Glucosylation of Deoxyribonucleic Acid by Enzymes from Bacteriophage-infected Escherichia coli*

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The deoxyribonucleic acid of bacteriophages T2, T4, and T6 contains glucose linked to the hydroxymethyl groups of hydroxymethylcytosine. The work of Günsulemter, Volkin, Jesaitis, and of Lichtenstein and Cohen (1-4) established the presence of glucosyl-hydroxymethylcytosine residues in the deoxyribonucleic acid and indicated that there is a characteristic glucose to hydroxymethylcytosine ratio for each phage. Lehman and Pratt (5) have now published a detailed analysis showing four types of hydroxymethylcytosine residues which have the following distribution in these phages:

<table>
<thead>
<tr>
<th>Glucosyl-{alpha}</th>
<th>in T2 25%</th>
<th>in T4 0</th>
<th>in T6 25%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucosyl-{beta}</td>
<td>in T2 70%</td>
<td>in T4 70%</td>
<td>in T6 3%</td>
</tr>
<tr>
<td>Glucosyl-1,2-glucosyl</td>
<td>in T2 0</td>
<td>in T4 30%</td>
<td>in T6 0</td>
</tr>
<tr>
<td>(diglucosyl)</td>
<td>in T2 5%</td>
<td>in T4 0</td>
<td>in T6 72%</td>
</tr>
</tbody>
</table>

To learn the enzymatic mechanism that determines these characteristic glucosylation patterns, we first undertook studies of T2-infected Escherichia coli and found an enzyme in these cells that transfers glucose from uridine diphosphate glucose to HMC in DNA (6). This enzyme was not detected in cells infected with T5, a phage that does not contain HMC in its DNA, nor in uninfected cells. The enzyme did not glucosylate T2 DNA (which is already partially glucosylated) nor unpolymerized HMC deoxynucleoside mono- or triphosphate, but acted on enzymatically prepared HMC-containing DNA. We have now extended the study of this enzyme and also initiated studies of enzymes from T4- and T6-infected cells.

The results reported here indicate that infection of the cell with a given phage gives rise to distinctive glucosylating enzymes that may in large measure account for the characteristic distribution of glucose residues in the DNA of that phage. The DNA-glucosylating enzyme found in T2 phage-infected cells transfers a monoglucosyl group to HMC in {alpha} configuration, which is the predominant form of the HMC residues observed in the DNA of T2. After T4 infection, two enzymes were found. One enzyme, like the enzyme of T2 infection, adds a monoglucosyl group in {alpha} linkage to HMC. The second also adds only a monoglucosyl group to the HMC, but the configuration in this case is {beta}. The enzymatic results are thus compatible with the known composition of T4 DNA, in which all the HMC residues have an {alpha}- or {beta}-monoglucosyl substituent. Two glucosylating enzymes were also found after T6 infection. One of these, like the enzyme of T2 infection, adds an {alpha}-monoglucosyl group to HMC. The second does not react with DNA's lacking glucose, but only with those containing monoglucosyl-HMC groups. Diglucosylated residues are produced in which the linkage between the glucose residues has the {beta} configuration. Here again the results are compatible with the composition of T6 DNA. In no case does an enzyme induced by a given phage add glucose to the DNA of that phage.

The three enzymes that produce {alpha}-linked, monoglucosyl groups—one each from the T2, T4, and T6 infections—will be referred to as {alpha}-glucosyl transferases: T2-HMC-{alpha}-glucosyl transferase, T4-HMC-{alpha}-glucosyl transferase, and T6-HMC-{alpha}-glucosyl transferase. The enzyme of T4 infection that produces {beta}-linked monoglucosyl residues will be termed T4-HMC-{beta}-glucosyl transferase, and the {alpha} glucosyl transferase of T6-infected cells that converts mono- to diglucosyl residues will be called T6-glucosyl-HMC-{beta}-glucosyl transferase, or the T6-{beta}-glucosyl transferase.

This report presents the evidence for distinguishing these enzymes and provides brief descriptions of their separation and partial purification. Intensive investigations of the purification and action of each of the enzymes are now in order and studies along these lines have been initiated.

