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Assembly Studies on Potato Virus Y and its Coat Protein

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SUMMARY

The effects of various reagents on the disassembly of potato virus Y are described and discussed. The virus can be disassembled in acetic acid, guanidine, LiCl, NaSCN and in a variety of other salts but is stable in NaCl, CsCl and NaF. Polymerization of coat protein from pH 3 to 11 in 0·1 to 0·5 M-NaCl was followed by analytical centrifugation. Extensive polymerization (with major proportions being 100 to 200S) was only found between pH 6 and pH 9 in 0·1 M-NaCl. A nucleoprotein with structural, density and stability properties similar to those of the virus, but less than one third as long, was obtained by the addition of RNA to polymerized protein at 20 °C at pH 7 to 8 at very low ionic strength. Possible modes of assembly are presented.

INTRODUCTION

Although rod-shaped viruses share the same basic architecture, their coat proteins require widely varying conditions of pH, ionic strength and temperature for assembly (Atabekov *et al.* 1968; Durham, Finch & Klug, 1971; Morris & Semancik, 1973; Erickson, Bancroft & Horne, 1976; McDonald, Beveridge & Bancroft, 1976). Similar wide variability has been reported in the conditions that permit their reconstitution (Fraenkel-Conrat & Williams, 1955; Semancik & Reynolds, 1969; Novikov, Kimaev & Atabekov, 1972; Abou-Haidar *et al.* 1973).

In a previous communication, we reported that potato virus Y (PVY), a virus with flexuous rod-shaped particles 730 nm long, could be dissociated and its isolated protein assembled into flexuous stacked-disc or ring particles (McDonald *et al.* 1976). We now describe further studies on the polymerization of the coat protein with and without RNA, and show that a nucleoprotein, resembling virus, can be assembled.

METHODS

Virus. The PVY (PVY-NC) isolate was kindly provided by E. Hiebert (University of Florida). Its propagation and purification were the same as described (McDonald *et al.* 1976).

Coat protein extraction. Protein was extracted by the LiCl method (Francki & McLean, 1968; McDonald *et al.* 1976). In addition, the acetic acid (Fraenkel-Conrat, 1957) and guanidine hydrochloride (Reichmann, 1960) methods were used. Extraction by acetic acid was performed by adding two volumes of glacial acetic acid to an ice-cold virus suspension (5 mg/ml). The mixture was incubated 1 h in an ice bath and then allowed to stand for 1·5 h at about 20 °C. The precipitated nucleic acid was removed by centrifugation at 15000 rev/min

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for 25 min and the protein-containing supernatant fluid was dialysed exhaustively against 10 mM-sodium citrate, 1 mM-dithiothreitol, pH 4 at 4 °C. Any contaminating virus was subsequently removed from the protein preparation by centrifugation at 45000 rev/min in a Beckman type 50 rotor for 5 h.

Extraction by guanidine hydrochloride was performed by adding an equal volume of 5 M-guanidine hydrochloride, 10 mM-EDTA at pH 8·4 to an ice-cold virus suspension (5 to 10 mg/ml) in 10 mM-tris-(hydroxymethyl) methylamine (tris), pH 8. The mixture was incubated 1 h in an ice bath. The precipitated nucleic acid was removed by centrifugation at 10000 rev/min for 15 min. The protein-containing supernatant was dialysed to 10 mM-tris, 0·5 M-NaCl, 1 mM-dithiothreitol, pH 8 at 4 °C.

Other salts were also examined for their ability to dissociate PVY. Virus (3 mg/ml) in 0·1 M-potassium phosphate, 5 mM-EDTA, pH 7, was added to equal volumes of 4 M-NaCl CsCl, or NaSCN, or to an equal volume of saturated NaF (final concentration about 1 M). Mixtures were either frozen in dry ice and kept overnight at −20 °C, or kept at 4 °C. They were then thawed and centrifuged at 10000 rev/min for 15 min to remove any precipitate. The contents of the supernatants were monitored by electron microscopy, by examining the u.v. absorption spectra and by testing for birefringence.

Nucleic acid extraction. PVY RNA was obtained by rehydrating the RNA precipitated during the guanidine hydrochloride extraction of coat protein. The precipitate was washed with one or more changes of water until the RNA remained soluble. A few drops of 1 M-NaCl and 0·5 M-EDTA (pH 7) were added to the RNA to improve its solubility. It was subsequently precipitated with two volumes of ethanol and kept at −20 °C overnight. The RNA precipitate was collected by centrifuging at 10000 rev/min for 15 min. It was then air dried, resuspended in water, and stored at −20 °C. Papaya mosaic virus (PMV) RNA, donated by J. Erickson (of this laboratory), was prepared in the same manner.

Protein sedimentation. Protein (about 1·8 mg/ml) prepared by the LiCl method was dialysed for 24 h against a variety of buffer systems, all containing 1 mM-dithiothreitol, and was centrifuged in a Beckman Model E analytical ultracentrifuge. Sodium azide (2 mM) was added to the buffers to inhibit microbial growth. The S values were corrected for temperature and concentration of NaCl and phosphate but not for the other buffers, which were used at 0·01 M.

