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GLUCOSYLATION OF THE DEOXYRIBONUCLEIC ACID IN HYBRIDS OF COLIPHAGES T2 AND T4

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SUMMARY

1. The 5-hydroxymethylcytosine nucleotides in the DNA of hybrid phages derived from crosses of T2 and T4 have been examined. One strain ($\overline{T2}$(1.0)) which is like T2 but with the glucose content of T4 has been found to have the same glucosylation pattern as T4. A second strain ($\overline{T2}$(0.85)) which has a glucose content intermediate between T2 and T4 is qualitatively similar to T2 DNA in its distribution of glucosylated 5-hydroxymethylcytosine nucleotides; however, significant quantitative differences were observed.

2. The glucosyl transferases induced by these hybrid phages on infection of susceptible bacteria can reasonably account for the glucosylation pattern observed in their DNA.

INTRODUCTION

Coliphages T2 and T4 contain glucose attached to the HMC of their DNA. In T2 the molar ratio of glucose to HMC is 0.8, in T4 it is 1.0 (see refs. 1–5). Analysis of the phage DNA's has shown that these ratios reflect a characteristic distribution of nonglucosylated, α- and β-monoglucosyl, and α-gentiobiosyl HMC nucleotides[6,7]. As shown by KORNBERG *et al.*[8–10], glucosyl transferases are present in extracts of *E. coli* infected with T2 or T4, which to some extent account for the pattern of glucosylation observed in these DNA's.

In making genetic crosses between T2 and T4, STREISINGER AND WEIGLE[11] and WEIGLE *et al.*[12] found that all of the progeny, including those which otherwise resemble the T2 parent, have a glucose to HMC ratio of 1.0. However, in crosses in which bacteria are infected with T2, then super-infected after an appropriate interval with a T2-like phage having the glucose content of T4, progeny are found which have a glucose content intermediate between that of T2 and T4.

In the present study we have examined the HMC nucleotides in the DNA of these hybrid strains and found that their distribution is compatible, as in the parent strains, with the glucosyl transferases they induce on infection.

MATERIALS AND METHODS

Dr. STREISINGER kindly provided us with strains $\overline{T2}$ LBx9 No. 3, a T2-like strain which has a glucose to HMC ratio of 1.0, and $\overline{T2}$ L.V.G. 111 h, which has a ratio of

Abbreviations: HMC, hydroxymethylcytosine; UDPG, uridine diphosphate glucose.

0.85 (see below). The first strain will be referred to as $\overline{T2}$(1.0), the second as $\overline{T2}$ (0.85). Stocks of these phages were grown from single plaque isolates and found to have the properties described by STREISINGER *et al.*[11,12] DNA isolated from the phages was enzymically degraded, and the HMC nucleotides were separated by chromatography of the digests on Dowex-1 acetate, using methods previously described[6]. The configuration of the glucosidic linkages in the monoglucosylated nucleotides was established with the use of α- and β-glucosidases.[6]

RESULTS AND DISCUSSION

Composition of hybrid DNA's

The analysis and distribution of the HMC nucleotides in $\overline{T2}$ (1.0) and $\overline{T2}$(0.85) DNA is given in Fig. 1 and Table I; the data for T2 and T4 are included for comparison. $\overline{T2}$(1.0) DNA appears to be the same as T4 DNA, having 100 % of its HMC residues monoglucosylated, 70 % in α-configuration and 30 % in β-configuration. $\overline{T2}$(0.85) DNA is qualitatively similar to T2 DNA in that the monoglucosylated HMC contains glucose only in the α-configuration, and nonglucosylated and diglucosylated HMC residues are also present. However, it is quantitatively different since it con-

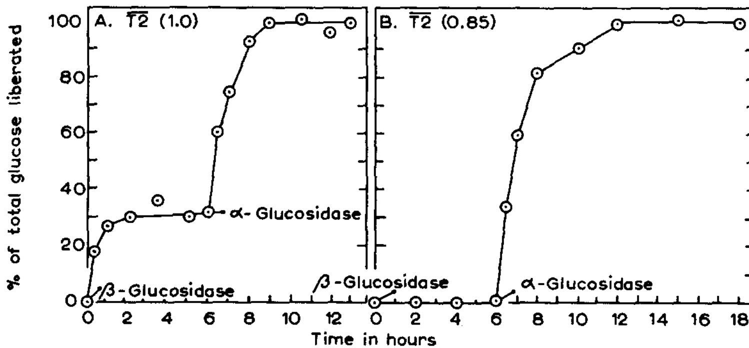

Fig 1. Enzymic release of glucose from monoglucosylated HMC nucleotides from $\overline{T2}$(1.0) DNA, and $\overline{T2}$(0.85) DNA. The assays were carried out as described in ref. 6.

TABLE I

PROPORTIONS OF GLUCOSYLATED HMC NUCLEOTIDES IN PARENT AND HYBRID PHAGE DNA'S

	Phage strain				
	*T2**	*$\overline{T2}$(0.85)***		*$\overline{T2}$(1.0)*	*T4**
	percent of total HMC				
		1	*2*		
Nonglucosylated	25	16.3	17.7	0	0
α-Glucosyl	70	82.4	80.8	70	70
β-Glucosyl	0	0	0	30	30
α-Gentiobiosyl	5	1.3***	1.5***	0	0

* Data from Refs. 6 and 7.
** Exp. 1 and 2 represent the analyses of DNA from two different phage isolates.
*** The disaccharide in this case was not identified but is presumed to be gentiobiose as in T2.

tains a higher proportion of monoglucosylated residues (81 %) and a lower proportion of nonglucosylated and diglucosylated residues (17.5 % and 1.5 %, respectively).

