INTERACTION OF TOBACCO MOSAIC VIRUS PROTEIN WITH LIPID MEMBRANE SYSTEMS

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1. Introduction

Tobacco mosaic virus (TMV) is a rod-shaped virus composed of a single-stranded RNA encapsulated in a protein capsid made up of the repetition of an unique protein [1]. Although the virus structure has been extensively studied [2], little is known about its mode of penetration into the host cell. The interaction between the protein of the capsid and the cell membrane will be the first step of this penetration process.

Here, we report on the capacity of the isolated TMV protein to interact with model membranes and to destabilize the lipid bilayer organization. Our results suggest that the hydrophobic TMV protein region could be a privileged site for TMV fixation on the host cell as confirmed by recent data on plant virus attachment to plant cell walls.

2. Materials and methods

Dipalmitoyl phosphatidylcholine (DPPC), distearoyl phosphatidylcholine (DSPC), dioleyl phosphatidylcholine (DOPC), dimyristoyl phosphatidylcholine (DMPC), cardiolipin (cardio), egg yolk phosphatidylcholine (Eg PC) and glycerol monolate (GMO) were purchased from Sigma Co. (St Louis, MO). Phosphatidyl serine (PS) was a Koch-Light product and carboxyfluorescein an Eastman-Kodak product. TMV was a gift of Professors R. Jeener and J. Urbain (Centre de Biologie Moléculaire, Université Libre de Bruxelles). TMV protein was obtained by elevating the pH of the TMV solution initially at pH 7 to pH 9 [3]. All reagents were of analytical grade. Water was triple distilled.

2.1. *Monolayers*

Lipids were dissolved in chloroform and spread at the air–water interface on a subphase containing 50 ml buffer (0.01 M Tris–HCl, 0.15 M NaCl, pH 7). The surface was limited by a Teflon ring (12 cm diam.). The surface pressure was recorded by the Wilhelmy plate technique, using a Cahn RG electrobalance.

2.2. *Planar lipid bilayers*

Planar lipid bilayers were formed from a solution of GMO dissolved in decane on a 1.3 mm diam. aperture in a Teflon cell separating 2 aqueous compartments [4–7]. *n*-Decane was redistilled before use. The aqueous phase was a 0.01 M Tris–HCl, 0.15 M NaCl, pH 7 buffer. Measurements were made at 20°C. Potential differences V_m (mV) were imposed by two platinum Ag/Ag Cl electrodes. Membrane conductances were determined by measuring the specific current I_m (A/cm^2) using a Keithley electrometer as a function of the imposed potential difference. Membrane formation was observed under reflected light with a low power microscope. The virus protein was added in the two compartments. The aqueous phase was a 0.01 M Tris–HCl, 0.15 M NaCl, pH 7 buffer.

2.3. *Preparation of liposomes [8–10]*

Lipids were dissolved in chloroform. The solvent was evaporated under a nitrogen flow and the lipid film dried overnight in vacuum. Multilamellar liposomes were obtained by mechanical stirring (Vortex mixer) of the lipid film to which a 0.01 M Tris-HCl, 0.15 M NaCl, pH 7 buffer was added. Small unilamellar liposomes (SUV) were obtained by submitting the multilamellar liposomes to ultrasonication in a Branson Sonifier (50 W for 20 min) in a heat-controlled water bath. The temperature was maintained slightly above the transition temperature of lipid.

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2.4. *Permeability experiments [11–13]*

Self quenching property of carboxyfluorescein (CF) was used to demonstrate the release of CF from liposomes. Indeed, the fluorescence of CF is quenched by 96% at 100 mM in phosphatidylcholine vesicles [12]. The CF release induces a drastic fluorescence increase which can be followed without separation of vesicles from released solute. The experiment consisted in exciting the CF at 470 nm and following its emission at 520 nm with a Jobin-Yvon spectrofluorometer.

In our procedure, the lipid film was submitted to vortex agitation followed by ultrasonication in a solution containing 200 mM of CF. The SUV were then eluted through a Sephadex G-50 column by a 0.01 M Tris–HCl, 0.15 M NaCl, pH 7 buffer to separate SUV and free CF.

3. Results and discussion

3.1. *Monolayers*

In our experiments, the lipid monolayer was spread at an initial pressure of 20 dynes/cm. At such a surface pressure, we can reasonably assume that the lipids exist almost exclusively in a close-packed state. In these conditions, the surface pressure increase can be attributed to protein penetration into the lipid monolayer and not to spontaneous protein adsorption into some lipid-free sites at the interface or to simple electrostatic interaction without penetration.

Fig.1 shows the surface pressure increase as a function of time for a monolayer of DPPC under which TMV protein was injected. This surface pressure increase at constant area indicates to what extent a protein can overcome lateral pressure between the lipids in order to insert itself between them. Such a surface pressure increase has already been observed with other membrane proteins such as melittin [14]. These proteins have hydrophobic segments which penetrate between the hydrocarbon chains of the lipid bilayer. Fig.1 indicates that the drastic penetration is not significatively related to the fluidity of the lipidic monolayer or to the charge of the polar head groups. No clear sequence of the role of the fluidity and of the charge can be unequivocally deduced.