Nucleoprotein assembly. Protein was dialysed to whatever buffer conditions were being tested and RNA (in H_2O) was added to the protein in approximately stoichiometric proportions (1 to 20 by weight, to give a RNA + protein concentration of about 0·5 to 1·0 mg/ml). Appropriate protein and RNA controls were also prepared. The pH values of the buffers were not corrected for the addition of RNA. The products were assayed by electron microscopy (followed by optical diffractometry) as before (McDonald *et al.* 1976), and by sedimentation on sucrose density gradients. For the latter procedure, unless otherwise stated, preparations were dialysed for 24 h against a protein dissociation buffer (D buffer) containing 20 mM-tris, 0·5 M-NaCl, 1 mM-dithiothreitol, pH 7·5, at 4 °C and then layered on 10 to 40 % (w/v) sucrose density gradients in the same solution but without the dithiothreitol. The gradient columns were centrifuged at 36000 rev/min in an SW 50.1 rotor and scanned at 254 nm in an ISCO fractionator. Infectivity was tested by inoculation to the local lesion host *Chenopodium amaranticolor* Coste & Reyn.

Equilibrium centrifugation. Preparations were mixed with CsCl to give a final density of about 1·31 g/ml. They were centrifuged for 18 h at 44770 rev/min at 20 °C in a Beckman Model E analytical ultracentrifuge.

Table 1. *Effects of molarity and pH on the sedimentation of PVY coat protein aggregates in sodium chloride**

Molarity (M) of NaCl	Sedimentation coefficients (S) of sedimenting species								
	pH 3 (glycine buffer)†	pH 4 (citrate buffer)†	pH 5 (acetate buffer)†	pH 6 (MES‡ buffer)†	pH 7 (MES buffer)†	pH 8 (tris buffer)†	pH 9 (tris buffer)†	pH 10 (CAPS§ buffer)†	pH 11 (CAPS buffer)†
0·5	—	2	—	22	—	7	—	—	—
				3		3			
0·3	—	50	—	35	—	16	—	12	—
								3	
0·2	—	35	—	142‖	—	56	—	—	—
				63		19			
0·1	20‖	35‖	35	206	195	209	202	15	2
	9	22		108	91	133	95	4	
				49	36	29	23		

* Protein dialysed for 24 h at room temperature and sedimented at 20 °C.
† All buffered at 10 mM.
‡ MES = sodium 2[*N*-morpholine] ethane sulphonate.
§ CAPS = cyclylhexylaminopropane sulphonic acid.
‖ Just barely discernible.

RESULTS

Protein extraction

The protein obtained by the acetic acid and guanidine hydrochloride methods appeared similar in every way to the products obtained using the LiCl and $CaCl_2$ methods (McDonald *et al.* 1976). The absorption spectra were typical of protein with E_{280}/E_{250} ratios of 2·4 to 2·6. When the proteins were dialysed to conditions of moderate to low ionic strength ($\mu \leqslant 0{\cdot}1$), between pH 6 and 9, polymerized stacked-ring particles were seen in the electron microscope.

PVY was not dissociated by 2 M-NaCl, CsCl, or saturated NaF, but was dissociated by 2 M-NaSCN, the latter observation being made by electron microscopy because this salt fails to precipitate RNA.

Protein polymerization

Table 1 lists major sedimenting species observed when PVY protein was sedimented in NaCl in the molarity and pH ranges of 0·1 to 0·5, and 3 to 11 respectively. The protein was highly polymerized when NaCl was at 0·1 M between pH 6 and pH 9, and appeared to form distinctive species near 100 and 200S. As the molarity increased the degree of polymerization decreased.

The effect of temperature on the degree of protein polymerization was examined. Fig. 1 shows Schlieren pictures of sedimenting protein that had been dialysed at 4° and at 20 °C in 10 mM-MES, 0·2 M-NaCl, pH 6 and subsequently sedimented at 5·9° and at 21·2 °C respectively. At 5·9 °C, 3, 11 and 73S species were evident (the 3 and 11S species are not resolved in this particular time frame), whereas at 21·2 °C, 63 and 142S species predominated.

Specific ion effects on protein polymerization were also noted. Fig. 2 shows Schlieren pictures of sedimenting protein that had been dialysed in NaCl and sodium phosphate, respectively, at the same ionic strength ($\mu = 0{\cdot}4$). As evidenced by the faster sedimenting species, the presence of phosphate promoted polymerization.

