Symposium on Phagocytosis, Tokyo, Japan.
Karle, I. L., Karle, J., Wieland, T., Burgermeister, W., Faulstich, H., & Witkop, B. (1973) *Proc. Natl. Acad. Sci. U.S.A. 70*, 1836.
Konopińska, D. (1978) *Pol. J. Chem. 52*, 953.
Konopińska, D., Nawrocka, E., Siemion, I. Z., Szymaniec, St., & Slopek, S. (1976) in *Peptides 1976* (Loffet, A., Ed.) pp 535–539, Editions de l'Université de Bruxelle, Belgique.
Konopińska, D., Siemion, I. Z., Szymaniec, S., & Slopek, S. (1978) *Pol. J. Chem. 52*, 573.
Konopińka, D., Siemion, I. Z., Szymaniec, S., & Slopek, S. (1979) *Pol. J. Chem. 53*, 344.
Kopple, K. O., Ohnishi, M., & Go, A. (1969) *Biochemistry 8*, 4087.
Kopple, K. O., Go, A., & Philipauskas, D. R. (1975) *J. Am. Chem. Soc. 97*, 6830.
London, R. E. (1978) *J. Am. Chem. Soc. 100*, 2678.
London, R. E., Stewart, J. M., Cann, J. R., & Matwiyoff, N. A. (1978) *Biochemistry 17*, 2270.
Martinez, J., Winternitz, F., & Vindel, J. (1977) *Eur. J. Med. Chem. Chim. Ther. 12*, 511.
Najjar, V. A. (1974) *Adv. Enzymol. Relat. Areas Mol. Biol. 41*, 129.
Najjar, V. A. (1977) *Exp. Cell Biol. 46*, 114.
Najjar, V. A. (1979a) in *Advance in Experimental Medicine and Biology, International Congress of the Reticuloendothelial Society, 7th* (Escobar, M., Ed.) Plenum Press, New York (in press).
Najjar, V. A. (1979b) in *Biochemistry of the Reticuloendothelial System* (Sbarra, A. J., & Strauss, R. R., Eds.) Vol. II, Plenum Press, New York (in press).
Najjar, V. A., & Nishioka, K. (1970) *Nature (London) 228*, 672.
Najjar, V. A., & Constantopoulos, A. (1972) *RES, J. Reticuloendothel. Soc. 12*, 197.
Najjar, V. A., & Schmidt, J. J. (1979) in *Lymphokine Reports* (in press).
Nishioka, K. (1979a) *Gann 69* (in press).
Nishioka, K. (1979b) *Br. J. Cancer* (in press).
Nishioka, K., Constantopoulos, A., Satoh, P., & Najjar, V. A. (1972) *Biochem. Biophys. Res. Commun. 47*, 172.
Nishioka, K., Constantopoulos, A., Satoh, P. S., Mitchell, W. M., & Najjar, V. A. (1973a) *Biochim. Biophys. Acta 310*, 217.
Nishioka, K., Satoh, P. S., Constantopoulos, A., & Najjar, V. A. (1973b) *Biochim. Biophys. Acta 310*, 230.
Patel, D. J., & Tonelli, A. E. (1974) *Biochemistry 13*, 788.
Stabinsky, Y., Bar-Shavit, Z., Fridkin, M., & Goldman, R. (1979) *Mol. Cell. Biochem.* (in press).
Sucharda-Sobczyk, A., Shemion, I. Z., & Konopińska, D. (1979) *Eur. J. Biochem. 96*, 131.
Tzehoval, E., Segal, S., Stabinsky, Y., Fridkin, M., Spirer, Z., & Feldman, M. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3400.
Urry, D. W., Ohnishi, M., & Walter, R. (1970) *Proc. Natl. Acad. Sci. U.S.A. 66*, 111.
Vold, R. L., Waugh, J. W., Klein, M. D., & Phelps, D. E. (1968) *J. Chem. Phys. 48*, 3831.
Waedler, S., Lee, L., & Redfield, A. G. (1975) *J. Am. Chem. Soc. 97*, 2927.

Partial Purification and Characterization of a Ribonucleic Acid N^2-Guanine Methyltransferase Associated with Avian Myeloblastosis Virus†

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ABSTRACT: A nucleic acid methylase, N^2-guanine ribonucleic acid (RNA) methyltransferase, which is associated with type C RNA tumor viruses, has been purified from avian myeloblastosis virions by gel filtration on Sephadex G-200, followed by chromatography on hydroxylapatite. The molecular weight estimated by gel filtration is 220 000, and the methylase activity has a pH optimum of 7.6–7.9. Magnesium and ammonium ions both stimulate activity 1.5-fold at 9.5 mM and 0.36 M, respectively, but apparently neither is essential for activity. Both daunomycin and adriamycin, antineoplastic drugs, also increase activity 1.5-fold at 1 mM. The enzyme was purified 120-fold from the virions and the activity is partially stabilized by dithiothreitol, but large losses were sustained during 24-h dialysis. The purified enzyme retains 75% of its activity on storage at −25 °C for 2 months in buffer containing 50% glycerol. *Escherichia coli* tRNA^{Phe} and tRNA^{Val} are preferred substrates with methylation occurring at position 10 of *E. coli* tRNA^{Phe}.

