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TOBACCO MOSAIC VIRUS PROTEIN INDUCES FUSION OF LIPOSOME MEMBRANES

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The fusogenic properties of tobacco mosaic virus (TMV) coat protein were investigated. Tobacco mosaic virus protein induces membrane fusion of a population of L-α-dimyristoylphosphatidylcholine (DMPC) and DL-α-dipalmitoylphosphatidylcholine (DPPC) vesicles giving rise to larger particles as seen by a drastic absorbance increase of the liposomal solution. Differential scanning calorimetry spectra demonstrate complete mixing of the acyl chains of the lipids during fusion. Electron micrographs indicate that the fused entities are multilamellar.

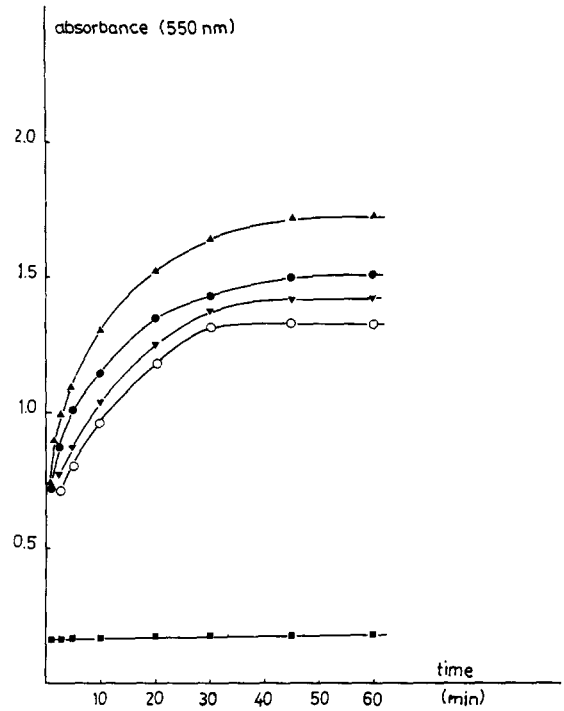
There is now a general consensus for paramyxoviruses that induction of cellular infection requires fusion of virus particles with the cellular plasma membrane [1]. This property is correlated with the presence of glycoprotein in the virus envelope but it has not yet been possible to induce cell fusion by adding pure preparation of this glycoprotein to cell cultures. Probably some specific organisation of the lipid matrix is needed to allow this fusion [2]. Here we report about the capacity of a protein isolated from a plant virus (tobacco mosaic virus) to induce the fusion of lipid membranes. Because this protein is one essential constituent of this plant virus, the results reported could be correlated to the tobacco mosaic virus infection process.

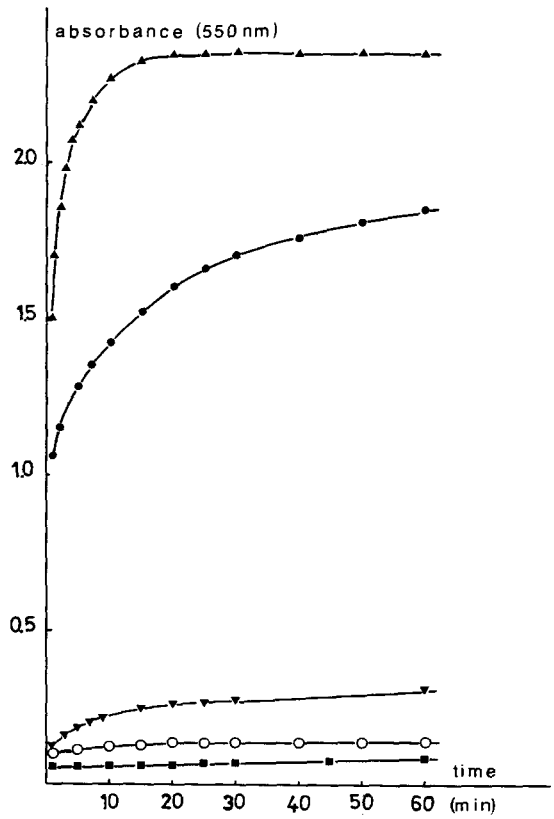
Figs. 1 and 2 show the absorbance increase over a period of 1 h of a solution of unilamellar liposomes of L-α-dimyristoylphosphatidylcholine (DMPC) (Fig. 1) and DL-α-dipalmitoylphosphatidylcholine (DPPC) (Fig. 2) to which tobacco mosaic virus protein has been added. Protein was obtained by the method of Fraenkel and Conrat [3] by elevating the pH of the viral solution initially at pH 7 to pH 9. Liposomes were prepared as previously described [4–6]. Control experiments with no protein added were equally performed. The absorbance of the liposomal solution increases very rapidly after addition of the tobacco mosaic virus protein indicating an increase in size of the lipid particles.

