PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Stabilization of Brome Mosaic Virus

P. Pfeiffer, M. Herzog and L. Hirth

Phil. Trans. R. Soc. Lond. B 1976 **276**, 99-107
doi: 10.1098/rstb.1976.0100

Phil. Trans. R. Soc. Lond. B. **276**, 99–107 (1976)
Printed in Great Britain

RNA VIRUSES

Stabilization of brome mosaic virus

By P. Pfeiffer, M. Herzog and L. Hirth
Institut de Biologie Moléculaire et Cellulaire, 15, *rue Descartes*,
67000 *Strasbourg*, *France*

[Plate 19]

The relative importances of protein–protein and RNA–protein interactions in stabilizing the architecture of brome mosaic virus particles are discussed in the light of the following experimental evidence: (*a*) disassembly pathways of the virus particles, (*b*) reassembly of the virus and self-association capacity of the protein moiety, and (*c*) the role of divalent cations in virus stabilization, and their relevance to localization of the RNA in the virus particles. Evidence is given that the capsid of BMV is primarily stabilized by hydrophobic bonds at low pH, but not around and above neutrality where RNA–protein electrostatic interactions are essential to the integrity of the virus particles. A model is proposed for the structure of BMV in the different configurational states.

Introduction

Small spherical plant viruses are generally obtained in high yield as the complete nucleoprotein. In a few instances (turnip yellow mosaic virus and related viruses), empty protein shells can however be readily isolated from the infected plant. Free viral RNA remains an exceptional feature existing only in the case of viral mutants with a defective coat protein and in actual fact does not represent a convenient source of native viral RNA. Thus, the first step in any experiment aimed at the study of virus assembly involves disassembly of the nucleoprotein to yield the isolated nucleic acid and protein. This is generally realized by interfering rather drastically with the stabilizing interactions which hold the virus particle together, for instance, by increasing the pH to extreme values (Schramm, Schuhmacher & Zillig 1955), raising the ionic strength of the medium (Yamazaki & Kaesberg 1963; Hiebert, Bancroft & Bracker 1968), or introducing high concentrations of protein denaturants such as urea, guanidinium hydrochloride (Zelazo & Haschemeyer 1969) or acetic acid (Fraenkel-Conrat 1957).

As a general rule, reassociation is performed by reversion to initial conditions of stability of the virus, i.e. removing the perturbant either rapidly by dilution or slowly by dialysis depending on the virus material. Under these conditions, co-aggregation of the RNA and protein moieties occurs, with hopefully restoration of the initial set of interactions stabilizing the virus structure.

Conditions required for *in vitro* morphogenesis are, with a few exceptions, quite different from the physiological conditions under which synthesis and assembly of the viral components occurs in an infected cell. This led us to focus our interest in the study of the stabilizing forces in native BMV in order to understand why non-physiological conditions were apparently required for *in vitro* morphogenesis, and to search for moderate conditions of assembly in the light of our data on BMV structure and stabilization in the various configurational states. A model of BMV structure consistent with the body of experimental evidence is presented.

Material and results

BMV is essentially a pH-sensitive virus. Although it is found in a compact 'native' form at pH values ranging from 3 to 6 (Bockstahler & Kaesberg 1962) in which the RNA is protected from the action of nucleases, it expands radially above pH 6.5 (Incardona & Kaesberg 1964). In the swollen form, BMV is sensitive to RNase (which degrades the particles into small $T = 1$ particles (Kassanis & Lebeurier 1969)), and also to trypsin (Agrawal & Tremaine 1972) and chymotrypsin (Pfeiffer & Hirth 1975).

This conformational change has been analysed in detail by Incardona, McKee & Flanegan (1973) who were able to demonstrate that BMV underwent first a true pH-induced configurational change with no temperature-dependence, followed by a thermal expansion step which can be completely abolished by the presence of Mg^{2+} ions. The optically detectable changes in the viral RNA *in situ* led these authors to postulate that Mg^{2+} prevented the thermal transition by interacting with the RNA. We found subsequently (Pfeiffer & Hirth 1975) that the partially swollen, Mg^{2+}-stabilized BMV was as resistant to RNase treatment as native virus, thus demonstrating that RNA became accessible to nucleolytic enzymes only after completion of the thermal expansion step.

BMV can thus exist in 3 different conformations: (i) the 'native' compact form, (ii) the partially expanded, Mg^{2+}-stabilized configuration, and (iii) the completely expanded form. In the latter case, BMV is almost exclusively stabilized by ionic linkages between the positively charged side-groups of the protein subunits and the anionic polyphosphate backbone of the RNA. The integrity of the RNA chain is apparently mandatory for stability of the swollen virus, since its cleavage by RNase causes collapse of the virus architecture. In this configuration, BMV is exceedingly sensitive to the anionic detergent sodium dodecylsulphate (SDS) (Boatman & Kaper 1976) and to high salt concentration (Hiebert *et al.* 1968), which reinforces the assumption of electrostatic interactions as major stabilizing forces.

BMV does not give rise to *in vivo* production of empty capsids, which is seemingly an argument for RNA-protein interactions as compulsory for capsid stability.

However, Bancroft, Wagner & Bracker (1968) have shown that isolated BMV protein was capable of *in vitro* self-assembly in the absence of RNA into empty capsids built from 180 subunits arranged, as in the virus, according to an icosahedral symmetry (Finch & Bancroft 1968).

Since these empty protein shells are only stabilized by protein–protein interactions, they provide an interesting model by analogy and contrast to the virus, and we investigated in detail the forces responsible for the formation and stabilization of the capsids.