Enzymatic methylation of nucleic acids is a widespread cellular phenomenon. One biological function of DNA methylation in some bacteria has been clearly demonstrated to confer resistance to cleavage of the DNA by specific restriction nucleases (Meselson & Yuan, 1968). RNA methylation has been implicated in cellular regulation and differentiation (Sharma et al., 1971), and 7-methylguanine at the 5′-phosphate end of mRNA has been shown (Muthukrishnan et al., 1975) to be a requirement for translation in some cases, though not all (Rose & Lodish, 1976). Further, aberrant nucleic acid methylation has been suggested as a fundamental event in some kinds of malignant transformation (Borek & Kerr, 1972).

N^2-Guanine RNA methyltransferase has been shown to be associated with the avian myeloblastosis virion (Gantt et al., 1971) and subsequently several other oncogenic RNA viruses

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(Gantt et al., 1973) including Rous sarcoma virus, Rous-associated virus-1, and the murine leukemia sarcoma complex. Further, evidence has been reported (Gantt et al., 1975) indicating the guanine methylase is in the core structure of murine mammary tumor virions and intracytoplasmic A particles, though it was not found in the core structure of avian myeloblastosis virions under the experimental conditions employed (Gantt et al., 1972). This report describes the purification and some selected properties of this enzyme from avian myeloblastosis virus.

Materials and Methods

Standard Methylase Incubation. The standard incubation contained one-half volume of a solution of 50 mM Tris-HCl,[1] pH 8.3 at 37 °C, 35 mM NaCl, 13 mM magnesium acetate, 20 mM dithiothreitol, 600 μg of *Escherichia coli* B tRNA (Schwartz/Mann or Calbiochem), and 15 μCi of [*methyl*-^{3}H]-*S*-adenosyl-L-methionine (New England Nuclear, 3.38–4.54 Ci/mmol) and one-half volume of enzyme solution with a protein concentration (Lowry et al., 1951) between 0.01 and 6.0 mg/mL. The final pH of the reaction was 7.9 at 37 °C in a total volume of 0.3 mL. One unit of enzyme is defined as that amount which transfers 1 pmol of methyl group to guanine in *E. coli* tRNA in 2 h at 37 °C. The reaction is linear from 0.31 to at least 3.4 mg of protein per mL.

Determination of N^2-Methylguanine. After incubation, 4 mg of carrier yeast tRNA was added, and the radioactive components were characterized by one of two procedures. In the first method, 2 mL of 1 M Tris-HCl, pH 9.1 at 0 °C, and 1 mL of 50% trichloroacetic acid were added, the mixture was centrifuged, the pellet was solubilized in 2 mL of 1 M Tris-HCl, and the procedure was repeated a total of 3 times. In the second method, the incubation was precipitated with 85 μL of 0.5% cetyltrimethylammonium bromide, centrifuged, and washed 2 times with 4.25 mL of H_2O and the pellet was extracted with 1.7 mL of 2 M NaCl, reprecipitated with 4.25 mL of absolute ethanol, allowed to stand 1 h at −15 °C, and centrifuged. The final precipitate from either procedure was hydrolyzed at 100 °C for 30 min in 1 M HCl, cooled, and applied directly to a Dowex-50 column in the acid form (Gantt et al., 1971) or the NH_4^+ form (Gantt & Julian, 1973) or to an AA-15 (polystyrenesulfonic acid resin, Beckman) high-resolution column (Gantt et al., 1975). The fractions were assayed for radioactivity by counting in 10 mL of Aquasol (New England Nuclear) at an efficiency of 35%.

Since recovery of the hydrolyzed purine bases and pyrimidine nucleotides was somewhat variable after the multiple precipitations (usually ~95%, occasionally as low as 80%), the radioactivity of the N^2-methylguanine peak was normalized to the highest adenine absorbance units recovered when a series of incubations were directly compared to each other.

Solubilization of Enzyme Activity. Avian myeloblastosis virus was obtained from ~300 mL of viremic chick serum stored at −80 °C. Typically, the titer of the sera varied from (10–16) × 10^{11} ATPase units/mL, from which we obtained 3–5 mg of virus per mL. The serum was clarified by centrifugation at 35000*g* for 1 h, and the pellet was washed in 10 mL of buffer containing 50 mM Tris-HCl, 1 mM EDTA, and 100 mM NaCl adjusted to pH 6.8 at 22 °C (SET buffer).

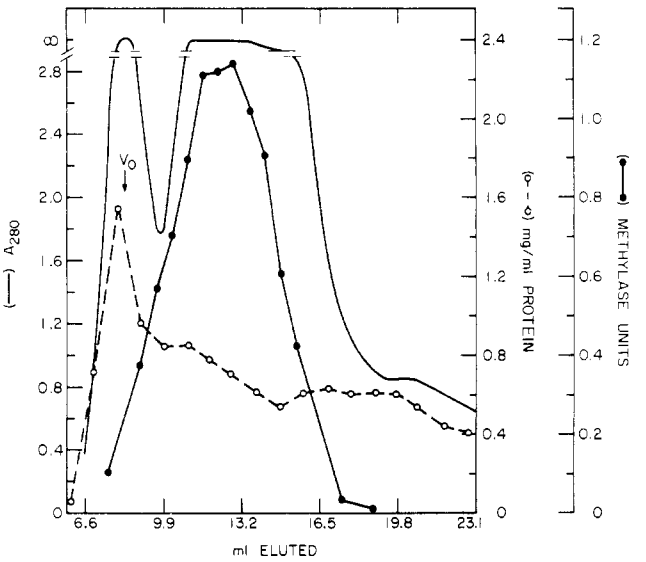

FIGURE 1: Gel filtration of Triton X-100 virus extract. 1 mL of the concentrated enzyme extract was applied to a 0.7 × 32.5 cm Sephadex G-200 column which had been equilibrated at 5 °C with buffer containing 10 mM Tris, pH 8.3, 60 mM NaCl, and 1 mM dithiothreitol; the flow rate was 1.8 mL/h. The excluded volume (V_0) was determined with Blue Dextran, and the methylase activity was measured with 0.15-mL samples in the standard assay. Protein determinations were uncorrected for Triton X-100.