If absorbance measurements do not allow to distinguish between membrane fusion and aggregation, differential scanning calorimetry (DSC) strongly suggests that tobacco mosaic virus protein induces the formation of multilamellar structures. Calorimetric measurements were recorded on a Setaram 111 using 100 μl inox cells. In Figs. 3, 4, we see that the DSC spectrum of the small unilamellar vesicles, characterized by a broad transition peak, has been replaced, after tobacco mosaic virus protein addition, by a new spectrum strongly similar to that of multilamellar vesicles.

Comparison of Figs. 3(2) and 3(3) indicates clearly that addition of tobacco mosaic virus protein to unilamellar vesicles induces the formation of multilamellar structures.

Abbreviations: DMPC, L-α-dimyristoylphosphatidylcholine; DPPC, DL-α-dipalmitoylphosphatidylcholine.





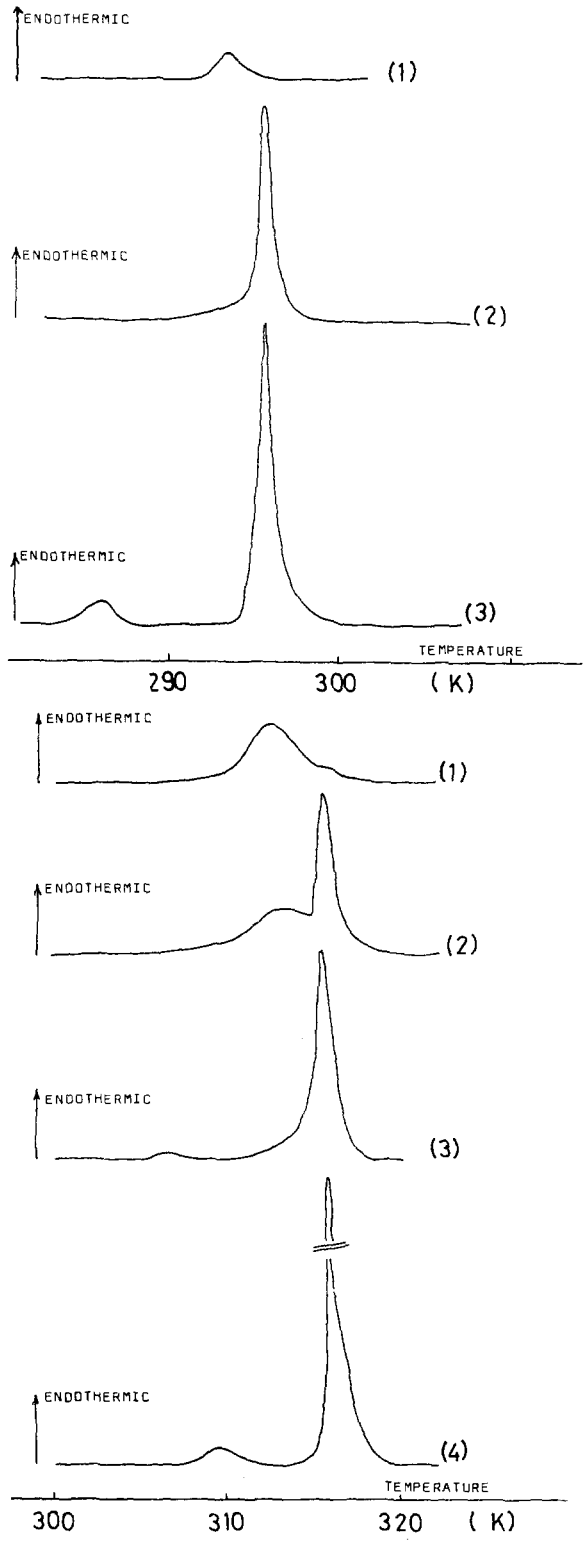
Electron micrographs obtained on an electron microscope Elmiscop I by the technique of negative staining [9] clearly confirm that the particles obtained after addition of tobacco mosaic virus protein to small unilamellar vesicles are multilamellar (Fig. 5). This multilamellar membrane differs from the single limiting membrane classically observed in cellular fusion. This process has been observed by others [7,9] but the comprehension of its mechanism requires further investigation.