**EXPERIMENTAL PROCEDURE**

Glucose-labeled UDP-glucose (3.2 X 10⁶ c.p.m. per pmole) was prepared from uniformly labeled C⁶ glucose (Isotopes Specialties Company, Inc.) by first producing glucose 6-phosphate via the hexokinase reaction, and then causing this product to react with UTP in the presence of phosphoglucomutase, glucose 6-phosphate, and UDP-glucose pyrophosphorylase (7, 8). For routine enzyme assays the C⁶-UDP-glucose was diluted to a specific radioactivity of 2.2 X 10⁶ c.p.m. per pmole with UDP-glucose purchased from Sigma Chemical Company. Streptomycin sulfate was a gift from Merck and Company, Inc., and protamine sulfate from Eli Lilly and Company. DEAE-cellulose was purchased from Brown and Company (Berlin, New Hampshire).

Bacteriophage and E. coli DNA's were prepared according to Lehman's directions (9) or were gifts from Dr. Lehman. Thy- mus DNA was prepared according to Kay, Simmons, and Dounce (11). Synthetic DNA containing HMC was prepared by the action of DNA polymerase (10) on the deoxyribonucleoside triphosphates of adenine, guanine, thymine, and HMC, with the use of primer DNA from several sources. Concentrations of DNA are expressed as equivalents of nucleotide phosphorus.

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† National Science Foundation Predoctoral Fellow.

The abbreviations used are: HMC, 5-hydroxymethylcytosine; dHMP, 5-hydroxymethyl deoxyxystidlate; EDTA, ethylenediaminetetraacetate, sodium salt.
TABLE I

Fractionation of HMC-α-glucosyl transferases from bacteriophage-infected E. coli

<table>
<thead>
<tr>
<th>Enzyme source</th>
<th>Fraction</th>
<th>Total activity</th>
<th>Activity yield</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T2 infection</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude extract</td>
<td>58</td>
<td>(100)</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Streptomycin super-</td>
<td>76</td>
<td></td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>-natant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DEAE-cellulose</td>
<td>20</td>
<td>38</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>148</td>
<td>(100)</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>DEAE-cellulose</td>
<td>55</td>
<td>37</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>83</td>
<td>(100)</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>DEAE-cellulose</td>
<td>72</td>
<td>87</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>DEAE-cellulose</td>
<td>37</td>
<td>45</td>
<td>1.3</td>
<td></td>
</tr>
</tbody>
</table>

Enzymatic degradation of DNA to mononucleotides and ion exchange chromatography of the digests were carried out according to Lehman and Pratt. The mono- and digluosyl-DHMP used as reference materials in chromatography were gifts from Dr. Lehman, as were the diesterase and the α- and β-glucosidase. In several experiments HMC derivatives in the digests were resolved by paper chromatography. Descending chromatography on Whatman No. 3 paper in isobutyric acid ammonia yielded the following Rf values: dHMP = 0.94; monogluosyl-DHMP = 0.78; digluosyl-DHMP = 0.58.

The bacteria were grown and infected with T2r+ bacteriophage as described previously (reference 6, footnote 19). T4r+ and T6r+-infected cells were similarly prepared except that L-tryptophan (20 mg per liter) was added at the time of infection and the chloramphenicol was added 15 minutes after the phage addition.

Protein was measured by the method of Lowry et al. (13).

The glucosyl transferases used in all experiments were the most purified fractions obtained from the purification procedures to be described below.

**Assays for Glucosyl Transferases**

The enzymes were assayed by measurement of the amount of radioactivity of glucose-labeled C14-UDP-glucose converted to an acid-insoluble product in the presence of a suitable DNA. The acceptor DNA used was a synthetic HMC-containing DNA for the three α-glucosyl transferases, T2 DNA for the β-glucosyl transferase of T4-infected cells, and T4 DNA for the T6-glucosyl-HMC-β-glucosyl transferase. One unit of enzyme is defined as that amount that transfers 1 micromole of glucose in 1 hour.

**α-Glucosyl Transferases**—The assay mixture (0.2 ml) contained 100 mM Tris buffer, pH 7.5, 20 mM glutathione, 0.025 mM C14-UDP-glucose, 0.015 to 0.03 mM HMC-DNA, and 0.0002 to 0.001 unit of enzyme. (Enzymes were diluted in 20 mM phosphate buffer.) After 15 minutes at 30°, the DNA was precipitated, washed, and plated exactly as described for the above assay.