After centrifugation again, the pellet was extracted with 14 mL of SET buffer containing 1% (v/v) Triton X-100 and centrifuged at 102000*g* for 1 h. The floating lipid layer was aspirated off and the remaining supernatant decanted. Centrifugation of the supernatant was repeated, and after again aspirating off any remaining lipid the supernatant was shell-frozen and stored at −70 °C. An additional one-third of the total activity could be solubilized by a second extraction of the virus pellet.

Two-milliliter samples of the thawed supernatant containing ~6 mg of protein per mL were made 1 mM in dithiothreitol and then concentrated to 1 mL by removal of water with Aquacide (Calbiochem) through a washed Visking membrane, 2 cm in diameter, held onto a bottomless vial by a screw cap with the center cut out; at 5 °C it required about 15 h to effect a twofold concentration.

Results

Enzyme Purification. One milliliter of the Triton X-100 extracted, concentrated, virus preparation was chromatographed on a Sephadex G-200 column. The distribution of the methylase activity can be seen in Figure 1. Control experiments demonstrated that the A_{280} which results from the Triton X-100 does not appear in the void volume but closely parallels the latter two-thirds of the broad peak which encompasses the methylase activity.

The peak methylase fractions of two successive Sephadex G-200 elutions were combined and adsorbed onto a freshly poured, equilibrated hydroxylapatite column. After the initial A_{280} peak (largely Triton X-100) washed through the column, the phosphate gradient was started, resulting in the profile shown in Figure 2. Two clearly resolved enzyme activities are evident, both of which have only N^2-guanine methylase activity. It is not yet known whether they are different forms of the same enzyme (e.g., an aggregate) or if they are actually different enzymes. The activity of the smaller peak is consistently between 25 and 40% of the larger peak. The pH, Mg^{2+}, and NH_4^+ optima were performed on enzyme from the larger peak. After storage for 2 months at −25 °C, 75% of

[1] Abbreviations used: SET buffer, 50 mM Tris-HCl, 1 mM EDTA, and 100 mM NaCl adjusted to pH 6.8 at 22 °C; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; DEAE, diethylaminoethyl; RNase T_1, Takadiastase 1 (EC 3.1.4.8); AMV, avian myeloblastosis virus.

Table I: Summary of Purification Steps

purifn step	sp act. (cpm per mg of protein)	protein mg/mL	protein total	purifn factor	recovery (% total act.)
crude supernatant of extract	290	6.2	24.8	1	100
Sephadex G-200	382	0.64	7.13	1.32	38
hydroxylapatite	35 240	0.011	0.022	121.6	11

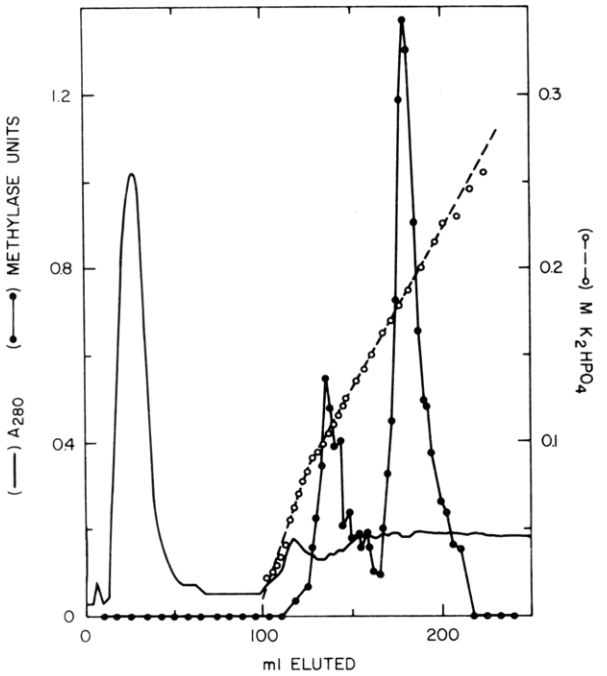

FIGURE 2: Hydroxylapatite chromatography of N^2-guanine RNA methyltransferase activity. Fractions of 10–15 mL were pooled from two successive gel filtration elutions and passed through a freshly prepared hydroxylapatite column (2 cm diameter × 3.8 cm high) which had been equilibrated with SET buffer containing 1 mM dithiothreitol at a flow rate of 20 mL/h. The phosphate gradient was formed by mixing SET buffer containing 0.5 M K_2HPO_4 and 1 mM dithiothreitol with a constant volume (200 mL) of the same buffer without phosphate. Each fraction (2.5 mL) was dialyzed for 3 h at 5 °C against a buffer containing 0.05 M Tris, pH 8.3 at 37 °C, 6 mM magnesium acetate, 60 mM NaCl, 1 mM dithiothreitol, and 50% glycerol (v/v). The enzyme activity was determined in the standard methylase assay, and the phosphate concentration was estimated by comparison of the conductivity to a standard curve.

