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INTERACTION OF INFLUENZA VIRUS PROTEINS WITH PLANAR LIPID BILAYERS: A MODEL FOR VIRION ASSEMBLY

Igor G. Kharitonenkov[1], Salam ElKaradahgi[2], Doris J. Bucher[3], Juri A. Zakomirdin[1], and Vsevolod A. Tverdislov[2]

1. Ivanovskii Institute of Virology, Gamaleya St. 16, Moscow, USSR.
2. Physical Department of Moscow State University, USSR.
3. Department of Microbiology, Mount Sinai School of Medicine of the City University of New York, N.Y., USA.

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SUMMARY

Changes in conductance of oxidized cholesterol planar lipid bilayers were measured following the incorporation of isolated surface glycoproteins; hemagglutinin and neuraminidase (HA+NA) or matrix protein (M-protein) of influenza virus. The conductance dependence of the lipid bilayers on the HA+NA or M-protein concentrations indicates different mechanisms of interaction of these viral proteins with the lipid bilayer. Adsorption of M-protein molecules on one side of the lipid bilayer affects the character of the HA+NA interaction with the opposite side. Planar lipid bilayers can be a useful model for investigation of the assembly of influenza virions and other enveloped viruses.

Assembly of influenza virus on the infected cell surface may involve "recognition" of patches of surface glycoproteins by matrix protein (M-protein) (1). The surface glycoproteins, hemagglutinin(HA) and neuraminidase(NA), are located on the outer surface of the virus particle and interact with the lipid bilayer through hydrophobic regions. M-protein forms a continuous layer covering the inner surface of the viral membrane (2). The molecular events of the interaction of the surface glycoproteins and M-protein with the lipid bilayer are unknown. M-protein has been reported to be closely associated with the viral lipids and is assumed to change the molecular organization of the lipid bilayer (3-5). On the other hand, Landsberger et al.(6) and Kharitonenkov et al.(7) using the spin probe method showed that HA and NA do not penetrate deeply into the lipid bilayer of influenza virus.

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"Recognition" between the surface of glycoproteins and M-protein can be explained on the basis of direct or indirect interaction between viral protein molecules. Wiley and associates (8) were unable to show close steric association or direct interaction of surface proteins with inner proteins in the influenza virus particle through the use of cross-linking reagents. Individual viral proteins and lipid bilayers may serve as a model for assembly of enveloped virions in the infected cell; experiments involving isolated viral proteins and liposomes have been reported (9-13).

The purpose of this communication is to report experimental results on the interaction of surface glycoproteins and M-protein isolated from influenza virions with planar lipid bilayer membranes. We used the method developed by Mueller and coworkers (14) which consists of incorporation of viral proteins into the lipid bilayer separating two adjacent compartments and measuring ionic current through this membrane. This method is widely used for the investigation of protein-lipid interaction (15-17).

MATERIALS AND METHODS

Virus. The MRC-11 strain of influenza H3N2 virus (the biological recombinant of A_2/Port Chalmers/I/73 and A/PR/8/34) was grown in 9-day-old embryonated eggs and purified as previously described (6). Purity was monitored by SDS-polyacrylamide gel electrophoresis (18) and electron microscopy.

Surface glycoproteins of influenza virus were obtained as follows: virus suspension (2.5 mg/ml) in distilled water was incubated 10 minutes at 37°C with 4% octylglucoside (Sigma). This treatment solubilizes both hemagglutinin and neuraminidase (10). Viral cores were separated from surface glycoproteins by centrifugation in the SW-65 rotor (centrifuge L5-65, 20 min, 40,000 rpm, t=4°C). Detergent was removed by exhaustive dialysis against distilled water.

Matrix protein of influenza virus was isolated by preparative electrophoresis of total viral proteins (after disruption of virus in 1% SDS) in cylindrical polyacrylamide gels (1.5 x 10cm) in 0.1% SDS without reducing agents (19). Detergent was removed by continuous ultrafiltration as described earlier (13).

Purity of viral protein preparations was checked by SDS-polyacrylamide gel electrophoresis. Protein concentration was determined by the Lowry assay using bovine serum albumin (Koch Light) as a standard (20).

Planar lipid bilayers were formed from oxidized cholesterol (Sigma) in n-decane (Sigma) on a 1 mm diameter aperture in a teflon partition separating two stirring thermostated compartments (14,21) containing 0.1 M NaCl+0.1M HCl+5 mM Tris-HCl, pH 7.4, buffer. The membrane formation was observed under reflected light with a low power microscope. Electrical measurements were made with Ag/AgCl electrodes connected with both compartments via 3M KCl-agar bridges in series with Pasteur pipettes containing agar in 0.1 M NaCl. A high-impedance operational amplifier circuit was used to clamp the membrane voltage (15 mv) and monitor the transmembrane current. Current vs. time plots were made on an X-Y recorder. The experiments were carried out at 25°C.