We previously reported that stacked-ring particles appeared more organized in phosphate than in other salts (McDonald *et al.* 1976). However, further investigation of the appearance

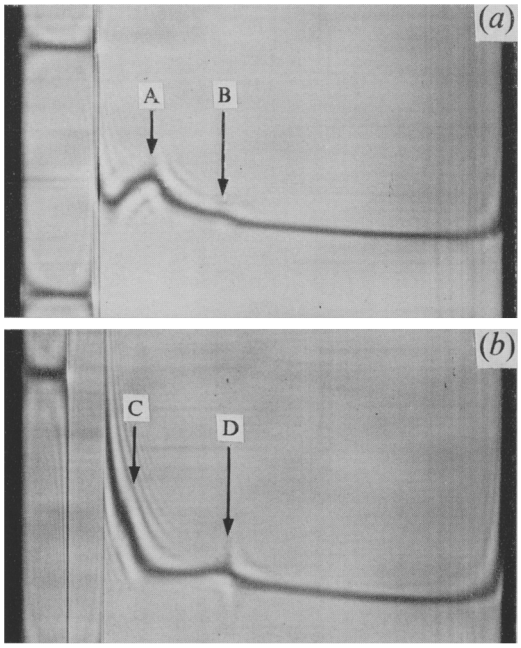

Fig. 1. Schlieren patterns of PVY coat protein in 10 mM-MES, 0·2 M-NaCl, pH 6. (*a*) At 21·2 °C, after 2 min at 42040 rev/min, containing 63S (A) and 142S (B) species. (*b*) At 5·9 °C, after 8 min at 42040 rev/min, containing 3 and 11S (C) and 73S (D) species.

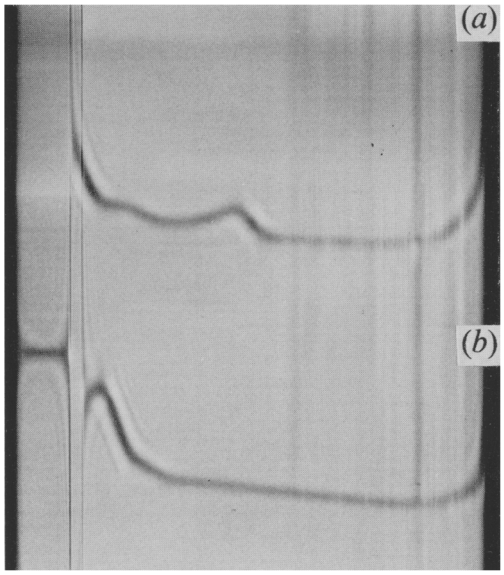

Fig. 2. Schlieren patterns of PVY coat protein at 20 °C, after 6 min at 52640 rev/min. (*a*) In 0·17 M-sodium phosphate, pH 7. (*b*) In 0·4 M-NaCl, 10 mM-MES, pH 7.

