Template Properties of Glucose-Deficient T-Even Bacteriophage DNA

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The vegetative DNA isolated from T4-infected *Escherichia coli* W4597 (UDPG PPase⁻) was about two to six times more active in stimulating protein synthesis in cell-free extracts than that isolated from T4-infected *E. coli* B06. This suggested that nonglucosylated vegetative DNA may be a better template than the glucosylated form. This view was supported by experiments measuring RNA synthesis on mature T-even DNAs with a range of glucose contents. The extent of ¹⁴C-GTP polymerization was inversely proportional to the glucose content of the DNA. Differences were also observed in both the kind and quantity of polypeptides produced in response to these DNAs.

The T-even bacteriophages contain hydroxymethylcytosine (HMC) instead of cytosine in their DNA (27). This modified pyrimidine can be partially or totally glucosylated (23), the extent and type of glucosylation being specific for the particular T-even phage (16). Glucosylation of the HMC residues in the polymerized T4 DNA molecule is carried out by phage-induced enzymes, alpha- and beta-glucosyl transferases (13), which use host-synthesized uridine diphosphoglucose (UDPG) as the glucosyl donor.

Since at some stage in T4 replication the DNA is nonglucosylated, it was of interest to see what effect glucosylation might have on the ability of the DNA to serve as a template for in vitro RNA and protein synthesis.

MATERIALS AND METHODS

Bacteriophage methods. Liquid media for culture of the host bacteria contained the following components in 1 liter: (i) minimal medium (1) consisting of Na₂HPO₄ (anhydrous), 6.0 g; KH₂PO₄ (anhydrous), 3.0 g; NH₄Cl, 1.0 g; NaCl, 0.5 g; MgSO₄·7H₂O, 0.26 g; glucose, 4.0 g; (ii) nutrient broth consisting of tryptone (Difco), 10.0 g; yeast extract, 5.0 g; NaCl, 5.0 g; glucose, 1.0 g; 1 M NaOH, 2.5 ml.

Wild-type T4D and T2L bacteriophage were grown on *Escherichia coli* B06 in minimal medium by the standard procedures of Adams (1). The plating efficiency of T4 on *E. coli* B06 compared to *Shigella dysenteriae* Sh16 was 0.94.

The nonglucosylated phage, T*4, were obtained by one-step growth of T4D on *E. coli* W4597 (UDPG PPase⁻) in broth shake cultures at 37 C. At a density of 5×10^8 cells/ml, wild-type T4 phage were added to give 3 to 4 PFU/bacterium. At 6 min after infection the culture was chilled by pouring on crushed ice, and the infected cells were collected by centrifugation at

$9,000 \times g$ for 10 min. The cell pellets were resuspended in a small volume of cold medium and then diluted into warm medium to give twice the original cell concentration. The culture was shaken for an additional 2 to 3 h at which time chloroform was added to complete the already visible lysis. The modified phage plated on *E. coli* B06 with an efficiency of about 0.05 compared to *S. dysenteriae* Sh16.

Bacteriophage T4 *agt*gt (referred to simply as T4gt) was produced in a manner similar to that for wild-type phage. *S. dysenteriae*, strain Sh16, was grown at 37 C in shake cultures of nutrient broth. At a cell concentration of 2×10^8 to 3×10^8 cells/ml, sufficient T4gt phage were added to give a multiplicity of 0.2 PFU/ml. Shaking was continued for 6 h at which time chloroform was added. The efficiency of plating for this mutant on *E. coli* B06 compared to *S. dysenteriae* Sh16 was 5.7×10^{-8} .

Bacteriophage T4 tsA90x5 was grown on *E. coli* B06 in minimal medium at 39 C.

Preparation of mature DNA. Phage were purified by the method described by Yamamoto et al. (28). The DNA was extracted from phage suspensions (20 to 40 A₂₆₀/ml) by the method of Thomas and Abelson (24) using redistilled phenol neutralized with 0.01 M Tris-chloride, pH 7.4. The aqueous phase was dialyzed exhaustively against 0.01 M Tris-chloride (pH 7.4), 0.10 M KCl. The final preparations had A₂₆₀/A₂₈₀ ratios of 1.80 to 1.95.

