De Novo and Maintenance DNA Methylation by a Mouse Plasmacytoma Cell DNA Methyltransferase*

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A DNA methyltransferase of $M_r = 140,000$ that is active on both unmethylated and hemimethylated DNA substrates has been purified from the murine plasmacytoma cell line MPC 11. The maximal rate of methylation was obtained with maintenance methylation of hemimethylated Micrococcus luteus or M13 DNAs. At low enzyme concentrations, the highest rate of de novo methylation occurred with single-stranded DNA or relatively short duplex DNA containing single-stranded regions. Strong substrate inhibition was observed with hemimethylated but not unmethylated DNA substrates. Fully methylated single-stranded M13 phage DNA inhibited neither the de novo nor the maintenance reactions, but unmethylated single-stranded M13 DNA strongly inhibited the maintenance reaction. The kinetics observed with hemimethylated and single-stranded substrates could be explained if the enzyme were to bind irreversibly to a DNA molecule and to aggregate if present in molar excess. Such aggregates would be required for activity upon hemimethylated but not single-stranded DNA. For de novo methylation of duplex DNA, single-stranded regions or large amounts of methyltransferase appear to be required. The relative substrate preference for the enzyme is hemimethylated DNA $> $ fully or partially single-stranded DNA $> $ fully duplex DNA.

5-Methylcytosine, the only modified base detected in mammalian DNA, occurs at a frequency of 2-7% of cytosine bases (1), mostly in the sequence CG (2). However, not all CG sites in eukaryotic DNA are methylated, so that various methylation patterns exist for many genes among different cell types. The exact function and origin of this variability are not understood, though DNA methylation has been inversely correlated with the degree of expression of certain genes (3-5).

The mechanism by which particular methylation patterns are maintained has been the focus of considerable attention. CG sites are methylated within 3 h of DNA replication (6, 7) by a maintenance methyltransferase activity that recognizes the hemimethylated product of semi-conservative DNA replication and modifies the appropriate cytosine residues on the newly replicated strand (8). Though modification of previously unmethylated sites has been hypothesized to be catalyzed by a different de novo methyltransferase activity (9, 10), there is no direct evidence for distinct maintenance and de novo methylases. Likewise, little is known of mechanisms by which particular regions of DNA become unmethylated, though such changes could arise by direct demethylation (11), by blocking maintenance activity for one round of replication, or by DNA repair events which fail to restore pre-existing methylation patterns.

A number of mammalian DNA methyltransferases have been partially purified and characterized (4), and all of the preparations tested utilize both unmethylated and hemimethylated DNAs as substrate. However, the relative rates of reaction on these two substrates vary considerably, depending on the source and method of purification of the enzyme (12-14). In addition, while the kinetics of the de novo methylation reaction have been studied in some detail using a partially purified rat liver methylase (15-17), analogous studies of maintenance methylation have only recently been initiated (4, 14). In this paper we report the isolation of a DNA methyltransferase from the murine plasmacytoma cell line MPC 11 and properties of both its de novo and maintenance activities.

EXPERIMENTAL PROCEDURES

The assay and purification of the DNA methyltransferase from the murine plasmacytoma cell line MPC 11 and the preparation of DNA substrates are described in the Miniprint Supplement. Except where noted, the concentrated phenyl-Sepharose pool of enzyme (9000 units/mg of protein with activated M. luteus DNA) was utilized.

RESULTS

Purification of DNA Methyltransferase—Although DNA methyltransferase activities from many mammalian sources have been studied (4), only enzyme from murine erythroleukemia cells and human placenta appears to have been extensively purified. The former was separated into three related species of molecular weights 150,000, 175,000, and 190,000 (18), and the latter might also be present in multiple forms since several placential polypeptides of molecular weights between 56,000 and 159,000 bound to monoclonal antibodies directed against a murine DNA methylase (13).

The Miniprint Supplement describes a method used to purify methyltransferase from the murine plasmacytoma cell
M. luteus reactions containing Sepharose fraction were incubated for the times indicated in 50 μl of M. luteus standard deviation of less than when estimated by usual weight of the peptide agrees well with the size of the activity during glycerol gradient sedimentation and appeared electrophoresis. This polypeptide distributed with enzyme line, MPC 11. The most purified fraction (Fraction 9, Fig. 1S) contained several polypeptides. The major one, constituting about 50% of the sample, had an Mr of approximately 140,000 when estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. This polypeptide distributed with enzyme activity during glycerol gradient sedimentation and appeared to be selected for throughout the purification. As the molecular weight of the peptide agrees well with the size of the methyltransferase estimated from the rate of sedimentation through glycerol (Mr = 130,000), it is believed to be the major active species in the most purified fraction. Two additional polypeptides (Mr = 102,000 and 110,000), present at very low levels in the methyltransferase preparation, also appear to distribute with activity across the glycerol gradient.