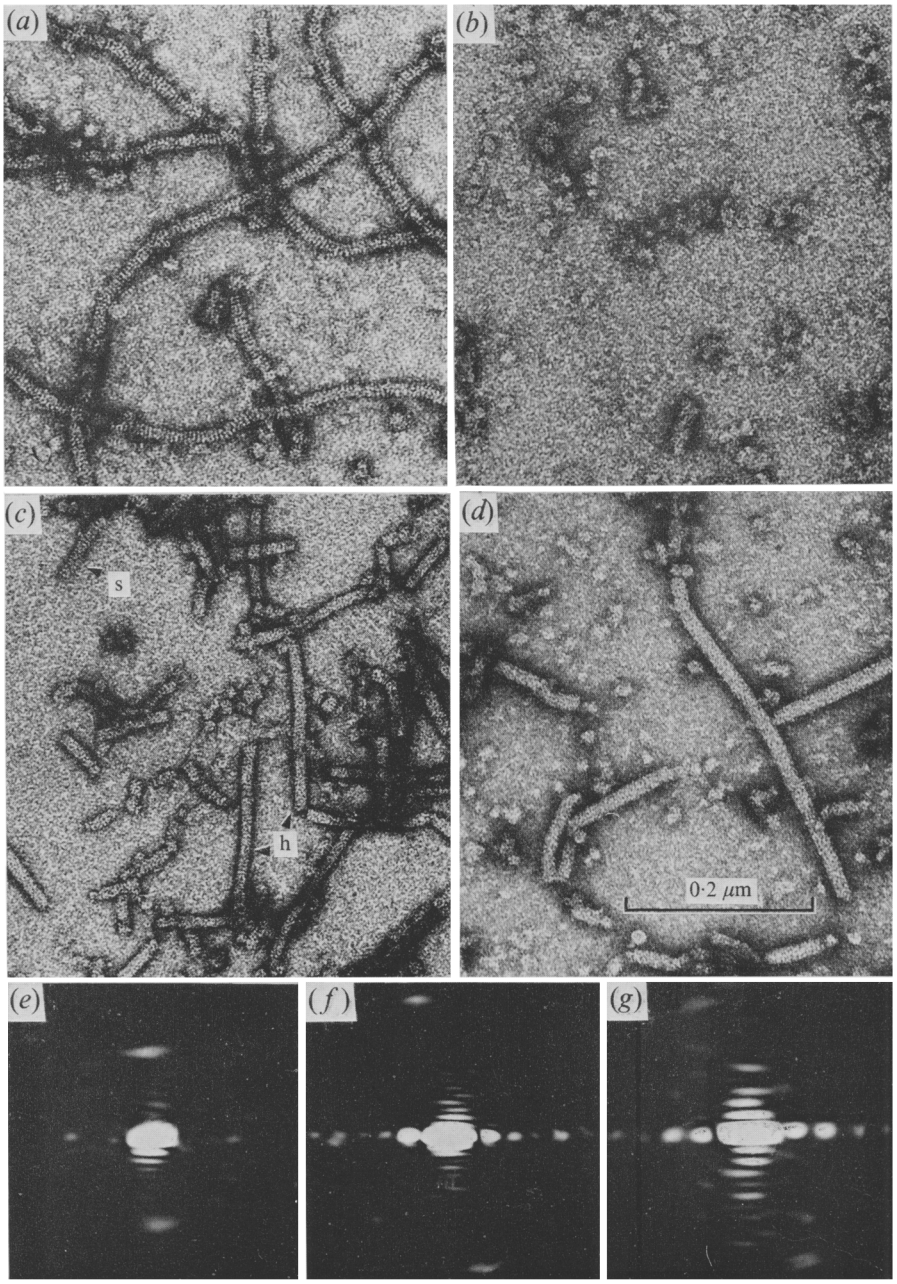

Fig. 3. PVY coat protein polymerized to stacked-ring particles in 10 mM-MES, pH 7 (*a*), and dissociated in D buffer (*b*). Assembled nucleoprotein (h), with PVY RNA and coat protein, and remnant stacked-ring particles (s), in 10 mM-MES, pH 7 (*c*). Assembled nucleoprotein and dissociated protein in D buffer (*d*). Optical transforms of stacked-ring protein (*e*), PVY particles (*f*) and nucleoprotein particles assembled with PVY RNA and coat protein (*g*) showing the repeat and pitch reflections.

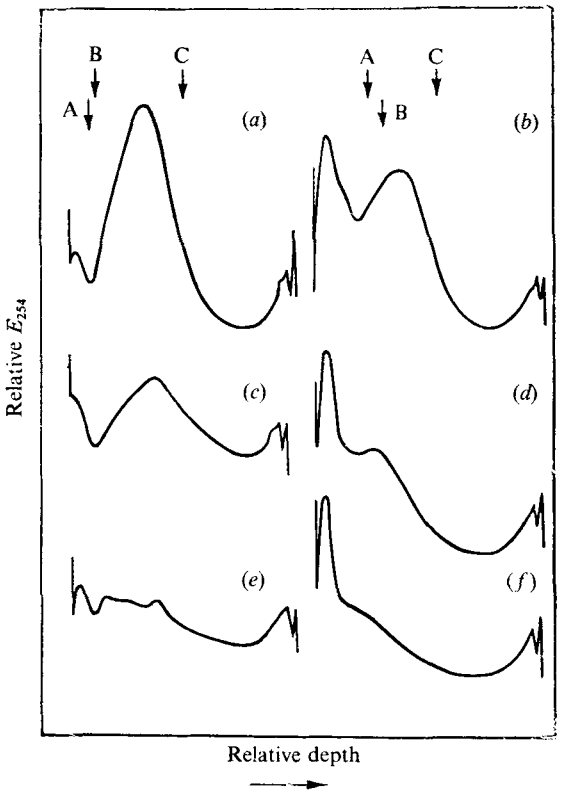

Fig. 4. Sucrose density gradient profiles of nucleoprotein assembly mixtures containing PVY RNA and coat protein (*a* and *b*), nucleoprotein assembly mixtures containing PMV RNA and PVY coat protein (*c* and *d*), and control PVY protein (*e* and *f*), after assembly for 2 h in 10 mM-MES, 1 mM-dithiothreitol, pH 7, at 20 °C. Preparations were split into two equal halves. One set was dialysed overnight against 10 mM-MES, 1 mM-dithiothreitol, pH 7, at 4 °C and sedimented on gradients containing 10 mM-MES, pH 7 (*a*, *c* and *e*)). The other set (*b*, *d*, *f*) was dialysed overnight against D buffer at 4 °C and sedimented on gradients of the same ionic strength and pH as D buffer. Arrows A, B and C mark the positions of PMV RNA, PVY RNA and PVY, respectively, in sister tubes. Centrifugation was at 36000 rev/min for 75 min at 4 °C in the SW 50.1 rotor.

of stacked-ring particles in the absence of phosphate indicated that this was a variable phenomenon, and that often well organized particles could be seen in the absence of HPO_4^{2-} (Fig. 3*a*). As phosphate apparently favours polymerization it is possible that the stacked-ring particles are less labile in this buffer and are therefore more likely to retain a well organized appearance in the electron microscope.

