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Interaction of non-enveloped plant viruses and their viral coat proteins with phospholipid vesicles

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The interaction of the non-enveloped plant viruses TMV (rod-shaped) and CCMV (spherical) and of their coat proteins in several well-defined aggregation states, with artificial membranes was investigated to study the early stages of the cellular infection process. Information about the separate steps in the interaction mechanisms was obtained by employing three assays, performed as a function of vesicle size, net membrane charge, pH and ionic strength. The assays allow to discriminate between aggregation of vesicles (turbidity assay) and membrane destabilization (vesicle leakage assay and lipid mixing assay). The aggregation of the vesicles is a result of electrostatic interactions between the viral material and vesicles surface (cross-linking), while the destabilization of the membrane is a result of penetration or bilayer disruption by hydrophobic protein domains. TMV virions and its coat protein, and CCMV virions, due to their net negative charge, predominantly interact with positively charged membranes. The coat protein of CCMV was found to interact with negatively charged membranes, an interaction that can be assigned to its basical N-terminal sequence. Changing the aggregational state of the viral coat proteins yielded most significant interactions in case of TMV coat protein aggregated in the disk form and CCMV coat protein aggregated in empty capsids with oppositely charged membranes. These protein aggregates are found to be the best compromise between efficiency (capacity of the protein to bridge vesicles and destabilize their membranes) and concentration of protein aggregates. The results are discussed with respect to previously proposed biological models of the early stages of plant virus infection.

Introduction

The early stages of non-enveloped plant virus infection involve attachment of virus particles to cells, the entry into the cytoplasm and the uncoating of the viral genome. Knowledge of these initial interactions is scarce and inconclusive (for a review, see Ref. 1). Several mechanisms have been proposed, on the basis of studies involving either protoplast membranes [2–5], isolated plasma membranes [6–8], or artificial phospholipid structures [9–13].

In absence of specific receptor or attachment sites on the plant cell membrane [5] and any contribution of endocytosis to in vivo infection [14], the infectious entry of a plant virus into a plant cell is generally thought to be facilitated by external factors, such as mechanical wounds or arthropods [15] or to occur via intracellular plasmodesmata. At present, a mechanism is favoured in which whole virus particles are internalized into the cell and possibly destabilized by membrane or cell wall components. These destabilized viral particles can be subsequently involved in a process of cotranslational disassembly, as has been shown for TMV [16] and CCMV in vitro [17,18].

The study of interactions between virus particles and model membrane vesicles can provide important information about the molecular mechanisms involved in virus–membrane interactions in vivo. For example,

Abbreviations: TMV, tobacco mosaic virus; CCMV, cowpea chlorotic mottle virus; SUV, small unilamellar vesicle(s); LUV, large unilamellar vesicle(s); RET, resonance energy transfer; *N*-Rh-PE, *N*-(lissamine Rhodamine B sulphonyl)phosphatidylethanolamine; *N*-NBD-PE, *N*-(7-nitrobenz-2-oxa-1,3-diazole-4-yl)phosphatidylethanolamine; DOPC, dioleoylphosphatidylcholine; DOPA, dioleoylphosphatidic acid; Palchol, palmitoylcholine iodide.

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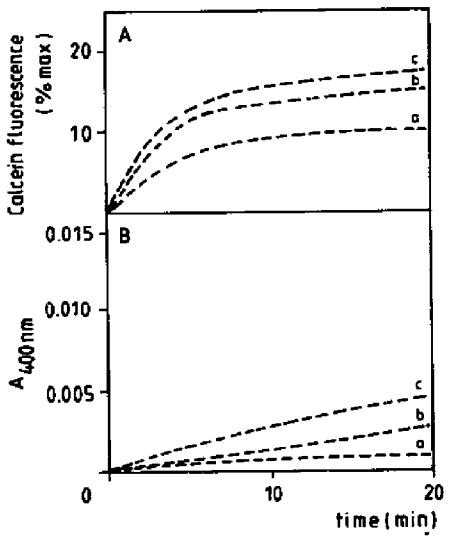

Fig. 2. The release of calcein (A) and change in turbidity (B) during the interaction of TMV coat protein with neutrally charged small unilamellar vesicles (DOPC) at various pH values. The experiments were carried out in 50 mM sodium acetate, 150 mM NaCl (pH 5.0) and in 50 mM Tris-HCl, 150 mM NaCl (pH 7.0 and pH 9.0) at final concentrations lipids and coat protein of 0.1 mM and 10 μg/ml, respectively. a: pH 5.0 (helical protein aggregate); b: pH 7.0 (disk-like protein aggregates); c: pH 9.0 (oligomeric protein aggregates, A-protein).