If two populations of small unilamellar vesicles (one made of DMPC and the other made of DPPC) are mixed together at 18°C, addition of tobacco mosaic virus protein is followed by the formation of a new component with a melting point identical to that of multilamellar liposomes made of an equimolar mixture of the two lipids (Fig. 6). Incubation of the same lipid suspension for a similar period of time in the absence of tobacco mosaic virus protein indicated no detectable mixing of the lipid peaks. In conclusion, DSC measurements show that the tobacco mosaic virus protein induces fusion of lipid membranes.

The fact that the fusion was observed at 18°C indicates that this process can be obtained between solid

Fig. 1. Change in absorbance of liposome suspensions of L-α-dimyristoylphosphatidylcholine (DMPC) (4 mg lipid/ml buffer) after tobacco mosaic virus protein addition. Protein was obtained by the method of Fraenkel-Conrat [3] by elevating the pH of the viral solution (10 mg/ml) initially at pH 7 (0.01 M Tris-HCl/0.15 M NaCl buffer) to pH 9 (by adding small quantities of 0.1 N NaOH). Small volumes of this protein solution was added to liposome suspensions. At time $t = 0$, 0.1 ml of a solution containing different quantities of protein was added to 1 ml of DMPC small unilamellar vesicles: ▲, 1 mg of protein; ●, 0.5 mg; ▼, 0.01 mg; ○, 0.001 mg; ■, control preparation not exposed to tobacco mosaic virus protein. The pH of the liposomal suspension was controlled to remain at pH 7 after addition of the protein solution. All experiments were carried out at 18°C in a 0.01 M Tris-HCl/0.15 M NaCl, pH 7 buffer.

Fig. 2. Change in absorbance of liposome suspensions of DL-α-dipalmitoylphosphatidylcholine (4 mg lipid/ml buffer) after addition of tobacco mosaic virus protein. At time $t = 0$, 0.1 ml of a solution containing different quantities of protein was added to 1 ml DPPC small unilamellar vesicles: ▲, 1 mg or protein; ●, 0.5 mg; ▼, 0.01 mg; ○, 0.001 mg; ■, control preparation not exposed to tobacco mosaic virus protein. All experiments were carried out at 18°C in a 0.01 M Tris-HCl/0.15 M NaCl, pH 7 buffer. Protein was obtained and added as described in the legend to Fig. 1.



vesicles: indeed, both DMPC and DPPC are below their transition temperatures. This result differs strongly from other works on liposome fusion [7,8] which suggest that a successful fusion of DMPC and DPPC vesicles required the lipids to be in a liquid state above their mutual transition temperature.

Although Ca^{2+} and acidic lipids have been supposed to be essential for membrane fusion, it is conceivable that tobacco mosaic virus protein may organize the lipid layer in a way that can favour fusion. The fusogenic properties of several hydrophobic membrane proteins have moreover been reported recently [8,10].

Experimental evidence has been presented that proteins bound to membranes can produce domain boundaries with conformation that are intermediate between the fluid and solid lipid states. These domain boundaries might act as sites of initiation of membrane fusion. We think that the present investigation has some relevance to cellular events associated to the infection process induced by tobacco mosaic virus. This view is supported by the fact that experiments on tobacco protoplasts revealed that tobacco mosaic virus does not infect spontaneously the cells but that

Fig. 3. Differential scanning calorimetry spectra of liposomes of DMPC exposed to tobacco mosaic virus protein. (1) Small unilamellar vesicles of DMPC (8 mg lipid/0.15 ml buffer) with no protein added. (2) Small unilamellar vesicles of DMPC (8 mg lipid/0.15 ml buffer) to which 0.5 mg of tobacco mosaic virus protein was added. Spectra were recorded after 1 h incubation. (3) Multilamellar vesicles of DMPC (8 mg lipid/0.15 ml buffer) with no protein added. All experiments were carried out in a 0.01 M Tris-HCl/0.15 M NaCl, pH 7 buffer. Incubation temperature: 18°C. Protein was obtained and added as described in the legend to Fig. 1.

