one experiment. The resulting values were 123,000 units mg of protein using the *de novo* substrate and reaction conditions employed by Wang et al [8] and 60,800 units/mg under the conditions employed by Pfeifer et al [3]. Thus, the enzyme described here is more than 28-fold higher in specific activity than that obtained with these two alternative preparation methods. Storage of fraction VII at −20°C resulted in the loss of 50% of the activity in 8 weeks. The purification is summarized in Table II. The final product (fraction VII) was purified 38,000-fold over crude extracts, with an apparent 8% recovery of the total enzyme activity.

An SDS polyacrylamide gel analysis of the steps in the purification is shown in Figure 3. Increasing purification is seen with each step in the procedure. Fraction VII

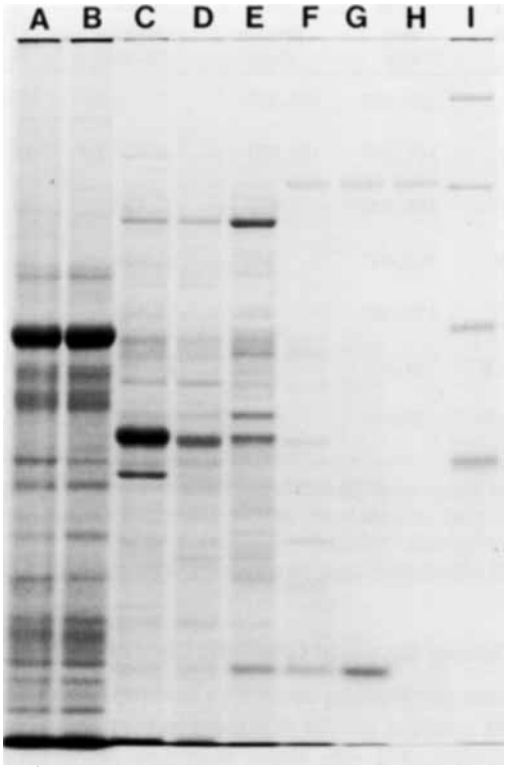

Fig. 3. Polyacrylamide sodium dodecyl sulfate slab gel electrophoresis of the fractions obtained in the purification of DNA methyltransferase. Sodium dodecyl sulfate slab gel electrophoresis and staining conditions were as described in Materials and Methods. The gel shown contains 10% polyacrylamide. Lane A) 250 μg fraction I; lane B) 250 μg fraction II; lane C) 50 μg fraction III; lane D) 50 μg fraction IV; lane E) 50 μg fraction V, lane F) 20 μg pooled dodecyl agarose peak; lane G) 20 μg fraction VI; lane H; 8.0 μg fraction VII. Lane I molecular weight markers: myosin, 205,000; β-galactosidase, 116,000; bovine serum albumin, 66,000; and ovalbumin, 45,000.

shows one major band of protein at about 126,000 daltons, suggesting it is a major component of the enzyme.

Additional evidence for purity is seen from the elution profile for fraction VII on heparin sepharose (Fig. 4). This matrix gives a fivefold purification of enzyme from fraction IV. Total protein and enzyme activity from fraction VII chromatographed on heparin sepharose. Neither protein nor activity were detected in the flow-through or wash fractions. Moreover, the SDS polyacrylamide gel profile of the pooled peak fractions from this profile show no detectable difference between this material and that of fraction VII (data not shown), suggesting that the enzyme was very highly purified before chromatography on heparin sepharose.