3C). The second increase is probably due to protein redistribution as a result of the very slow establishment of an equilibrium between protein oligomers and disks [31,40,41]. After removal of the disk protein due to binding to SUV, the oligomers in the sample will form new disks to restore the equilibrium. These new disks may then further enhance the lipid bilayer mixing process.

Interaction of CCMV and its (modified) coat protein with SUV

A schematic representation of the spherical CCMV virion and the two states in which the coat protein is organized (spherical, empty capsids (consisting of 180 units) at pH 5 and dimers at pH 7.5, as described by Verduin [32]) is given in Fig. 1.

As found for TMV virions, interaction of CCMV virions occurs only with positively charged SUV (DOPC/Palchol, 80:20 mol/mol). For CCMV almost no leakage and a small increase of turbidity is observed at pH 7.5 only (data not shown). CCMV coat protein with or without the N-terminal arm at pH 5 and 7.5 does not give leakage of vesicle contents or an increase of turbidity with positively charged SUV.

Incubation of CCMV coat protein with neutrally charged SUV does not give any effect within a time period of 20 min. After a prolonged incubation time (15 h) some lipid bilayer mixing (5%), but no leakage is observed (data not shown).

The interaction of CCMV coat protein with negatively charged SUV, composed of DOPC/DOPA 80:20 mol/mol, is shown in Fig. 4. In all assays the effects at pH 5 (empty capsids) are more pronounced as compared to those at pH 7.5 (coat protein dimers). However, CCMV protein lacking the N-terminal arm shows much less leakage, increase of turbidity and lipid bilayer mixing. The N-terminal peptide of CCMV protein induces a slight leakage and hardly any increase of turbidity, but a significant small amount of lipid bilayer mixing at pH 5.

Interaction of TMV coat protein disks and CCMV coat protein capsids with charged LUV

The extent of interaction between viral material and LUV is expected to be much smaller as compared to the interaction with SUV. This arises because LUV lack the defects in packing of the lipid molecules, which are present in SUV due to the strong bilayer curvature. It is known that bilayer curvature is one of

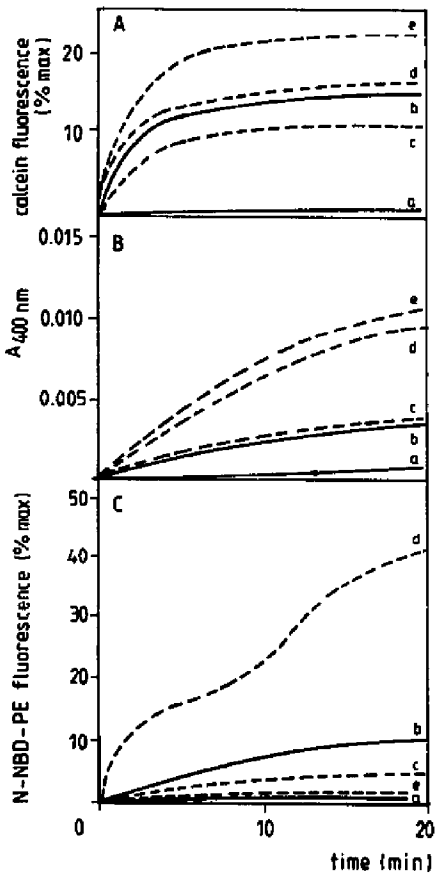

Fig. 3. Kinetics of the interaction of TMV (solid lines) and TMV coat protein (dashed lines) with positively charged small unilamellar vesicles (80:20, mol/mol DOPC/Palchol), as monitored by the leakage of calcein from the vesicle interior (A), change in turbidity at 400 nm (B) and lipid bilayer mixing (C). Conditions were as described in the legend of Fig. 2. a: TMV pH 5.0; b: TMV pH 7.0; c: TMV coat protein pH 5.0; d: TMV coat protein pH 7.0; e: TMV coat protein pH 9.0.