Nucleoprotein assembly

Addition of PVY or PMV RNA to stacked-ring protein in 10 mM-MES, 1 mM-dithiothreitol, pH 7, at 20 °C resulted in the assembly of short virus-like particles (Fig. 3*c*). Such preparations usually contained a mixture of the virus-like particles and short stacked-ring protein particles. The pitch of the virus-like particles (Fig. 3*f*) was the same as for PVY (Fig. 3*g*; 31 to 33Å), but was significantly smaller than the repeat of the stacked-ring particles (Fig. 3*e*; 40 to 42 Å). When such preparations were sedimented in 10 mM-MES, pH 7, on sucrose density gradients it was not possible to discriminate between the protein and nucleoprotein particles because they sedimented at similar rates. However, dialysis

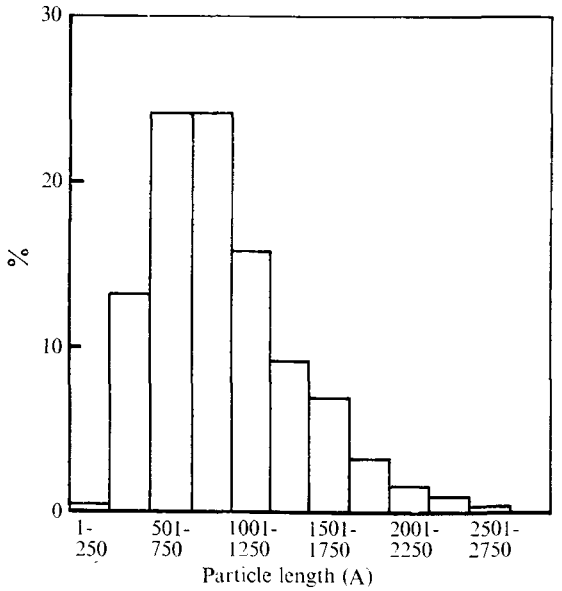

Fig. 5. Histogram showing the length distribution of 302 particles assembled with PVY RNA and coat protein as a percentage of the total. Assembly was performed for 2 h at 20 °C in 10 mM-MES, 1 mM-dithiothreitol, pH 7. The preparation was subsequently dialysed overnight at 4 °C against D buffer and samples were negatively stained and examined in the electron microscope.

against D buffer dissociated the stacked-ring protein particles (Fig. 3*a*, *b*; 4*e*,*f*), but left assembled nucleoprotein intact (Fig. 3*c*, *d*; 4*a*, *b*, *c*, *d*).

Under these protein-dissociating conditions, the nucleoprotein sedimented at about 100S (as determined by analytical centrifugation) compared to about 145S for PVY nucleoprotein particles (Huttinga & Mosch, 1974). Three hundred and two particles were measured and the distribution of lengths is shown in Fig. 5. Results from equilibrium centrifugation in CsCl showed that the density of the assembled nucleoprotein corresponded to that of PVY (1·32 g/ml). However, it was not infectious, nor was the RNA control although it was infectious before being mixed with PVY protein.

The kinetics of the assembly process at 20 °C were examined by terminating the reaction after 15 min, 30 min, 1 h, 2 h, and 8 h by dialysis against D buffer. As judged by sedimentation profiles (Fig. 6), no additional assembly could be detected after 1 h. The reason for the apparent decrease in assembled product at 8 h is not known. Assembly of nucloprotein at 4 °C was also observed. However, these particles were shorter and less numerous than those found at 20 °C.

The effects of pH and ionic strength were examined. At 20 °C with either PVY or PMV RNA, the efficiency of assembly was about the same at pH 7 and 8 (Fig. 7*d*, *g*, *e*, *h*), but was poor at pH 9 (Fig. 7*j*, *k*). Addition of protein to RNA at pH 6 resulted in a precipitate composed of large dense amorphous masses as viewed in the electron microscope. These were presumably nucleoprotein aggregates because peaks corresponding to free nucleic acid were absent when such preparations were sedimented on sucrose density gradients (Fig. 7*a*, *b*). The aggregates had not dissolved after dialysis to pH 7. However, nucleoprotein assembled at pH 7 remained soluble when dialysed to pH 6 just as native virus nucleoprotein does. Nucleoprotein assembly was markedly sensitive to ionic strength. The efficiency was poor in 0·05 M-NaCl (Fig. 8*d*, *e*) and only very slowly sedimenting particles were made in 0·1 M-NaCl (Fig. 8*g*, *h*).