**T6-Glucosyl-HMC-β-Glucosyl Transferase**—The assay mixture (0.2 ml) contained 100 mM Tris buffer, pH 7.5, 40 mM 2-mercaptoethanol, 25 mM MgCl2, 0.05 mM C14-UDP-glucose, 0.08 mM T4 DNA, and 0.0002 to 0.001 unit of enzyme. (The enzyme was diluted in 20 mM phosphate buffer.) The mixture was incubated for 15 minutes at 30°, and then treated as above.

**Purification of Enzymes**

All the procedures were carried out at 0-5°. The centrifugations were at 10,000 to 12,000 X g.

T2, T4, and T6-HMC-α-Glucosyl Transferases (Table I)—T2-infected cells (12 g) suspended in 54 ml of 0.05 mM glycollycine buffer, pH 7.0, were treated for 20 minutes in a 10-kc Raytheon sonic oscillator. Of the supernatant fluid, 28 ml after centrifugation was at 10,000 to 12,000 X g.

**T4-HMC-β-Glucosyl Transferase** (Table II)—Crude extract (64 ml), prepared as above, was mixed with an equal volume of 1 N perchloric acid and centrifuged. The precipitate was dissolved in 0.3 ml of 0.2 N NaOH, precipitated with 0.3 ml of cold 1 N perchloric acid, diluted with 4 ml of cold water, and centrifuged again. The precipitate at this state was sometimes washed with 2 ml of cold 1 N acetic acid and recentrifuged. The precipitate was dissolved and washed into a planchet with two or three 0.4-ml portions of 2 N NH4OH. The planchet was dried and the radioactivity measured with a gas flow counter. Control assays, performed either in the absence of enzyme or without incubation, measured 5 c.p.m. or less over the background count of 15 c.p.m.

**T4-β-Glucosyl Transferase**—The assay mixture (0.2 ml) contained 100 mM potassium phosphate buffer, pH 7.8, 0.05 mM C14-UDP-glucose, 0.055 mM T2 DNA, and 0.0002 to 0.001 unit of enzyme. (The enzyme was diluted in 20 mM phosphate buffer.) After 15 minutes at 30°, the DNA was precipitated, washed, and plated exactly as described for the above assay.

*2 We are grateful to Dr. Margeris A. Jesaitis for suggesting the use of this solvent.*
0.05 M glycyglycine buffer, pH 7.0, then with 38 ml of a 1% solution of protamine sulfate, and centrifuged. The active precipitate was washed with 100 ml of 0.05 M potassium phosphate buffer, pH 6.8, and the enzyme was then eluted with 65 ml of 0.15 M potassium phosphate, pH 7.4 (protamine eluate). In subsequent fractionation with ammonium sulfate, large losses by coprecipitation were avoided by stepwise removal of early inactive fractions. Protamine eluate fraction (60 ml) was first treated with 13.4 g of ammonium sulfate and allowed to stand for 5 minutes. It was then centrifuged for 5 minutes and the precipitate discarded. This was repeated next with 5.5 g and then with 4.2 g of the salt. Finally 6.3 g of ammonium sulfate were added to the supernatant fluid. The precipitate that had developed after 5 minutes was collected by centrifugation for 5 minutes and dissolved in 1.5 ml of 0.05 M potassium phosphate, pH 7.4. Additional precipitate formed when the solution was kept at −10°C overnight was also collected, dissolved in 0.5 ml of the phosphate buffer, and combined with the previous fraction (ammonium sulfate I).

Ammonium sulfate I (1.5 ml) diluted with the same buffer to 6 ml was stirred with 1.5 g of ammonium sulfate. After 5 minutes the suspension was centrifuged and the precipitate discarded. Ammonium sulfate (0.4 g) was added to the solution and, after 5 minutes, the resulting precipitate was collected by centrifuging for 5 minutes. It was dissolved in 1 ml of 0.05 M potassium phosphate, pH 7.4 (ammonium sulfate II).