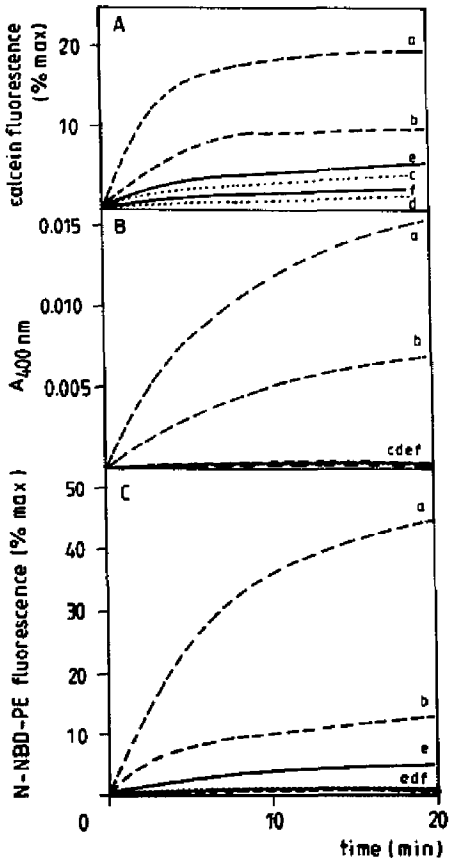

Fig. 4. Kinetics of the interaction of CCMV coat protein (dashed lines), CCMV coat protein lacking the N-terminus (dotted lines) and the N-terminal sequence only (solid lines) with negatively charged small unilamellar vesicles (80:20, mol/mol DOPC/DOPA) as monitored by the release of calcein (A), the change in turbidity (B) and the lipid bilayer mixing (C). The experiments were carried out in 50 mM sodium acetate, 200 mM NaCl (pH 5.0) and in 50 mM Tris-HCl, 200 mM NaCl (pH 7.5). a: CCMV coat protein at pH 5.0 (empty capsids); b: CCMV coat protein at pH 7.5 (protein dimers); c: CCMV coat protein lacking the N-terminal arm at pH 5.0 (empty capsid); d: CCMV coat protein lacking the N-terminal arm at pH 7.5 (dimers); e: N-terminal peptide at pH 5.0; f: N-terminal peptide at pH 7.5.

the driving forces for membrane interaction and fusion [42]. In addition, the vesicle concentration (at equal lipid concentration) of LUV is approximately 30-fold smaller than that of SUV. Therefore, only for the two most active systems, TMV protein at pH 7 (disk) with positively charged vesicles and CCMV protein at pH 5 (empty capsid) with negatively charged vesicles, the RET assay was applied. In the case of TMV protein with positively charged LUV, no interaction was found, even if the protein concentration was raised 20-fold. CCMV protein induces a small amount of lipid bilayer mixing of negatively charged LUV. As compared to the interaction with SUV, the observed rate is very slow: after 2 h of incubation a fluorescence increase of about 3% was observed. However, an increase in fluorescence of about 30% was obtained if the concentration of coat protein was raised 20-fold after 15 h of incubation, indicating that bilayer mixing is continuously proceeding.

Effect of ionic strength

The leakage of carboxyfluorescein from DOPC LUV, induced by TMV coat protein oligomers, was measured as a function of the NaCl concentration. The release of the vesicle contents of the neutrally charged LUV decreases at higher ionic strength of the medium (data not shown).

Discussion

As compared to the results of the turbidity assay [13], we have now investigated several steps in the total interaction process of viral material with membranes. In general, the observations are in agreement with the commonly accepted theory of vesicle fusion [22,26]. In the present work it has been possible to discriminate between aggregation of vesicles (turbidity assay) and membrane destabilization (vesicle leakage and lipid mixing). The aggregation of the vesicles is a result of electrostatic interactions between the viral material and the vesicles surface (cross-linking), while the destabilization of the membrane is a result of penetration or bilayer disruption by hydrophobic protein domains.

Electrostatic interactions

The nature of interaction between viral material and membranes is initially electrostatic, even in case of vesicles that are net neutrally charged. The viral material is then likely to be associated to the headgroups of the lipids. Indications for the electrostatic nature of this first attaching process are: (1) The viral material interacts with oppositely charged membranes (charge neutralization); (2) The interaction decreases when the ionic strength increases (data not shown; [13]).

In case of positively charged membranes, interaction is observed with TMV (Fig. 3) and CCMV virions (data not shown), which are both negatively charged above their isoelectric point at pH 3.5–3.6 [43,44]. Similar effects are observed for the coat protein of TMV, which is negatively charged above pH 4.7 [45] and the coat protein of CCMV, which is net negatively charged above pH 5 (personal communication, Verduin, B.J.M.). In case of negatively charged membranes (Fig. 4) an interaction is found in the presence of the highly positively charged CCMV N-terminal peptide (9 of the 25 amino acid residues are basic [46]) and with intact CCMV coat protein, containing this N-terminus. Clearly the interaction observed arises from this positively charged N-terminal domain, which should, thus, be accessible from the outside of the empty capsid; after enzymatic cleavage of this domain the interaction is almost completely lost (Fig. 4).

the membrane fusion reaction as the key event in the infectious entry of several enveloped animal viruses into their host cells has been investigated extensively using phospholipid vesicles as target membranes [19–22]. In case of the predominantly non-enveloped plant viruses, the study of interactions between intact virions or isolated viral components with artificial lipid vesicles may provide insight into molecular details, not only of the process of the infectious cellular entry of the particular virus, but also of the process of destabilization and cotranslational disassembly at the often membrane-associated ribosomes [23] and the process of association of TMV coat proteins with thylakoid membranes of infected plant cells [24].