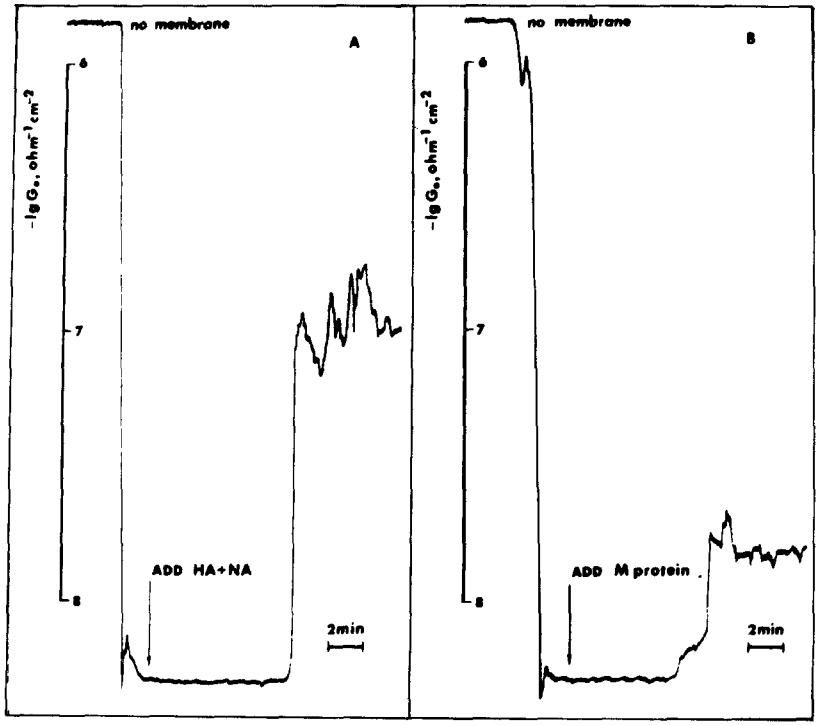

Fig. 1. Addition of surface glycoproteins HA+NA and M-protein of influenza virus to a cholesterol lipid bilayer. (A). 6.6×10^{-3} µg/ml of HA+NA was added to the inner compartment of the electric cell. (B). 1.8×10^{-3} µg/ml of M-protein was added to the inner compartment of the electric cell.

RESULTS AND DISCUSSION

The specific conductance of unmodified cholesterol lipid bilayers is very small, generally less than 10^{-8} ohm^{-1}cm^{-2}. When the surface glycoproteins or M-protein were added to the solution bathing the membrane, the conductance increased dramatically (Fig.1). This effect can be explained by incorporation of viral proteins into the lipid bilayer with formation of conducting deformations or ionic channels. The log time (about 8 minutes) probably depends on the rate of diffusion of the proteins during their incorporation into the membrane.

The curve of increasing conductance after addition of the surface glycoprotein mixture in the bathing solution (Fig.2A, lines 1 and 2) is very similar to the typical curve for interaction of hydrophobic proteins with the

