Fatty Acid Covalently Linked in Vivo to the Glycoprotein of Vesicular Stomatitis Virus*

(Received for publication, December 1, 1980, and in revised form, January 20, 1981)

William A. Petri, Jr.,+ Ranajit Pal, Yechezkel Barenholz, and Robert R. Wagner$  

From the Department of Microbiology, The University of Virginia School of Medicine, Charlottesville, Virginia 22908 and the Department of Biochemistry, School of Medicine, Hebrew University, Jerusalem, Israel

The covalently-attached fatty acid of the membrane glycoprotein (G) of vesicular stomatitis virus was fluo-  
erescently labeled biologically by isolating vesicular stomatitis virus from infected baby hamster kidney  
clone 21 cells that had been grown in the presence of 16(9-anthroyloxy)palmitate. The fluorescent labeling  
was specific for the G protein; the other viral membrane protein, the matrix (M) protein, was not labeled. Steady state fluorescence anisotropy of the 16(9-anthroyloxy)palmitate-labeled G protein reconstituted into dipalmitoylphosphatidylcholine vesicles indicated that the fatty acid attached to G protein is located in a dipalmitoylphosphatidylcholine domain that does not undergo the gel to liquid-crystalline phase transition.

Vesicular stomatitis virus is unique among the enveloped viruses in that its membrane contains only a single intrinsic glycoprotein (1). The G protein forms the spikes seen on the viral surface, has a molecular weight of 69,000 (1), and is anchored in the viral membrane by a thermolysin-resistant tail fragment (2, 3). The nucleotide sequence of a cDNA clone representing the 3' end of G mRNA reveals a region of the G protein 29 amino acids from the COOH terminus that contains 20 consecutive hydrophobic amino acids, by which the G protein presumably spans the viral membrane (4).

The G protein has one to two molecules of covalently bound fatty acid which are resistant to extraction with organic solvents and boiling in sodium dodecyl sulfate but are released by mild alkali treatment of the G protein (5). The site of fatty acid acylation has tentatively been identified as a serine residue (5). We have recently shown that the covalently attached fatty acid is located solely in the tail fragment of the G protein left in the viral membrane after thermolysin digestion (6). The 66-amino-acid thermolysin-resistant tail fragment contains near its center the 20-amino-acid hydrophobic domain that is thought to span the membrane (4). There are 3 serine residues in the hydrophobic domain, but there are also 3 serine and 2 threonine residues on either side of this domain that could be possible sites of fatty acid acylation (4).

In the present studies, we demonstrate that the fatty acid covalently attached to the G protein can be made fluorescent by isolating VS virus from infected BHK-21 cells that were grown in the presence of 16(9-anthroyloxy)palmitate. The environment of the fatty acid attached to the G protein reconstituted in dipalmitoylphosphatidylcholine vesicles was then characterized by steady state fluorescence depolarization.

Baby hamster kidney clone 21 cell monolayers were trypan-  
sized and passaged at a 1:5 dilution directly into Dulbecco’s modified Eagle’s medium (GIBCO, Grand Island, NY) containing 10% salt-fractionated calf serum (7) and 40 μg/ml of 16(9-anthroyloxy)palmitate (Molecular Probes, Plano, TX). The 16(9-anthroyloxy)palmitate was added to the medium in ethanol so that the final ethanol concentration of the medium was less than 0.5%. After 24 to 48 h of growth, the confluent cell monolayers were infected with plaque-purified VS virus of the Indiana serotype at a multiplicity of 0.1–1 plaque-forming unit/cell at 31 °C in medium containing 40 μg/ml of 16(9-anthroyloxy)palmitate. The virus was harvested from the tissue culture medium 18 to 21 h postinfection and purified by differential, rate zonal, and equilibrium centrifugation (8).

The VS virus glycoproteins and lipids were released from the virus by incubation with 30 mM octyl glucoside (octyl-β-D-glucopyranoside) (Calbiochem, La Jolla, CA) in salt-free 10 mM Tris (pH 7.5) as previously described (9). The G protein was purified from the viral lipids by equilibrium centrifugation in a 15–30% sucrose gradient containing 60 mM octylglucoside, 0.5 M NaCl, and 50 mM Tris, pH 7.5 (9). The M protein was purified from Triton X-100-disrupted virions on a phosphocellulose column (10). Phosphatidylcholine labeled with 16(9-anthroyloxy)palmitate was isolated from the infected BHK-21 cells by thin layer chromatography (7). Fluorescence excitation and emission spectra of the purified M and G proteins were performed on an SLM fluorescence spectrophotometer (Urbana, IL).