Isolation of vegetative DNA. For the experiment presented in Table 1; parallel cultures of *E. coli* B06 and *E. coli* W4597 (UDPG PPase⁻) were grown in nutrient broth at 30 C and infected with wild-type T4 (multiplicity of infection [MOI] = 12) at cell densities of 1.3×10^8 and 0.9×10^8 cells/ml, respectively. At 20 min past infection the cultures were chilled by pouring over a 0.5 volume of crushed ice or ice cubes of 0.15 M NaCl so that the temperature dropped to 0 C within a few seconds. The chilled cells were collected by centrifugation at $10,000 \times g$ for 10 min. The

supernatant fluid was discarded and the pelleted cells were resuspended in a small volume of buffer (0.01 M Tris-chloride, [pH 7.4], 0.005 M $MgCl_2$, 0.05 M KCl, 0.005 M 2-mercaptoethanol) to give approximately 3×10^{10} to 6×10^{10} cells/ml. Egg white lysozyme and EDTA were added to give final concentrations of 300 $\mu g/ml$ and 5×10^{-4} M, respectively, and the mixture was incubated at 37 C for 30 min. At this time sodium dodecyl sulfate (SDS) was added to a final concentration of 0.1% and incubation at 37 C was continued for 5 min more. The suspension was then subjected to three cycles of freezing and thawing in a dry ice-acetone bath. The resulting lysate was centrifuged at $5,000 \times g$ for 10 min, and the supernatant fluid was dialyzed overnight at 4 C against 1 liter of 0.01 M Tris-chloride (pH 7.4) containing 0.15 M NaCl.

For the experiment presented in Fig. 6 *E. coli* B cells were grown in minimal medium to 5×10^8 cells/ml, infected with 5 PFU/cell, harvested at 20 min past infection, and resuspended at a concentration of 3×10^{10} cells/ml in 0.01 M Tris-chloride (pH 7.8), 0.10 M NaCl, 0.01 M NaN_3 . To 0.8 ml of cells was added 0.2 ml of a solution containing 0.10 M Tris-chloride (pH 8), 0.05 M EDTA (pH 8), and 4 mg of egg white lysozyme per ml, and incubation was carried out at 30 C for 10 min. Then, 0.2 ml of a solution containing 4 mg of Pronase per ml was added and incubation was continued for another 15 min, followed immediately by the addition of 0.8 ml of 0.4% SDS and a further 10 min of incubation. After the mixture cleared, it was extracted two times with buffer-saturated phenol and dialyzed as described for mature DNA.

Preparation of cell-free extracts. *E. coli* B06 or *S. dysenteriae* Sh16 cells were grown at 37 C in 15 liters of nutrient broth with vigorous aeration, harvested during the early logarithmic phase of growth (10^8 to 4×10^8 cells/ml) by pouring over crushed ice, collected by continuous flow centrifugation, washed once in standard buffer (0.01 M Tris-chloride [pH 7.4], 0.01 M $MgCl_2$, 0.05 M KCl, 0.005 M 2-mercaptoethanol), and used for the preparation of S-30 extracts similar to that of Nirenberg and Matthaei (18). The S-30 was preincubated as described by these authors and used for in vitro DNA-dependent amino acid incorporation.

The crude preparation of RNA polymerase was prepared by pelleting the ribosomes at $145,000 \times g$ for 3 h from the S-30 fraction. The top two-thirds of the supernatant solution was removed, and nucleic acids were removed by precipitation with protamine sulfate (26). After centrifugation the supernatant fluid was dialyzed against standard buffer and stored at -65 C.

In vitro protein synthesis. The reaction mixtures for protein synthesis contained the following in micromoles per milliliter: Tris-chloride (pH 7.8), 50; $MgCl_2$, 10; NH_4Cl , 33; KCl, 100; 2-mercaptoethanol, 4.7; phosphoenolpyruvate, 12.5; ATP, 2.0; GTP, CTP, UTP, 0.50 each; 19 ^{12}C -amino acids, 0.20 each; pyruvate kinase, 33 μg ; S-30 protein, 0.90 to 1.30 mg; DNA, 5 to 100 μg ; and ^{14}C -amino acid as indicated in the legends to the figures and tables. Incubations were usually for 20 min at 37 C in a final volume of 0.150 ml. Reactions were terminated with 7% trichloroacetic acid and filtered on cellulose nitrate filters as described previously (4).