Due to the instability and insufficient quantity of the most purified fraction, the phenyl-Sepharose pool from the preceding step of the purification was used in the analysis of substrate specificity. As estimated from Coomassie Blue-stained sodium dodecyl sulfate-polyacrylamide gels, the 140-kD protein made up approximately 5% of the total protein in this sample. A very rough estimate for the methyltransferase concentration in the phenyl-Sepharose fraction would then be 300 pmol of enzyme molecules/µg of protein.

De Novo Methyltransferase Activity—Two substrates, activated and native M. luteus DNA, were utilized to monitor DNA methylase activity during purification of the enzyme. The relative activity with these two substrates varied greatly during purification, though higher or equal levels were always detected with the activated DNA. Activities with the two substrates partitioned similarly during the purification scheme and preferred similar reaction environments, however (see Miniprint Supplement).

When time courses for the methylation of 200 μM native or activated M. luteus DNAs were followed, the reactions were linear for about 15 min, then leveled off, with little increase in methylation after 30 min (Fig. 1). The limits of the reaction were 7- and 13-pmol methyl groups transferred to native DNA and to activated DNA, respectively. Each assay contained 1.2-nmol CG sites, so the limit was probably not due to saturation of potential methylation sites. From a very rough estimate of enzyme purity, it was calculated that approximately 1 pmol

**TABLE I**

Methylation of various fd DNA substrates

| DNA substrate | Amount of enzyme utilized (µg) | 0.3 µg | 1.3 µg | 2.6 µg | 5.1 µg |
|---------------|--------------------------------|-------|-------|-------|-------|
| fdDNAs       |                                | pmol methyl groups transferred |       |       |       |       |
| RF I         |                                | 0.8   | 1.9   | 2.7   | 4.2   |
| RF II        |                                | 0.1   | <0.05 | 2.2   | 3.1   |
| RF III       |                                | 0.7   | 2.0   | 2.6   | 3.6   |
| ss circular  |                                | 0.6   | 1.0   | 2.1   | 2.4   |
| ss linear    |                                | 0.4   | 1.0   | 2.4   | 4.0   |
| Activated M. luteus DNA |               | 0.7   | 4.4   | 10.7  |
of enzyme molecules was added to each assay, in which case the methyltransferase would have turned over only 7 times with native DNA and 13 times with activated DNA during 30 min.

Peculiar effects were noted during the methylation of both activated and native DNA with varying enzyme concentrations (Fig. 2). At the lower enzyme concentrations, the rate of methylation of activated DNA increased roughly linearly with increased enzyme, proceeding at a higher rate than that with the native substrate. However, at higher enzyme concentrations, the reaction with activated DNA substrate reached saturation. In contrast, native DNA was not an efficient substrate at low enzyme concentrations but became progressively a more efficient substrate at the higher concentrations.

The difference in the efficiencies of methylation of the two substrates at low enzyme concentration could indicate that the enzyme prefers DNA that contains nicks, breaks, single-stranded regions, and/or gaps for activity. To explore such a preference, unit length forms of phage fd DNA of the following structures were utilized as substrate: supercoiled circles, nicked circles, double-stranded unit length linear molecules, single-stranded circles, and single-stranded unit length linear molecules (Table I).

Activated M. luteus DNA was generally a more efficient methyl acceptor than any of the fd DNA substrates. The frequency of CG sites in M. luteus DNA is three times higher than that in fd DNA, a fact which might help to account for the preference. Supercoiled and linear duplex DNAs had similar efficiencies as substrate for the methylase at all enzyme concentrations as did linear and circular single-stranded DNA. Form II DNA was active as a substrate at high enzyme concentrations but was virtually inactive at low concentrations. This substrate is analogous to native M. luteus DNA in that both substrates should have a minimum of single-stranded regions and are relatively long. (Form I DNA is presumed to have some single-strand character due to the supercoiling.)

It appears, given the similarities between native M. luteus DNA and nicked circular DNA and between activated M. luteus DNA and the other forms of fd DNA, that, at low concentrations of enzyme, competent substrates for the purified methyltransferase must have single-stranded regions or be relatively short. At higher concentrations such requirements diminish. DNA ends appear not to be required even at low enzyme concentrations.