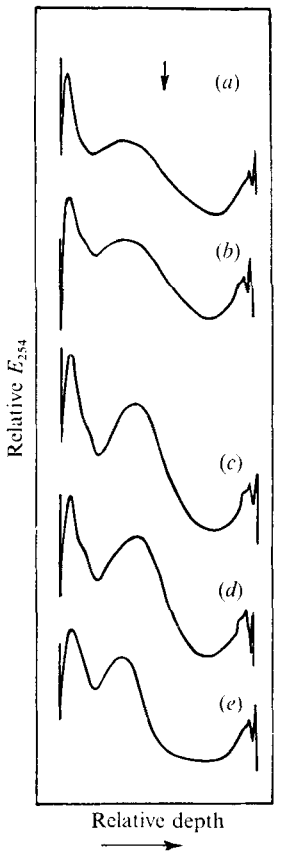

Fig. 6

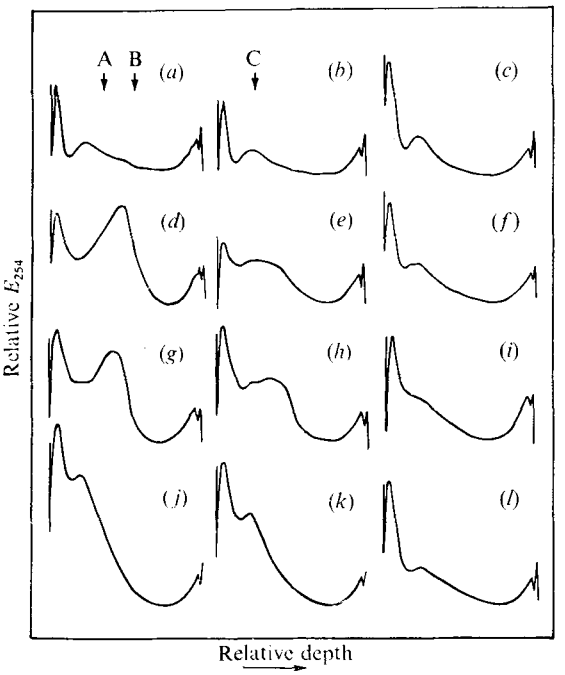

Fig. 7

Fig. 6. Sucrose density gradient profiles of nucleoprotein assembly mixtures containing PVY RNA and coat protein. After (*a*) 15 min, (*b*) 30 min (*c*) 1 h, (*d*) 2 h, (*e*) 8 h incubation in 10 mM-MES, pH 7, at 20 °C. Mixtures were subsequently dialysed overnight against D buffer and were sedimented at the same pH and ionic strength. Centrifugation was at 36000 rev/min for 75 min at 4 °C in the SW 50.1 rotor. The arrow marks the position of PVY in a sister tube.

Fig. 7. Sucrose density gradient profiles of nucleoprotein assembly mixtures containing PVY RNA and coat protein (*a*, *d*, *g* and *j*), nucleoprotein assembly mixtures containing PMV RNA and PVY coat protein (*b*, *e*, *h* and *k*), control PVY coat protein (*c*, *f*, *i* and *l*). Preparations were incubated for 2 h at 20 °C, and were subsequently dialysed overnight against D buffer and sedimented at the same pH and ionic strength. Incubations, all in 1 mM-dithiothreitol, were made in 10 mM-MES, pH 6 (*a*, *b* and *c*), 10 mM-MES, pH 7 (*d*, *e* and *f*), 10 mM-tris, pH 8 (*g*, *h* and *i*) and 10 mM-MES, pH 9 (*j*, *k* and *l*). Arrows A, B and C, mark the positions of PVY RNA, PVY, and PMV RNA respectively, in sister tubes. Centrifugation was at 36000 rev/min for 75 min at 4 ° C in the SW 50·1 rotor.

DISCUSSION

Although the forces which disassemble a quaternary association of proteins and RNA, like those found in a virus, are not necessarily the same as those which cause it to be formed initially, their study can sometimes yield information on what holds a polymer together. For example, acetic acid disassembles particles of PVY (Table 2) as well as of a number of other viruses. Acetic acid decreases surface tension and has a lower dielectric constant than water so its action may be primarily concerned with the dissolution of hydrophobic or entropic unions. This type of interpretation may be strengthened, but not unequivocally,

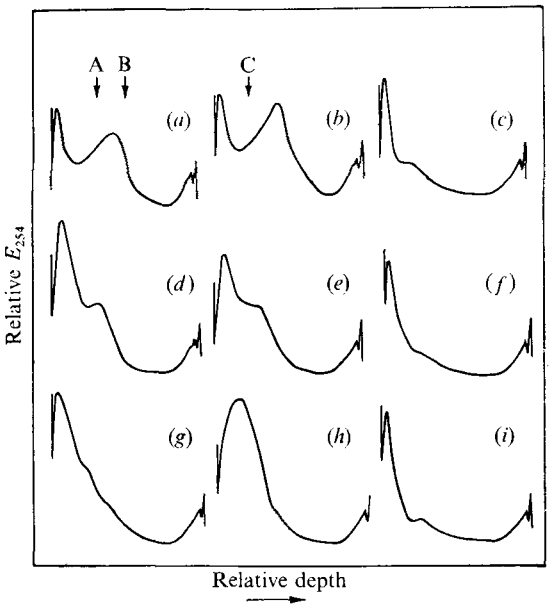

Fig. 8. Sucrose density gradient profiles of nucleoprotein assembly mixtures containing PVY RNA and coat protein (*a*, *d* and *g*), nucleoprotein assembly mixtures containing PMV RNA and PVY coat protein (*b*, *e* and *h*), and control PVY protein (*c*, *f* and *i*). Preparations were incubated for 2 h at 20 °C and were subsequently dialysed overnight against D buffer and sedimented at the same pH and ionic strength. Incubations, all in 1 mM-dithiothreitol, were made in 10 mM-MES, pH 7 (*a*, *b* and *c*), 10 mM-MES, 0·05 M-NaCl, pH 7 (*d*, *e* and *f*), 10 mM-MES, 0·1 M-NaCl, pH 7 (*g*, *h* and *i*). Arrows, A, B and C, mark the positions of PVY RNA, PVY, and PMV RNA, respectively, in sister tubes. Centrifugation was at 36000 rev/min for 75 min at 4 °C in the SW 50.1 rotor.