Reaction mixtures for RNA synthesis. The reaction mixtures for RNA synthesis contained the following in micromoles per milliliter: Tris-chloride (pH 7.8), 50; $MgCl_2$, 10; KCl, 167; 2-mercaptoethanol, 4.7; ATP, CTP, UTP, 0.50 each; S-145 protein, 0.2 to 0.5 mg; DNA, 5 to 100 μg ; and ^{14}C -GTP as indicated in the legends to the tables and figures. The final volume of incorporation mixtures was 0.150 ml and incubation was at 37 C for 5 to 10 min. The reactions were stopped by adding 2 ml of ice-cold 7% trichloroacetic acid and placing the tubes in ice. The precipitates were collected on 2.4-cm Whatman glass fiber filters (GF/C) and washed with 20 ml of ice-cold 7% trichloroacetic acid. The washed filters were placed in scintillation vials, dried in an oven, covered with 15 ml of a scintillation fluid containing 5.0 g of 2,5-diphenyloxazole (PPO) and 0.25 g of 1,4-bis-[2-(5-phenyloxazolyl)]-benzene (POPOP) in 1 liter of reagent grade toluene, and counted at 60 to 90% efficiency.

Enzymatic glucosylation of T-even DNAs. The transfer of ^{14}C -glucose from UDP- ^{14}C -glucose in the presence of T4 beta-glucosyl transferase was determined in reaction mixtures containing 12.5 μmol of Tris-chloride (pH 7.7), 1.25 μmol of 2-mercaptoethanol, 5.1 μmol of $MgCl_2$, 6.8 nmol of UDP- ^{14}C -glucose (5 $\mu Ci/\mu mol$), 2 to 10 μg of DNA, and 230 μg of beta-glucosyl transferase fraction in a final volume of 0.25 ml. The source for glucosyl transferase was an S-145 fraction prepared from T4 *amN122*-infected *E. coli* B06 at 20 min past infection (30 C). This fraction also contains alpha-glucosyl transferase, but its activity is inhibited by the addition of magnesium ions to the reaction mixture (5). Incubation was for 30 min at 37 C after which time 250 μg of salmon sperm DNA was added as carrier followed by 2 ml of ice-cold 7% trichloroacetic acid. Precipitates were collected on glass fiber pads (Whatman GF/C, 2.4 cm), washed with 15 ml of ice-cold 7% trichloroacetic acid, dried, and counted in the toluene-based scintillation fluid at 60% efficiency.

Polyacrylamide gel electrophoresis and autoradiography. The SDS-polyacrylamide gel electrophoresis was carried out basically as described by Laemmli (15). Incubations were stopped by adding 0.100 ml of a solution containing 0.1 M Tris-chloride (pH 6.8), 8% (wt/vol) SDS, 20% (vol/vol) 2-mercaptoethanol, and 40% (vol/vol) glycerol to in vitro reaction mixtures that were scaled up twofold (0.300 ml). Hot trichloroacetic acid-insoluble material was determined in a 50- μ liter sample. Samples were heated in a boiling water bath for 90 s just before application to the gels.

The final concentrations in the stacking gel were as follows: 2.5% (wt/vol) acrylamide, 0.625% (wt/vol) *N,N'*-methylenebisacrylamide (BIS), 0.575% (vol/vol) *N,N,N',N'*-tetramethylethylenediamine (TEMED), and 0.1% (wt/vol) riboflavin to catalyze polymerization. The separating gels contained 10% (wt/vol) acrylamide, 0.2% (wt/vol) BIS, 0.0288% (vol/vol) TEMED, 0.1% (wt/vol) SDS, 0.375 M Tris-chloride (pH 8.8), and 0.07% (wt/vol) ammonium persulfate to catalyze polymerization. The electrode buffer was 0.025 M Tris-chloride (pH 8.3), 0.192 M glycine, and 0.1% (wt/vol) SDS.