Maintenance Methyltransferase Activity—One substrate utilized to analyze maintenance methylase activity was hemimethylated M. luteus DNA prepared by nick translation of nicked M. luteus DNA in the presence of deoxy-5-methylcytidine triphosphate to the extent that 35% of the dCMP residues were replaced by deoxy-5-methylcytidine monophosphate. Assuming that the nicking had been random, 46% of

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**Fig. 4. Effect of methyltransferase concentration on methylation of M13 DNA substrates.** Increasing amounts of the concentrated phenyl-Sepharose fraction (roughly 360 fmol of enzyme molecules/μg of protein) were incubated for 15 min in reactions containing the following DNA substrates at 10 μM: hemimethylated ds M13 DNA (35-fmol circles, 10.5-pmol hemimethylated site/assay, ○—○); unmethylated ss M13 DNA (69-fmol circles, 21-pmol CG sites/assay, □—□); unmethylated ds M13 DNA (35-fmol circles, 21-pmol CG sites/assay, ▲—▲). The lower panel is an expanded portion of the curves between 0 and 1 μg of enzyme.

**Fig. 5. Methylation of M13 DNAs.** A, aliquots of either 75 ng (roughly 27 fmol of enzyme molecules) or 225 ng (roughly 80 fmol of enzyme molecules) of the concentrated phenyl-Sepharose fraction were incubated for 15 min in reactions containing the indicated concentrations of hemimethylated M13 DNA. B, reactions were carried out as in A, except that single-stranded unmethylated M13 DNA was the substrate. Values are the average of duplicate assays with a standard deviation of less than 0.1 pmol (curve A) or 0.05 pmol (curve B). Points where the ratio of enzyme molecules to DNA molecules are estimated to be 2 (2E:O) or 1 (1E:O) are indicated.
Aliquots (225 ng) of the concentrated phenyl-Sepharose fraction were assayed under standard conditions as indicated. Rates in parentheses probably do not represent initial rates due to the extent of the reaction. ND, not determined. Where indicated, results are the mean ± S.D. of two determinations.

| Hemimethylated M13 DNA concentration | CG sites/assay | Methyl groups transferred |
|--------------------------------------|---------------|----------------------------|
|                                      | 1-min incubation | 3-min incubation | 15-min incubation |
| µM DNA nucleotide | pmol | pmol/min | pmol | pmol/min | pmol | pmol/min |
|-------------------|-------|----------|------|----------|------|----------|
| 0.5               | 0.52  | 0.05 ± 0.02 | 0.05 | 0.16 ± 0.10 | 0.05 | 0.47 ± 0.01 |
| 0.8               | 0.84  | 0.30 ± 0.02 | 0.30 | 0.58 ± 0.01 | 0.13 | 0.50 ± 0.06 |
| 1.2               | 1.26  | ND       | ND   | 0.65 ± 0.06 | 0.22 | 1.2 ± 0.1 |
| 2.0               | 2.10  | ND       | ND   | 1.2 ± 0.2 | 0.38 | 1.7 ± 0.1 |
| 7.0               | 7.35  | ND       | ND   | 1.1 | 0.37 | 2.1 |
| 15.0              | 15.8  | ND       | ND   | 0.73 | 0.24 | 1.2 |
| 30.0              | 31.5  | ND       | ND   | ND | 0.10 | 0.007 |

The shape of the curve of Fig. 3B was very reproducible and was nearly identical when the less pure DEAE II fraction was used. However, because the M. luteus DNA substrate was a mixture of hemimethylated, fully methylated, and unmethylated sequences, a more definitive analysis of this methylation reaction was not possible with this substrate.

Therefore, the methylation reactions with unmethylated ss, unmethylated ds, or fully hemimethylated ds M13 DNAs, each at DNA nucleotide concentrations of 10 µM, were compared with increasing concentrations of enzyme (Fig. 4). The duplex DNAs were both nicked (Form II) circles, and the DNA concentrations correspond to 35 fmol of ds circle or 69 fmol of ss circles, and 21 pmol of unmethylated CG sites or 10.5 pmol of hemimethylated sites present per reaction. Sigmoidal curves were generated by varying enzyme concentration with hemimethylated DNA and probably unmethylated duplex DNA but not with single-stranded DNA. With 0.5 µg (roughly 180 fmol) of enzyme/reaction, the ratio of activities on hemimethylated DNA to unmethylated ss DNA to unmethylated ds DNA was 19:7:1. At enzyme amounts greater than 2 µg (roughly 720 fmol of enzyme) per assay, the theoretical limit of 10.5 pmol of methyl groups transferred per assay was reached with hemimethylated DNA; however, limits were not achieved with the unmethylated substrates.