**T6-Glucosyl HMC-β-glucosyl Transferase**—This enzyme was obtained in the course of the isolation of the α-glucosyltransferase from T6-infected cells. T6 crude extracts contained about the same order of the glucosyl-β-glucosyl transferase activity as the α-glucosyltransferase—0.1 unit per mg of protein. Of the glucosyl-β-glucosyl transferase activity, 35% remained in the streptomycin supernatant fraction described above. When this preparation was then fractionated on DEAE-cellulose to isolate the T6-α-glucosyl transferase, the glucosyl-β-glucosyl transferase remaining in the streptomycin supernatant fraction became separated from the α-glucosyltransferase. Of the glucosyl-β-glucosyl transferase 30% was not held by the column; 60% appeared early in the elution, between 57 and 80 ml of eluant; and none was found in the later fractions in which the α-glucosyltransferase was eluted (115 to 126 ml of eluant). The glucosyl-β-glucosyl transferase used in the experiments that follow was the preparation eluted as just noted. It was dialyzed before use against 0.05 M Tris buffer (pH 7.5) containing 0.02 M glutathione. The activity of the preparation was 0.4 unit per mg of protein.

### RESULTS

**Comparison of Fractionation Behavior of Enzymes**

Five different enzymes that catalyze the glucosylation of DNA have been fractionated as described above from extracts of *E. coli* cells infected with bacteriophages T2, T4, or T6; one enzyme from the T2 infection; and two each from the T4 and T6 infections.

Certain similarities of the enzyme from the T2-infected cells and of one of the enzymes from the T4- and T6-infected cells, the α-glucosyl transferases, are apparent from the descriptions of the purification steps (Table I). The activity measured was the ability to glucosylate synthetic HMC-DNA. These three enzymes were not readily precipitated from the extracts by streptomycin sulfate, were quantitatively held by DEAE-cellulose, and were eluted from this adsorbent in similar yields and at similar salt concentrations.

The α-glucosyl transferase isolated from T4-infected cells glucosylated T2 DNA to only a slight extent (this will be discussed in detail in a later section). Crude extracts of T4-infected cells, on the other hand, were capable of more extensive glucosylation of T2 DNA. It was observed in studying this reaction of crude extracts that the addition of phosphate stimulated this activity 10-fold or more whereas, as will be seen later, the α-glucosyltransferase is inhibited by phosphate. Fractionation (Table II) of the crude extracts for the phosphate-stimulated glucosylating activity (T4-β-glucosyl transferase) showed that this enzyme was more readily precipitated by streptomycin sulfate and by protamine, and was separated from α-glucosyltransferase early in the purification procedure.

The α-glucosyltransferase prepared from T6-infected cells did not transfer glucose to T4 DNA, whereas the crude extract did. Fractionation of the crude extract on the basis of glucose transfer to T4 DNA (the T6-glucosyl-HMC-β-glucosyl transferase) showed this enzyme to be largely precipitated from the extracts with streptomycin sulfate, weakly held by DEAE-cellulose, and therefore readily separated from the α-glucosyl transferase (see "Purification of Enzymes").

**Effects of Some Salts and Sulfhydryl on Enzymes**

The α-glucosyltransferases were alike in their requirement for a protective sulphydryl reagent, their inhibition by phosphate buffer and by MgCl₂, and their insensitivity to EDTA (Table III). The β-glucosyl transferase of T4-infected cells, by contrast, could be fractionated and assayed in the absence of a sulphydryl reagent and was stimulated by phosphate buffer and MgCl₂. The phosphate buffer stimulation of the T4-β-glucosyl transferase shown in the table was obtained in the presence of 25 mM MgCl₂; under the assay conditions given above for this enzyme, MgCl₂ is not present and the enzyme is virtually inert in the absence of the phosphate buffer. The glucosyl-HMC-β-glucosyl transferase from T6-infected cells, like the α-glucosyl transferases, required a sulphydryl reagent but, like the T4-β-glucosyl transferase, was relatively inert in the absence of MgCl₂.