The G protein purified free from viral lipids was reconstituted with DPPC (Avanti, Birmingham, AL) at a 1:1000 protein to lipid molar ratio by octylglucoside dialysis. Phosphatidylcholine-G protein vesicles formed by octylglucoside dialysis contain glycoprotein spikes protruding in the same external orientation as in the VS virus membrane (9). Proteolytic digestion of these vesicles with thermolysin leaves a hydrophobic glycoprotein tail fragment in the vesicle membrane that migrates identically on polyacrylamide gels with the tail fragment from VS virus treated with thermolysin (9). The use of 16(9-anthroyloxy)palmitate as a probe of DPPC liposomes has been described by Cadenehead et al. (11). Of the anthroyloxy fatty acids, 16(9-anthroyloxy)palmitate has been shown to have the least perturbing effect on the DPPC bilayer as judged by transition temperature lowering (11). Its fluorescence anisotropy is sensitive to the gel to liquid-crystalline phase transition of DPPC with the fluorescence anisotropy of 16(9-anthroyloxy)palmitate higher in the gel state than in the liquid-crystalline state of DPPC (11). Steady state fluorescence depolarization of the 16(9-anthroyloxy)palmitate-labeled G protein and phosphatidylcholine was performed on a
modified Perkin-Elmer MPF3 fluorescence spectrophotometer with polarizers in the excitation and emission beams (12, 13). The samples for the fluorescence depolarization were excited at 285 nm and the fluorescence emission was measured at 455 nm, using the MPF3 430-nm cutoff filter to reduce scattered light. The fluorescence anisotropy was calculated from the fluorescence depolarization as previously described (12).

Fig. 1 shows the fluorescence spectra of G and M proteins from VS virus isolated from infected BHK-21 cells grown in the presence of 16(9-anthroyloxy)palmitate. The M protein had a peak of tryptophan absorption at 285 nm and the G protein at 292 nm (Fig. 1A). The anthroyloxy excitation and emission spectra recorded at about a 10-fold greater sensitivity than the tryptophan spectra demonstrated that only the G protein contained the 16(9-anthroyloxy)palmitate, with characteristic excitation peaks of 348, 365, and 385 nm (Fig. 1B) and a broad emission peak at 441 nm (Fig. 1C). The peaks present in the G and M spectra at 394 nm (Fig. 1B) and 414 nm (Fig. 1C) are Raman lines, and not due to fluorescence. It was not surprising that only the G protein was labeled in vivo with the 16(9-anthroyloxy)palmitate, since only the G protein contains covalently attached fatty acid, 80% of which is palmitic acid (5). The anthroyloxy fluorescence in the G protein was not due to contaminating viral lipids as the anthroyloxy-palmitate remained associated with the G protein in a sucrose gradient containing octylglucoside and was not extractable from the protein with hexane (data not shown). The G protein purified from VS virus isolated from BHK-21 cells grown in the presence of anthracene-9-propionic acid (Molecular Probes) or anthracene-9-decanoic acid (a gift from A. Dagan, Hebrew University School of Medicine, Jerusalem, Israel) was not fluorescently labeled, suggesting that only a longer chain fluorescent fatty acid such as 16(9-anthroyloxy)palmitate could be covalently linked in vivo to the virus glycoprotein (data not shown).

Fig. 2 depicts the fluorescence anisotropy as a function of temperature of 16(9-anthroyloxy)palmitate-labeled G protein and 16(9-anthroyloxy)palmitate-labeled phosphatidylcholine in unlabeled G protein-DPPC vesicles. The fluorescence anisotropy of the anthroyloxy-G protein solubilized in octylglucoside was very low and almost temperature-independent. The anthroyloxy-G protein reconstituted at a level of 0.1 mol % into DPPC vesicles and a broad emission peak at 441 nm (Fig. 1C). The peaks present in the G and M spectra at 394 nm (Fig. 1B) and 414 nm (Fig. 1C) are Raman lines, and not due to fluorescence. It was not surprising that only the G protein was labeled in vivo with the 16(9-anthroyloxy)palmitate, since only the G protein contains covalently attached fatty acid, 80% of which is palmitic acid (5). The anthroyloxy fluorescence in the G protein was not due to contaminating viral lipids as the anthroyloxy-palmitate remained associated with the G protein in a sucrose gradient containing octylglucoside and was not extractable from the protein with hexane (data not shown). The G protein purified from VS virus isolated from BHK-21 cells grown in the presence of anthracene-9-propionic acid (Molecular Probes) or anthracene-9-decanoic acid (a gift from A. Dagan, Hebrew University School of Medicine, Jerusalem, Israel) was not fluorescently labeled, suggesting that only a longer chain fluorescent fatty acid such as 16(9-anthroyloxy)palmitate could be covalently linked in vivo to the virus glycoprotein (data not shown).