Recently, we investigated the interaction of several non-enveloped plant viruses with model membranes, using a turbidity assay [13]. However, a change in turbidity does not yield detailed information about the separate steps in the interaction process, or about the nature of interaction. In this study, the interaction between intact plant viruses or their coat proteins, in well-defined aggregation states, with phospholipid vesicles have been investigated in detail, by varying the vesicles surface charge and size, under different conditions of pH and ionic strength. Three assays have been employed to study the interaction mechanisms: (1) binding or penetration processes [25] were studied by monitoring the leakage of vesicle contents; (2) binding of viral material to vesicles and cross-linking of vesicles [26] were followed by recording the change in turbidity; and (3) merging of lipids among different bilayers by fusion was measured by the RET assay [27,28]. The results are discussed with respect to previously proposed biological models of the early stages of plant virus infection.

Materials and Methods

Chemicals. *N*-(Lissamine Rhodamine B sulphonyl) phosphatidylethanolamine (*N*-Rh-PE), *N*-(7-nitrobenz-2-oxa-1,3-diazole-4-yl)phosphatidylethanolamine (*N*-NBD-PE) were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). Calcein and 5,6-carboxyfluorescein were obtained from Eastman Kodak and were purified as described [29]. Dioleoylphosphatidylcholine (DOPC), dioleoylphosphatidic acid (DOPA) and palmitoylcholine iodide (Palchol) were purchased from Sigma and Triton X-100 from Bio-Rad. All other chemicals were of pro-analytical grade.

Viral material. TMV was grown and purified as described [30]. TMV coat protein was prepared as reported by De Wit et al. [31]. CCMV and its coat protein was purified as described [32]. Virus concentrations were determined spectrophotometrically using an extinction coefficient at 260 nm of 3.0 ml/mg per cm for TMV and 5.87 ml/mg per cm for CCMV. For the coat protein determination an extinction coefficient at 280 nm of 1.27 ml/mg per cm for TMV and CCMV coat protein was used. The N-terminal part of the CCMV coat protein was removed enzymatically as described by Vriend et al. [33]. The N-terminal peptide of the CCMV coat protein was synthesized by Ten Koortenaar et al. [34].

Vesicle preparation. Small unilamellar vesicles (SUV) were prepared by sonication of a multilamellar vesicle dispersion under a flow of nitrogen with a Branson Sonifier B15 during 6 min (duty cycle 50%, output 50 W), keeping the suspension on ice. Vesicle suspensions were centrifuged (10 min, $8800 \times g$) to remove titanium particles from the tip of the sonicator and multilamellar structures, if present.

Large unilamellar vesicles (LUV) were prepared by the reverse-phase evaporation technique [35] modified by Wilschut et al. [36]. The vesicles were sized by extrusion through a 0.2 μm Unipore polycarbonate filter (Bio-Rad) under a nitrogen pressure of 5 atm [37].

Vesicles destined for the leakage experiments were loaded with 40 mM calcein (sodium salt) in the appropriate buffers (mentioned in the figure legends) with a reduced concentration of NaCl to avoid leakage due to difference in osmolarity. The osmolarity of 40 mM calcein equals that of 100 mM NaCl. Non-encapsulated calcein was removed by gel filtration chromatography on a Bio-Gel P10 column (1.5×20 cm) in the buffers mentioned in the figure legends. Lipid contents were determined by measuring the phospholipid phosphorous by the procedure of Bartlett [38].

Leakage experiments. Release of vesicle content was monitored at room-temperature in a Hitachi Perkin-Elmer fluorescence spectrophotometer MPF-2A by adding virus or viral coat protein (final concentration 10 μg/ml, unless stated otherwise) to 3 ml vesicle suspension in the appropriate buffer (final lipid concentration 0.1 mM). The excitation and emission wavelengths were adjusted to 490 nm and 515 nm, respectively. Residual fluorescence of the vesicles was taken as the zero level and the fluorescence after lysis of the vesicles with Triton X-100 (0.5% v/v) as the maximum value. Controls were performed under all conditions.

Absorption measurements. The turbidity of the samples, including controls, (final concentrations lipid and viral material 0.1 mM and 10 μg/ml, respectively) was measured in a Kontron Uvikon 810 spectrophotometer at 400 nm at 18°C.