Behaviour of BMV protein in the absence of a nucleating agent: control of the self-assembly

BMV protein dialysed from pH 7.4 and 1.5 M NaCl to pH 5 and 0.2 M NaCl polymerizes into protein shells with the same morphology and structure as the native virus (Finch & Bancroft 1968). In these experiments, however, *both* pH and ionic strength (I) were varied at the same time; and it has been our experience that this can lead to formation of aggregates which, once formed, will be stable under conditions under which they would not form. This led us to investigate in detail the influence of pH and I on shell formation from BMV protein. We found (Pfeiffer & Hirth 1974*b*) that at I above 0.25, BMV protein is essentially found either as a small aggregate sedimenting with 2.7S (called 3S protein for convenience) or as empty

capsids (called 'pseudo-top component' or PTC) sedimenting with 52S. The capsid is found at pH values below pH 5.5 (and also at slightly higher pH values with increasing I), and the dissociated protein above these pH values (figure 1). Polymerization of BMV protein into capsids can be induced either by lowering the pH at constant I, or by increasing the I at constant pH. In both cases, there is apparently a direct conversion of dissociated protein into shells with no detectable intermediate, and this is a fully reversible process (Pfeiffer & Hirth, 1974*b*) on which temperature has very little effect if any (Incardona *et al.* 1973; Pfeiffer, unpublished results).

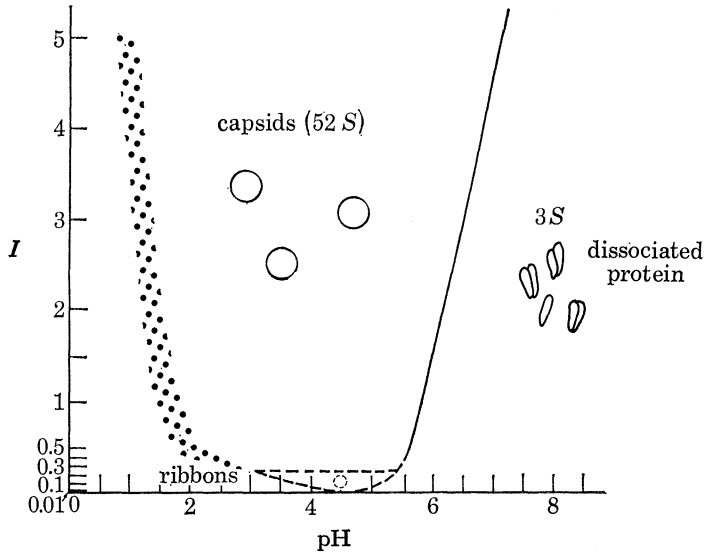


FIGURE 1. Occurrence of the main BMV protein aggregates as a function of pH and ionic strength (I) at 22 ± 1 °C, and for protein concentrations 1–5 mg/ml. Notice displacement of the threshold of reassociation into capsids toward higher pH values with increasing I. The aggregates found in the range delimited by broken lines (broken circle) sediment in the range 5–40S and correspond to intact and broken capsids with a loose structure (see figure 2). The material found at low I and more acidic pH consists mainly of ribbons of protein. BMV protein precipitates in the dotted area.

At I below 0.25, however, the situation becomes much more complicated in the range of pH where aggregation into shells occurs at higher I values. The protein is still found in the dissociated state at high pH in low I, and tends to aggregate at low pH into particles similar to PTC, but with a very loose structure (figure 2, plate 19). Attempts to tighten their configuration by further lowering the pH or varying the I failed and led to their precipitation or disruption into ribbons of protein which could no longer be used for capsid formation.

As a general rule, increased I results in a higher degree of association of BMV protein subunits. This suggests that hydrophobic forces play a major role in the polymerization process, although the 'switch' itself is certainly of an electrostatic nature. In view of this non-negligible role of hydrophobic forces in capsid stabilization, the question could be raised about the existence of conditions under which the virus could be capsid-stabilized by protein–protein interactions, which had proved to be sufficient to maintain an architecture very similar to that of the native virus. This condition was realized at low pH where the virus was found to be stable in conditions of high I which caused its breakdown above neutrality (Pfeiffer & Hirth 1974*a*).

This feature suggested a second question: can one find a transitory situation where the virus is on the threshold of collapse owing to ionization of the critical groups responsible for

the conformational change, but where hydrophobic forces would still be sufficient to maintain the integrity of the capsid? In other words, can one derive empty capsids directly from the virus, as this has already been done for turnip yellow mosaic virus (Kaper 1960; Jonard & Hirth 1966) and the related viruses (Bouley 1975)?

Formation of empty capsids (artificial top component) from BMV

Figure 3 shows that our expectation was confirmed and we found that BMV particles incubated at high ionic strength were able to release their RNA in a critical range of pH without collapse of the protein capsid (Pfeiffer & Hirth 1974*a*). Thus, RNA–protein interactions can be severed while protein–protein interactions of mainly hydrophobic nature are sufficient to maintain the structural integrity of the capsid, in a way similar to that already described for TYMV and the related viruses.