Fig. 2. Fluorescence anisotropy of 16(9-anthroyloxy)palmitate-labeled G protein reconstituted in dipalmitoylphosphatidylcholine vesicles. The 16(9-anthroyloxy)palmitate-labeled G (anthroyloxy-G) protein and 16(9-anthroyloxy)palmitate-labeled phosphatidylcholine (anthroyloxyphosphatidylcholine) were isolated and individually reconstituted into DPPC vesicles as described in the text. The fluorescence anisotropies were measured as a function of temperature on a modified Perkin-Elmer MPF3 fluorescence spectrophotometer. ◊, anthroyloxy-G protein in octylglucoside; □, anthroyloxy-G protein reconstituted at a level of 0.1 mol % in DPPC vesicles; ○, unlabeled G protein reconstituted at a level of 0.1 mol % in DPPC vesicles containing 1 mol % anthroyloxyphosphatidylcholine.

Fig. 1. Fluorescence spectra of G and M proteins of VS virus isolated from infected BHK-21 cells grown in the presence of 16(9-anthroyloxy)palmitate. VS virus was purified from the infected cell culture medium and the G and M proteins were isolated and purified as described in the text. A, tryptophan excitation spectra (emission wavelength = 340 nm); B, anthroyloxy excitation spectra (emission wavelength = 455 nm); and C, anthroyloxy emission spectra (excitation wavelength = 364 nm) of the G and M proteins. The anthroyloxy excitation and emission spectra were recorded at approximately 10-fold higher sensitivity than was the tryptophan spectra.
the DPPC vesicles containing 0.1 mol % of unlabeled G protein detected a sharp DPPC gel to liquid-crystalline phase transition (Fig. 2). That the 16(9-anthroyloxy)palmitate-labeled phosphatidylcholine did detect the DPPC phase transition also indicates that the lack of a detectable DPPC phase transition, when the anthroyloxy fatty acid was attached to G protein, is not due simply to a perturbation of the bilayer by the bulky anthroyloxy group.

The fluorescence anisotropy of the 16(9-anthroyloxy)palmitate-labeled G protein reconstituted in DPPC vesicles was higher than that of the G protein solubilized in octylglucoside (Fig. 2). This difference in anisotropies was likely a reflection of both a decreased rotational motion of the reconstituted G protein and a restriction of the protein-independent motion of the covalently bound 16(9-anthroyloxy)palmitate upon reconstitution of the G protein into the DPPC bilayer. While the fluorescent fatty acid attached to G protein did not detect the DPPC phase transition, the probe's anisotropy became markedly temperature-dependent upon reconstitution (Fig. 2), demonstrating the effect of the DPPC on the mobility of the covalently attached fatty acid.

The ability to metabolically label the G protein of VS virus with a fluorescent fatty acid provides additional information about the covalently attached fatty acids that are present on many eukaryotic intrinsic membrane proteins (15) and presents a novel way of fluorescently labeling membrane proteins. The exact location of the fatty acid ester linkage in the G protein tail segment is not known, but the temperature dependence of the fluorescence anisotropy of the reconstituted G protein (Fig. 2) indicates that the fatty acid is located in the DPPC bilayer. The G protein has been shown to disrupt the DPPC phase transition, decreasing the enthalpy change and transition temperatures as measured by differential scanning calorimetry (14). That the 16(9-anthroyloxy)palmitate attached to G protein did not detect the DPPC phase transition (Fig. 2) demonstrates that the covalently attached fatty acid is located in a domain of DPPC that is perturbed by the G protein so as to remove the DPPC from the phase transition.

Acknowledgments—We are grateful to Thomas Markello for recording the fluorescence spectra and to Jack Zakowski for isolating the M protein.

REFERENCES