Lipid bilayer mixing experiments. In the RET assay, described by Struck et al. [27], 0.6 mol% each of *N*-NBD-PE and *N*-Rh-PE was incorporated in the bilayer of half of the vesicle population. Typically 2 ml vesicle suspension in the appropriate buffer (final lipid concentration 0.1 mM) was thermostated at 18°C in a cuvette and stirred continuously. The reaction was

initiated by adding virus or coat protein solution (final concentration 10 μg/ml, unless stated otherwise). Fluorescence was monitored in a Perkin-Elmer LS-5 luminescence spectrometer. The excitation and emission wavelengths were 465 and 530 nm, respectively. Complete dilution of *N*-NBD-PE and *N*-Rh-PE was established by addition of Triton X-100 (final concentration, 0.5% v/v). A fluorescence increase value of 100 is taken as the maximum fluorescence increase possible. This value is determined after the addition of Triton X-100 to the samples and correction for the 30% decrease in quantum yield of *N*-NBD-PE in the presence of Triton. Controls were performed under all conditions.

Results

The interaction of TMV, CCMV or isolated viral components with phospholipid vesicles was studied by following vesicle aggregation, leakage of vesicle contents and mixing of bilayer lipids, under various conditions of pH and ionic strength. In all cases SUV were used; only for the most active systems additional experiments on LUV were performed. In all cases, the kinetics of the processes observed is dependent on the concentration of the vesicles and the viral material. Therefore, to enable a comparison between the different parameters studied in a particular system, the overall concentration of the lipids and viral material was kept constant. It should be noted that in experiments with the N-terminal peptide of the CCMV coat protein, a molar concentration equal to that in the intact coat protein is used.

Interaction of TMV and TMV coat protein with SUV

Depending on salt concentration and pH, three aggregational states of the TMV coat protein can be distinguished. At the fixed NaCl concentration used (150 mM), the coat protein is organized in the so-called A-protein (oligomeric; 1–8 units) at pH 9, disks (34 coat protein units) at pH 7 or helix (several hundreds units) at pH 5 [39]. The various aggregation states of the coat protein are illustrated in Fig. 1.

The interaction of TMV coat protein with neutrally charged DOPC SUV is given in Fig. 2. TMV coat protein induces a leakage of calcein from the vesicle interior and gives an increase in turbidity at 400 nm. This effect increases at higher pH values. Lipid bilayer mixing, as monitored by the RET assay, is not observed in a time period of 20 min and hardly after prolonged incubation. TMV itself induces only very small amounts of leakage of vesicle contents or change in turbidity at pH 5 or 7 in DOPC SUV systems (results not shown).

Interaction of TMV or its coat protein with net negatively charged SUV, composed of DOPC/DOPA (80:20, mol/mol), is negligible.

In Fig. 3 the results for TMV and TMV coat protein with positively charged SUV (DOPC/Palchol, 80:20 mol/mol) are given. The effect of TMV at pH 7 is much larger than at pH 5, where the effect is almost negligible. TMV coat protein is more active than the virion. The oligomeric A-protein (pH 9) induces a leakage of the vesicle contents and gives an increase in turbidity, but induces hardly any lipid bilayer mixing. Disk aggregates (pH 7) are very efficient in bilayer mixing. It should be noted that the lipid bilayer mixing has a strong increase at $t = 0$ and at $t = 10$ min (Fig.

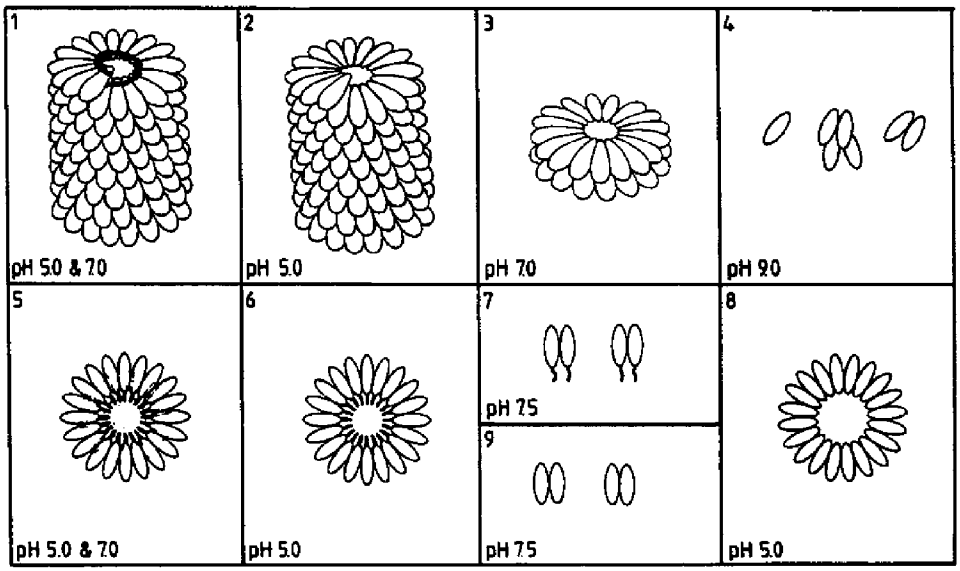

Fig. 1. Schematic representation of TMV and CCMV and various aggregation states of their coat proteins as a function of pH. Rod-shaped TMV virions (1). TMV protein in helix-form (2), in disk-form (3) and as oligomers or A-protein (4). Spherical CCMV virions (5). CCMV coat protein in empty capsids (6) and in dimers (7). CCMV coat protein without the N-terminal sequence in empty capsids (8) and in dimers (9).