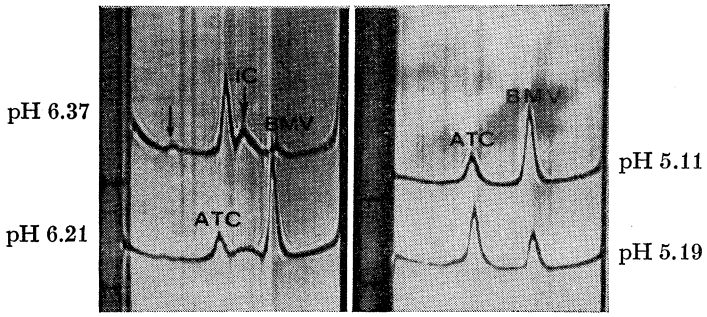
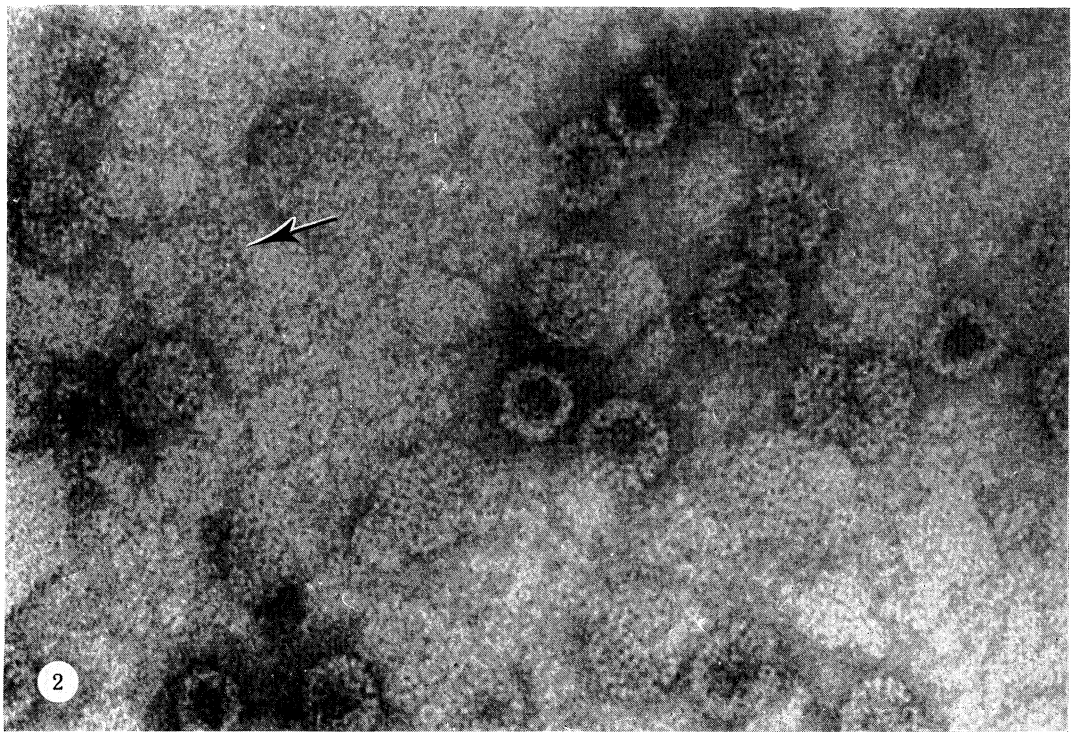


FIGURE 3. Degradation of BMV at high I and low pH. The intermediate component (IC) can be readily seen on the sedimentation patterns when degradation is performed in 1.5 M NaCl, but co-migrates with the peak of artificial top component (ATC) in 1 M $CaCl_2$. Arrow: peak of $T = 1$ particles.

We have analysed in detail the release of the various RNA species from BMV incubated in 1 M $CaCl_2$ (Herzog, Pfeiffer & Hirth 1976), and found that virions containing one molecule of RNA released it in one step, while those containing one copy of each 0.3 and 0.7×10^6 RNA species underwent a two-step degradation. The 0.7×10^6 was released first, with formation of a defective nucleoprotein which could be readily seen on sedimentation patterns in 1.5 M NaCl, but not in 1 M $CaCl_2$ where it was shown to co-migrate with the peak of empty capsids. In the latter case, purification of this component showed that it was in a swollen state, and its study can be valuable in understanding the mechanism of release of the RNA from the virions and in analysing the preferential interaction of the 0.3×10^6 RNA with the coat protein (for which it codes).

Thus degradation of BMV induced by high-salt treatment can be considered as a two-step process with transitory formation of empty capsids which are stable at low pH but dissociate into protein subunits upon increase in pH.

This finding suggested the possibility that *in vitro* morphogenesis of the virus from its isolated components – with possible extension to *in vivo* morphogenesis – could proceed in a similar fashion, i.e. entry of the RNA into a preformed capsid, rather than nucleation by the RNA molecule with subsequent arrangement of protein subunits around the nucleation complex so as to yield finally a complete nucleocapsid. Such a procapsid has now been found to exist for many complex bacteriophages and for poliovirus, but its existence is generally associated



