DNA methylation of viruses infecting a eukaryotic *Chlorella*-like green alga

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ABSTRACT

The genomic DNAs of the eukaryotic *Chlorella*-like green alga, strain NC64A, and eleven of its viruses all contain significant levels of 5-methyldeoxycytidine. In addition, the host DNA as well as six of the viral DNAs also contain N⁶-methyldeoxyadenosine. At least some of the methylated bases in the host reside in different base sequences than the methylated bases in the viruses as shown by differential susceptibility to restriction endonuclease enzymes. This suggests that the viruses encode for DNA methyltransferases with sequence specificities different from that of the host enzyme.

INTRODUCTION

We have recently discovered a number of viruses in fresh water ponds and rivers in Illinois, North Carolina, and South Carolina which form plaques on lawns of a unicellular, eukaryotic, *Chlorella*-like green alga (1). All of these viruses are polyhedrons (160 to 190 nm in diameter) and contain large dsDNA genomes (ca. 300 kbp as estimated by summing restriction fragments). Although these viral DNAs exhibited extensive homology with the previously described PBCV-1 virus DNA (1, 2), the viruses can be distinguished from one another and from PBCV-1 by at least one of the following characteristics: plaque size, DNA restriction patterns, or resistance to certain DNA restriction endonucleases. We have grouped the viruses into 5 classes based on the sensitivity or resistance of their DNAs to 15 restriction endonucleases (1). The resistance of the viral DNAs to certain restriction enzymes suggested that the viral DNAs might contain modified bases.

The present report describes base analyses of eleven of these viral DNAs as well as the host *Chlorella* DNA and establishes the presence of 5-methyldeoxycytidine (m⁵dC) in

host nuclear DNA and all the viral DNAs. The host DNA, as well as six of the viral DNAs, also contain N⁶-methyldeoxyadenosine (m⁶dA). Finally we show that at least some of the methylated bases in the host reside in different base sequences from those in the viruses.

MATERIALS AND METHODS

Preparation of viral and host DNAs.

The production and purification of the viruses (PBCV-1, NC-1A, NC-1B, NC-1C, NC-1D, SC-1A, SC-1B, Il-2A, Il-2B, Il-3A, and Il-3D) and the growth of the host *Chlorella* NC64A on MBBM medium have been described (1, 2). Viral DNAs were isolated on CsCl gradients from purified viruses as described (3). Host nuclear DNA was also isolated and separated from chloroplast DNA on CsCl gradients as described (4) except that the cells were disrupted in a mortar and pestle and the DNA extracts were not intentionally sheared.

The purified DNAs were enzymatically digested (to avoid potential acid degradation of any unusual bases) and the resulting deoxynucleosides were analyzed by both ion-exclusion and reverse-phase high performance liquid chromatography as described (5).

The DNAs were treated with restriction endonucleases according to manufacturers' recommendations and fragments were separated by electrophoresis on 1.2% agarose gels in 0.08 M Tris-phosphate and 0.008 M EDTA, pH 8.5.

To determine if m⁶dA was responsible for the resistance of PBCV-1 DNA to the restriction endonuclease *Mbo*I, we used plasmid pLG164, which contains a 16 kbp *Bam*HI fragment of PBCV-1 (designated B6) cloned into pBR322. This plasmid was purified from its original host (*E. coli* strain LE392) and transformed into *E. coli* strain GM2163 using standard procedures (6). *E. coli* GM2163, kindly provided by Dr. Martin Marinus, lacks both *dam* and *dcm* methylating activity (7). The plasmid was restricted with *Bam*HI and the viral fragment B6 insert DNA was recovered from low melting agarose gels by repeated phenol and phenol:chloroform extractions. The purified fragment was nick-translated with α -³²P dCTP (800 Ci/mmol) using a nick translation kit (Bethesda Research Labs) according to the manufacturer's recommendations. This labeled fragment was used to probe Southern blots (8) of PBCV-1 virus

DNA and pLG164/GM2163 DNA which had been sequentially digested with restriction endonucleases BamHI and either DpnI, MboI, or Sau3AI.

RESULTS

Base compositional analysis.

The percentages of modified bases found in eleven viral DNAs and the host nuclear DNA are summarized in Table 1. Although all the viral DNAs contained readily detectable levels of m^5dC , viruses NC-1A, NC-1B, IL-2A, IL-2B, IL-3A, and IL-3D contained high levels of this nucleoside (7.0 to 13.4%). With the exception of NC-1D (0.33% m^5dC), the remaining four viruses contained intermediate levels (1.7 to 2%) of m^5dC . The host Chlorella nuclear DNA contained 21.2% m^5dC .

In addition to m^5dC , m^6dA was present in the host nuclear DNA (0.6%) and in six of the seven viruses from North and South Carolina (1.5 to 8.2%). The virus NC-1D and the four Illinois viruses lacked detectable m^6dA . The lower limit of detection in these experiments was about 0.02%.

The overall G + C content of the host nuclear DNA was 67 mole percent and the viral DNAs ranged from 39 to 41 mole percent. There was no indication that the viruses contained modified bases other than m^5dC and m^6dA .

Table 1. Concentration of methylated bases in the viral DNAs and their host, Chlorella NC64A.

<u>Virus</u>	<u>Percent Methylated Bases</u>	
	m^5dC^a	m^6dA^b
PBCV-1	1.86 (6) ^c	1.45 (3)
NC-1A	7.06 (5)	7.32 (2)
NC-1B	13.36 (4)	8.19 (2)
NC-1C	1.72 (4)	1.59 (2)
NC-1D	0.33 (4)	<0.08 (2)
SC-1A	1.94 (6)	7.34 (3)
SC-1B	2.04 (4)	7.45 (2)
IL-2A	9.38 (4)	<0.01 (2)
IL-2B	10.85 (6)	<0.08 (4)
IL-3A	9.67 (4)	<0.03 (2)
IL-3D	12.62 (5)	<0.01 (3)
Host nuclear	21.23 (6)	0.59 (4)

^apercent m^5dC per dC + m^5dC + deoxyuridine.

