STUDIES ON THE DNA OF A VIRUS FROM *GALLERIA MELLONELLA*

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1. Introduction

A small spherical virus, Galleria Free virus, (GFV), and empty protein shells (top component) have been isolated from the insect, *Galleria mellonella*. GFV has been shown to contain DNA [1]. We wish to describe some unusual features of the DNA which appears to have a partially single-stranded conformation in the virus and which, when isolated, adopts a highly ordered structure characteristic of native double-stranded DNA.

2. Experimental

GFV and its associated top component were purified as previously described [1]. Unless otherwise stated all samples were suspended in a standard buffer of 0.1 M NaCl, 0.7 mM sodium phosphate, pH 7.0. DNA was prepared by phenol-extraction of virus essentially as described by Sinsheimer [2].

The effect of 2% formaldehyde on the ultraviolet absorption spectrum of virus and DNA at 37°C was examined by a method similar to that of Crawford [3].

Thermal denaturation of virus and DNA was studied at 260 nm as previously described [4]. For virus and top component readings at 320 to 350 nm were recorded to correct the reading at 260 nm for background scattering. Hyperchromism is defined as $[(OD_T/OD_{20°})^{-1}]$ × 100 per cent, where $OD_{T°}$ is the optical density at 260 nm at temperature T° and $OD_{20°}$ is the optical density at 260 nm at 20°C. The melting temperature, T_m, is defined as the temperature at which 50% of the total hyperchromism is attained.

Ultracentrifuge studies were carried out at 20°C in a Spinco Model E analytical ultracentrifuge fitted with both Schlieren and ultraviolet absorption optics. Molecular weights of virus and top component were determined by sedimentation equilibrium using short columns of solution as described by Yphantis [5]. The concentration distribution was measured over the entire length of the column and the results were analyzed by the method of Lamm [6] and Van Holde and Baldwin [7].

Concentrations were determined from the refractive index of the solutions using a specific refractive index increment for both virus and top component of 0.186/g/ml [8]. The extinction coefficient of the DNA in the virus was calculated assuming a DNA: protein ratio of 37:63 [1] after correcting for the spectral contribution of the protein to the viral spectrum.

3. Results

The addition of 2% formaldehyde to virus produced an increase in absorption over a period of 24 hr, and a shift in absorption maximum from 261 nm to 265 nm (fig. 1). The total increase was about 10% at the maximum. Formaldehyde had no effect on the sedimentation properties of the virus neither did it affect the spectrum of top component in the range 320–240 nm. The magnitude of this increase may be compared with the 12 per cent increase found for ϕ X 174 DNA on reaction with formaldehyde. This latter DNA has been shown to exist in a single-stranded conformation in which the amino groups on the nucleotides are not involved in specific hydrogen bonding [2]. Thus a large proportion of the amino groups on GFV DNA appear to be accessible to reaction with formaldehyde. This implies that the DNA in GFV is partially single-stranded. Similar spectral changes have been observed in the case of two other small DNA viruses, ϕ X 174

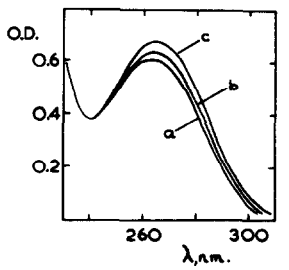

Fig. 1. The effect of 2% formaldehyde on the spectrum of GFV: (a) at zero time; (b) one hr after addition of formaldehyde; (c) 24 hr after addition of formaldehyde.

[2] and Minute Virus of Mouse [3], and were also attributed to the presence of single-stranded regions of DNA in the virus.

By contrast, 2% formaldehyde had no effect on the absorption spectrum of isolated GFV DNA or on native calf thymus DNA which is known to have a double-helical conformation. This suggested that there were no reactive amino groups in the GFV DNA, a property which is characteristic of the native double-stranded conformation.

The melting profiles of virus and DNA are shown in fig. 2. The optical density of virus remained nearly constant up to 55°C. Heating to 55°C followed by cooling did not change the sedimentation coefficient of the virus. Above this temperature the optical density decreased by 13–18% and the sedimentation pattern of the virus showed that breakdown had occurred. The decrease was a time-dependent process. It was followed by a sharp increase of about 30% beginning at 74°C. DNA melted sharply with an overall hyperchromism of 35% over the same temperature range, 74–85°C, as virus. DNA partly renatured on slow cooling. In both cases the T_m of the overall increase was 79.5°C. The sharpness and magnitude of the hyperchromism imply that the DNA is in a double-stranded helical form. This is consistent with the lack of reaction with formaldehyde, and is in marked contrast to the melting behaviour of isolated ØX 174 DNA which is single-stranded [2]. In 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0, the melting temperature of DNA was 85°C, which corresponded to a G plus C content of 40 per cent [11,17]. The similarities in the melting profiles of virus and DNA suggest that the following sequence of events occur on heating virus. Above 55°C the virus is disrupted and liberates partially single-stranded DNA. This then adopts a more ordered conformation as shown by the decrease in optical density. On further heating the more ordered conformation melts at a higher temperature in a manner characteristic of double-stranded native DNA, which implies that the DNA liberated on heating virus assumes a double-stranded conformation. The hypochromism observed on disrupting virus also shows that the conformation of the DNA in the virus is more hyperchromic than the double-helical form it adopts when liberated or isolated. Since hyperchromism is thought to reflect primarily the extent of interaction between stacked nucleotides, there is less interaction between the bases of the DNA in the virus than between the bases of native double-stranded DNA. This would be consistent with the DNA having a single-stranded conformation when it is in the virus as suggested by the formaldehyde results.