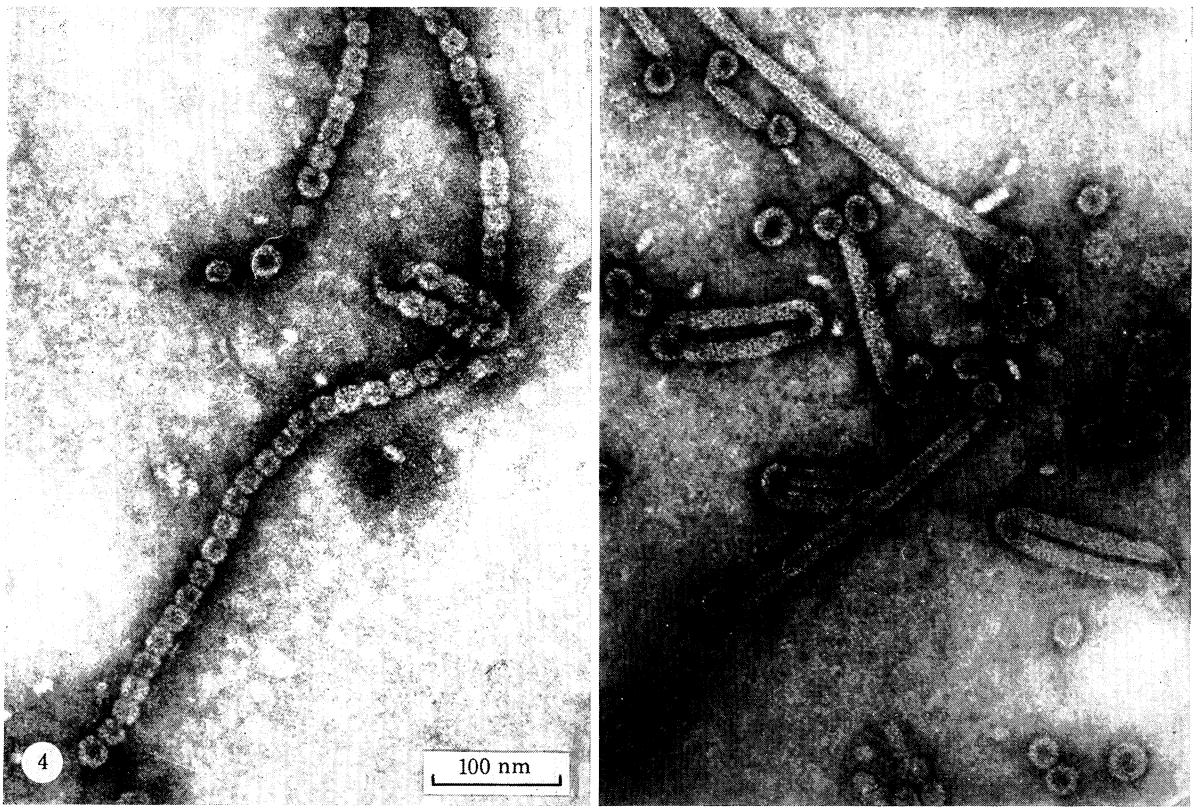


FIGURE 2. Electron micrograph of the material formed from BMV protein at pH 4.5 and 0.1 *I*. Capsids with a loose structure (sometimes partially collapsed) are seen. The arrow points to a pentamer surrounded by 5 hexamers, a cluster similar to those depicted in the model on figure 5. (Magn. × 400 000.)

FIGURE 4. Structures formed by encapsidation of *E. coli* DNA by BMV protein. Left: BMV protein contaminated with 3–5% residual (short chain) RNA; right: pure BMV protein.

(*Facing p.* 102)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

with that of scaffolding proteins or precursors of the coat protein which are subsequently cleaved at specific sites.

A prerequisite for such a mechanism for BMV would be existence of stable capsids (yet not 'frozen' in a configuration, but rather susceptible to conformational changes so as to allow entry of the RNA) under 'physiological' conditions, i.e. in a range of pH 6–8, and at I 0.1–0.2 at room temperature. Unfortunately, neither would BMV protein form capsids, nor would such capsids even be stable under such conditions. This renders the 'procapsid' model rather unlikely for *in vivo* BMV morphogenesis, at least if coat protein as such were used to build this precursor capsid.

Assembly of BMV protein in the presence of a nucleating agent

Virus assembly from the newly synthesized components takes place in an infected cell under conditions where protein alone does not form capsids: the RNA is thus likely to be the nucleating agent required for particle formation.

However, BMV protein is able *per se* to form structures with the same symmetry and morphology as native virus, and it thus seems that the presence of RNA does not cause drastic changes in protein conformation. Rather, the role of the RNA is to help the dissociated protein to overcome the nucleation barrier at higher pH values, probably by neutralizing repulsive charges and allowing small conformational changes and/or positioning of the subunits.

In contrast to TMV (Durham & Klug 1971; Butler & Klug 1971) no evidence has been reported so far for a discrete protein aggregate playing a critical role in shell formation of small spherical plant viruses. Although the trimer of the protein subunit would actually represent the best candidate for the formation of an icosahedral shell (Finch, Crowther, Hendry & Struthers 1974), dissociated BMV protein is mainly found in the form of dimers (Stubbs & Kaesberg 1964), such as that of the closely related cowpea chlorotic mottle virus (Adolph & Butler 1974); however, trimers may be present in low amount in equilibrium with the dimers.

Although the pathways of assembly are not yet known for BMV, the favouring role of polyanions on shell formation at high pH and low ionic strength is obvious (Bancroft, Hiebert & Bracker 1969). In actual fact, the presence of polyanions seems to promote specifically the formation of pentamers: reassembly of BMV protein with short length RNA gives rise preferentially to $T = 1$ icosahedral particles exclusively composed of pentamers, and BMV protein contaminated with 3–5% residual short chain RNA, to series of icosahedral shells along a DNA molecule. Pure protein gives rise to tubular structures (probably tubes of hexamers) under the same conditions (figure 4, plate 19). It seems thus that local neutralization of excess positive charges near or at the contact surfaces of the morphological subunits is mandatory for closing the constrained ring of the pentamer.

Reconstitution of BMV and related viruses is usually performed in conditions of pH and I (typically around pH 7.4 with I below 0.1) where the reconstituted virus is in a swollen form, requiring stabilization by lower pH (Bancroft & Hiebert 1967). Under these conditions, the virus particles are essentially stabilized by salt-linkages; in view of the non-negligible participation of hydrophobic forces in the stabilization of the capsid and of BMV at low pH, we investigated reconstitution at high I.

Contrary to earlier reports (Hiebert & Bancroft 1969), we found that BMV, RNA and protein can interact at high I, provided that reassociation is performed at a high protein concentration. However, the particles formed in this case are defective, since they sediment

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

in the range 60–55S, and buoyant density centrifugation showed that their density was lower than that of native virus. Polyacrylamide gel electrophoresis indicated that the smaller RNA species (0.3×10^6, and to a lesser extent the 0.7×10^6) were preferentially encapsided at high I, and that the larger RNAs (1.0 and 1.1×10^6) were encapsided in increasing amounts with decreasing I. That this was not merely an effect of chain size could be demonstrated by competition experiments with t-RNA which is scarcely encapsided in the presence of BMV RNA (Herzog, Pfeiffer & Hirth, to be published).

We found however that all RNAs were correctly encapsided whatever the I, when reconstitution was performed at low protein concentration.