Table 2. *Summary of results for the dissociation of PVY*

Dissociation obtained at 4 °C	Dissociation obtained only at −20 °C	No dissociation obtained either at 4° or −20 °C
$BaCl_2$*	LiCl*	NaF
$CaCl_2$*		NaCl
$MgCl_2$*		CsCl
$SrCl_2$*		
NaSCN		
Guanidine HCl		
Acetic acid		

* Results reported previously (McDonald *et al.* 1976; Goodman *et al.* 1976).

by the fact that whereas PVY is stable in NaF and NaCl, it is unstable in NaSCN (Table 2). The F^- orders water (structure-maker) whereas SCN^- is a water structure-breaker and consequently destabilizes hydrophobic unions. The order of effectiveness of these ions in virus dissociation follows the Hofmeister or lyotropic series as well as the order of anti-chaotropic → chaotropic ions which have been correlated with decreasing water structure (Hatefi & Hanstein, 1969; Dandliker & de Saussure, 1971). However, Li^+ is more structure-forming than Na^+ and Cs^+, yet PVY was dissociated in LiCl but not in NaCl or CsCl. Robinson & Jencks (1965*b*) and Nagy & Jencks (1965) have interpreted the solubilization of protein in concentrated salt solutions in terms of the effects of cations on the activity

coefficients of exposed amide and peptide groups. In favouring solubilization they found that $Ba^{2+} > Ca^{2+} > Mg^{2+} > Li^{+} > Cs^{+} \sim Na^{+} \sim K^{+} \sim Rb^{+}$, which is consistent with our results for those cations that we have tested on PVY (Table 2). Although guanidine can decrease surface tension, there is considerable evidence that it also solubilizes proteins by decreasing the activity coefficients of amide and peptide groups (Robinson & Jencks, 1965*a*). Thus, the identification of what solubilizes PVY and certain other flexuous viruses can be as much a reflection of how the reagents used are regarded as it is a specification of the forces involved.

There is a gross architectural similarity between the stacked-disc rods formed by TMV protein and the polymer composed of PVY protein in that neither particle is helical. Apart from this characteristic, the particles are quite different in their appearance. The TMV protein particle is rigid and generally limited in length whereas that composed of PVY protein is flexuous and long. Possible reasons for specific length distributions without nucleic acid have been presented by Lauffer (1975). The TMV stacked-disc particle is slowly assembled near neutrality from double-discs (e.g. Durham *et al.* 1971) at ionic strengths approaching 0·5. The polymer has been shown to be made from protein which seems to have undergone limited proteolysis (Durham, 1972) and once formed is quite stable (Durham & Finch, 1972) although there is an indication that this proteolysis may not be obligatory (Champness *et al.* 1976). The PVY protein particle, on the other hand, assembles rapidly from pH 6 to 9 at low ionic strengths and its formation is inhibited by 0·5 M-NaCl. It is not composed of degraded protein and can readily be disassembled and reassembled (McDonald *et al.* 1976). The same product is made from protein obtained in a variety of ways from the virus and there is no reason to believe that the PVY particle is composed of abnormal protein. It is not known if a subassembly aggregate is involved in the formation of the PVY protein particle, even though it would seem likely that one exists although it would not necessarily have to be a double-disc since the polymer is not rigid. In view of these differences and since the term stacked-disc rod has definite technical connotations related to a subassembly step, we shall name the PVY protein aggregates stacked-ring particles.

The results presented in Table 1 may be interpreted as the influence of pH on the assembly of PVY protein at different concentrations of NaCl. The possibility of the different buffers producing ion effects is not discounted. However, we believe these effects were minimal because the buffers were used at low concentration (0·01 M), and similar sedimenting species were produced in both MES and tris at 0·1 M-NaCl.

The wide pH range (pH 6 to 9) over which assembly occurs suggests that more than minimum net charge is involved. The isoelectric point of the protein subunit, or whatever subassembly aggregate is needed, is very probably lower than pH 6 yet polymerization is limited below that level. Assembly occurs rapidly at pH 6, which is about where the imidazole group of a histidyl residue (about 4 histidine residues per subunit; Makkouk & Gumpf, 1975) will lose a proton (pK $\sim$ 6·5) indicating that the condition of some histidine side-chains may be critical to the assembly process. Assembly does not occur, or can be reversed (McDonald *et al.* 1976) above pH 9 where the α amino group of lysyl residues (about 13 per subunit; Makkouk & Gumpf, 1975) with pK $\sim$ 9·5 lose protons thereby increasing the net negative charge significantly. The phenolic hydroxyl group of tyrosine residues (about 5 per subunit; Makkouk & Gumpf, 1975), with about the same pK as the side-chain of lysine, will also lose its proton but will have less effect than lysine in terms of net charge. The polymerization/depolymerization pattern of PVY protein can thus be viewed in terms of the usual ionization behaviour of the side-chains of a few amino acids. But, there are more