3.2. *Planar lipid bilayers*

Interaction of TMV protein with planar lipid bilayers offers another way to confirm the capacity of TMV protein to interact with lipids. Addition of TMV protein to each side of a planar lipid bilayer induces a 10-fold increase in conductance (fig.2). This conductance increase can be attributed to protein penetration into planar lipid bilayers as observed with other

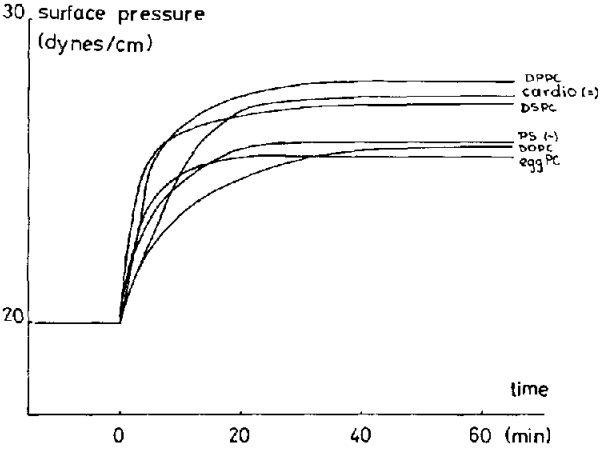

Fig.1. Interaction of TMV protein with lipid monolayers. Initial surface pressure, 20 dynes/cm. At $t = 0$, 1 mg TMV protein was injected in the subphase and the variation of surface pressure was recorded as a function of time. Subphase: 50 ml of 0.01 M Tris–HCl, 0.15 M NaCl, pH 7 buffer. Temperature was maintained at 20°C.

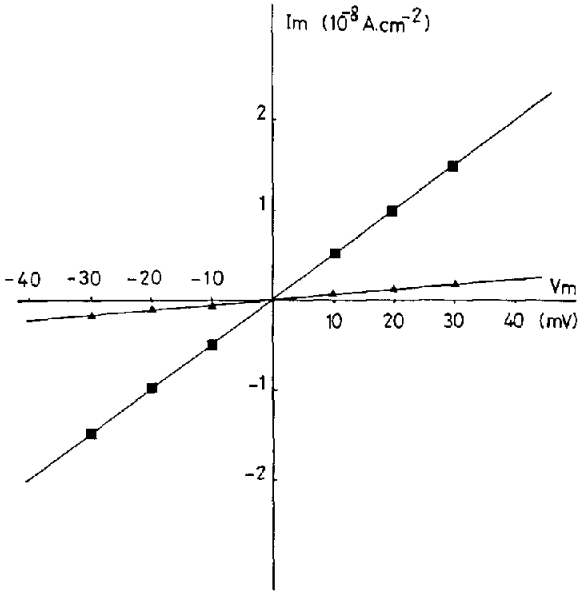

Fig.2. Current voltage characteristics of GMO planar bilayer membranes alone (▲) and in presence of TMV protein (■). The aqueous phase bathing the bilayers was a 0.01 M Tris–HCl, 0.15 M NaCl, pH 7 buffer. Final TMV protein concentration: 0.5 mg/10 ml. Temperature was maintained at 20° C.

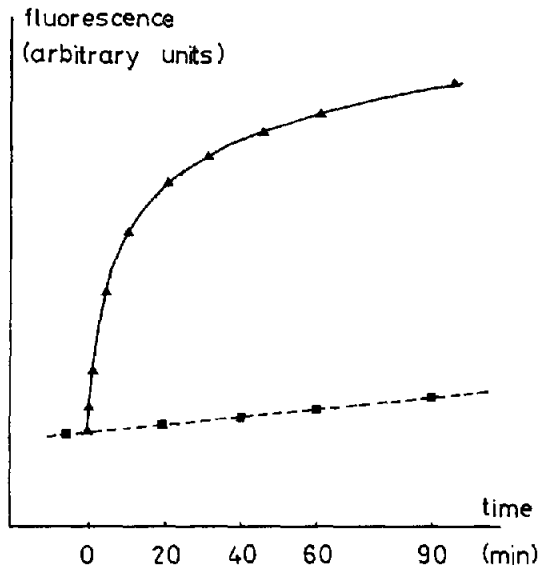

Fig.3. Carboxyfluorescein (CF) release. Fluorescence increase as a function of time for small unilamellar liposomes (SUV) of DPPC alone (■) and in the presence of TMV protein (▲). CF was entrapped in SUV at 200 mM. The final lipid concentration of the solution was 4 mg/3 ml and that of protein was 0.5 mg/4 mg lipid. The experiments were carried out in a 0.01 M Tris–HCl, 0.15 M NaCl, pH 7 buffer at 20° C.

proteins bearing hydrophobic sites [15], or with proteins that acquire a hydrophobic site after interaction with a receptor included in the bilayer [6,7].

3.3. *Permeability to carboxyfluorescein (CF)*

The presence of TMV protein alters the release of CF which has been sequestered inside small unilamellar liposomes. Fig.3 shows the fluorescence increase of CF as a function of time for liposomes of DPPC. In agreement with the monolayer penetration experiments, an important release is immediately observed in the presence of the TMV protein indicating a very fast interaction with the lipid bilayer.

In conclusion, our experiments demonstrate that TMV protein interacts hydrophobically with model membranes and perturbs their permeability properties. It is known that the forces which maintain the protein monomers together in the entire virus are essentially hydrophobic [2]. For this reason, the extremity of the cylindrical virus with its hydrophobic sites exposed could be a privileged region for attachment to the host membrane. Data obtained by electron microscopy [16] show precisely that there occurs an end-on attachment of rod-shaped plant viruses to plant cell walls. The attachment step is immediately followed by a virus degradative phase where the rod shortens down. Moreover, experiments on protoplasts revealed that TMV does not spontaneously infect the cells but that agents such as polyethylene glycol are needed to promote the infection [17]. Polyethylene glycol is an agent capable to destabilize the lipid membrane [18] and to induce cell fusion [19].

This work and the data in [20] demonstrate that TMV protein, an essential component of the plant virus, possesses these destabilizing and fusogenic properties [20]. How the isolated TMV protein determines the cell infection process remains to be investigated.

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