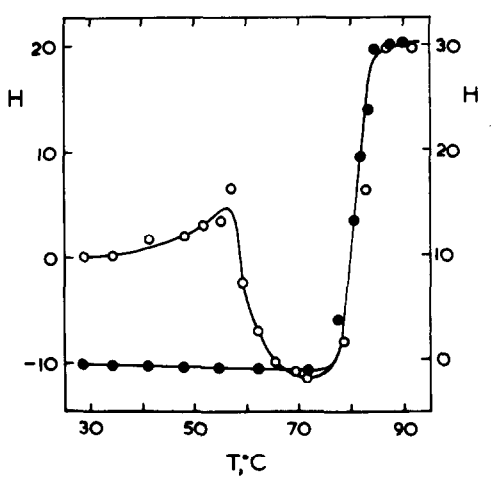

Fig. 2. The melting profiles of GFV and GFV-DNA in 0.1 M NaCl, 0.7 mM sodium phosphate pH 7.0: –o– GFV; –•– DNA. H = hyperchromism expressed as a percentage. Left-hand ordinate for GFV, right-hand ordinate for DNA.

The extinction per mole of DNA phosphorus at 260 nm for virus, allowing for absorption due to the protein moiety was 7.9×10^3. This is considerably higher than the value of 6.6×10^3 usually found for native, double-stranded DNA [9], and provides further evidence that the conformation of the DNA in the virus, although probably highly ordered, is more

like that of denatured DNA than native double-helical DNA.

The DNA sedimented as a single sharp boundary with $S^{\circ}_{20,w}$= 16 S. Using the relation between sedimentation coefficient and molecular weight developed by Eigner and Doty [10] and assuming the DNA to have the conformation characteristic of native DNA this corresponds to a molecular weight of 4.0×10^6. These findings are similar to those of Truffaut et al. [11]. The molecular weights of virus and top component measured by sedimentation equilibrium and extrapolated to zero concentration were 5.7×10^6 and 3.5×10^6 respectively [12]. By difference this gives the molecular weight of the DNA in the virus as 2.2×10^6, in marked contrast to the value estimated from the sedimentation coefficient. Thus the molecular weight of the isolated DNA is double that of the DNA in the virus.

4. Discussion

The spectral studies show that in the virus the DNA is partly single-stranded. This is not unexpected in view of the small size of the spherical virus particle which is only about 20 nm in diameter [1], and which could not accommodate an essentially rigid linear double helix. However, on isolation in solutions of high ionic strength or when liberated from the virus by thermal denaturation, the DNA appears to have properties identical with those of native double DNA in which the two strands are joined together by complementary base pairing. Furthermore, the molecular weight of the isolated DNA is twice that of the DNA encapsulated in the virus. Two possible explanations for these observations may be considered. Firstly, the DNA in a single virus particle has an arrangement of nucleotides which is almost completely complementary but which only forms specific base pairs when the DNA is free. In this case the molecule would form one 'hairpin' loop of molecular weight two million and would sediment with a sedimentation coefficient very much lower than that observed unless the hairpin itself was more flexible than an equivalent length of native DNA. Secondly, DNA molecules from different virus particles are complementary. This latter possibility although unlikely is supported by the observation that the apparent molecular weight of DNA deduced from its sedimentation behaviour is double that of the DNA in the virus.

A similar situation appears to arise in the case of the Adeno-associated viruses, AAV-1 and AAV-4. Thus, AAV-4 reacted with acridine orange as though the virus contained single-stranded DNA [13], whereas the extracted DNA was found to be double-stranded [14]. The DNA from AAV-1 has also been shown to be double-stranded [15] and it has been argued that the molecular weight of the isolated DNA is twice that of the DNA in the virus [16]. On this evidence it has been suggested that the two complementary strands are present in different virus particles [16]. Unless the isolated GFV DNA has hydrodynamic properties unlike those of native DNA, which would invalidate the estimation of the molecular weight based on the sedimentation coefficient, then we conclude that GFV also exists as a mixed population of particles each containing a complementary strand of DNA which is in a partly single-stranded conformation in the virus. On isolation these strands unite to form a double-helical structure. This is the first observation of such behaviour associated with a virus capable of free replication in the host.

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