In addition to the electrostatic effect discussed above, an effect, which could clearly be assigned to pH, is observed in the case of the virions. TMV induces more leakage, increase in turbidity and lipid bilayer mixing with positively charged SUV at pH 7 than at pH 5. Since the concentration of vesicles and virions is constant and hardly extra buried domains are exposed at higher pH (no dissociation of the virions within this range) the increase in negative charge at higher pH explains this result. It should be noted that the net charge of the vesicles is hardly affected due to the absence of titrational groups on the lipid molecules within this pH range. In case of CCMV virions a similar pH effect with positively charged vesicles is found, which can be explained in a comparable way.

Membrane destabilizing interactions

After the initially electrostatic interactions, responsible for the observed attachment of the viral material to the membranes and bridging or cross-linking of vesicles, further membrane destabilization processes (monitored as penetration, disruption or mixing of the lipid bilayers) can occur. For example, lipid bilayer mixing is clearly demonstrated for the TMV coat protein and CCMV coat protein with oppositely charged SUV (Figs. 3 and 4), where the electrostatic interactions give rise to the initial attachment. However, in absence of strong electrostatic attachment, as is the case with neutrally charged SUV and TMV coat protein (Fig. 2A), vesicle leakage can be observed, but hardly any lipid bilayer mixing occurs. This suggests a single membrane penetration or disruption process, in which the mixing of vesicle lipids is negligible. TMV coat protein can penetrate one bilayer, but is unable to attach and mix the lipids in more bilayers.

In general, membrane destabilization by proteins is facilitated by exposure of hydrophobic domains of the molecules [10,11,13]. Hydrophobic forces are known to be involved in the bilayer penetration or merging of lipid bilayers [47,48]. However, hydrophobic interaction does not explain the bilayer mixing effect, observed for the N-terminal peptide in Fig. 4. In this case, there are no hydrophobic domains present on this small peptide and only electrostatic interactions exist. This result is in agreement with results obtained with certain polycations [49,50]. A possible mechanism, that explains the observed effect, may be the strong local absorbance to negatively charged groups on the membrane surface of the highly positively charged peptide, which would thereby create membrane distortions and result in exposure of hydrophobic domains on the, thus, destabilized bilayer, similar to the effect of divalent cations on negatively charged phospholipid vesicles [22,48].

It should be noted that, due to the strong bilayer curvature, SUV are known to show defects in lipid packing, thereby exposing hydrophobic lipid domains. This results in a greater tendency for SUV to fuse as compared to larger vesicles [26]. This also explains the high rates of bilayer mixing found in the experiments with SUV, as compared to LUV.

Effect of aggregational state

For the coat proteins of both TMV and CCMV, an increase in pH not only produces an increase of the number of negative charges, but also a change in the aggregational state of the proteins, as illustrated in Fig. 1. Dissociation of protein aggregates results in an increased number of exposed protein domains, and a higher concentration of smaller protein aggregates.

The effect of the aggregational state of the viral coat proteins can best be understood by observing the effect of the coat protein during the lipid bilayer mixing experiments, which represents the most sensitive assay for monitoring protein–vesicle interaction described in this paper. On changing the pH for TMV coat protein from 5 to 9, an increase of the electrostatic as well as hydrophobic interaction towards positively charged vesicles is expected, because of the increase of the number of negative charges and exposed domains, but also because of the higher concentration of protein aggregates. Thus, for the experiments shown in Fig. 3C, a gradual increase of bilayer mixing could be predicted. However, disk aggregates (curve d) are much more effective than the oligomers (A-protein, curve e). A corresponding effect is observed for the interaction of CCMV coat protein with negatively charged vesicles (Fig. 4C, curve a), where the empty capsids have a greater efficiency than the dimers (curve b). This means that the disk aggregates for TMV coat protein and the empty capsid aggregates for CCMV coat protein are the best compromise between efficiency (capacity of the protein to bridge vesicles and to destabilize their membranes) and the concentration of the protein aggregates. This effect is illustrated by the experiments with LUV as well, where lipid bilayer mixing is only observed in case of the disk aggregates of TMV coat protein with positively charged LUV and in case of empty capsid aggregates of CCMV coat protein with negatively charged LUV.