Electrophoresis was carried out toward the anode with a current of 3 mA per gel until the bromophenol blue marker had migrated 10 cm. After the gels were removed from the tubes, the proteins were fixed in the gel with 50% trichloroacetic acid for at least 2 h followed by "dialysis" against 10% acetic acid overnight.

The gels were then prepared for autoradiography as described by Fairbanks et al. (7).

Materials. Bacteriophage T2L, *S. dysenteriae* Sh16, and *E. coli* B06 were gifts from E. Six. Bacteriophage T4D was a gift from W. Bode, and T4 α gt57 β gt14 and *E. coli* W4597 (UDPG, pyrophosphorylase deficient) were kindly provided by H. Revel. Bacteriophage T4 tsA90x5 (dCTPase, gene 56) was obtained from E. Kutter. R. Edgar kindly furnished T4 amN122 (dCMP hydroxymethylase, gene 42).

Specialized material and their suppliers were as follows: J. T. Baker Chemical Co., polyethylene glycol (PEG, molecular weight 6,000 to 7,500); Eastman Organic Chemicals, all reagents for polyacrylamide gel electrophoresis; General Biochemicals, deacylated *E. coli* tRNA; Millipore Corp., 1-inch cellulose nitrate membranes (0.45 μ m pore size); New England Nuclear, UDP-¹⁴C-glucose; P-L Biochemicals, nucleoside triphosphates; Schwarz/Mann Inc., uniformly labeled ¹⁴C-lysine, GTP-8-¹⁴C; Sigma Chemical Co., pancreatic DNase I (electrophoretically pure), protease (Pronase), pyruvate kinase, phosphoenolpyruvate (PEP), and amino acids; Whatman, glass fiber filters (GF/C). All other chemicals were purchased from commercial sources and were reagent grade.

RESULTS

In preliminary experiments the activities of normal and glucose-deficient vegetative T4 DNAs as templates for in vitro protein synthesis were compared. These experiments were performed with preparations of vegetative DNA obtained from wild-type T4-infected *E. coli* W4597, a strain deficient in UDPG pyrophosphorylase, and compared to similar preparations derived from infected cultures of *E. coli* B. The DNA of phage grown in *E. coli* W4597 is glucose deficient, since UDPG, an obligatory cofactor for the phage glucosyl transferases, is absent. As seen in Table 1, the relative specific activities of the templates show the non-glucosylated form to be about six times more active than the glucosylated form and in fact more active than the mature DNA used as a control. Sauerbier and Bräutigam (22) have made similar observations in vivo in which they showed RNA production in a T4-infected UDPG⁻ host was 2.5 times greater than in the T4-infected wild-type cells. These observations could mean that nonglucosylated DNA is a better template for transcription, both in vitro and in vivo.

To determine whether this phenomenon was restricted to DNA actively engaged in replica-

TABLE 1. Template activities from wild-type T4-infected *E. coli* B06 and *E. coli* W4597^a

Template	¹⁴ C-lysine incorporation (counts/min)	Relative specific activity
Mature T4 DNA, 10 μ g	9,980	1.0
Crude vegetative DNA		
T4-infected <i>E. coli</i> B06, 1 μ g	698	0.7
T4-infected <i>E. coli</i> W4597, 0.6 μ g	2,825	4.7

^a Isolation of vegetative DNA and measurement of amino acid incorporation are described in Materials and Methods. Reaction mixtures for protein synthesis in a final volume of 0.150 ml contained 1.27 mg of *E. coli* S-30 protein, 14 nmol of ¹⁴C-lysine (20 μ Ci/ μ mol), and the quantities of DNA as indicated. Incubation was at 37 C for 20 min. Specific activities, defined as counts per minute of ¹⁴C-lysine incorporated per microgram of DNA, were normalized to that of mature T4 DNA. Preparations of vegetative DNA saturate S-30 extracts at 2 to 3 μ g of S-30 protein per mg, whereas mature DNA becomes saturating at 12 to 20 μ g of S-30 protein per mg (G. S. Cox, Ph.D. thesis, University of Iowa, Iowa City, 1973).