Role of Mg^{2+} in BMV stabilization and their effect on the quaternary structure of the virus

The divalent cation requirement for *in vitro* BMV reconstitution (Hiebert & Bancroft 1969) is not yet clearly understood. At least part of the virus can be reassembled in their absence, and Mg^{2+} has been demonstrated to have no effect on the formation and stability of BMV capsid (Incardona *et al.* 1973), yet at a pH different from that required for reconstitution with RNA. We cannot rule out the possibility suggested by Bancroft *et al.* (1967) of Mg^{2+} serving as an ionic bridge to position two carboxylate groups in the configuration they will occupy in the compact form of the virus, where they form a carboxyl-carboxylate pair. On another hand, it is now ascertained from direct experimental evidence by neutron scattering analysis (Jacrot & Pfeiffer, to be published) that Mg^{2+} interferes with the localization of BMV RNA. We found that the localization of the RNA in BMV remains basically the same in the partially swollen Mg^{2+}-stabilized BMV as in the native virus, with RNA loops protruding only some 0.5–0.8 nm into the protein shell. When Mg^{2+} ions are removed, and the virus allowed to undergo the thermal expansion step, the RNA loops were found to expand much further into the capsid (about 3 nm which brings the RNA loops 2–2.5 nm from the outer surface of the virus particle). Only the completely swollen form of BMV was found to become sensitive to RNase and at the same time to trypsin and chymotrypsin (Pfeiffer & Hirth 1975).

This explained the early results of Brakke (1963) who found that Mg^{2+} stabilized the infectivity of BMV above neutrality: BMV RNA becomes accessible *in situ* to RNase only after completion of the thermal expansion step which is abolished by Mg^{2+}.

Furthermore, unmasking of the specific cleavage site for trypsin, Arg_{25} (identified by Tremaine, Ronald & Agrawal (1974)) requires changes in accessibility achieved only upon the thermal expansion step which is now demonstrated to be intimately correlated with radial expansion of the RNA loops.

In a similar fashion, completely swollen BMV becomes sensitive to chymotrypsin, but in this case, there are apparently two sites of cleavage which become unmasked. Chymotrypsin-converted BMV (Chy-BMV) retains many of the characteristic features of BMV; it is still capable of undergoing a pH-induced conformational change with the same dependence upon Mg^{2+} ions as BMV, and it still becomes sensitive to trypsin and RNase in the completely swollen state. Further, the protein isolated from Chy–BMV is capable of self-assembly into the same structures as native BMV protein. The sites for chymotrypsin cleavage are currently under investigation, but it has already been ascertained that a peptide localized at the surface of the particle is lost, while the regions of the protein which harbour the side-groups responsible for conformational changes and control of protein self-assembly are conserved.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Concluding remarks

BMV has long been considered as essentially stabilized by RNA–protein interactions of salt type, involving the polyanionic backbone of the RNA and the positively charged lysyl and arginyl residues of the protein subunit. This view depended on many experimental features, e.g. the sensitivity of BMV to high salt concentrations, the collapse of the particle after RNase treatment which caused *in situ* breakage of the RNA chain, and the high sensitivity of BMV to anionic detergents such as SDS.

Successful reconstitution of BMV from its isolated components seemed to require low I, which reinforced the model of ionic linkage stabilization.

However, our own work on the polymerization of BMV protein pointed out the important role played by hydrophobic forces in capsid stabilization, and the behaviour of BMV towards high salt concentrations turned out to depend strongly on the pH at which the reaction proceeds and thus on the conformational state of the virus.

Hence, it must be kept in mind that most pieces of evidence suggesting that ionic RNA-protein interactions represent the major stabilizing forces in BMV are concerned with the completely swollen form of the virus. In native BMV however, it seems that hydrophobic protein–protein interactions play a non-negligible role in virus stabilization and become weakened only when the hydrophobic interfaces of the protein subunits are repelled from each other at higher pH values, as the probable consequence of ionization of carboxyl-carboxylate pairs into repulsive carboxylate groups.

The use of proteolytic and nucleolytic enzymes as a probe of conformational changes in BMV indicated further that the most important structural changes, both at the level of the RNA and of the protein subunit occurred upon the thermal expansion step and were intimately related. Tremaine *et al.* (1974) found that trypsin treatment under these conditions released a large peptide (25 residues) from the *N*-terminus of the protein subunit, thus causing the collapse of the virus structure. Since this peptide comprises 1 lysyl and 8 arginyl residues, it becomes a good candidate for a major site of interaction with the viral RNA *in situ*; its lack of accessibility in the compact form of the virus indicates an internal localization.

The data obtained in neutron scattering experiments, together with the body of experimental evidence we have presented, led us to propose the following models for the structure of BMV under the various conformational changes: we believe that hexamers and pentamers exist in BMV not only as structural but also as physical entities. Indeed, isolated pentamers, hexamers, and clusters of 5 hexamers surrounding a pentamer can be seen in the electron microscope, when BMV is stained with acidic stain. By analogy with the model of TYMV proposed by Klug, Longley & Leberman (1966), we assume that RNA loops protrude up to 3 nm from the surface of the particle into the central hole of the morphological subunits where they interact strongly with the *N*-terminal region of the protein molecule. This situation is preserved in the partially-swollen, Mg^{2+}-stabilized form of BMV, since the localization of the RNA remains that of the native virus, and the sites for action of trypsin and chymotrypsin remain hidden. The capsomeres of the partially expanded virus would thus retain a compact configuration, and the swelling of the virus would affect only inter-capsomere bonds.

The partial swelling of the virus could thus be interpreted as a repulsion of the capsomeres which would stay in a compact configuration themselves. In contrast, upon thermal expansion

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

the RNA comes closer to the surface of the particle, and the structure of the capsomere itself becomes looser and allows both access of the RNA loops to nucleases and unmasking of the trypsin and chymotrypsin-sensitive sites (figure 5). Under these conditions, the hydrophobic faces of the protein subunits which were in intimate contact are exposed to the solvent, and high salt concentrations can now play their dissociating role.

