Structural Transitions of Cowpea Chlorotic Mottle Virus

By K. W. ADOLPH

Medical Research Council Laboratory of Molecular Biology,
Hills Road, Cambridge CB2 2QH, U.K.

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SUMMARY

Particles of cowpea chlorotic mottle virus (CCMV) underwent an abrupt structural transition as the pH was raised to near pH 6.75. At low ionic strength (I = 0.2) the transition was observed as a decrease from 83S to 73S in the sedimentation coefficient of the virus particle. At high ionic strength (I = 1.0) the virus disassembled to components sedimenting at about 40S. These observations suggest a structural role for the RNA in maintaining virus stability. As ionic strength at pH 7.5 was increased, a critical point was reached (I = 0.4) at which the virus disassembled to the 40S aggregates. The effect of raising the temperature of both unswollen and swollen virus was to gradually, but substantially, reduce the sedimentation coefficient.

INTRODUCTION

A decrease in the sedimentation coefficient of cowpea chlorotic mottle virus (CCMV), which was interpreted as resulting from an increase in its radius, was first noted by Bancroft et al. (1967). At pH 5.0 the virus was stable and sedimented at 88S. But at pH 7 the sedimentation coefficient had dropped to 78S while the mol. wt. was essentially unchanged (Bancroft et al. 1968).

The details of the conformational changes due to varying the solution conditions have been investigated more closely for brome mosaic virus (BMV; Incardona & Kaesberg, 1964; Incardona, McKee & Flanagan, 1973). BMV is structurally very similar to CCMV (Bancroft, 1970) and is reported to be serologically related (Scott & Slack, 1971). Incardona et al. (1973) were able to distinguish two independent contributions to the structural rearrangement responsible for swelling: a pH induced transition and a thermal effect.

These studies on BMV were confined to low ionic strength. A recent paper has demonstrated that an artificial top component (the protein shell) can be produced for BMV in 1.5 M-NaCl around pH 6.4 (Pfeiffer & Hirth, 1974a).

This communication relates the structural changes in CCMV which resulted from varying the pH, ionic strength and temperature.

METHODS

Cowpea chlorotic mottle virus was isolated from the sap of infected cowpea plants (Vigna unguiculata (L.) Walp. var. Blackeye) by differential sedimentation following polyethylene glycol precipitation. The purified virus was stored at 4 °C in pH 5, I = 0.1 sodium acetate buffer. Concentrations of CCMV were estimated spectrophotometrically from the extinction coefficient $E_{260}^{\text{cm}} = 5.87$ (Bancroft et al. 1968) and were normally about 0.1 mg/ml in the sedimentation experiments.
Buffers were prepared to $I = 0.1$ and made up to the required ionic strength by the addition of 3 M-KCl. Formic acid-sodium formate buffer was used below pH 4.0, acetic acid-sodium acetate between pH 4.0 and pH 5.75, $Na_2HPO_4 - NaH_2HPO_4$ between pH 6.0 and pH 7.5, and boric acid-sodium hydroxide at pH 8.0 and above. The analytical grade reagents which were used to prepare these buffers were obtained from British Drug Houses Ltd, Poole, England. Dialysis in the various buffers was for two days to ensure that the virus was at equilibrium.

Sedimentation coefficients were obtained with the photoelectric scanning system of an MSE Analytical Ultracentrifuge Mark II. Ultraviolet absorption optics were employed throughout at a wavelength of 260 nm. The use of double-sector cells (10 mm light path) superimposed, with solvent in one sector and sample in the other, the base line and the sample. The solutions containing virus were kept for at least 1 h in the rotor at the required temperature before centrifuging. The temperature of the rotor as indicated on the control panel is stated by the manufacturers to be accurate to within $\pm 0.1 \, ^\circ C$ and is maintained to better than $\pm 0.05 \, ^\circ C$. A least squares programme allowed the rapid calculation of the sedimentation coefficients from plots of $\ln(r)$ against $t$ and the correction of these values to $s_{20,w}$. The sedimentation coefficients that are plotted are the average of at least two measurements.

**RESULTS**

*Effect of pH upon the sedimentation coefficients*

The sedimentation behaviour of CCMV upon varying the pH was characterized by an abrupt transition in the range pH 6.5 to 7.0. In solutions of low ionic strength ($I = 0.2$) at 5 $^\circ C$, the sedimentation coefficient remained constant at 82 to 83 S between pH 3.5 and pH 6.5 (Fig. 1b and Table 1). But above pH 6.5 the $s_{20,w}$ value dropped, at first sharply by pH 6.75 and then more gradually to 73 S at pH 8.0.