tion or if it was also characteristic of mature DNA, several DNAs were prepared which contained HMC residues glucosylated to various extents (summarized in Table 2). The number of ¹⁴C-glucose residues accepted by the different DNAs was assumed to be equal to the number of unmodified (i.e., glucose-free) HMC residues in that DNA sample. It is pointed out that although the alpha/beta ratio in T4 DNA is 70 to 30, the beta-enzyme can in fact glucosylate all the available HMC, even in a synthetic HMC-DNA (5). The data show fairly close agreement between predicted and observed HMC contents for T4, T2, and T4gt DNAs; it was not possible to predict a value for T*4 DNA. In regard to this latter point, Fukasawa and Saito (8) showed the glucose content of DNA from T4 phage grown on *E. coli* W4597 was about 10% of that from T4 phage grown in a normal host *E. coli* W3110. Furthermore, the UDPG pyrophosphorylase activity in the mutant was also about 10% that of the wild-type. The observed glucosylation (approximately 41%) for T*4 in this study is thus somewhat higher than expected but probably accounts for the higher plating efficiency observed with lysates of T*4 in this work (0.05) compared to those of the above authors (0.003).

The ability of these DNAs to serve as substrates for the *E. coli* DNA-dependent RNA polymerase present in the S-145 extracts was measured by increasing the DNA concentration while maintaining a constant amount of S-145

TABLE 2. Degree of glucosylation of some T-even DNAs^a

Phage DNA	HMC residues containing glucose (%)	
	Predicted	Observed ^b
T4	100 ^c	99.9
T2	75 ^c	73.7
T*4		41.3
T4gt	3 ^d	10.0

^a The DNAs were prepared from purified phage suspensions by phenol extraction as described in Materials and Methods. The glucose content of each DNA was determined indirectly by determining the amount of glucose accepted by the DNA. This value, which represents the free HMC residues, is subtracted from the total HMC content of the DNA to give the amount of glucosylated HMC present. The transfer of glucose from UDP-¹⁴C-glucose to DNA by T4 beta-glucosyl transferase was assayed as described in Materials and Methods. The amounts of DNA assayed and glucose transferred were as follows (DNA, micrograms, counts per minute): T4, 10.5, 250; T*4, 11.9, 27,600; T2, 13.3, 13,800; T4gt, 4.2, 14,900.

^b These values were calculated from the glucosylation data assuming the T4 genome is equivalent to 2×10^{-10} μ g of DNA and contains 68,000 HMC residues (5). The percentage of HMC residues containing no glucose was determined and then subtracted from 100%.

^c In T4 100% of the HMC residues are glucosylated and in T2 75% of the HMCs contain glucose (11).

^d This value is predicted from the work of Georgopoulos (8), whose direct determination of the glucose content of DNA from this phage showed that it contained 2 to 3% as much glucose as the DNA from wild-type phage.

protein. The results (Fig. 1) show that the DNAs elicit a response which is dependent on the state of glucosylation of the template. A more quantitative representation of this relationship is presented in Fig. 2. Here it is seen that the capacity of the template for transcription is directly proportional to its capacity for accepting glucose. That is to say, the glucose-deficient templates are more active for RNA synthesis than the glucosylated ones. The maximum difference observed with extracts from *E. coli* B06 was about twofold whereas extracts from *S. dysenteriae* Sh16, although less active overall for ¹⁴C-GMP incorporation, showed an eightfold stimulation by T4gt DNA over T4+ DNA (Fig. 3). The reason for this is unknown. It may simply be due to intrinsic species differences in the RNA polymerase molecules from the two sources, or it might be explained by the fact that *E. coli* B06 is a restrictive host whereas *S. dysenteriae* Sh16 is a permissive host for glucose-deficient phage.