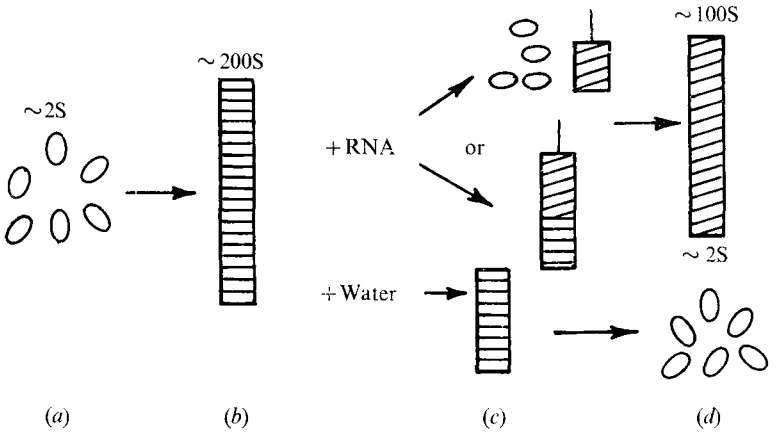

Fig. 9. Summary of the assembly procedure. (*a*) Dissociated PVY coat protein in 0·5 M-NaCl, 20 mM-tris, 1 mM-dithiothreitol, pH 8, at 4 °C (~ 2S). (*b*) PVY coat protein assembled to stacked-ring particles in 10 mM-MES, 1 mM-dithiothreitol, pH 7, at 20 °C (~ 200S). (*c*) Formation of helically constructed nucleoprotein after addition of stoichiometric amounts of PVY RNA (1 : 20 by weight). Possible mechanisms are either by dissociation of the stacked-ring protein particle or by penetration of the RNA into the stacked-ring tube. (*d*) Dialysis to 20 mM-tris, 0·5 M-NaCl, 1 mM-dithiothreitol (D buffer), pH 7·5, at 4 °C leaves the assembled nucleoprotein intact (~ 100S), but dissociates the stacked-ring protein particles (~2S).

than electrostatic factors involved. Polymerization is favoured by HPO_4^{2-} more than Cl^-. This could mean that water structure (Dandliker & de Saussure, 1971) or activity coefficient (Robinson & Jencks, 1965*b*) effects are involved and specific ion series important to these aspects of protein behaviour require investigation. Further, there is an indication, which probably can be improved with more attention to pH and ionic strength, that the extent of polymerization increases with temperature, again pointing to a solvent entropic effect. The polymerization of undegraded proteins of other tubular viruses such as TMV (e.g. Lauffer, 1975), tobacco rattle virus (Morris & Semancik, 1973), papaya mosaic virus (Erickson *et al.* 1976) and narcissus mosaic virus (Robinson *et al.* 1975) all seem to have an endothermic component of varying importance depending on the protein and ionic conditions in assembly and PVY seems to be no exception.

Proof of reconstitution can only come from infectivity experiments. These were unsuccessful with PVY possibly because the RNA was digested during reconstitution. Nevertheless, a nucleoprotein with physical properties similar to those of the virus was made, even though it was shorter than the virus particles. The pitch of the helix of the reconstituted particles was that of the virus; the densities were the same, and both the virus and reassembled material were, unlike the polymerized protein, stable in 0·5 M-NaCl, pH 7. The latter type of result is analogous to the stability of reconstituted viruses such as TMV (Caspar, 1963), cowpea chlorotic mottle virus (Bancroft, 1970) and papaya mosaic virus (J. W. Erickson & J. B. Bancroft, unpublished data) under conditions in which their nucleic acid-free coats or capsids are unstable. In the case of TMV, the relative stability of the reconstituted particle has been attributed to a decrease in enthalpy (e.g. Lauffer, 1975).

We do not know how PVY assembles. Unlike any other simple virus, its coat protein rapidly and efficiently forms a linear polymer under the same conditions as required for reconstitution. This may be a coincidence, because our conditions are incorrect. However, as indicated in Fig. 9, the stacked-ring particle may actually be a novel intermediate in

assembly, the RNA being threaded into the small central cavity of the protein polymer thereby causing a conformational shift from the stacked-ring to the helical form. If coat protein was made in excess during PVY infection, careful examination leaf-dip preparations might show stacked-ring particles. Alternatively, the virus may be directly formed from protein monomers or small subassembly aggregates, the stacked-ring particles being disassembled by the RNA before reassembly around it. Whatever the mechanism, the specificity is limited because papaya mosaic virus RNA as well as PVY RNA is encapsidated. It was noted, however, that the quantity of nucleoprotein from homologous assembly was not always the same as from heterologous assembly (e.g., compare Fig. 7*d* and *e* with Fig. 8*a* and *b*.) The reasons for this variation have not been determined.

The nucleoprotein made from PVY protein and RNA assembled best near neutrality at low ionic strength and there is an indication that there is a favourable response to an increase in temperature, although it is not of the same magnitude as found for TMV. Thus, the endothermic factor in the assembly process may be relatively minor. The salt levels required by TMV are inhibitory to the assembly of the PVY nucleoprotein and those of potato virus X (Novikov *et al.* 1972; Goodman, Horne & Hobart, 1975) and papaya mosaic virus (J. W. Erickson & J. B. Bancroft, unpublished data). All of the flexuous viruses so far examined seem to require about the same conditions for most rapid assembly.

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