Deoxyribonucleic Acid of Adeno-associated Satellite Virus

HEATHER D. MAYOR, LIANE JORDAN, AND MICHIO ITO

Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas 77025

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Staining patterns suggest that in the adeno-associated satellite virion there exist quasi single-stranded regions which are renatured after extraction to exhibit double strandedness.

The deoxyribonucleic acid (DNA) of the adeno-associated satellite virus has a number of unusual properties. First, it is functionally defective and can lead only to production of satellite virions in the presence of replicating adenoviruses (1, 4, 14). Second, in smears prepared from purified and concentrated satellite virions, the DNA develops staining reactions with acridine orange (AO) consistent with a single-stranded secondary structure (11). However, biochemical analysis and hydrodynamic data obtained on DNA extracted from satellite virions are consistent with a double-stranded configuration (13, 16).

One explanation of these conflicting results would be a possible structural difference between the state of the DNA in the mature virion and after extraction. In the present study, we have compared reactions of satellite virions and satellite DNA with diluted formaldehyde and with AO with those of a number of purified viruses and viral nucleic acids in which the secondary structure of the latter is reasonably well established. The results of these experiments are reported here.

Purified viruses and nucleic acids were prepared as dried-down droplets for staining with 0.01% AO at pH 4 as described previously (9). Materials were fixed in Carnoy's fluid or in 1% acrolein (10), or they were examined in the unfixed state. Suspensions used for vital staining were incubated with 0.01% AO in water for 1 hr in the dark. Fluorochroming of agar-gel immunodiffusion bands was carried out as described by Ohlson (12). When diffusion lines were well established, the plates were fixed in Carnoy's fluid and stained with AO.

For fluorochroming preparations of the larger virions (adenovirus, reovirus), concentrations were adjusted to approximately 10^{10} particles/ml, as determined by electron microscopic particle counts. Concentrations of the smaller virions (poliovirus, satellite, ϕ X 174) were adjusted to

10^{11} particles/ml. Therefore, the nucleic acid content of microdroplets was equivalent. Nucleic acid preparations were diluted to contain 1 μ g of DNA per ml, and 2 μ g of RNA per ml. All fluorochromed preparations were examined with a Zeiss fluorescence microscope filtered to emit in the blue-violet range. Techniques for fluorescence microscopy used in this laboratory have been described (8).

The staining reactions with acridine orange of the following materials were studied: satellite virions, satellite DNA, bacteriophage ϕ X 174, ϕ X 174 single-stranded DNA, adenovirus double-stranded DNA, bacteriophage R17 single-stranded RNA, and calf thymus DNA. Except for satellite virion preparations, the other nucleic acids gave typical results, indicative of their expected secondary structure. Choice of fixative (Carnoy's or 1% acrolein), drying of smears before or after their reaction with AO, or fluorochroming undried preparations (immunodiffusion bands) did not influence the final staining reactions. Double-stranded DNA (adenovirus) and double-stranded RNA (replicative form of bacteriophage R17) could also be readily distinguished by fluorochroming procedures and enzyme digestion tests. Satellite virions gave staining patterns with acridine orange consistent with a single-stranded structure (red fluorescence), whereas those obtained on extracted satellite DNA confirmed a double-stranded helix (yellow-green fluorescence). Apparently, "quasi" single-stranded regions exist in the virion which are renatured to form a firmly hydrogen-bonded macromolecule after extraction.

In the formaldehyde experiments, conditions similar to those described by Sinsheimer (17) were used. Equal volumes of virus or nucleic acid in phosphate buffer (0.1 M, pH 7.5) were mixed with 3.6% formaldehyde to give a final concentration of 1.8% HCHO. The mixtures were incubated in stoppered tubes for the required time interval and the absorption spectrum was

determined. Ultraviolet absorption measurements were carried out in a DK2 spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.) equipped with microcuvettes (approximately 0.2 ml).