Kutter and Wiberg (15) have described a T4

mutant with a temperature-sensitive dCTPase (gene 56) in which cytosine replaces 20% of the HMC residues when the phage is grown at an intermediate temperature (39 C). It was of interest to examine the template properties of the DNA from this mutant since replacement of HMC by cytosine effectively reduces the glucose content of the genome. Figure 4 shows that the DNA from T4 tsA90 (39 C) is somewhat more active than T4+DNA and slightly less active than T2 DNA for overall GTP polymerization. This is in agreement with the free HMC residues in these templates, namely 0, 20, and 25% for T4, T4 tsA90 (39 C), and T2, respectively.

Brody et al. (3) have shown that RNA transcribed in vitro from T*4 DNA is of an early class, and likewise (in experiments not shown) we have observed no difference in the abilities of glucose-deficient or fully glucosylated DNA to direct the synthesis of polypeptides capable of precipitation with anti-T4 serum. Therefore, it was considered doubtful that the increased activity was due to the synthesis of any late RNA species, and that the differences in template activity of these DNAs probably meant either a quantitative or qualitative difference in certain species of the early messengers being

FIG. 1. Stimulation of RNA synthesis by mature T-even DNAs of various glucose content. The preparations of mature DNA are those used for the experiment described in Table 2. Reaction mixtures for RNA synthesis contained 2.6 nmol of ¹⁴C-GTP (19.1 μ Ci/ μ mol), 0.375 mg of *E. coli* S-145 protein as the source of DNA-dependent RNA polymerase, and DNA as indicated in a final volume of 0.150 ml. Incubation was for 7 min at 37 C. The DNAs added were: T4 (●), T2 (○), T*4 (▲), T4gt (△).

FIG. 2. Effect of glucose content on the template activity of some mature T-even DNAs. The values for the abscissa were calculated from the data given in Table 2. The ordinate values (nanomoles of GMP incorporated per microgram of DNA) were calculated from the curves in Fig. 1 at a DNA concentration of 1 μ g. Specific activities of isotopes are given in Materials and Methods and in the legends to the tables and figures.

transcribed. In an attempt to determine which of these possibilities might be occurring, the proteins directed by each DNA were analyzed by polyacrylamide gel electrophoresis. The autoradiograms of these gels showed the spectrum of proteins to be somewhat similar for each DNA, yet definite differences existed in some of the bands. To simplify the analysis, only part of the densitometer tracings of these autoradiograms is presented in Fig. 5A. The pattern for T2 is not shown since it is genetically different from the other phages and differences could not be attributed to glucosylation. Both partially glucosylated DNAs give rise to a new band (shoulder) at position B which is not present with T4⁺ DNA. Also, by analyzing the tracings on a Dupont curve resolver it was observed that the area under individual bands A, C, D, and E remained relatively constant (within 2%) whereas that under F represented 47% of the whole for T4⁺, 40% for T*4, and 31% for T4gt (Fig. 5B). Taken together these results suggest that the glucose content of T4 DNA can to some degree influence both the type and quantity of proteins synthesized in vitro.

The significance of these observations for T4 DNA undergoing normal replication was tested by determining whether or not vegetative DNA was fully glucosylated. Using the technique of Cs₂SO₄ equilibrium centrifugation, Erikson and Szybalski (6) were able to show that non-glucosylated DNA banded at a lower density than glucosylated DNA and that T6 DNA appeared fully glucosylated by 9 min after infection. However, it was thought that a more sensitive assay to detect glucose-deficient HMC residues would be to measure their enzymatic glucosylation in vitro. This experiment was performed with deproteinized vegetative DNA extracted 20 min after infection. Figure 6 shows

FIG. 3. Transcription of glucose-deficient DNA in extracts from *E. coli* and *S. dysenteriae*. The DNA preparations are the same as those described for Table 2 and Fig. 1. The RNA synthesis was determined in reaction mixtures containing 2.6 nmol of ¹⁴C-GTP (19.1 μ Ci/ μ mol); 0.267 mg of *E. coli* S-145 protein or 0.283 mg of *S. dysenteriae* S-145 protein; 5.25 μ g of T4 DNA, 5.32 μ g of T2 DNA, 5.95 μ g of T*4 DNA, or 5.25 μ g of T4gt DNA; all in a final volume of 0.150 ml. Incubation was at 37 C for 10 min. Extracts for this experiment were prepared without DNase. Activities were expressed as picomoles of GMP incorporated per microgram of DNA per milligram of S-145 protein and normalized to T4 DNA (ordinate). The amounts of glucose accepted per microgram of DNA was taken from Fig. 2 and normalized to T4gt DNA (abscissa).