**Configuration of Glucosyl Linkages Produced by Transferases**

The transferases have been classified here as α- or β-transferases. The experimental evidence that each of these DNA-glucosylating enzymes acts specifically as an α- or β-glucosyl transferase is given in Table IV. DNA was glucosylated with C4-UDP-glucose by the different enzymes as shown, then isolated and degraded to nucleosides. These nucleosides were tested for susceptibility to splitting by specific α- and β-glucosidases, by determining the amount of C4-glucose released from

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total activity</th>
<th>Activity yield</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>129</td>
<td>100</td>
<td>0.7</td>
</tr>
<tr>
<td>Protamine eluate</td>
<td>95</td>
<td>74</td>
<td>0.49</td>
</tr>
<tr>
<td>Ammonium sulfate I</td>
<td>60</td>
<td>31</td>
<td>2.1</td>
</tr>
<tr>
<td>Ammonium sulfate II</td>
<td>23</td>
<td>18</td>
<td>5.9</td>
</tr>
</tbody>
</table>
all experiments, except those in which the phosphate buffer (pH 7.4) is specified, were carried out in the presence of Tris buffer (100 mM, pH 7.4). MgCl₂, 25 mM, was used in the experiments with the T₄-P-glucosyl and the TB-glucosyl-HMC-P-glucosyl transferases, in the assay; when mercaptoethanol was absent from the assay, the sulfhydryl compound was also omitted from the enzyme diluent. Other conditions and procedures are those described under "Assays for Glucosyl Transferases."

The values show the limits of β-glucose transfer reached under conditions of excess enzyme and UDP-glucose concentration and extended time of incubation. The values are expressed as a percentage of the calculated total number of HMC residues in the acceptor DNA added to the reaction mixture. Synthetic HMC-DNA's were enzymatically prepared with primer DNA's from T₂, T₄, T₆, E. coli, and calf thymus, and tested with all the glucosylating enzymes except the last enzyme; only T₂-primed DNA was used with the T₆-β-glucosyl transferase. Ps₂-labeled 5-hydroxymethyl deoxycytidine triphosphate was used in the synthesis for ease of measuring the amount of HMC in the DNA's. When the Ps₂ had decayed and its count was no longer measurable, the above experiments were carried out. The number of HMC residues in the phage DNA's was calculated as 0.16 mole of HMC per mole of phosphorus (15).
ference in the preparation. This experiment also shows that α-glucosyl transferase from T4-infected cells did not add glucose to T6 DNA, which β-transferase does.

The amount of glucose transferred to the DNA of T2 and T6 phages by the β-glucosyl transferase of T4-infected cells was close to the amount of unglucosylated HMC in these DNA's. It did not react at all with T4 DNA, as expected from the absence of unglucosylated HMC residues. The extensive reaction with synthetic HMC-DNA was not anticipated, in view of the fact that only 30% of the glucosyl residues in T4 DNA have the β configuration. Studies of T4 phage infection indicate that at about 10 minutes after infection, midway in the latent period, some phages are already completed while new DNA is still being synthesized (17). This suggests that the β-glucosyl transferase does not arise later in the infection than the α-glucosyl transferase, and it seemed worthwhile to obtain quantitative information on this question. The experiment summarized in Fig. 2 shows that the rate of appearance of the two transferases is about the same during the course of infection.

The T6-glucosyl-HMC-β-glucosyl transferase did not bring about any detectable transfer of glucose to synthetic HMC-DNA, whereas the enzyme from T4-infected cells glucosylated glucosyl-HMC residues of either α or β configuration. This is shown by the experiment in Table VI in which a sample of synthetic HMC-DNA was glucosylated to its limit with either of the two enzymes from T4-infected cells. α-Glucosyl-HMC was produced by one enzyme, β-glucosyl-HMC by the other (Table IV), yet the T6-glucosyl-HMC-β-glucosyl transferase when added in excess transferred to both types of monoglucosyl- and diglucosyl-HMC nucleotides.