Maintenance and *De Novo* Enzyme Activity

The ratio of enzyme activity on DNA that contains hemimethylated sites to that on double-stranded unmethylated DNA was determined for each stage in the purification. The results (Table II) show that the DNA methyltransferase has a clear

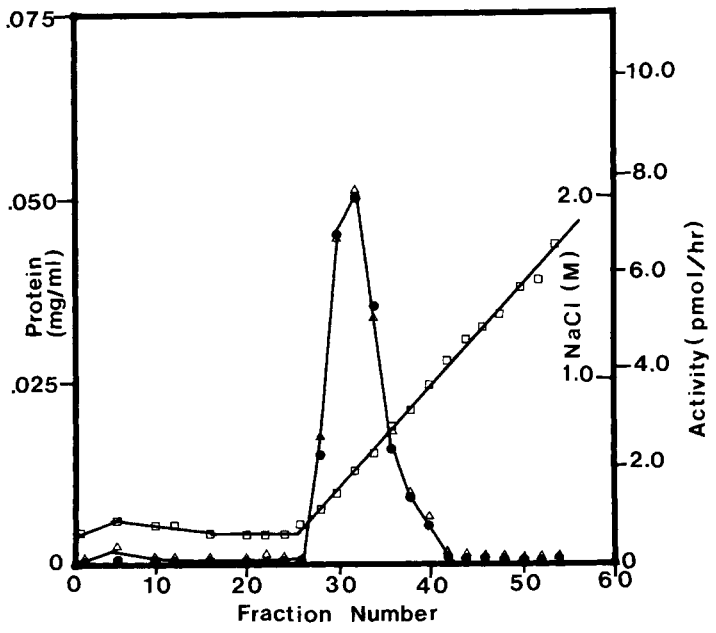

Fig. 4. Cochromatography of total protein and enzyme activity. Peak fractions from the glycerol gradient were pooled (fraction VII), dialyzed against buffer A containing 50 mM NaCl, and applied to a 0.50-ml column of heparin sepharose. After washing the column with five column-volumes of equilibration buffer, it was eluted with a linear gradient from 50 mM to 2.0 M NaCl in buffer A (5.0 ml total). Fractions (0.20 ml) were collected at a flow rate of 1.0 ml/hr. (□), NaCl concentration; (△), protein concentration; (●), methyltransferase activity.

preference for hemimethylated DNA. Activity ratios for fractions I and II could not be determined accurately. The observation of the relatively low activity ratio for fraction V was reproducible.

The results of analytical chromatography and sodium dodecyl sulfate gel electrophoresis on fraction VII also suggest that the enzyme is nearly homogeneous. The SDS gel profile of fraction VII shows that it is composed primarily of a single polypeptide chain of 126,000 daltons. If the enzyme is a typical globular protein, then the observed sedimentation coefficient of 6.3S suggests that it is composed of a single polypeptide chain. However, two additional minor bands of 57,000 and 66,000 account for about 5% of the total protein based on the results of densitometric scanning of Coomassie-stained gels. These two bands were also seen in gels after analytical heparin sepharose column chromatography. Gels stained with silver by the method of Merril et al [35] showed no additional bands.

The DNA methyltransferase purified by this procedure was able to catalyze both the *de novo* and maintenance methylation reactions. Both catalytic activities copurify with the 126,000-dalton polypeptide, and both are demonstrable at each stage in the purification. This result strengthens suggestions [3,8,34,36] that these activities reside on the same enzyme molecule. The maintenance activity of the most highly purified material, as measured by the rate of transfer of methyl groups to hemimethylated M13 duplex DNA, is about 12-fold higher than the *de novo* activity

on unmethylated M13 duplex DNA. At each stage in the purification, the rate of maintenance methylation was higher than the *de novo* rate by an average factor of 9.4.

Since this enzyme may carry out *de novo* methylation *in vivo* and since it seems likely that the selectivity of this process must be highly regulated, it is possible that the reaction conditions could be optimized *in vitro* to permit higher rates of activity on double-stranded duplex DNA. The availability of the enzyme in pure form and in relatively large amounts should permit studies of this type on both the maintenance and *de novo* activities. Moreover, studies of the structure, function, regulation, and expression of DNA methyltransferase activities can now be performed.

ACKNOWLEDGMENTS

This work was supported by the following grants from the N.I.H.: S07RR05841 (S.S.S.), GM32863 (S.S.S.), GM312163 (A.D.R.), and CA2652905 (J.R. Benfield). We also acknowledge support from the Council for Tobacco Research U.S.A., Inc. (grant 1571 to S.S.S.). In addition, we thank Sally Wilkins for technical assistance.

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