FIG. 4. Incorporation of ^{14}C -GMP as a function of DNA added. The DNA was prepared from wild-type T2, wild-type T4, and T4 tsA90x5 grown at 39 C as described by Kutter and Wiberg (12). Reaction mixtures for RNA synthesis were as described in Materials and Methods and contained 2.6 nmol of ^{14}C -GTP (19.1 $\mu\text{Ci}/\mu\text{mol}$), 0.375 mg of *E. coli* S-145 protein as the source of RNA polymerase, and the amounts of DNA shown in a final volume of 0.150 ml. Incubation was at 37 C for 10 min.

that this DNA was unable to accept any glucose from UDP- ^{14}C -glucose whereas approximately 72% of the available HMC in the T2 DNA was glucosylated after incubation with T4 beta-glucosyl transferase for only 2 min. This suggests that the replicative form of T4 DNA is glucosylated very shortly after it is polymerized. However, the possibility that small regions remain transiently unglucosylated *in vivo* cannot be ruled out since residual glucosylation could take place during the collection of cells and extraction of the DNA.

DISCUSSION

These results suggest that the extent of glucosylation can influence the template activity of either vegetative T4 DNA or mature T-even phage DNAs for *in vitro* RNA or protein synthesis (Table 1 and Fig. 1).

It is generally believed that the predominant biological role of DNA glucosylation is to give the T-even phage a survival advantage in cer-

FIG. 5. Analysis of proteins made *in vitro* in response to various T-even mature DNAs. A, Reaction mixtures for protein synthesis in a final volume of 0.300 ml contained 0.5 μCi of ^{14}C -lysine (310 $\mu\text{Ci}/\mu\text{mol}$), 0.5 μCi of ^{14}C -arginine (312 $\mu\text{Ci}/\mu\text{mol}$), 1.95 mg of *E. coli* S-30 protein, and 10 μg of the appropriate DNA. After a 30-min incubation period at 37 C the reactions were terminated as described in Materials and Methods, and radioactivity in hot trichloroacetic acid-insoluble material was determined in a 50- μl sample from each sample. Electrophoresis in SDS-polyacrylamide gels and autoradiography were performed as described in Materials and Methods. Migration of the tracking dye was 10 cm. Amounts of radioactivity (counts per minute) applied to gels were as follows: T4+, 432,000; T*4, 432,000; and T4gt, 435,000. Dried gels were exposed to X-ray film for 6 days, and densitometric tracings of the developed films were obtained on a Beckman DU spectrophotometer equipped with a Gilford scanning and recording device. B, The data presented in A were analyzed on a Dupont curve resolver where peaks A, B, C, D, E, and F were set equal to 100%. The percentage of the total area was determined for each peak. The results are presented graphically.

tain hosts which are able to degrade infecting phage DNA which is insufficiently glucosylated (5, 19). Nevertheless, several studies have been made to examine possible roles of glucosylated DNA in phage development other than protection against restriction enzymes. A normal

FIG. 5B

course of induction of phage early enzymes and of growth of T* phage in permissive hosts indicate equivalence of glucosylated and non-glucosylated DNA as far as the genetic code is concerned (8). Hybridization-competition studies with RNA synthesized in infected cells or in vitro with the complete sigma-containing *E. coli* RNA polymerase have suggested that partial glucosylation of the DNA template does not lead to a preferential transcription of either early or late genes (3, 21, 22).

Several investigators have shown that the mRNA transcribed from glucose-deficient DNA by the complete *E. coli* RNA polymerase is of the early class (3, 5). However, Hall et al. (10) found that 14% of the RNA made from non-glucosylated DNA in response to T4 polymerase (*E. coli* polymerase modified after T4 infection) corresponded to late sequences. There was also a concomitant increase in symmetric transcription. Since the T4 polymerase is not only devoid of sigma but is also modified in the core subunits (2, 25), it is difficult to know which of these changes account for the differences in specificity.