Clearly, a high local concentration of opposite charges (with respect to the membrane charge), as achieved by these types of aggregates, is necessary to enable effective binding and cross-linking of two or more vesicle bilayers. If the aggregates are too large (TMV coat protein in helical aggregates at pH 5) the large aggregate could possibly prohibit the binding process. However, also the concentration of the aggregates is reduced and so is the number of charges and exposed domains. For small aggregates (TMV A-protein and the N-terminal peptide of CCMV coat protein), obviously the local charge concentration is too small for a strong effect.

Biological implications

TMV and CCMV virions, and also TMV coat protein, are found to interact predominantly with positively charged membranes. Since phospholipids in plant cellular membranes are net neutrally or negatively charged, such an interaction is unlikely to play a role in the interaction of the virions with plant membranes. A possible interaction could be with positively charged (receptor) sites on the membrane. For example, the coat proteins of TMV, which are found to be present with thylakoid membranes from chloroplasts [24] might possibly be associated to membrane-bound sites. However, up till now evidence for the presence of such sites on plant cellular membranes has never been reported.

On the other hand, a small positively charged domain, the N-terminal sequence of the net negatively charged CCMV coat protein, is found to be of crucial importance for the interaction of the coat protein with negatively charged membranes. It should be noted that the observed electrostatic interactions are not specific and that the lipid composition of the vesicles used differs from that found in plant cell membranes.

Based on the extreme conditions used in our experiments (small, stressed SUV and high concentration of viral material) and the relatively slow time dependences observed, it can be concluded that the coat proteins of plant viruses are probably not designed to induce membrane fusion, since the observed fusogenic activity, as compared to the activity of fusion proteins of animal viruses [19,20], is very small.

As has been stated before, the entry of the plant virus into the cytoplasm of the plant cell is presumably facilitated by damage of the cell membrane [51]. The strong interaction of CCMV coat protein with the negatively charged membranes may be the basis for a possible interaction of the coat protein with the negatively charged membranes *inside* the cell. Similar results have been obtained for the coat proteins of brome mosaic virus (BMV) and southern bean mosaic virus (SBMV), which also have a strong basic N-terminus [13]. This effect is a possible indication for the involvement of the N-termini in processes during the uncoating of the viral genome on the ribosomes, which are often associated with membranes [16–18]. The viral coat proteins could be stripped-off from the viral RNA, when the protein-RNA interactions are replaced for protein–ribosome interactions [52] and protein–lipid interactions [53]. In this view, the reactive *N*-terminus of CCMV coat protein can, depending on the conditions, release the RNA in favour of another negatively charged cell component, such as membranes.