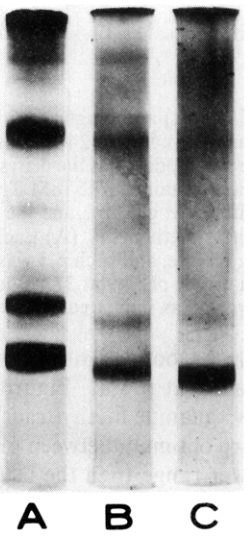

FIGURE 3: Acrylamide gel electrophoresis of the crude enzyme extract and the gel-filtrated enzyme fraction. The electrophoretic procedure is discontinuous (Davis, 1964) with a stacking gel of 1.25% and a separating gel of 7%, pH 8.9. The samples, 230 μL containing 13% glycerol, were layered on top of the stacking gel, and a current of 3 mA/tube was applied until the samples reached the separating gel where 5 mA/tube was applied until the dye front was ~5 mm from the end. The gels were stained for 16 h with Coomassie blue (0.05% in 12% trichloroacetic acid) and destained in 7% acetic acid. Gels A and B are crude high-speed supernatant extracts containing 60 and 10 μg of protein, respectively. Gel C contains 9 μg of the pooled methylase peak eluted from the Sephadex G-200 column.

the purified enzyme activity remains.

Table I summarizes the results of the purification procedure. It appears from the data that the gel filtration step is of questionable value considering a loss of nearly two-thirds of the total methylase activity with a purification of only ~1.3. However, we found by acrylamide gel electrophoresis that the gel filtration removed at least three protein components which apparently elute with the second methylase peak from hydroxylapatite. The hydroxylapatite step reduces the total protein about 325-fold with a 90-fold increase in specific activity, resulting in an overall purification of about 120-fold and a total recovery of ~11%. This does not take into account the activity in the smaller peak of hydroxylapatite which would tend to increase the yield 25–40%.

Efforts to assess the purity of the final enzyme preparations by acrylamide gel electrophoresis were unsuccessful. No activity in the gels was detected, and the only stained material found was near the interface of the stacking gel and the running gel. Comparison of the crude extract with material eluted from Sephadex G-200 is shown in Figure 3. Gels A and B are identical except for protein concentration. Gel C, the material purified on Sephadex, contains the same protein concentration as the crude material on gel B. After chromatography on Sephadex, all the discernible bands are diminished in intensity except the fastest moving band, which appears more intense, and the material at the top of the running gel (7.5%), which appears to be about the same. When the Sephadex-purified material was chromatographed on hydroxylapatite and the active peak was pooled and then analyzed by electrophoresis in the same gel system, no discernible bands were present in the running gel. These results are the same as those previously published on the N^2-guanine RNA methylase from chicken embryos (Izzo & Gantt, 1977). However, because the enzyme was available from chicken embryos in much larger quantities, a small amount of methylase activity could be detected in the band at the top of the running gel, but this experiment was not feasible with the limited amounts of virus-associated enzyme.

Determination of pH, Mg^{2+}, and NH_4^+ Optima. Several laboratories have reported studies on the pH optima of eucaryotic RNA methylases. In general, they tend to be broad with optima in the range of pH 8.8–8.9 (Kaye & Leboy, 1968; Rodeh et al., 1967), though Chan & Fraser (1972) found that a N^2-dimethylguanine methylase from an Ehrlich ascites cell had an optimum pH at 7.5–8.5 while Zeleznick (1967) found that the results depended on the methyl group acceptor. It should be noted that often more than one methylase activity is present during these measurements which clearly complicates the results if the different activities are not assayed separately. Recently, Glick et al. (1978) showed that N^2-guanine and

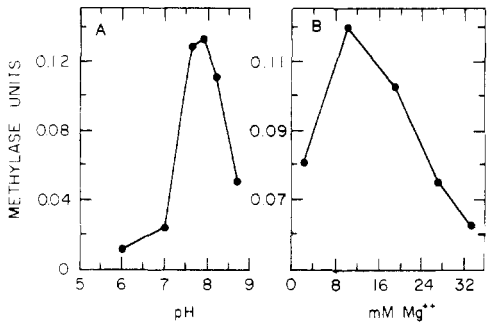

FIGURE 4: Effect of pH (A) and magnesium ion concentration (B). Purified enzyme was assayed after 24 h of dialysis rather than the standard 3 h. By the assumption that the dialysate was at equilibrium, the incubation solution contained 37.5 mM Tris-HCl, 45 mM NaCl, 20.5 mM dithiothreitol, 3 M glycerol, and the routine concentrations of tRNA and *S*-adenosylmethionine. (A) Each incubation contained 9.5 mM magnesium acetate, and each pH was determined at 37 °C. (B) Each incubation was at pH 7.9 at 37 °C, and magnesium acetate concentration was varied as indicated.

1-guanine methylases, both highly purified, had broad pH optima which peaked at pH 8. Figure 4A shows that the virus-associated N^2-guanine methyltransferase activity has a comparatively sharp optimum between pH 7.6 and 7.9, which is a somewhat lower range than the usual literature values.