Sinsheimer (17) showed that the single-stranded DNA of bacteriophage ϕ X 174 can react with diluted formaldehyde at 37 C even in the intact virion. The reaction was characterized by an increase in optical density and a shift of the absorption maximum to a longer wavelength. Double-stranded DNA does not react under these conditions and shows no change in absorption properties. The results of similar experiments are shown in Fig. 1-4. As shown in Fig. 1, satellite 4 virus gave a 20-hr reaction pattern with dilute formaldehyde similar to that of ϕ X 174 (approximately a 25 to 30% increase in optical density and a 6-nm shift in wavelength maximum). Control preparations of double-stranded calf thymus DNA were unaffected by formaldehyde.

When purified preparations of naked viral nucleic acids were reacted with formaldehyde under similar conditions, typical single-stranded reactions were obtained with R17 RNA (Fig. 2). DNA from adenovirus 2 was virtually unaffected (Fig. 3), consistent with its double-stranded

FIG. 1. Effect of formaldehyde (1.8%) upon the ultraviolet absorption spectra of satellite virus and ϕ X 174 virus. The formaldehyde reactions were carried out at 37 C for 20 hr.

FIG. 2. Effect of formaldehyde (1.8%) upon the ultraviolet absorption spectrum of the single-stranded RNA from bacteriophage R17. The formaldehyde reaction was carried out at 37 C for 20 hr.

structure, and the extracted DNA from satellite virus 4 was also unaffected (Fig. 4).

These results indicate that, in the virion, free amino groups on satellite DNA are available for reactions with dilute formaldehyde. Once the DNA is extracted, these reaction sites are no longer available and are most probably hydrogen-bonded into a stable double-stranded helix. Thus, substantial regions of partially denatured, or "quasi" single-stranded, DNA seem to exist in satellite virions. Renaturation of this DNA occurs on release from the capsid.

Recently, interest has been focused on an emerging group of small DNA-containing "picodna" viruses (7, 11). There have been conflicting reports on the secondary structure of the DNA of a number of these viruses.

Crawford (2) found an increase in absorption and a shift of the maximum to a longer wavelength for MVM virus after reaction with formaldehyde, indicating that the amino groups were available for reaction and the nucleic acid presumably single-stranded in virio. Hydrodynamic data obtained on extracted MVM DNA was also consistent with a single-stranded DNA.

FIG. 3. Effect of formaldehyde (1.8%) upon the ultraviolet absorption spectrum of DNA prepared from adenovirus type 2. The formaldehyde reaction was carried out at 37 C for 20 hr.

FIG. 4. Effect of formaldehyde (1.8%) upon the ultraviolet absorption spectrum of DNA prepared from satellite virus type 4. The formaldehyde reaction was carried out at 37 C for 20 hr.

Unfortunately, formaldehyde curves were not obtained on the extracted DNA. Recently (3), chemical analysis of MVM DNA has shown a base composition consistent only with a single-stranded structure and a molecular weight of no more than 1.7×10^6 daltons for the DNA—the smallest reported value for an autonomous DNA animal virus. By contrast, May and colleagues (7) found narrow-range melting characteristics, orthochromatic fluorochroming patterns, and failure to react with formaldehyde for the DNA from K rat virus, properties consistent with a double-stranded helix. More recently, Robinson and Hetrick (15) have obtained convincing evidence that rat virus contains single-stranded DNA. Single-stranded DNA has also been reported for X14 rat virus (5). Both MVM and K rat virus appear to be able to replicate in susceptible cells without assistance from adenoviruses (2, 6). In addition, the DNA of K rat virus appears to have a molecular weight of 2×10^6 daltons. This low molecular weight, together with its relatively low guanine + cytosine content of 45% (7), could contribute to a

more stable secondary structure both within and outside the capsid.

The adeno-satellite viruses are the only members of this group known to be biologically defective. It is unlikely that this defect is due to a paucity of DNA as they appear to contain approximately 3×10^6 daltons (13, 16) as compared with the 2×10^6 daltons for K rat virus (7). However, if satellite viruses contain single-stranded DNA in the virion which somehow becomes double-stranded on extraction, it is possible that the population comprises particles containing complementary single-strands in different particles. This possibility has been suggested by Crawford (3), although he concluded that it was unlikely. In this case, the DNA molecular weight would be only about 1.5×10^6 daltons. Its small amount could be a contributing factor in defectiveness. Further experiments are planned to elucidate these points.

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