**TABLE VI**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Glucose transferred</th>
<th>Glucose incorporated</th>
<th>T4-α</th>
<th>T4-α + T6-β</th>
<th>Δ due to T6-β</th>
<th>T4-β</th>
<th>T6-β + T6-β</th>
<th>Δ due to T6-β</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.3</td>
<td>0.2</td>
<td>1.4</td>
<td>1.6</td>
<td>2.9</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.3</td>
<td>0.2</td>
<td>0.7</td>
<td>1.7</td>
<td>2.3</td>
<td>0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.8</td>
<td>1.1</td>
<td>0.3</td>
<td>0.8</td>
<td>1.2</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Glucose transfer by HMC-α-glucosyl transferase of T4-infected cells to T2 DNA but not to T6 DNA. Reactions were carried out under assay conditions, amounts of enzyme, and DNA acceptor as shown. Time of incubation on T2 DNA curve: 10 minutes with 1 µg of enzyme, 15 minutes with 30 and 60 µg of enzyme; on T6 DNA curve: 12, 25, and 50 minutes at each point.

Fig. 2. Levels of α- and β-glucosyl transferases after infection of E. coli with T4+ bacteriophage. The study was done as described previously (Method II, reference (6)). Assays for α-glucosyl transferase were as described under "Experimental Procedure." For β-glucosyl transferase, incubation mixtures contained, in 0.2 ml: 100 mM Tris buffer (pH 7.5), 25 mM MgCl₂, 0.05 mM C¹⁴-UDP-glucose, 0.055 mM T2 DNA, and 7 to 15 µg of crude extract protein.

**DISCUSSION**

At the time that the DNA-glucosylating enzyme from T2-infected cells was described, it was known that the enzyme adds glucose only to HMC residues that are part of a DNA chain (6). It was not apparent then whether the distribution of glucose characteristic of T2, T4, and T6 DNA's would prove to be a result of this one enzyme acting on the different DNA's or to be due to additional enzymes produced in each infection. The experiments reported here demonstrate the latter alternative. Distinctive enzymes are found as a consequence of T2, T4, or T6 infections. The DNA isolated from one bacteriophage may...
Enzymatic transfer of C14-glucose from C14-UDP-glucose to acceptor DNA's was carried out in the reaction mixtures described for each enzyme. Enzyme was added in excess of that necessary to glucosylate to the limit the quantity of DNA used. Glucosylated DNA was isolated as described in the legend to Fig. 3, degraded completely to mononucleotides (5), and chromatographed on paper (see "Experimental Procedure"). After the chromatograms were developed, ultraviolet-quenching spots were cut out, eluted with 2.5 ml of 0.1 N HCl, and counted. No detectable radioactivity (<5%) was found at the starting line or in zones adjacent to and between the "mono" and "di" spots. Zero values are given where no reaction was detectable (see Table V) and no chromatograms were run.

**Table VII**

<table>
<thead>
<tr>
<th>Transferase</th>
<th>Synthetic HMC-DNA</th>
<th>T2</th>
<th>T4</th>
<th>T6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monoglucosyl-DHMP</td>
<td>&lt;5</td>
<td>&gt;95</td>
<td>&gt;95</td>
</tr>
<tr>
<td>T2-HMC-α-glucosyl</td>
<td>*</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T4-HMC-α-glucosyl</td>
<td>&gt;95</td>
<td>&lt;5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T6-HMC-α-glucosyl</td>
<td>&gt;95</td>
<td>&lt;5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T4-HMC-β-glucosyl</td>
<td>&gt;95</td>
<td>&gt;95</td>
<td>&lt;5</td>
<td>0</td>
</tr>
<tr>
<td>T6-glucosyl-HMC-β-glucosyl</td>
<td>0</td>
<td>&lt;5</td>
<td>&gt;95</td>
<td>&lt;5</td>
</tr>
</tbody>
</table>

*See Fig. 3.*

**Fig. 4.** Ion exchange separation of C14-diglucosyl-dHMP from a digest of T4 DNA after glucosylation by crude extract of T6-infected cells. Reaction mixture (3.0 ml) contained 100 mM Tris buffer, pH 7.5, 200 mM glutathione, 0.05 mM C14-UDP-glucose (300,000 c.p.m.), 0.05 mM T4 DNA, and 2.8 mg of enzyme protein; incubation time, 1 hour. DNA was isolated and degraded to mononucleotides (see legend of Fig. 3). The digest (41,000 c.p.m.) was chromatographed (16) with a mixture of authentic monogluco-syl-dHMP (1.4 μmoles) and 0.8 μmole of the diglucosyl analogue. Recovery of C14 was 92% of that put on the column.