The various eucaryotic RNA methylases respond to ions in a complex way where divalent ions may stimulate some and inhibit other methylases. Leboy (1971) showed in rat liver extracts that Mg^{2+} both increased and decreased methylase activity, depending on the particular methylated base. Further, when N^2-guanine methylase was tested in the presence of NH_4^+ ion, Mg^{2+} had very little effect. In other systems, Mg^{2+} has been reported to be generally stimulatory (Hancock & Eleftherion, 1971) or to have little or no stimulation (Bjork & Svensson, 1969). Figure 4B demonstrates an optimum stimulation of about 1.5-fold at 9.5 mM magnesium acetate, and activity decreases at higher concentrations. Because Mg^{2+} was included in the isolation procedure, a separate experiment was carried out with the crude extract in which Mg^{2+} was absent and 0.5 mM EDTA was added. The results demonstrated that methylase activity in the crude extract was not dependent on the presence of Mg^{2+}. The effect of ammonium acetate was examined at concentrations from 0 to 0.5 M, and an optimum at 0.19 M was found which stimulates 1.86-fold (data not shown). In each instance, N^2-methylguanine is the only base methylated.

Effect of Various Drugs on Methylase Activity. Because of the numerous reports of increased methylase activity after malignant conversion, several antineoplastic agents as well as some antibiotics and miscellaneous chemicals were tested for inhibition or stimulation of the virus-associated N^2-guanine methyltransferase. All the compounds were arbitrarily tested at 1 mM in 2-h incubations. Those compounds that stimulated the methylase activity included daunomycin (150%), adriamycin (167%), spermine tetrachloride (154%), and polylysine (139%). The spermine and polylysine have been reported to be much more potent stimulators (Leboy, 1971; Glick et al., 1978), but the duanomycin and adriamycin, which differ from each other in only one hydroxyl group, have not been previously tested.

Antibiotics and antineoplastic agents which were neither stimulators nor inhibitors included oxytetracycline, atractyloside, cycloheximide, azathioprine, colcemid, rifamycin, showdomycin, streptomycin, cyclophosphamide, mitomycin, aminopterin, and trenimon. Agents which were borderline inhibitors (activity is 70–80% of control) include rifampin, actinomycin D, *p*-aminobenzoic acid, and vincristine.

None of the following cofactors and miscellaneous compounds altered the methylase activity by more than 10% of the control when tested in the 1 mM range: nicotinamide, NAD, $NADH^+$, NADP, $NADPH^+$, FAD, thiamin, pyridoxine, vitamin B_{12}, purine derivatives, cAMP, emetine, poly(IC), epinephrine, catechol, tropolone, phenol, and guaicol. Similar to previously reported methylases, this enzyme was also inhibited by *S*-adenosylhomocysteine (99%), homocysteine (25%), selenomethionine (30%), and proflavin (43%).

Examination of Methylase Activity as a Lipoprotein. Since the N^2-guanine methylase activity associated with AMV has an absolute dependence on surfactant addition (Gantt et al., 1971), the possibility that it may be a lipoprotein or perhaps tightly bound to a lipoprotein component in the absence of surfactant was examined. When suspensions of viremic sera were frozen and thawed, methylase activity was partially released, presumably due to rupture of virions. This activity remained in the supernatant even after high-speed centrifugation, indicating that it is a soluble component or perhaps a low-density particle. Serum containing 16% KBr (w/w) was centrifuged for 69 h at 102000*g* in the fixed angle Beckman no. 65 rotor at 2° (de Lalla & Gofman, 1954). The tube was separated into a pellet and four fractions containing 1.2 mL each. The small pellet was suspended in 1 mL of the standard incubation buffer, and all the fractions were dialyzed for 2 h and assayed for methylase activity with and without the surfactant, Sterox SL. Activity was found only in the 1.2 mL at the bottom of the tube, and ~32% of the total activity was recovered. These results indicate that unless the salt dissociates the activity from a lipid component, the activity is not a light lipoprotein particle and has a density under these conditions greater than 1.16 g/mL.

Molecular Weight. The virus-associated N^2-guanine RNA methyltransferase molecular weight was estimated to be 220 000 from a gel filtration calibration curve (Andrews, 1964). It should be noted that the methylase extract was applied to the Sephadex G-200 column in 1% Triton X-100. For determination of whether the surfactant affected the distribution of the protein standards in the gel matrix, *B*-phycoerythrin and cytochrome *c* were applied to the column with and without 1% Triton X-100. The elution profiles were indistinguishable. However, hemoglobin eluted as expected from its molecular weight in the presence of surfactant, while in the absence of surfactant it was dramatically retarded; perhaps the surfactant inhibits interaction of the aromatic heme group with the gel matrix.