Fig. 4. Differential scanning calorimetry spectra of liposomes of DPPC (8 mg lipid/0.15 ml buffer) exposed to tobacco mosaic virus protein. (1) Small unilamellar vesicles of DPPC with no protein added. (2) Small unilamellar vesicles of DPPC to which 0.5 mg of tobacco mosaic virus protein was added. Spectra were recorded after 1 h incubation. (3) Small unilamellar of DPPC to which 0.5 mg of tobacco mosaic virus protein was added. Spectra were recorded after 24 h incubation. (4) Multilamellar vesicles of DPPC with no protein added. All experiments were carried out at 18°C in a 0.01 M Tris-HCl/0.15 M NaCl, pH 7 buffer. Protein was obtained and added as described in the legend to Fig. 1.

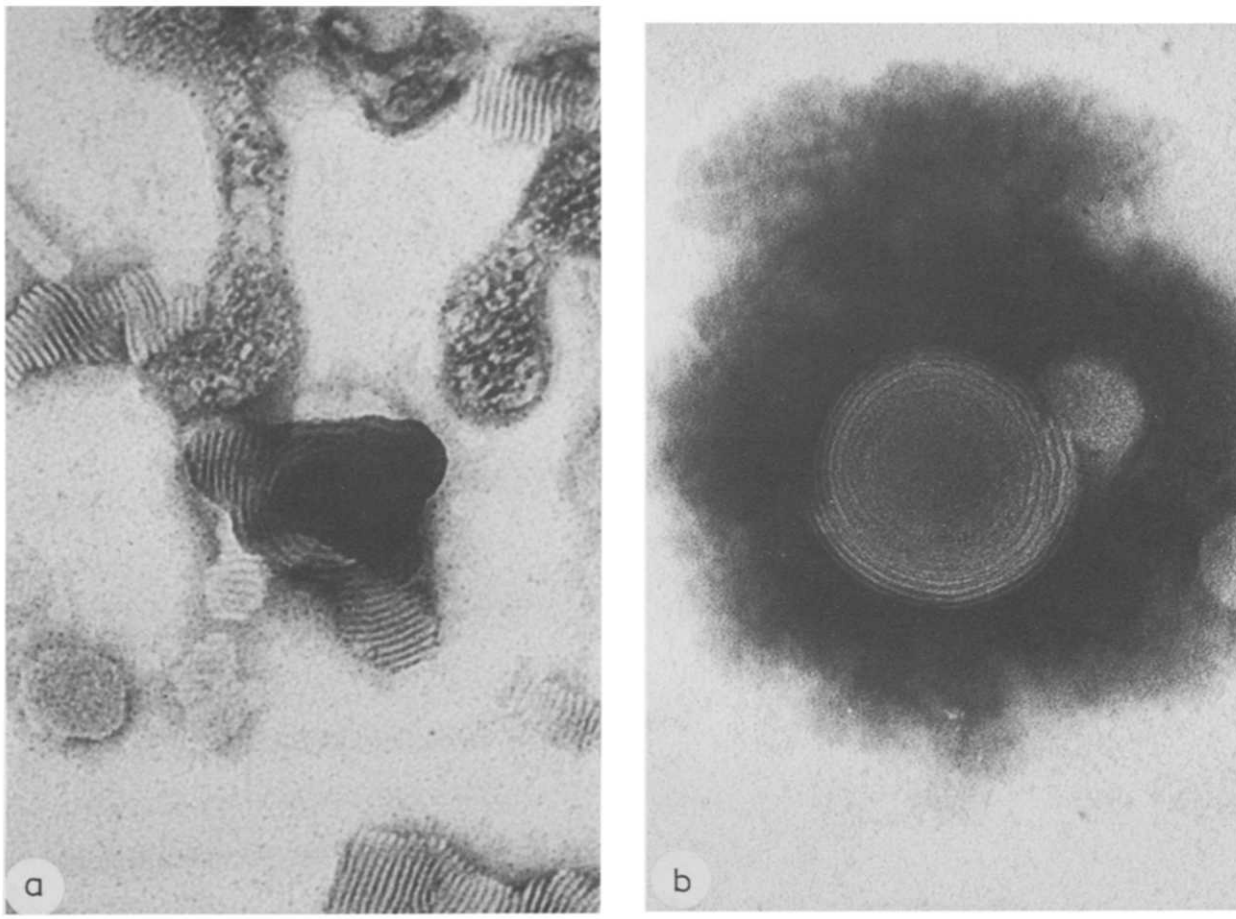


Fig. 5. Electron micrographs of: (a) typical shape of particles obtained after incubation of unilamellar vesicles of DMPC (4 mg lipid/ml buffer) with tobacco mosaic virus protein (0.5 mg protein/4 mg lipid) for 1 h at 18°C. (b) large multilamellar vesicle of DMPC at the same lipid concentration (with no protein added). Both micrographs are at a magnification of 200 000. All samples were prepared in a 0.01 M Tris-HCl/0.15 M NaCl, pH 7 buffer and stained with 1% sodium/potassium phosphomolybdate at pH 7.

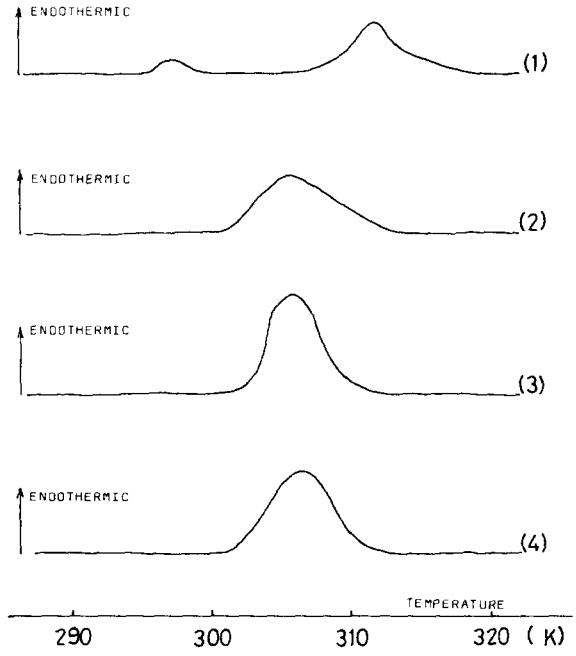