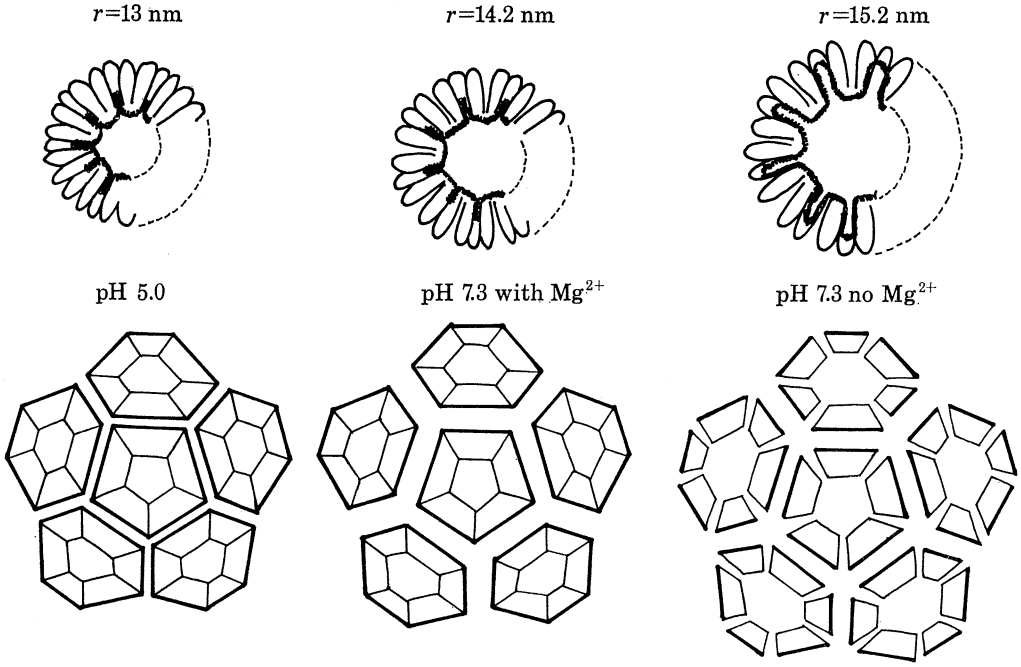


FIGURE 5. Model proposed for the mechanism of swelling of BMV around neutrality at 25 °C. The figures given are derived from data obtained in neutron-scattering experiments. Upper row: changes in RNA conformation *in situ* when passing from the compact native virus (left), to the partially expanded, Mg^{2+}-stabilized BMV (middle) and then to the completely swollen BMV after completion of the thermal transition (right). The corresponding changes in capsomere structure and arrangement are given in the lower row.

In view of the stabilizing role of divalent cations in BMV exposed to physiological pH values, one could wonder whether newly synthesized BMV would not contain a critical number of divalent cations (which would be lost upon purification), positioned so as to preserve the integrity of both viral RNA and protein under conditions otherwise adverse to virus stability. Further, upon infection of a host plant cell, the Ca^{2+} and Mg^{2+} binding substances existing there would tend to displace these ions from their specific binding sites (Durham, personal communication) and thus trigger the conformational change bringing the RNA loops closer to the surface of the particle, where they might interact with cell membranes and/or ribosomes for decapsidation and translation of the genetic message to start the infection and multiplication processes.

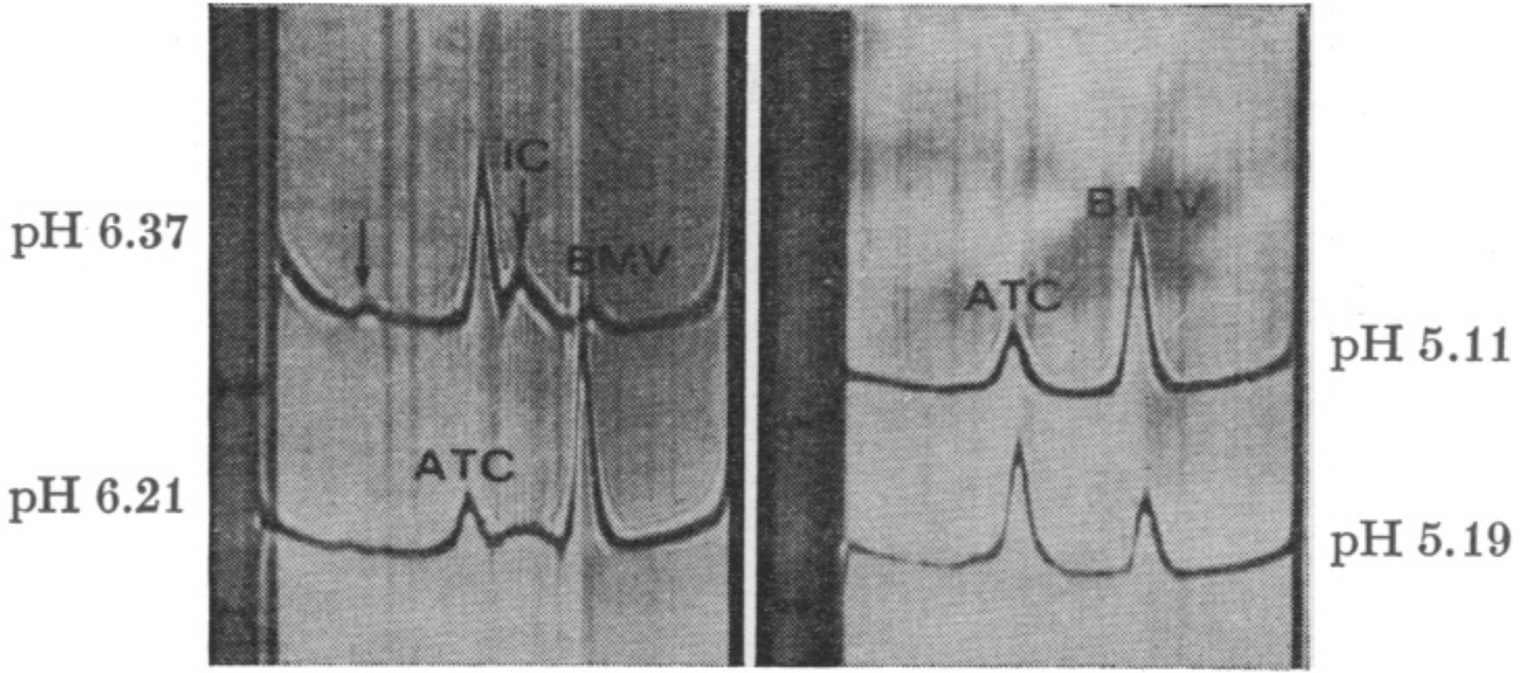
REFERENCES (Pfeiffer, Herzog & Hirth)

Adolph, K. W. & Butler, P. J. G. 1974 *J. molec. Biol.* **88**, 327–342.
Agrawal, H. O. & Tremaine, J. H. 1972 *Virology* **47**, 8–20.
Bancroft, J. B. & Hiebert, E. 1967 *Virology* **32**, 354–356.
Bancroft, J. B., Hiebert, E. & Bracker, C. E. 1969 *Virology* **39**, 924–930.
Bancroft, J. B., Wagner, G. W. & Bracker, C. E. 1968 *Virology* **36**, 146–149.
Boatman, S. & Kaper, J. M. 1976 *Virology* **70**, 1–16.