Chicken embryo N^2-guanine methyltransferase has a reported molecular weight of 77 000 (Izzo & Gantt, 1977), rat liver has a reported molecular weight of 69 000 (Glick et al., 1978), and both rat liver and leukemic spleen have reported molecular weights of 100 000 (Kraus & Stachelin, 1974). These three reports suggest a general weight range near 100 000 for the cellular enzyme, while the virus-associated enzyme is more than twice that molecular weight. Perhaps the virus-associated enzyme is aggregated or possibly an enzyme different from the chicken embryo enzyme.

tRNA Specificity. In contrast to eucaryotic tRNAs, a mixture of *E. coli* tRNAs serves as substrate for the virus-associated N^2-guanine methyltransferase. Since the natural substrate has not been defined, tRNAs which were available in high purity and whose sequences are known were tested. Table II shows that $tRNA^{Phe}$ and $tRNA^{Val}$ are methylated to the greatest extent and $tRNA^{Arg}$ to an intermediate extent. Leucine-4, leucine-1, and *N*-formylmethionine tRNAs are

Table II: Methyl Acceptor Activity of Some Purified tRNA Species

tRNA species	% purity[a]	N^2-methylguanine cpm[b]	% of Val act.
valine	100	2269	100
phenylalanine	95	2251	99
arginine	76	946	42
N-formylmethionine	100	421	19
leucine-4	98	313	14
leucine-1	85	460	20

[a] Percent purity as determined by the supplier (Miles Laboratories). [b] Total activity in a standard methylase incubation containing 30 μg of tRNA in 0.3-mL volume.

methylated the least and to about the same extent. These small amounts of activity may be due to small amounts of contaminating RNA species, or possibly the enzyme methylates these substrates slowly. The $tRNA^{Val}$ and $tRNA^{Phe}$ share an identical primary sequence of 16 bases. Thiouracil at position 8 through dihydrouracil-16 and dihydrouracil-21 through adenine-27 of $tRNA^{Val}$ (Yaniv & Barrell, 1969, 1971) occupy the same cloverleaf position as thiouracil-8 through dihydrouracil-16 and dihydrouracil-20 through dihydrouracil-26 of $tRNA^{Phe}$ (Barrell & Sanger, 1969). Arginine tRNA (Murao et al., 1972) has two exceptions to this same sequence: the base in the hydrogen-bonded position for guanine-10 and the 16th base adjacent to guanine-15. Possibly these positions are essential to maintain the proper conformation or binding specificity for enzyme optimum activity. The poor substrates, $tRNA^{Leu}$ (Dube et al., 1970) and $tRNA^{fMet}$ (Dube et al., 1968), on the other hand, differ in nine and seven of the sixteen nucleotides, respectively. $tRNA^{Phe}$, $tRNA^{Val}$, and $tRNA^{Arg}$ share a ten-base sequence similarity in the TΨC arm, whereas $tRNA^{Leu}$ and $tRNA^{fMet}$ share a similarity in only nine of these bases. Whether the TΨC sequence is necessary for the maintenance of tertiary structure during methylation has not been determined.

Location of the Methylated Base in $tRNA^{Phe}$. Forty A_{260} units of *E. coli* $tRNA^{Phe}$ were methylated in a standard 3-mL methylase incubation. The reaction mixture was passed through a DEAE-cellulose column (2.0 × 8.0 cm) equilibrated at 5 °C in 0.1 M NaCl. After the radioactivity returned to background, thus removing the free *S*-adenosylmethionine and most of the protein, the tRNA was eluted with 1 M NaCl and then precipitated with 2.5 volumes of ethanol at −20 °C and the precipitate was chromatographed on a RPC-5 column (Pearson et al., 1971). This tRNA was then digested with pancreatic ribonuclease and chromatographed on a DEAE-Sephadex–urea column at pH 7.6 (Tener, 1967), and the major fraction (88%) of the radioactivity appeared in the −4 trinucleotide peak, as shown in Figure 5. There are five trinucleotides generated by pancreatic RNase digestion of $tRNA^{Phe}$. When a Takadiastase 1 digestion of methylated $tRNA^{Phe}$ (obtained with hydroxylapatite-purified methylase) is chromatographed, the sole peak of radioactivity appears just before the −6 peak, suggesting a pentanucleotide. The only pentamer which is possible from the sequence of $tRNA^{Phe}$ is $CpUpCpApG^{15}p$. However, oligonucleotides containing thiouracil usually chromatograph with the next higher phospate series (Uziel & Gassen, 1969; Harada et al., 1971). Thus, the tetranucleotide $ApS^4UpApG^{10}p$ could also chromatograph in the pentanucleotide region. The Gp in both of these oligonucleotides appears in the trinucleotide peak of a pancreatic RNase digestion, and the nucleotide sequences are $ApG^{10}pCp$ and $ApG^{15}phUp$. These two trinucleotides were distinguished