To explore the effect of DNA concentration, 0.5–9 µM hemimethylated M13 DNA was treated for 15 min with either 75 or 225 ng of methylase (Fig. 5A). With this uniform substrate, results were more definitive than those obtained with the hemimethylated M. luteus DNA; high concentrations of the hemimethylated M13 DNA completely inhibited methyltransferase activity. Maximal amounts of methylation were observed near a molar ratio of enzyme to DNA molecules of roughly 2:1 at both enzyme concentrations. Moreover, the values for a ratio of 1:1 could be rationalized by assuming the control DNA, the half-maximal velocity was at concentrations less than or equal to 1 µM DNA nucleotide; however, in contrast to that DNA, concentrations above 3 µM DNA nucleotide were inhibitory. Interestingly, the ratio of enzyme to DNA fragments at 3 µM DNA was about 3:1. At DNA concentrations above 9 µM (corresponding to roughly one enzyme molecule per DNA fragment), the methylation rate remained at a constant level which was half the maximal rate observed.

The abbreviations used are: ss, single-stranded; ds, double-stranded; RF, replicative form; BSA, bovine serum albumin; dCTP, deoxy-5-methylcytidine triphosphate; dTTP, deoxythymidylate; PMSF, phenylmethylsulfonyl fluoride; TPCK, l-tosyl-2-phenylethylamide chloromethyl ketone; TLCK, N-α-tosyl-l-lysine chloromethyl ketone; SDS, sodium dodecyl sulfate.
random binding of enzyme to DNA molecules and that only those DNA molecules in the population which have bound more than one enzyme molecule were being methylated. Thus, it appears that the degree of methylation was dependent not only upon the enzyme concentration but also upon the enzyme:DNA ratio.

The methylation levels observed at the lowest concentrations of substrate in Fig. 5A probably do not reflect rates of reaction; for example, with 225 ng of enzyme, over 80% of the CG sites were methylated at DNA concentrations up to 0.8 μM. To measure a rate for the maintenance methylase reaction at these low DNA concentrations, assays containing 225 ng (roughly 80 fmol) of methyltransferase and various concentrations of hemimethylated M13 DNA were monitored at 1, 3, or 15 min (Table II). Presumably, the methylation of hemimethylated DNA at low DNA concentrations was non-linear with respect to time due to exhaustion of substrate. However, there was also a significant slowing between 1 and 3 min with higher DNA concentrations. The maximal initial reaction rate observed was estimated to be roughly 0.38 pmol of methyl transfer per min per 225 ng (80 fmol) of enzyme or roughly 5 methyl groups transferred per min per enzyme molecule. This rate is roughly 50-fold that observed for de novo methylation of ds M13 DNA (see Fig. 4). The half-maximal velocity for hemimethylated M13 DNA, estimated from the data in Table II, was approximately 1 μM, similar to that observed with other substrates.

**Methylation of ss M13 DNA**—Normal substrate saturation curves were obtained using ss M13 DNA and either 75 or 225 ng of the methyltransferase (Fig. 5B), i.e. no inhibition of methylase activity was observed at the higher DNA concentrations, and it would appear that saturation was obtained when the DNA:enzyme molar ratio was 1:1. The half-maximal velocity for methylation of the ss DNA was estimated from these data to occur between 1.5 and 2.5 μM DNA nucleotide.

**Inhibition of Maintenance Methylase by Various DNAs**—When ss M13 DNA which had been methylated to a limit enzymatically was preincubated with the methyltransferase for 5 min at 0 °C and then aliquoted into methylase reactions containing various amounts of unmethylated ss M13 DNA, methylation was not significantly inhibited even at high methylated:unmethylated DNA concentrations (Fig. 6). The fully methylated ss M13 DNA also did not inhibit methyltransferase activity with the hemimethylated substrate when both DNAs were present at 1 μM (data not shown). Pre-exposure of enzyme to poly(dA-dT) also had no effect on methylation (Fig. 6). It appears, therefore, that DNA which does not contain sites available for methylation does not appear to efficiently interact with the methylase.