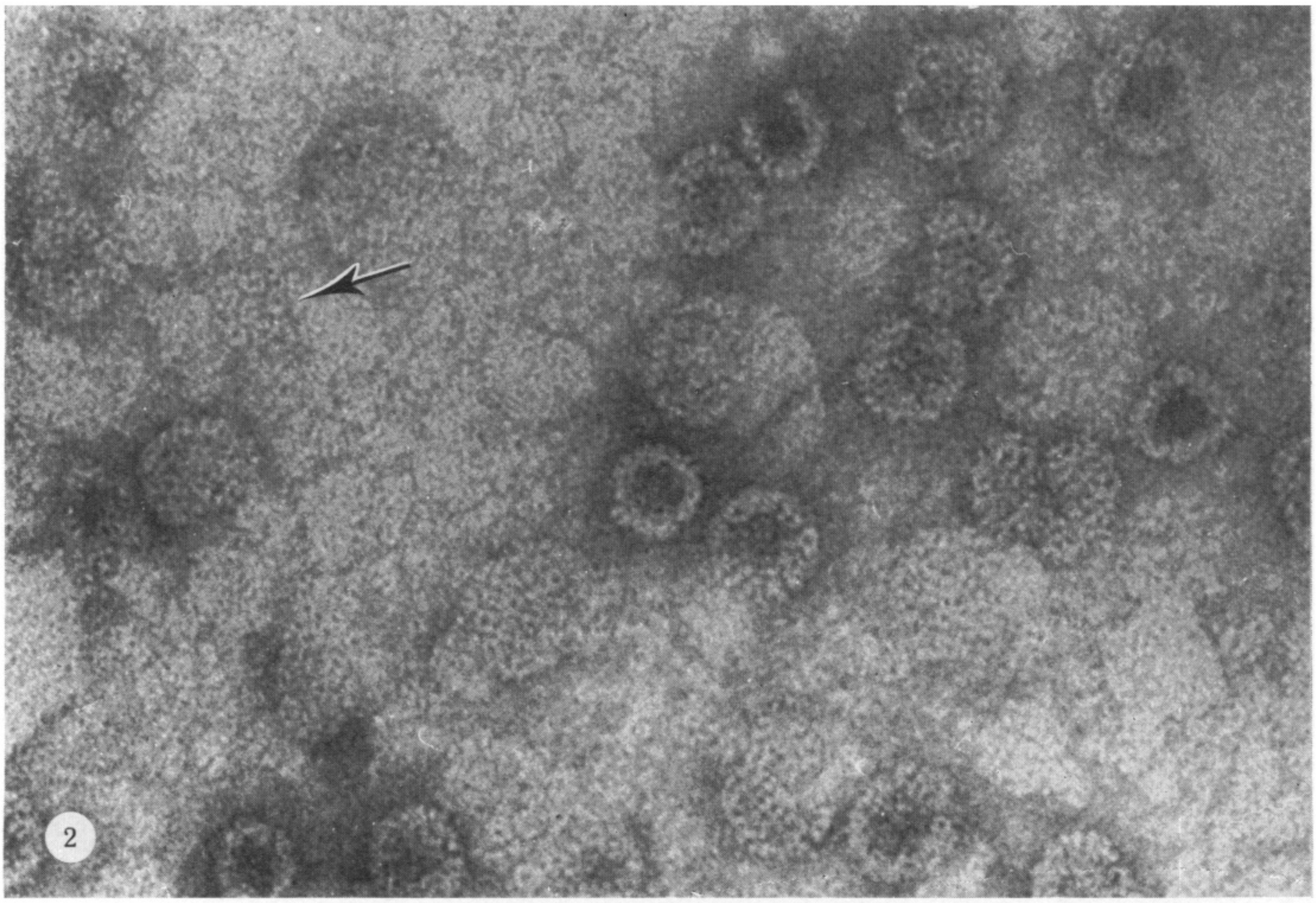
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Bockstahler, L. E. & Kaesberg, P. 1962 *Biophys. J.* **2**, 1–9.
Bouley, J. P. 1975 Ph.D. Thesis University of Strasbourg.
Brakke, M. K. 1963 *Virology* **19**, 367–374.
Butler, P. J. G. & Klug, A. 1971 *Nature, Lond.* **229**, 47–50.
Durham, A. C. H. & Klug, A. 1971 *Nature, Lond.* **229**, 42–46.
Finch, J. T. & Bancroft, J. B. 1968 *Nature, Lond.* **220**, 815–816.
Finch, J. T., Crowther, R. A., Hendry, D. A. & Struthers, J. K. 1974 *J. Gen. Virol.* **24**, 191–200.
Fraenkel-Conrat, H. 1957 *Virology* **4**, 1–4.
Herzog, M., Pfeiffer, P. & Hirth, L. 1976 *Virology* **69**, 394–407.
Hiebert, E. & Bancroft, J. B. 1969 *Virology* **39**, 296–311.
Hiebert, E., Bancroft, J. B. & Bracker, C. E. 1968 *Virology* **34**, 492–508.
Incardona, N. L. & Kaesberg, P. 1964 *Biophys J.* **4**, 11–21.
Incardona, N. L., McKee, S. & Flanegan, J. B. 1973 *Virology* **53**, 204–214.
Jonard, G. & Hirth, L. 1966 *C. R. hebd. Séanc. Acad. Sci. Paris* **263** *D*, 1909–1912.
Kaper, J. M. 1960 *J. molec. Biol.* **2**, 425–433.
Kassanis, B. & Lebeurier, G. 1969 *J. gen. Virol.* **4**, 385–395.
Klug, A., Longley, W. & Leberman, R. 1966 *J. molec. Biol.* **15**, 315–343.
Pfeiffer, P. & Hirth, L. 1974*a* *Virology* **58**, 362–368.
Pfeiffer, P. & Hirth, L. 1974*b* *Virology* **61**, 160–167.
Pfeiffer, P. & Hirth, L. 1975 *FEBS Lett.* **56**, 144–148.
Schramm, G., Schuhmacher, G. & Zillig, W. 1955 *Z. Naturf.* **10**b, 481–493.
Tremaine, J. H., Ronald, W. P. & Agrawal, H. O. *APS* 1974 Meeting Abstract 398.
Yamazaki, H. & Kaesberg, P. 1963 *J. molec. Biol.* **7**, 760–762.
Zelazo, P. O. & Haschemeyer, R. J. 1969 *Biochemistry* **8**, 3587–3592.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