The data presented in Table 1 and Fig. 1 show that protein and RNA synthesis are both higher for partially glucosylated DNA in the in vitro system, and that the increased activity is inversely proportional to the glucose content of the DNA (Fig. 2). The reason for this is not

known. A simple explanation would be that the binding of RNA polymerase to DNA is interfered with by the glucose moieties. Alternatively, the differences in template activities might result from the fact that glucose-deficient T-even DNA is more susceptible to some nucleases (17, 20). That is, the glucose-deficient DNA might be a better template for RNA synthesis because it has received more nicks from nucleases present in the infected cell extracts from which it was isolated or present in the extracts used for in vitro RNA synthesis. However, Hurwitz et al. (12) have reported that T-even DNA incubated for a short time with low levels of DNase exhibits decreased, not increased, template activity for RNA. Also, in experiments not shown, it was found that vege-

FIG. 6. Inability of T4 vegetative DNA to accept glucose in vitro. Total intracellular DNA was prepared from wild-type T4-infected cells as described in Materials and Methods. Incubation mixtures for T4 beta-glucosyl transferase were as described in the legend to Table 2. Each tube contained 0.23 mg of S-145 protein from T4 amN122-infected *E. coli* B and 10 μ g of the appropriate DNA. After incubation at 37°C for the times indicated, the amount of ¹⁴C-glucose (6,950 counts per min per nmol) transferred was determined as described in the legend to Table 2. Data were corrected for zero time incorporation (approximately 400 counts/min). Symbols: ●, mature T2 DNA; ○, mature T4 DNA; ▲, vegetative T4 DNA.

tative DNA isolated from cells infected with T4 amH39X (DNA ligase, gene 30) is less active for stimulating protein synthesis than vegetative DNA from wild-type infected cells (G. S. Cox, Ph.D. thesis, University of Iowa, Iowa City, 1973). Furthermore, when the template activities of the glucose-deficient DNAs are measured with purified *E. coli* RNA polymerase, the results are the same. A third possibility is suggested by the experiments of Mokulskaya and co-workers (17) which showed that the glucosylated DNA of T-even phages cannot revert from the B to the A conformation, whereas the nonglucosylated DNA of T2 can go through this transition. This suggests that the glucose located in the large groove of the B form sterically prevents it from narrowing, an event which must take place during reversion of the DNA to the A form. Thus, nonglucosylated DNA may possess greater structural lability and therefore be capable of assuming a larger number of conformations than glucosylated DNA.

It is postulated that this decreased rigidity may be associated with the increased sensitivity of the nonglucosylated phage DNA to cell nucleases (17). It may also be the basis for the observed increase in RNA synthesis (Fig. 1), although the exact mechanism is unclear. Although overall synthesis is higher on partially glucosylated templates, the transcription of some cistrons is reduced as evidenced by the fact that the decrease in glucosylation is paralleled by a decrease in radioactivity migrating as band F in Figure 5B.

It is questionable whether these effects observed in vitro with nonglucosylated DNA play any significant role in phage development. Nevertheless, it is worth pointing out that Sauerbier and Bräutigam (22) have observed a two- to threefold increase in total RNA synthesis but substantial decreases in T4 lysozyme synthesis and burst size in *E. coli* CR63 (UDPG PPase⁻) hosts. Also, Hausman and Gold (11) noted that the rate of induction of a DNA methylase was twofold higher in T*2-infected than in T2-infected *Shigella*. In spite of this, consideration of the plating efficiencies (see Materials and Methods) and template activities (Fig. 2) of T4+ and T4gt, specifically the 100,000-fold increase in phage production of T4+ over T4gt in certain restrictive hosts compared to the twofold decrease in the rate of gene transcription for its fully glucosylated DNA, suggests that the effect of glucosylation on template activity was subordinate to an evolutionary event of much greater significance to the survival of the phage, namely protection of the DNA against restriction nucleases.

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