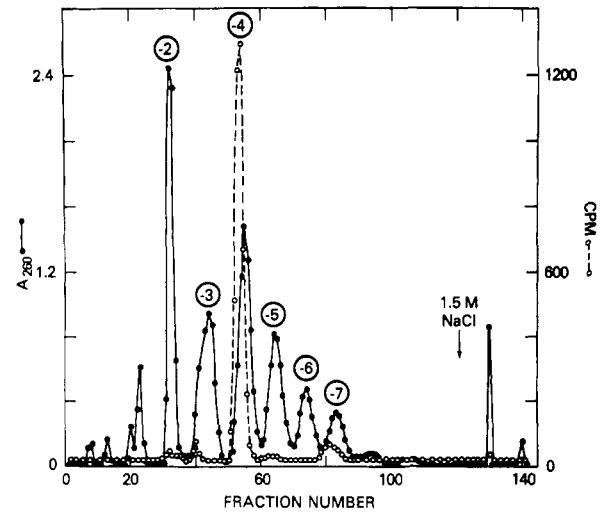

FIGURE 5: Chromatography of a pancreatic RNase incubation mix on DEAE-Sephadex A-25 at pH 7.6. The methylated $tRNA^{Phe}$ was made 0.35 M in NaCl and placed atop an RPC-5 column (0.5 × 25 cm) previously equilibrated with 0.01 M Tris-HCl, pH 7.5, 0.01 M Mg^{2+}, and 0.35 M NaCl. A NaCl gradient was formed by dropping 0.9 M NaCl into a 220-mL equilibrium buffer in a closed flask. The fraction corresponding to "intact" $tRNA^{Phe}$ was precipitated with 2 volumes of ethanol after adding 2 mg of *E. coli* tRNA carrier. Ethanol-precipitated intact $tRNA^{Phe}$ was then incubated in 0.1 M Tris, pH 7.25, 0.04 M EDTA, 1 drop of chloroform, and 20 g of pancreatic RNase (RAF, Worthington) for 2 h in a total volume of 1 mL. The mixture was diluted with 4 volumes of Tris–7 M urea and 100 OD units of 2′-AMP and cAMP standards and then placed atop a 0.9 × 26 cm DEAE-Sephadex A-25 column equilibrated with 0.005 M Tris (pH 7.6)–7 M urea. The salt gradient was generated with a closed mixing flask of equilibrating buffer (130 mL) and 0.35 M NaCl in equilibrating buffer dripping in the mixing flask. The flow rate was 3 mL/10 min.

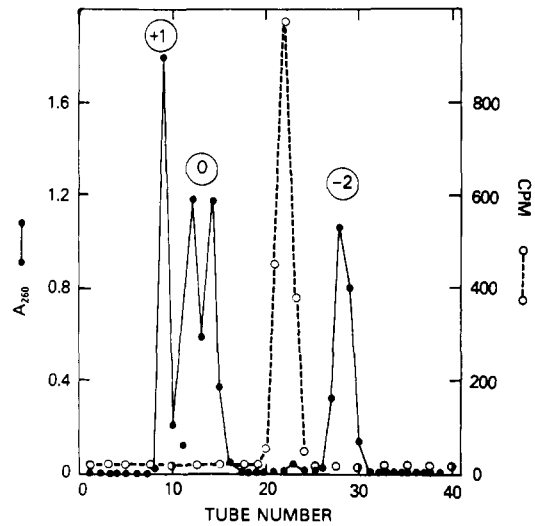

FIGURE 6: Net charge estimation of the radioactive trinucleotide. A fraction from the major radioactive peak in Figure 5 was diluted with 2 volumes of urea–HCl buffer to reduce the salt concentration. Charge standards adenosine (+1), cAMP (0), and UMP (−2) were added, and the sample was chromatographed at pH 2.7 on a DEAE-Sephadex A-25 column (0.9 × 63 cm). The elution gradient was formed in a closed mixing flask (130 mL) containing 7 M urea–HCl, pH 2.7, and the same buffer containing 0.2 M NaCl was added by dropping into the mixing flask.

from each other by comparison of the charge at pH 2.7 by DEAE-Sephadex chromatography (Rushizky et al., 1964) before and after RNase T_1 digestion. At pH 2.7, ApGpCp will have a net negative charge of about −1 while ApGphUp will have a charge of about −2. Figure 6 shows that the trinucleotide chromatographs with a −1 charge, demonstrating

that the G^{10} position is methylated. After RNase T_1 digestion of the trinucleotide, the radioactivity again eluted at the −1 position, confirming that the G^{10} position is methylated. If the G^{15} position were methylted, then the radioactive peak would have shifted because T_1 digestion of both trinucleotides gives the dinucleotide ApGp with a charge of about −1.

Discussion

The 120-fold enzyme purification reported here is comparable to the purification factors of other enzymes from viruses. For example, Kacian et al. (1971) demonstrated a 35-fold purification of reverse transcriptase from AMV with gel electrophoretic evidence indicating high purity, while Hurwitz & Leis (1972) purified the polymerase at least 80-fold. Martin et al. (1975) purified a guanine 7-methyltransferase from vaccinia virus 106-fold and presented evidence for homogeneity. These laboratories based their calculations on protein determinations of crude virus extracts, as we did.

It is not clear why our attempts to assess protein homogeneity failed on acrylamide gels. For certainty that protein of the estimated molecular weight of the methylase (220 000) readily enters the 7% running gel, *B*-phycoerythrin (270 000) was electrophoresed under the same conditions and it migrated 50% of the gel length in 2 h. The lightly stained upper third of the acrylamide gel which contained 10 μg of purified methylase protein (Figure 3), which smeared, may be the enzyme itself interacting with the gel matrix. If that were the case, sodium dodecyl sulfate–acrylamide gels would be expected to minimize such interactions, but an attempt using the methods of Weber & Osborn (1969) also failed to give any protein bands. Those electrophoretic results available with the virus-associated enzyme are comparable to the results obtained with the N^2-guanine methylase purified from chick embryos (Izzo & Gantt, 1977) where greater availability of material allowed more complete studies.