serve as glucose acceptor with enzymes induced by other phages. Thus the DNA's from T2 and T6 phages, in which about 25% of the HMC residues are unglucosylated, will accept about this amount of glucose by the action of a T4 infected cell enzyme; presumably this enzyme is responsible for synthesis of the completely glucosylated T4 DNA in vivo. Similarly the DNA's of T2 and T4, which contain monoglucosyl-HMC but few or no disaccharide units, can be further glucosylated by an enzyme from T6-infected cells to an extent approximating the diglucosyl-HMC content of T6 DNA. Broadly speaking, the glucosylating enzymes identified in a given infection carry out reactions appropriate to the DNA being produced during that infection and will act equally well on the DNA's of other types of phages if sites are still available. The major question still remains as to how a given glucosylating enzyme reaches a fixed limit short of the total number of groups available for glucosylation in the DNA acceptor provided. A plausible interpretation is that the arrangement of the HMC residues in the DNA chain determines whether these residues can be glucosylated by a given enzyme.

Additional questions remain or have been raised by these studies. First, the existence of a small percentage of disaccharyl units in T2 DNA suggests the presence of an enzyme in T2-infected cells comparable to the disaccharyl-producing activity found in T6-infected cells; thus far such an enzyme has not been demonstrated. Secondly, in T4 DNA, 70% of the glucose residues are α-linked and the remainder are β-linked. When it was found that there are indeed two enzymes in T4-infected cells, one producing α linkage and the other β, it was expected that the "α enzyme" would proceed to a limit of 70% of available HMC residues and the "β enzyme" to a 30% limit. However, the glucosylation by the β enzyme of an enzymatically synthesized HMC-DNA is more extensive than by the α enzyme and generally in the ratio of 100:70. These findings hold whether the
synthesis of the HMC DNA has been primed by any of several DNA's, including the DNA's of T2, T4, or T6. The kinetics of appearance of the two enzymes during infection is similar. The question of what controls are at the basis of this 70:30 proportion of \( \alpha \) to \( \beta \) linkages in T4 DNA remains to be answered.

The transferases described in this report transfer the \( \alpha \)-linked glucose residue in UDP-glucose with a retention of configuration of the glucosidic linkage in the case of the \( \alpha \)-glucosyl transferase and an inversion in the case of the \( \beta \)-glucosyl transferase. Although examples of retention of the \( \alpha \) configuration in glucosyl transfer from UDP-glucose are the most common, instances of inversion have been well documented (18).

**SUMMARY**

1. Infection of *Escherichia coli* with one of the bacteriophages of the series T2, T4, and T6 leads to the production of enzymes which transfer glucose from uridine diphosphate glucose to hydroxymethylcytosine (HMC) residues in deoxyribonucleic acid (DNA). Each infection results in the development of one or more distinctive glucosylating enzymes which may in large measure account for the glycosylation pattern characteristic of the phage.

2. An enzyme partially purified from T2-infected cells transfers glucose to form monoglucosyl units in \( \alpha \) linkage to a fixed fraction of the HMC residues of enzymatically synthesized DNA; no transfer is detectable to DNA from T2 phage. An enzyme purified from T4-infected cells and an enzyme from T6-infected cells are similar in properties and action to the enzyme from T2-infected cells, but are distinguishable from the latter by their ability to transfer a small amount of glucose to T2 DNA. These three enzymes have been designated the HMC-\( \alpha \)-glucosyl transferases.

3. Another enzyme purified from T4-infected cells, and physically separable from the \( \alpha \)-glucosyl transferase, differs in many properties and forms monoglucosyl units in \( \beta \) linkage. It reacts with HMC residues of enzymatically synthesized DNA, as well as with essentially all the available HMC residues of T2 DNA and T6 DNA.

4. T6-infected cells contain two physically separable enzymes; one, the \( \alpha \)-glucosyl transferase, and another which adds a second glucose unit in \( \beta \) linkage to a pre-existing monoglucosylated HMC residue. These two enzymes acting in succession could account for the diglycosylated HMC units that preceed in T6 DNA.

**REFERENCES**