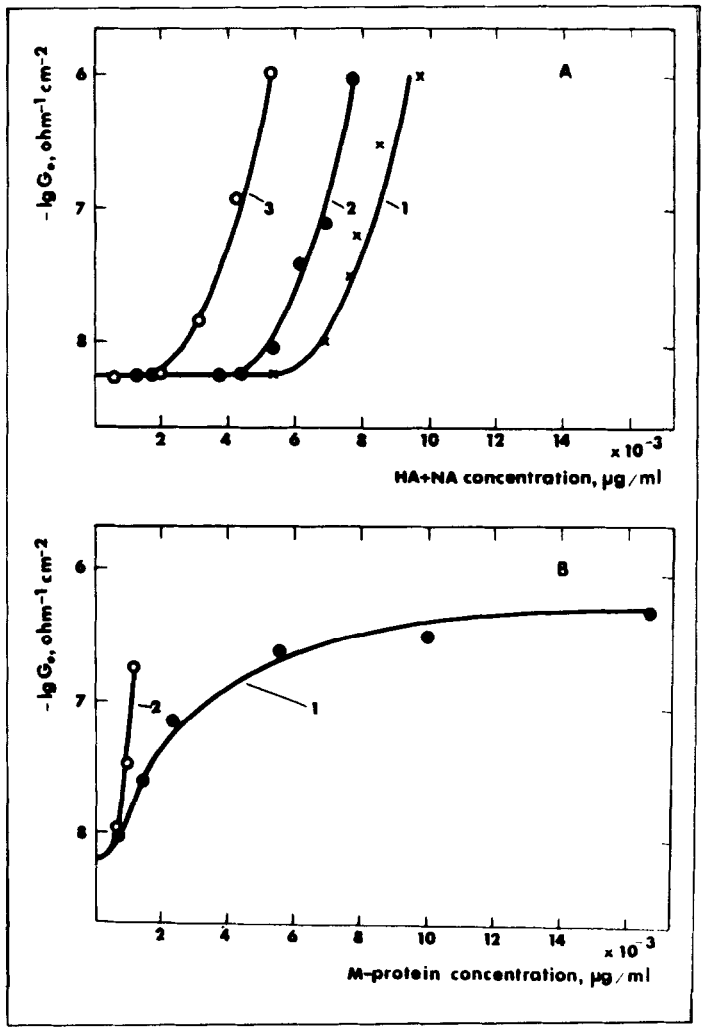

Fig. 2.A. Dependence of logarithm of ionic conductivity of the lipid bilayer in the presence of influenza virus surface glycoproteins in the inner compartment (curve 1) and in the inner and outer compartments (curve 2) of the electric cell. Curve 3 corresponds to the case when 0.48×10^{-3} μg/ml of M-protein was added to the outer compartment and HA+NA aliquots were placed in the inner compartment of an electric cell.

B. Dependence of logarithm of ionic conductivity of the lipid bilayer in the presence of influenza virus M-protein in the inner and outer compartments of electric cell (curve 1). Curve 2 corresponds to the case when 0.36×10^{-3} μg/ml of HA+NA was added to the outer compartment and M-protein aliquots were placed into inner compartment of electric cell.

lipid bilayer (22). Examination of the conductance-concentration curve reveals a region (0-4×10^{-3} μg/ml) where the surface glycoproteins did not

change the electrical properties of the membrane as well as a region where conductance dramatically increased. This type of conductance curve suggests that aggregation of viral surface glycoproteins is necessary for the appearance of conductance defects in the membrane. The glycoproteins can aggregate by lateral diffusion after incorporation in the lipid bilayer. This is in agreement with the idea of formation of clusters of viral glycoproteins on the infected cell membrane during assembly of myxoviruses (24).

The dependence of ionic conductance of the membrane on the concentration of M-protein has a Langmuir character (Fig.2B, curve 1). This type of curve suggests that M-protein molecules incorporate into the lipid bilayer in aggregate form and that the aggregates also exist in the bathing solution. This assumption is likely since M-protein of influenza virus has been shown to be very hydrophobic (1) and at high concentrations can form multimeric structures on the surface of liposomes (13).

The role of M-protein molecules preincorporated into planar lipid bilayers on the interaction of the surface glycoproteins (HA+NA) with the lipid bilayer was also examined. M-protein was added to the inner compartment of the electric cell at a concentration (0.48×10^{-3} µg/ml) too small to change the ionic conductance.

Subsequently, different amounts of surface glycoproteins were added to the outer compartment and the conductance of the planar lipid bilayer monitored. The results of this experiment is shown in Fig.2A (curve 3). In the presence of M-protein, the membrane conductance shows an increase at a concentration of HA+NA which is 2-3 fold lower than in the absence of matrix protein. This observation may be the result of several different mechanisms. [1] M-protein molecules are adsorbed on the membrane, alter the structure of the lipid bilayer and thus influence the lateral movement of viral surface glycoproteins with formation of clusters of HA+NA on the opposite side of the planar membrane. This view is supported by the decrease in fluidity of the lipid bilayer observed after M-protein incorporation (3,4,24). [2] M-protein

(or viral surface glycoproteins) may influence the lipid composition of the inner and outer surface of the lipid bilayer. The change of lipid asymmetry could be responsible for the ability of viral glycoproteins to interfere with the electrical properties of lipid membranes. [3] An alternative explanation of our results may be based on the direct interaction of surface glycoproteins and M-protein molecules on their penetration into the lipid bilayer. The analogous experiments on the influence of viral surface glycoproteins on the adsorption of M-protein were also performed and results are shown in Fig.2B (curve 2).

The main purpose of this work is to show the simplicity of the planar lipid bilayer method for investigation of the interaction of viral proteins with lipid membranes. This method may be helpful for direct measurement of the mechanism of interaction between an internal protein (M) and surface glycoprotein(s) during assembly of enveloped viruses on the lipid membranes of the infected cell. The possibility of using this method together with the fluorescent probe technique (25) increases the usefulness of the approach for investigation of the influence of viral proteins on the structure of the lipid bilayer.

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