^bpercent m^6dA per dA + m^6dA + deoxyinosine.

^cNumber of determinations

Figure 1. Electrophoresis of genomic PBCV-1 DNA treated with BamHI (lanes 1, 3, 5) or a 16 kbp PBCV-1 BamHI fragment, B6, cloned in an E. coli dam⁻, dcm⁻ host (lanes 2, 4, 6). All of the DNAs were first treated with BamHI followed by digestion with either MboI (lanes 1 and 2), DpnI (lanes 3 and 4) or Sau3AI (lanes 5 and 6). The bands were localized by probing with the ³²P-labeled PBCV-1 B6 fragment after Southern blotting. Molecular weights in kbp are indicated on the side of the figure. Note: the PBCV-1 DNA fragment B6 is digested by DpnI and resistant to MboI; after cloning fragment B6 in the dam⁻, dcm⁻ E. coli it is resistant to DpnI and digested by MboI.

Sequence specificity of the methylation.

We reported previously that the DNAs from PBCV-1, NC-1A, NC-1B, NC-1C, SC-1A, and SC-1B were resistant to cleavage by MboI and sensitive to DpnI (1) which suggests that m⁶da is present in the GATC sequence. To confirm that the resistance of PBCV-1 DNA to MboI and its sensitivity to DpnI was due to GATC methylation, plasmid pLG164, containing a PBCV-1 BamHI fragment, was grown in an E. coli dcm⁻, dam⁻ strain. Following re-isolation, the PBCV-1 fragment was now sensitive to MboI but resistant to DpnI (Fig. 1). This result indicates that during propagation in the dam host, the cloned PBCV-1 segment lost the GATC methylation present in the genomic virion DNA.

Host nuclear DNA shows a different pattern of

Figure 2. Electrophoresis of *Chlorella* nuclear DNA after treatment with either *Sau3AI* (lane 2), *MboI* (lane 3), *DpnI* (lane 4), *MspI* (lane 6) or *HpaII* (lane 7). The DNA in lanes 1 and 5 was untreated.

resistance/sensitivity to these restriction enzymes. The host DNA was cleaved by *MboI* (and *Sau3AI*) but was partially resistant to *DpnI* (Fig. 2, lanes 1 to 4). Likewise the viral DNAs which lack *m* *d* *A* exhibit the same sensitivity pattern as host DNA (1). These results clearly indicate that *m* *d* *A* is present in GATC sequences of PBCV-1, and probably in NC-1A, NC-1B, NC-1C, SC-1A and SC-1B viral DNAs but is absent in a major portion of the host nuclear GATC sequences.

In a parallel experiment the susceptibility of the host DNA to the restriction endonucleases, *MspI* and *HpaII*, was determined. These two enzymes recognize the sequence CCGG; however, *MspI* will not cleave this sequence if the external C is methylated, whereas *HpaII* will not cleave it if the internal C is methylated (9). All of the viral DNAs are cleaved by both

enzymes (1). In contrast, nuclear DNA was susceptible to restriction by MspI but poorly restricted by HpaII (Fig. 2, lanes 5 to 7). This suggests that at least some of the m⁵dCs in the host DNA, but not in viral DNAs, are located in the internal C of the sequence CCGG.

DISCUSSION

These results establish that the genomic DNA of the eukaryotic green alga, Chlorella, strain NC64A, and eleven of its viruses contain significant levels of methylated bases. Chlorella nuclear DNA contains high levels of m⁵dC (21.2%), as do certain higher plants (10, 11), but not other green algae (12, 13). This Chlorella strain also contains a low concentration (0.59%) of m⁶dA. The nuclear DNA of several other eukaryotic protists also contains m⁶dA. Examples include the green alga Chlamydomonas reinhardtii (13), the protozoa, Tetrahymena thermophila (previously named pyriformis) (14, 15) and Paramecium aurelia (16), and certain dinoflagellates (17).

While some bacteriophages commonly contain small amounts of methylated bases, viruses that infect eukaryotic organisms typically lack methylated bases (18,19). One exception is frog virus 3 (FV 3), an Iridovirus which contains approximately 20% m⁵dC in its genome (20). Recent experiments suggest that FV 3 also encodes for a unique DNA methyltransferase (21). Another animal virus containing m⁵dC is human papilloma virus (22, 23); in this instance, methylation occurs in specific CCGG and GCGC sequences.

All the viruses described in this report contain m⁵dC; the concentration of m⁵dC varies from 0.33% to 13.4%. The viral DNAs are not methylated at most of the CCGG sequences, since they are equally susceptible to HpaII and MspI restriction endonucleases (1). This is in contrast to the host Chlorella DNA which is almost completely resistant to HpaII, but sensitive to MspI, indicating that it contains m⁵dC in the sequence C^mCGG. Six of the Chlorella viruses also contain m⁶dA. In contrast to the host Chlorella, the viral DNAs contain m⁶dA in GATC sequences.

These results suggest that the viruses may also encode one or more unique DNA methyltransferases. In addition, the viral DNA does not appear to be a substrate for the host DNA methyltransferase(s) in vivo. We have examined PBCV-1 infected

cells for DNA methyltransferase activity and preliminary results indicate that infected cells contain much higher activity than uninfected cells. Characterization of this methyltransferase activity is in progress.

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