Fig. 6. Differential scanning calorimetry spectra of a mixture of two populations of liposomes (one of DMPC and one of DPPC) exposed to tobacco mosaic virus protein. Total concentration in lipids: 4 mg DMPC + 4 mg DPPC/0.15 ml buffer. (1) Small unilamellar vesicles of DMPC + Small unilamellar vesicles of DPPC with no protein added; (2) Small unilamellar vesicles of DMPC + Small unilamellar vesicles of DPPC to which 0.5 mg of tobacco mosaic virus protein was added. Spectra were recorded after 1 h incubation; (3) Small unilamellar vesicles of DMPC + Small unilamellar vesicles of DPPC to which 0.5 mg of tobacco mosaic virus protein was added. Spectra were recorded after 24 h incubation; (4) Multilamellar vesicles formed from a mixture of the lipids (DMPC/DPPC, 50 : 50) with no protein added. All experiments were carried out at 18°C in a 0.01 M Tris-HCl/0.15 M NaCl, pH 7 buffer. Protein was obtained and added as described in the legend to Fig. 1.

agents such as poly(ethylene glycol) are needed to promote the infection [11]. Poly(ethylene glycol) is precisely an agent capable to destabilize the lipid membrane [12] and to induce cell fusion [13]. Our experiments demonstrate that tobacco mosaic virus protein, an essential component of a plant virus, possesses the same fusogenic properties.

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