With solutions of high ionic strength ($I = 1.0$) the change in the sedimentation coefficient was more dramatic (Fig. 1a). Below pH 6.5 the $s_{20,w}$ values were almost constant at around 80S, with an increase of just 2S as the pH was raised from 3.5 to 6.5. But by pH 7.0 the majority of the material sedimented at less than 40S. At higher pH values almost all of the virus had been converted to this slowly sedimenting form, with sedimentation coefficients between 38S at pH 7.0 and 35S at pH 8.5.

*Effect of Mg$^{2+}$*

The effect of adding 0.01 M-MgCl$_2$ to the $I = 0.2$ buffers at 5 $^\circ C$ was to modify the swelling of CCMV but not to eliminate the decrease in $s_{20,w}$ (Fig. 2). The sedimentation coefficient dropped from 83S at low pH to 73S above neutrality. The transition from unswollen to swollen virus occurred more gradually with a lessening of the sharp decrease in $s_{20,w}$ values that was found at pH 6.5 to 6.75 in the absence of Mg$^{2+}$. The upward tails of the curve of Fig. 2 that are found below pH 4.0 and above pH 8.0 were unexpected. However, the tail below pH 4.0 is not surprising considering the anomalous behaviour of both the protein (Adolph & Butler, 1974) and the RNA (Adolph, 1975) below pH 4.0.

Preliminary experiments were also undertaken to discover the effect of Mg$^{2+}$ on the $s_{20,w}$ values at high ionic strength ($I = 1.0$). The results showed the transition from the fast form of the virus to the form sedimenting near 40S to be only slightly altered. The presence of Mg$^{2+}$ did not, therefore, stabilize the virus in either the unswollen or the swollen form.
Fig. 1. Sedimentation coefficients of CCMV upon varying the pH at 5 °C with (a) ionic strength 1·0 and (b) ionic strength 0·2. The virus was at a concentration of around 0·1 mg/ml and had been dialysed in the appropriate buffer for at least two days. In (b) the virus is in its fast, unswollen form below pH 6·5, while above this pH a radial expansion of the virus (swelling) has probably taken place. In (a) the CCMV particles partially disassemble at pH 7 and above.

Table 1. Sedimentation behaviour of CCMV at 5 °C*

<table>
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<td></td>
<td>38S</td>
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</tr>
<tr>
<td>0·2</td>
<td>82S</td>
<td>83S</td>
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</table>

* Derived from Fig. 1.

Effect of ionic strength

The conditions under which the various forms of CCMV and degraded CCMV could be found were further explored by varying the ionic strength at selected pH values. The selected low pH was pH 4·5, since both the virus and the protein capsid are stable at this pH. The representative high pH on the other side of the transition was taken as pH 7·5
**Fig. 2.** Sedimentation coefficients of CCMV at $I = 0.2$, at $5 \, ^\circ$C in the presence of $0.01 \, \text{M-MgCl}_2$. Compare with Fig. 1b.

**Fig. 3.** Sedimentation coefficients of CCMV at increasing ionic strengths (at $5 \, ^\circ$C). At pH 4.5 the only effect is a slight decline in the $s_{20,w}$ values, but at pH 7.5 the virus disassembles at about $I = 0.4$ to components which sediment near 40S. ○—○, pH 4.5; △—△, pH 7.5.
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Fig. 4. Effect of temperature upon the sedimentation coefficients at ionic strength 0.2. A substantial but gradual decline occurred at each pH value studied. ○—○, pH 4.5; □—□, pH 6.5; △—△, pH 7.5.

Table 2. Sedimentation behaviour of CCMV at 30 °C

<table>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>0.2</td>
<td>79S</td>
<td>82S</td>
<td>71S</td>
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</table>

disassembled
swollen

* Derived from Fig. 4 and 5.

where the properties are also stable. Raising the ionic strength from 0.1 to 1.0 at 5 °C had only a slight effect upon the sedimentation coefficient of the virus at pH 4.5 (Fig. 3). The sedimentation coefficients at I = 1.0 were similar to those of swollen virus at I = 0.2.

The breakdown of the virus was characterized more fully by varying the ionic strength at pH 7.5 (Fig. 3). Up to ionic strength 0.4, the sedimentation coefficient of the swollen virus gradually declined at a rate which paralleled that of the virus at low pH. But at the critical ionic strength of 0.4 two components were found, sedimenting at 70S and 43S, since the protein-protein and protein-RNA bonds were not sufficient to maintain the integrity of the virus. The 43S component comprised about two-thirds of the extinction profile. As the ionic strength was further increased, the $s_{20,w}$ value of the slowly sedimenting component declined gradually and linearly by about 9 units. Examination of this material in the electron microscope did not reveal a definite structure. This sedimentation species is not given a designation, such as '40S', in the manner that the protein capsid was designated '50S', because it is not certain that this form represents a fixed association
of RNA and protein. It can easily be imagined that as the ionic strength was increased, more and more protein was released to account for the decline in the sedimentation coefficient.