Recently Pierre et al. (1978) confirmed our results demonstrating the presence of N^2-guanine methyltransferase in avian myeloblastosis virus and Rous sarcoma virus. They concluded that the enzyme has a cellular origin based on comparison of substrate activity and similar kinetic constants, and they suggest that it, specifically, is enclosed within the virion envelope during budding of the virus because this methylase is localized near the cell membrane while the others are not. In this report we demonstrate that the viral-associated methylase transfers the methyl group to the guanine in position 10 of certain tRNAs. This is a common methylase in eucaryotic cells. The fact that the molecular weight is nearly 3 times the chick embryo methylase (Izzo & Gantt, 1977) and the pH optimum is 0.5 unit lower suggests that the virus-associated methylase is different from the chick embryo enzyme. However, the difference could simply be a difference in enzymes from chick embryo cells and leukemic chick myeloblasts. Therefore, the question of the origin of the virus-associated enzyme remains open. In spite of a possible cell origin, the enzyme may have an unexpected function in the virus life cycle. A precedent for this possibility is the presence of tryptophan tRNA, of cellular origin, that is associated with viral 70S RNA of Rous sarcoma virus and avian myeloblastosis virus (Dahlberg et al., 1974; Faras et al., 1974; Harada et al., 1975; Waters et al., 1975) which is an effective in vitro primer for synthesis of viral DNA from the virion RNA.

References

Andrews, P. (1964) *Biochem. J. 91*, 222.
Barrell, B. G., & Sanger, F. (1969) *FEBS Lett. 3*, 275.
Bjork, G. R., & Svensson, I. (1969) *Eur. J. Biochem. 9*, 207.
Borek, E., & Kerr, S. J. (1972) *Adv. Cancer Res. 15*, 163.
Chan, T-C., & Fraser, M. J. (1972) *Life Sci. 11*, 793.
Dahlberg, J. E., et al. (1974) *J. Virol. 13*, 1126.
Davis, B. I. (1964) *Ann. N.Y. Acad. Sci. 121*, 404.
de Lalla, O. F., & Gofman, J. W. (1954) *Methods Biochem. Anal. 6*, 459.
Dube, S. K., Marcker, K. A., Clark, B. F. C., & Cory, S. (1968) *Nature (London) 218*, 232.
Dube, S. K., Marcker, K. A., & Yudelevich, A. (1970) *FEBS Lett. 9*, 168.
Faras, A. J., et al. (1974) *J. Virol. 13*, 1134.
Gantt, R., & Julian, B. (1973) *Anal. Biochem. 54*, 283.
Gantt, R., Stromberg, K. J., & Montes de Oca, F. (1971) *Nature (London) 234*, 35.
Gantt, R., Stromberg, K., & Julian, B. (1972) *J. Virol. 9*, 1057.
Gantt, R., Smith, G. H., & Julian, B. (1973) *Virology 52*, 584.
Gantt, R., Julian, B., & Smith, G. (1975) *Cancer Res. 35*, 1847.
Glick, J. M., Averyhart, V. M., & Leboy, P. S. (1978) *Biochim. Biophys. Acta 518*, 158.
Hancock, R. L., & Eleftherion, B. E. (1971) *Physiol. Chem. Phys. 3*, 139.
Harada, F., Kimuura, F., & Nishimura, S. (1971) *Biochemistry 10*, 3269.
Harada, F., Sawyer, R. C., & Dahlberg, J. E. (1975) *J. Biol. Chem. 250*, 3487.
Hurwitz, J., & Leis, J. P. (1972) *J. Virol. 9*, 116.
Izzo, P., & Gantt, R. (1977) *Biochemistry 16*, 3576.
Kacian, D. L., Watson, K. F., Burny, A., & Spiegelman, S. (1971) *Biochim. Biophys. Acta 246*, 365.
Kaye, A. M., & Leboy, P. S. (1968) *Biochim. Biophys. Acta 157*, 289.
Kraus, J., & Stachelin, M. (1974) *Nucleic Acids Res. 1*, 1455.
Leboy, P. S. (1971) *FEBS Lett. 16*, 117.
Lowry, O. H., Roxbrough, N. J., Farr, A., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265.
Martin, S. A., Paoletti, E., & Moss, B. (1975) *J. Biol. Chem. 250*, 9322.
Meselson, M., & Yuan, R. (1968) *Nature (London) 217*, 1110.
Murao, K., Tanabe, T., Ishii, F., Namiki, M., & Nishimura, S. (1972) *Biochem. Biophys. Res. Commun. 47*, 1332.
Muthukrishnan, S., Both, G. W., Furuichi, Y., & Shatkin, A. J. (1975) *Nature (London) 255*, 33.
Pearson, R. L., Weiss, J. F., & Kelmers, A. D. (1971) *Biochim. Biophys. Acta 228*, 770.
Pierre, A., et al. (1978) *Biochem. Biophys. Res. Commun. 81*, 315.
Rodeh, R., Feldman, M., & Littauer, U. Z. (1967) *Biochemistry 6*, 451.
Rose, J. K., & Lodish, H. F. (1976) *Nature (London) 262*, 32.
Rushizky, G. W., & Sober, H. A. (1964) *Biochem. Biophys. Res. Commun. 14*, 276.
Sharma, O. K., Kerr, S. J., Lipshitz-Wiesner, R., & Borek, E. (1971) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 30*, 167.
Tener, G. M. (1967) *Methods Enzymol. 12A*, 398.
Uziel, M., & Gassen, H. G. (1969) *Biochemistry 8*, 1643.
Waters, L. C., et al. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 2155.
Weber, K., & Osborn, M. (1969) *J. Biol. Chem. 244*, 4406.
Yaniv, M., & Barrell, B. G. (1969) *Nature (London) 222*, 278.
Yaniv, M., & Barrell, B. G. (1971) *Nature (London), New Biol. 233*, 113.
Zeleznik, L. D. (1967) *Arch. Biochem. Biophys. 118*, 133.