Unmethylated ss and ds M13 DNA substrates, which are less efficient methyl acceptors than hemimethylated M13 DNA, were also tested for their ability to inhibit the maintenance methylase reaction. Methyltransferase activity was measured in 3-min assays with either the hemimethylated substrate, the unmethylated substrates, or mixtures of hemimethylated and unmethylated DNAs (Table III). With the enzyme level utilized (225 ng) and 1 μM DNA, the only efficient methyl acceptor was the unmethylated DNA (Experiment I). When present in equimolar concentrations, unmethylated ds M13 DNA did not inhibit methylation of the hemimethylated substrate and, therefore, apparently was not as efficiently recognized by the enzyme.

Single-stranded M13 DNA, on the other hand, was capable of completely inhibiting the maintenance methylase activity (Experiment I), suggesting a greater affinity of the enzyme for unmethylated ss DNA. To confirm that this was the case, methyltransferase activity was measured in reactions containing equimolar concentrations of single-stranded and hemimethylated M13 at enzyme and DNA concentrations sufficient to observe methylation of either substrate (Experiment II). At methylase levels which transferred 0.52-pmol methyl groups in 15 min to ss DNA and 1.77 pmol to hemimethylated DNA, the only significant inhibition observed was due to single-stranded M13 DNA. The degree of inhibition was dependent upon the concentration of single-stranded DNA and methyltransferase activity as measured in 3-mm assays with either the hemimethylated substrate or with unmethylated M13 DNA (Table III). The inhibition was greater at 15 min incubation than at 3 min (Table III). Presumably, the methylation of unmethylated M13 DNA (see Fig. 6) as compared to methylated M13 DNA would appear to be an irreversible reaction and therefore more resistant to inhibition.
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DNA, only 0.05-pmol methyl groups were transferred to a mixture of the two substrates, i.e. the mixture of substrates was less efficiently methylated than either one alone. This and other data in Table III indicate that the inhibition of maintenance methylation observed with unmethylated ss DNA cannot be accounted for simply by preferred binding and utilization of single-stranded DNA by the enzyme.

DISCUSSION

Most mammalian methyltransferase preparations are more active with single-stranded DNA substrates than with duplex DNA (4). Comparing various forms of M. luteus, fD, and M13 DNAs, it likewise appears that relatively long DNAs containing few single-stranded regions are not efficient substrates for low concentrations of the purified MPC 11 methylase. At high enzyme concentrations, however, the structure of the unmethylated substrate appears to have less effect upon the rate of methylation. This result, together with the observed nonlinear response of native DNA methylation to increasing enzyme concentration, suggests that more than one molecule from the methyltransferase preparation is required for methylation of long double-stranded DNA polymers. One trivial explanation for this observation would be the presence in the enzyme preparation of nuclease activity capable of “activating” native DNA. However, under conditions of the methylase assay, we were unable to detect any exonuclease activity, and an extremely low level of endonuclease activity that was detectable would not have been sufficient to produce a significant quantity of single-stranded regions or double-strand breaks.

Alternatively, it could be that DNA binding proteins present in the preparation might have facilitated methylation of the native DNA by introducing single-stranded regions into the DNA. However, we were unsuccessful in increasing methylation of native DNA by the addition of earlier fractions from the MPC 11 methylase purification extracts which contained DNA binding proteins.

A third explanation for the nonlinearity of methyltransferase activity with the native DNA substrates is a requirement for cooperativity between several enzyme molecules. This explanation is consistent with results we have obtained investigating the maintenance reaction as well. Unfortunately, our most purified fractions of MPC 11 methyltransferase were neither sufficiently stable nor concentrated to study this possibility directly.

The strong substrate inhibition observed with the hemimethylated DNA substrates suggests a requirement for cooperativity among methyltransferase molecules or between the methyltransferase and other proteins in the preparation. High concentrations of DNA would then effectively dilute the activity by preventing cooperativity. Conceivably, the substrate inhibition could have been due to the presence of methylated cytosine residues in the DNA substrate. However, fully en-

![Fig. 7. Summary of relative substrate efficiencies. Based upon the observations reported in the text, the following are proposed. A, hemimethylated DNA is the best substrate, and unmethylated DNA containing single-stranded regions is a better substrate than unmethylated fully duplex DNA. B, single-stranded molecules give maximal activity with a monomer subunit. Hemimethylated DNA requires a specific-sized aggregate for maximal activity. (A dimer of identical catalytic subunits is shown, but higher states of aggregation and/or heteroaggregates would also fit the observations.) With unmethylated duplex DNA, maximal activity is observed only at high enzyme concentrations, perhaps because aggregation is required to initiate stable binding at single-stranded regions, some of which may normally exist only transiently at termini or within supercoils.](image)