Effect of temperature

The effect of temperature on the sedimentation behaviour of CCMV was investigated at pH values of 4.5, 6.5 and 7.5. For an ionic strength similar to that of plant sap (I = 0.2), the general result of increasing the temperature from near 0 °C to 40 °C was to gradually decrease the sedimentation coefficient (Fig. 4 and Table 2). It thus appeared that increasing the temperature, as well as increasing the pH and ionic strength, resulted in weakening the various macromolecular bonds that are responsible for the virus structure. The sedimentation coefficient dropped about 8 Svedberg units at pH 4.5, 5 units at pH 6.5 and 5 units at pH 7.5. In all three cases the trend was for the $s_{20,w}$ values to level off at
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the highest temperatures. Only for the swollen virus (pH 7.5) could a region be defined, with a midpoint at 25 °C, where the decrease was especially sharp.

An investigation of the effect of temperature at high ionic strength (I = 1.0) revealed more dramatic changes in the sedimentation coefficients of CCMV at the same three pH values (Fig. 5a, b). The $s_{20,w}$ values dropped 8 Svedberg units and 14 units at pH 4.5 and pH 6.5, respectively. In both cases the change was from values characteristic of unswollen virus to values similar to those of virus which had undergone the pH induced structural transitions. With I = 1.0, the temperature region of greatest change was around 30 to 40 °C (Fig. 5) whereas with I = 0.2, the tendency was to plateau at the highest temperatures (Fig. 4). At I = 1.0, the greatest change occurred at pH 7.5, a steady decrease from about 34S at 0 °C to around 20S at 40 °C. The latter $s_{20,w}$ value was about what would be expected for the RNA of CCMV under these conditions. It therefore appeared that the effect of temperature was to strip the proposed RNA-protein complex of protein until at the highest temperature studied (40 °C) the complete degradation of the virus to its isolated RNA and protein components had finally been accomplished. Thus, in addition to the contributions to swelling which resulted from increasing the pH and ionic strength, an additional contribution due to raising the temperature was required to abolish the last of the macromolecular interactions which stabilized the native virus structure.

DISCUSSION

The abruptness of the structural transition upon raising the pH at low ionic strength suggests the operation of a well-defined switching mechanism and not just a general loosening of the structure. Another sharp transition was discovered in our earlier work on the isolated protein of CCMV (Adolph & Butler, 1974), which has also been found for BMV (Pfeiffer & Hirth, 1974b). The CCMV capsid was stable below pH 5.5, but disassembled to a low mol. wt. aggregate at the critical pH. It is likely that the two phenomena have the same origin and that the switching mechanism that results in the decrease in the sedimentation coefficient of the virus also controls the assembly of the protein capsid. The difference of one pH unit in the occurrence of the two phenomena could be due to an involvement of RNA in maintaining the structural integrity of the virus.

The results at high ionic strength are compatible with this interpretation. Varying the pH at I = 1.0 reveals a sharp transition between a fast sedimenting form of CCMV and a disassembly product sedimenting at less than 40S. It thus appears that the effect of high ionic strength is to diminish the strength of the protein-RNA interactions, leading to a partial breakdown of the virus.

For brome mosaic virus, Incardona & Kaesberg (1964) proposed that the pH induced structural transition represents a radial expansion of the virus. This interpretation was supported by the electron microscopic observation of Luftig (1967) that the diam. of brome mosaic virus increases from 271 Å at pH 6.1 to 299 Å at pH 7.5. A similar swelling of CCMV is the likely explanation for the substantial decrease in the sedimentation coefficient as the pH increases.

Varying the ionic strength as well as the pH allows at least two types of swelling to be distinguished. The first is the abrupt pH-controlled switching that occurs around pH 6.75 and which can be related to the mechanism responsible for the assembly-disassembly of the protein capsid. The second is the gradual loosening of the virus conformation with increasing ionic strength by interfering with the stabilizing salt-links. When both types
of swelling occur, at high pH and high ionic strength, the native conformation is disrupted and disassembly occurs, but not directly to 3S protein and isolated RNA. The initial product (after two days dialysis to equilibrium) of the high pH and high ionic strength disaggregation is a complex sedimenting at approx. 40S.

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REFERENCES


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