References

1 Shaw, J.G. (1985) in Molecular Plant Virology (Davies, J.W., ed.), Vol. 2, pp. 1–21, CRC Press, Boca Raton.
2 Kiho, Y. and Shimomura, T. (1976) Japan. J. Microbiol. 20, 537–541.
3 Watts, J.W. and King, J.M. (1984) J. Gen. Virol. 65, 1709–1712.
4 Hull, R. and Maule, A.J. (1985) in The Viruses (Francki, R.I.B., ed.), pp. 83–115, Plenum Press, New York.
5 Roenhorst, J.W., Van Lent, J.W.M. and Verduin, B.J.M. (1988) Virology164, 91–98.
6 Kiho, Y., Shimomura, T., Abe, T. and Nozu, Y. (1979) Microbiol. Immunol. 23, 735–748.
7 Kiho, Y., Abe, T. and Ohashi, Y. (1979) Microbiol. Immunol. 23, 1067–1076.
8 Durham, A.C.H. (1978) Biomedicin 28, 307–314.
9 Kiho, Y. and Abe, T. (1980) Microbiol. Immunol. 24, 617–628.
10 Banerjee, S., Vandenbranden, M. and Ruysschaert, J.M. (1981) Biochim. Biophys. Acta 646, 360–364.
11 Banerjee, S., Vandenbranden, M. and Ruysschaert, J.M. (1981) FEBS Lett. 113, 221–224.
12 Abdel-Salam, A., White, J.A. and Sehgal, O.P. (1982) Phytopathol. Z. 105, 336–334.
13 Datema, K.P., Spruijt, R.B., Verduin, B.J.M. and Hemminga, M.A. (1987) Biochemistry 26, 6217–6223.
14 Van Lent, J.W.M. and Verduin, B.J.M. (1986) in Developments in Applied Biology. 1. Developments and applications in virus testing (Jones, R.A.C. and Torrance, L., eds.), pp. 193–211, Association of Applied Biologists, Wellesbourne, U.K.
15 Lopez-Abella, D., Bradley, R.H.E. and Harris, K.F. (1988) Adv. Disease Vector Res. 5, 251–285.
16 Shaw, J.G., Plaskitt, K.A. and Wilson, T.M.A. (1986) Virology 148, 326–336.
17 Brisco, M.J., Hull, R. and Wilson, T.M.A. (1986) Virology 148, 210–217.
18 Roenhorst, J.W., Verduin, B.J.M. and Goldbach, R.W. (1989) Virology 168, 138–146.
19 White, J., Kielian, M. and Helenius, A. (1983) Q. Rev. Biophys. 16, 151–195.
20 Stegmann, T., Hoekstra, D., Scherphof, G. and Wilschut, J. (1985) Biochemistry 24, 3107–3113.
21 Stegmann, T., Hoekstra, D., Scherphof, G. and Wilschut, J. (1986) J. Biol. Chem. 261, 10966–10969.
22 Wilschut, J. (1988) in Energetics of Secretion Responses, Vol. II (Akkerman, J.W.N., ed.), pp. 63–80, CRC Press, Boca Raton.
23 Wilson, T.M.A. (1984) Virology 137, 255–265.
24 Reinero, A. and Beachy, R.N. (1986) Plant Mol. Biol. 6, 291–301.
25 Blumenthal, R., Seth, P., Willingham, M.C. and Pastan, I. (1986) Biochemistry 25, 2231–2237.
26 Nir, S., Bentz, J., Wilschut, J. and Düzgüneş, N. (1983) Progr. Surf.Sci. 13, 1–124.
27 Struck, D.K., Hoekstra, D. and Pagano, R.E. (1981) Biochemistry 20,4093–4099.
28 Hoekstra, D. (1982) Biochemistry 21, 2833–2840.
29 Ralston, E., Hjelmeland, L.M., Klausner, R.D., Weinstein, J.N. and Blumenthal, R. (1981) Biochim. Biophys. Acta 649, 133–137.
30 Leberman, R. (1966) Virology 30, 341–347.
31 De Wit, J.L., Hemminga, M.A. and Schaafsma, T.J. (1978) J. Magn. Reson. 31, 97–107.
32 Verduin, B.J.M. (1978) J. Gen. Virol. 39, 131–147.
33 Vriend, G., Hemminga, M.A., Verduin, B.J.M., De Wit, J.L. and Schaafsma, T.J. (1981) FEBS Lett. 134, 167–171.
34 Ten Koortenaar, P.B.W., Krüse, J., Hemminga, M.A. and Tesser, G.I. (1986) Int. J. Peptide Protein Res. 27, 401–413.
35 Szoka, F. and Papahadjopoulos, D. (1978) Proc. Natl. Acad. Sci. USA 75, 4194–4198.
36 Wilschut, J., Düzgüneş, N., Fraley, R. and Papahadjopoulos, D. (1980) Biochemistry 19, 6011–6021.
37 Olson, F., Hunt, C.A., Szoka, F.C., Vail, W.J. and Papahadjopoulos, D. (1979) Biochim. Biophys. Acta 557, 9–23.
38 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468.

39 Durham, A.C.H. (1972) J. Mol. Biol. 67, 289–305.
40 De Wit, J.L., Alma, N.C.M., Hemminga, M.A. and Schaafsma, T.J. (1979) Biochemistry 18, 3973–3976.
41 Hilhorst, H.W.M., Postma, U.D. and Hemminga, M.A. (1982) FEBS Lett. 142, 301–304.
42 Blaurock, A.E. and Gamble, R.C. (1979) J. Membr. Biol. 50, 187–204.
44 Fraenkel-Conrat, H. and Nariba, X. (1958) in Symposium on Protein Structure (Neuberger, A., ed.), pp. 249, Methuen, London.
44 Bancroft, J.B. (1971) Adv. Virus Res. 16, 19–134.
45 Fraenkel-Conrat, H. (1957) Virology 4, 1–4.
46 Rees, M.W. and Short, M.N. (1982) Virology 119, 500–503.
47 Ohki, S. (1982) Biochim. Biophys. Acta 689, 1–11.
48 Wilschut, J. and Hoekstra, D. (1986) Chem. Phys. Lipids 40, 145–166.
49 Gad, A.E., Silver, B.L. and Eytan, G.D. (1982) Biochim. Biophys. Acta 690, 124–132.
50 Uster, P.S. and Deamer, D.W. (1985) Biochemistry 24, 1–8.
51 Roenhorst, J.W. (1989) PhD. Thesis, Agricultural University,Wageningen, The Netherlands.
52 Wengler, G. (1987) Arch. Virol. 94, 1–14.
53 Hatta, T. and Matthews, R.E.F. (1976) Virology 73, 1–16.