A. 

inactive: enzyme subunit

inactive: methylation strand

inactive: unmethylated strand

Maximally Active (on per enzyme basis)

Less Active (on per enzyme basis)

Enzyme subunit

Methylated strand

Unmethylated strand
zynamically methylated as M13 DNA had no inhibitory effect on either maintenance or de novo methylation. Alternatively, some unusual local structure (such as Z-DNA) could have formed as a consequence of modification of every cytosine residue in one strand of hemimethylated DNA. But, unless the methylase preferentially bound to those sites, complete inhibition of activity could not have occurred.

An attractive hypothesis, then, would be that methylation of hemimethylated DNA and possibly unmethylated ds DNA as well requires a dimer (or multimer) of methylase proteins to methylate or at least to initiate a processive methylase reaction on a particular DNA molecule. Because enzyme aggregation was not observed during velocity sedimentation and inhibition was not observed by pre-exposure of enzyme to poly(dA-dT) or methylated ds DNA, substrate DNA would be required for such assembly. Monomeric methyltransferase, on the other hand, may be sufficient to modify unmethylated single-stranded or activated DNA. These suggestions are summarized and elaborated upon in Fig. 7.

The dependence of the maintenance methylation rate upon the enzyme:DNA ratio is consistent with this hypothesis, since maintenance activity was increased at ratios estimated to be less than 2:1. This hypothesis also accounts for the fact that methylation was not completely inhibited at high concentrations of hemimethylated DNA. The methylase observed under those conditions could be attributable to methylation at single-stranded regions of that heterogeneous substrate.

Pedrali-Noy and Weissbach (19) utilized poly(dI-dC) and its hemimethylated derivative poly(d-5'C)dC) poly(d-I-dC) as substrates for the HeLa DNA methyltransferase. In contrast to studies reported here with the MPC 11 enzyme, slight inhibition by high concentrations of unmethylated DNA was found. It is possible that in the experiments reported here the methylase preferentially bound to those sites, complete inhibition of activity could not have occurred. These suggestions are summarized and elaborated upon in Fig. 7.
Table 1. Purification of Nic 11 DNA methyltransferase

| Sample | Volume (ml) | Protein mg/ml | activity (pmol/min/mg) | specific activity (pmol/min/mg) | total activity (pmol/min) | specific activity (pmol/min/mg) |
|--------|-------------|---------------|------------------------|------------------------|------------------------|------------------------|
|        |             |               | total                  | specific                | total                  | specific                |
|        |             |               | activity (pmol/min/mg) | activity (pmol/min/mg)  | activity (pmol/min/mg) | activity (pmol/min/mg)  |
| 1      | 1.0         | 0.0004        | 1.0                    | 0.0004                  | 1.0                    | 0.0004                  |
| 2      | 0.5         | 0.0008        | 0.5                    | 0.0008                  | 0.5                    | 0.0008                  |
| 3      | 0.25        | 0.0016        | 0.25                   | 0.0016                  | 0.25                   | 0.0016                  |
| 4      | 0.125       | 0.0032        | 0.125                  | 0.0032                  | 0.125                  | 0.0032                  |
| 5      | 0.0625      | 0.0064        | 0.0625                 | 0.0064                  | 0.0625                 | 0.0064                  |

**Figure 2** (Top) Glycerol gradient sedimentation of DNA methyltransferase. A fraction of the cell extract (0.1 ml) was assayed as described in Experimental Procedures and contained in 0.2 ml Fractions from the bottom of the tube. Activity was determined using activated Nic 11 DNA as substrate. Individual fractions contained less than 10 protein per ml and no detectable activity on native DNA. The sedimentation velocities of the heavy and light bands (4,000 to 9,000 Svedberg units) are indicated by dots and bars, respectively.

**Figure 3** (Middle) Glycerol gradient sedimentation of RNA methyltransferase. The purified enzyme was assayed using two different substrates: 1) native DNA and 2) the heavy band of RNA. The activity was determined using Nic 11 DNA as substrate.

**Figure 4** (Bottom) Glycerol gradient sedimentation of DNA methyltransferase. The purified enzyme was assayed using two different substrates: 1) native DNA and 2) Nic 11 DNA. The activity was determined using Nic 11 DNA as substrate.