Glucosyl transferase activities in bacteria infected with hybrid phages

Extracts of *E. coli* infected either with T2 or with T4 contain α-glucosyl transferases which transfer glucose in α-configuration from UDPG to HMC-containing DNA. T4 infection also results in the formation of a β-glucosyl transferase, which adds glucose to HMC in the β-configuration. The α-glucosyl transferase from T2-infected cells will not further glucosylate DNA from T2, even though there are nonglucosylated HMC residues available, while both the α- and β-glucosyl transferases from T4-infected cells will completely glucosylate the remaining HMC residues in T2 DNA[8–10].

As shown in Table II, the glucosyl transferase activity of extracts of cells infected with $\overline{T2}$(1.0) is indistinguishable from that of T4-infected cell extracts and contains

TABLE II

GLUCOSYL TRANSFERASE ACTIVITY IN EXTRACTS OF PHAGE-INFECTED *E. coli*

Sonic extracts were prepared as described by KORNBERG, ZIMMERMAN AND KORNBERG[8] and assayed under the conditions described by JOSSE AND KORNBERG[10]. T2 DNA, 103 mμmoles of nucleotide (4.5 mμmoles of nonglucosylated HMC residues), or $\overline{T2}$(0.85) DNA, 117 mμmoles of nucleotide (3.6 mμmoles of nonglucosylated HMC residues), were used per assay. In each case, 0.02 ml of a sonic extract containing about 20 mg protein/ml was added. Under conditions for measurement of the α-glucosyl transferases, less than 0.5 % of the activity of the β-glucosyl transferase can be detected, while under the conditions for assay of the β-transferase about 10 % of the activity of the α-enzymes is expressed[10]. The values given represent approximations of initial rates since incorporation of [^{14}C]glucose into the DNA substrate was found to proceed linearly for only the first 10 min of incubation.

Extract of cells infected with	*α-Glucosyl transferase activity on*		*β-Glucosyl transferase activity on*	
	T2 DNA	*$\overline{T2}$(0.85) DNA*	*T2 DNA*	*$\overline{T2}$(0.85) DNA*
	mμmoles of glucose incorporated/15 min			
T2	0.05	$<$0.05	$<$0.05	$<$0.05
$\overline{T2}$(0.85)	0.93	$<$0.05	0.17	$<$0.05
$\overline{T2}$(1.0)	0.82	$<$0.05	2.7	0.47
T4	0.86	0.05	2.2	0.23

both α- and β-glucosyl transferases. The extract of $\overline{T2}$(0.85)-infected cells contains α-glucosyl transferase activity which is like that found in T4-infected extracts since it is able to glucosylate T2 DNA further. Moreover, it does so at about the same rate and to about the same extent as the T4-infected extract. A small amount of β-glucosyl transferase activity also appears to be present in the extract. This may, however, be attributable to α-transferase activity measured under conditions used for assay of the β-transferase.

On the basis of these results it would appear that, as with the parent phages, the glucosylation patterns of the DNA derived from the hybrid phages can be reasonably accounted for by the glucosyl transferases which they induce on infection. Thus, $\overline{T2}$(1.0) DNA contains both α- and β-linked glucosyl residues and the corresponding transferases are induced on infection of *E. coli* with this phage. Similarly, the DNA

from the $\overline{T2}$(0.85) strain which is qualitatively like T2 in that it does not contain β-glucosyl residues, appears to induce, on infection, only an α-glucosyl transferase.

It is of interest, however, that the α-glucosyl transferase induced by $\overline{T2}$(0.85) functionally resembles that from T4 rather than from T2 in its ability to glucosylate T2 DNA. Since the $\overline{T2}$(0.85) DNA does not contain β-glucosyl residues, it appears that such residues are not required for the induction of a T4-like α- transferase in $\overline{T2}$ (0.85)-infected cells. It might therefore follow that β-glucosyl residues in T4 DNA are not required for induction of the α-transferase in T4-infected cells. This argument is based on two assumptions: (a) The functional similarity in the T4 and $\overline{T2}$(0.85) α-transferases reflects a chemical and physical similarity in the two enzymes. (b) The cistron in T4 DNA directing the synthesis of the T4 α-glucosyl transferase contains β-glucosyl residues. The latter assumption is reasonable only if the β-glucosyl residues in T4 DNA are randomly distributed throughout the T4 genome.

It is also of interest that $\overline{T2}$(0.85) DNA, while qualitatively similar to T2 DNA in the distribution of its HMC nucleotides, differs significantly from it in its reduced ability to serve as a substrate for the α- and β-glucosyl transferases induced by T4. It has been suggested that the specificity of these glucosylating enzymes may be related to the secondary and tertiary structure of their DNA substrates[10]. If so, the insertion of part of the DNA of T4 into the DNA of T2 may have changed the secondary or tertiary structure of the resulting DNA sufficiently to produce an altered receptivity to glucosylation.

It seems likely that the genes controlling the formation of the α-glucosyl transferases of T2 and T4 are allelic (although the possibility has not been excluded that both enzymes are present in $\overline{T2}$ (0.85)-infected extracts). If the genes controlling the formation of the diglucosylating enzyme of T2 and the β-glucosylating enzyme of T4 are also allelic, one other type of recombinant between T2 and T4 would be possible; that is, a phage which would induce on infection the α-glucosyl transferase of T2 and the β-glucosyl transferase of T4. If, on the other hand, the genes are not allelic, a number of other combinations of glucosylating enzymes might be found. It would be of interest to look for such recombinants.

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