IGURE 3. Degradation of BMV at high I and low pH. The intermediate component (IC) can be readily seen on the sedimentation patterns when degradation is performed in 1.5 M NaCl, but co-migrates with the peak of artificial top component (ATC) in 1 M $CaCl_2$. Arrow: peak of $T = 1$ particles.



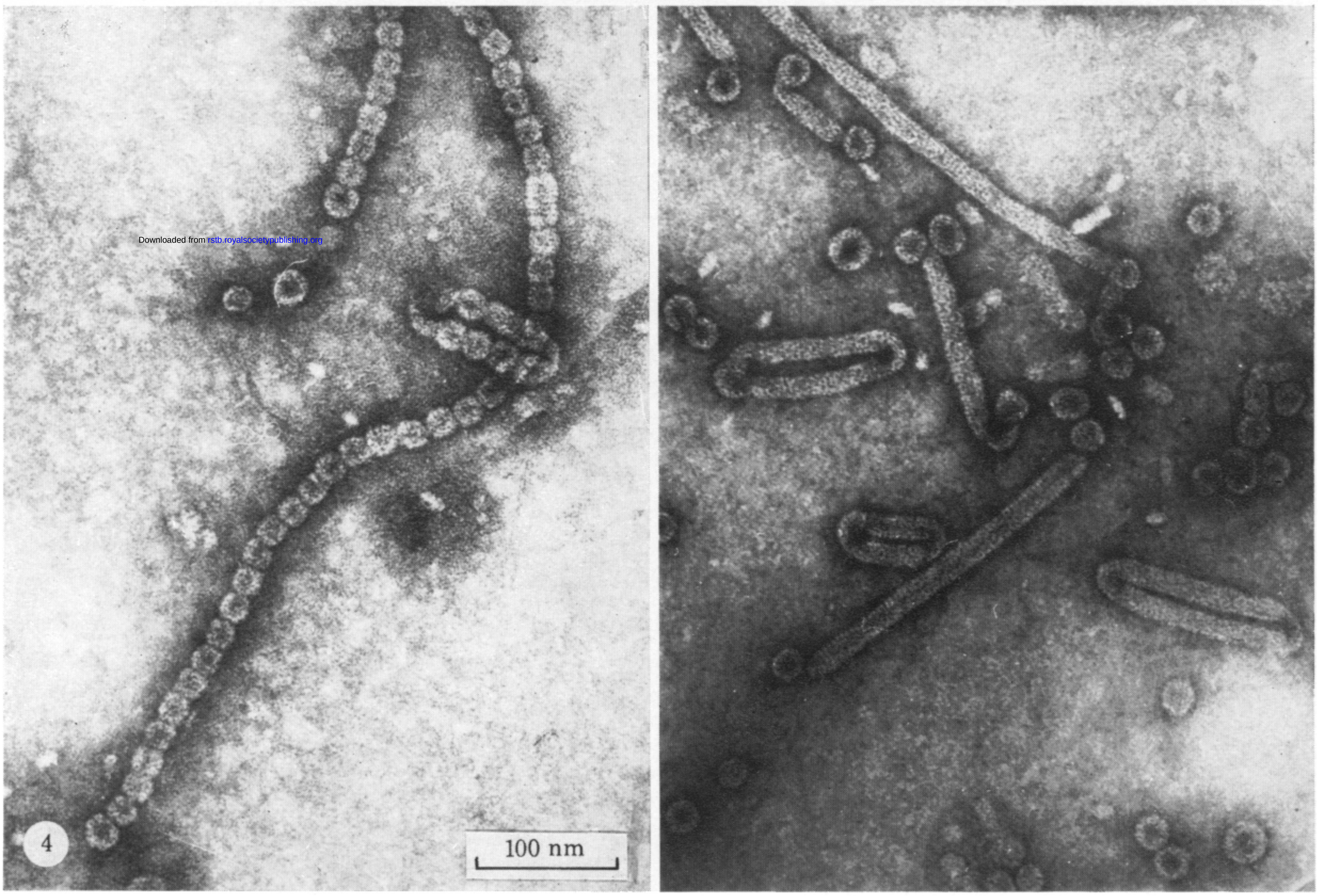


FIGURE 2. Electron micrograph of the material formed from BMV protein at pH 4.5 and 0.1 *I*. Capsids with a loose structure (sometimes partially collapsed) are seen. The arrow points to a pentamer surrounded by 5 hexamers, a cluster similar to those depicted in the model on figure 5. (Magn. × 400 000.)

FIGURE 4. Structures formed by encapsidation of *E. coli* DNA by BMV protein. Left: BMV protein contaminated with 3–5% residual (short chain) RNA; right: pure BMV protein.